DANMAP 2022

Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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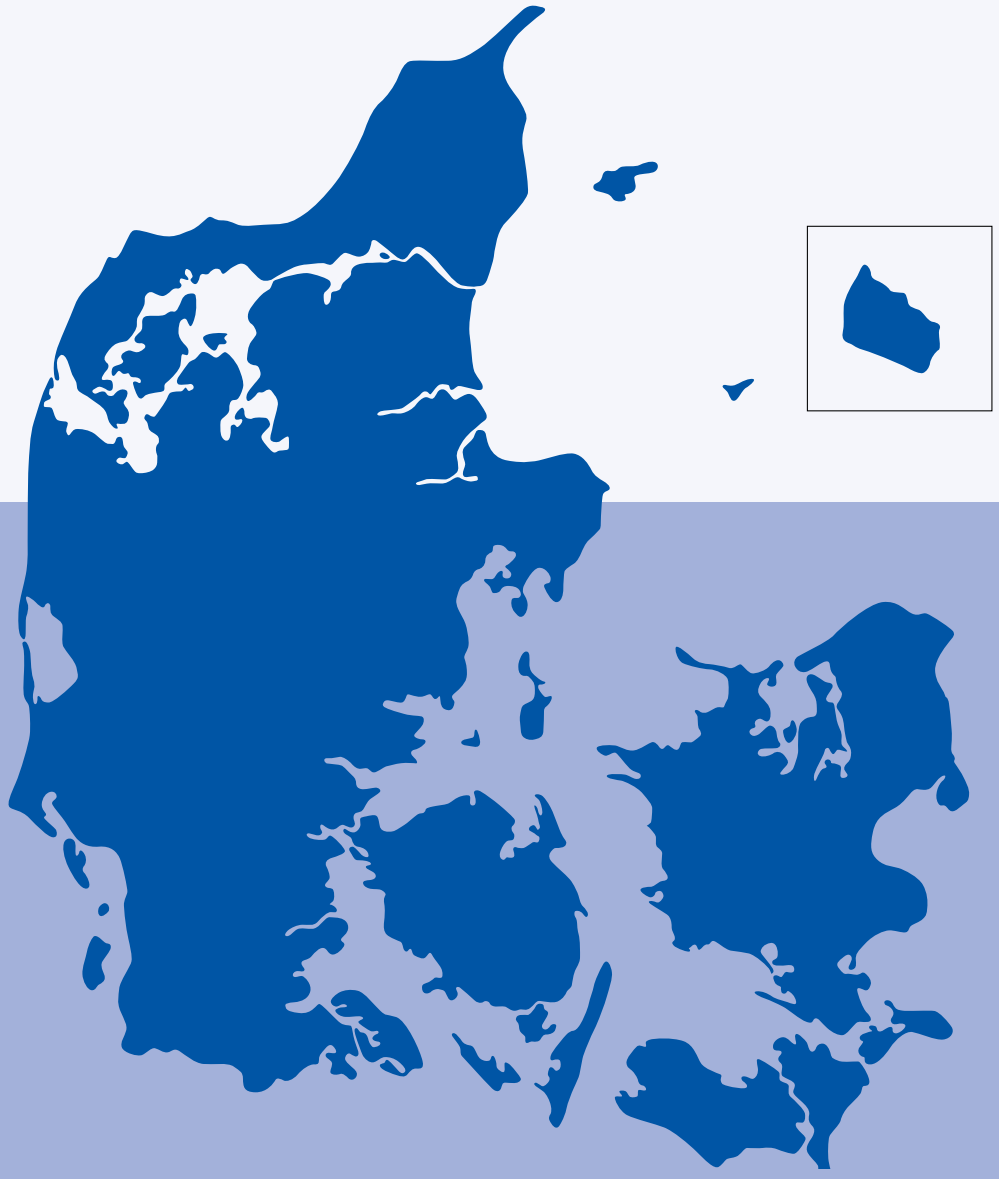

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DANMAP 2022

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DANMAP 2022

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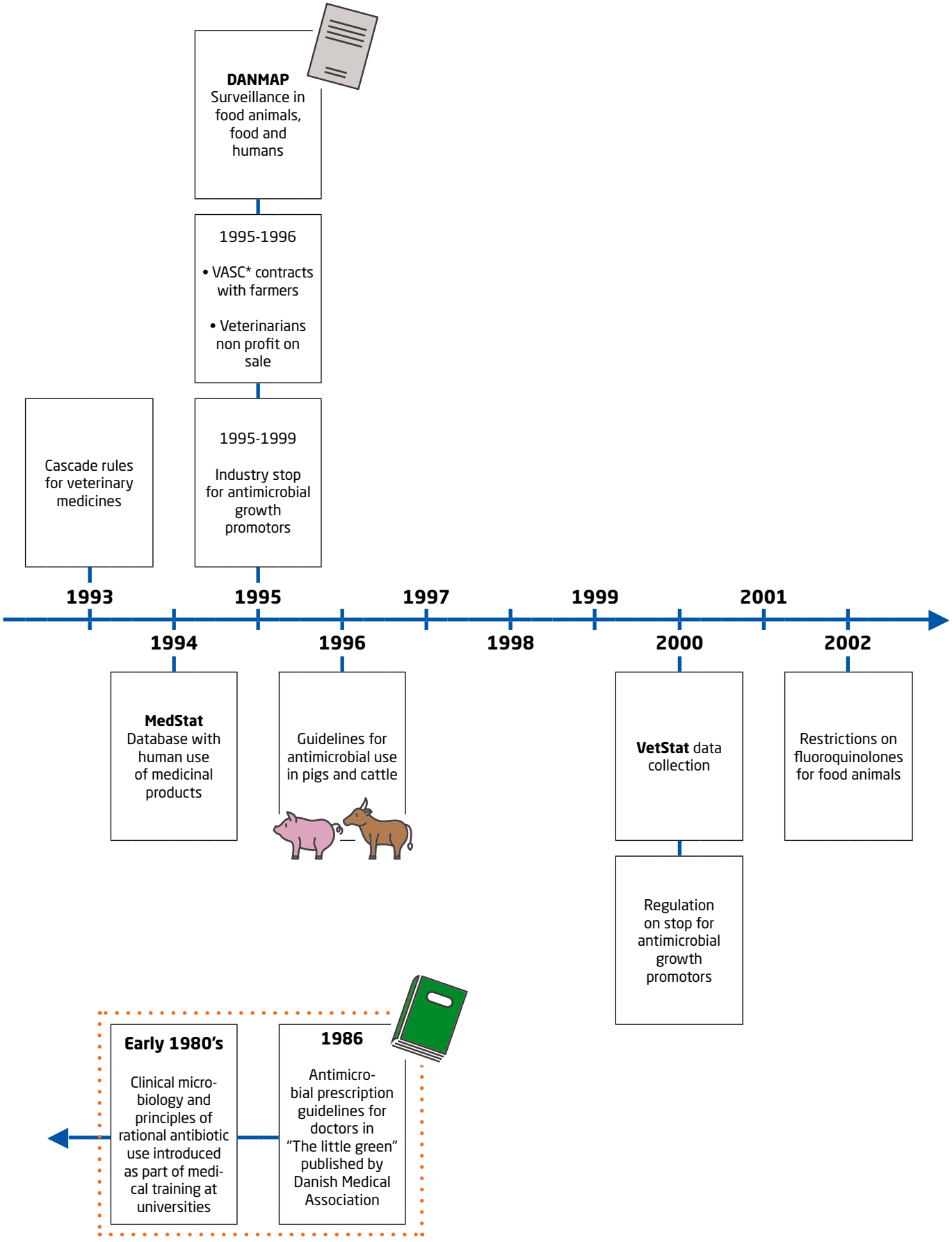
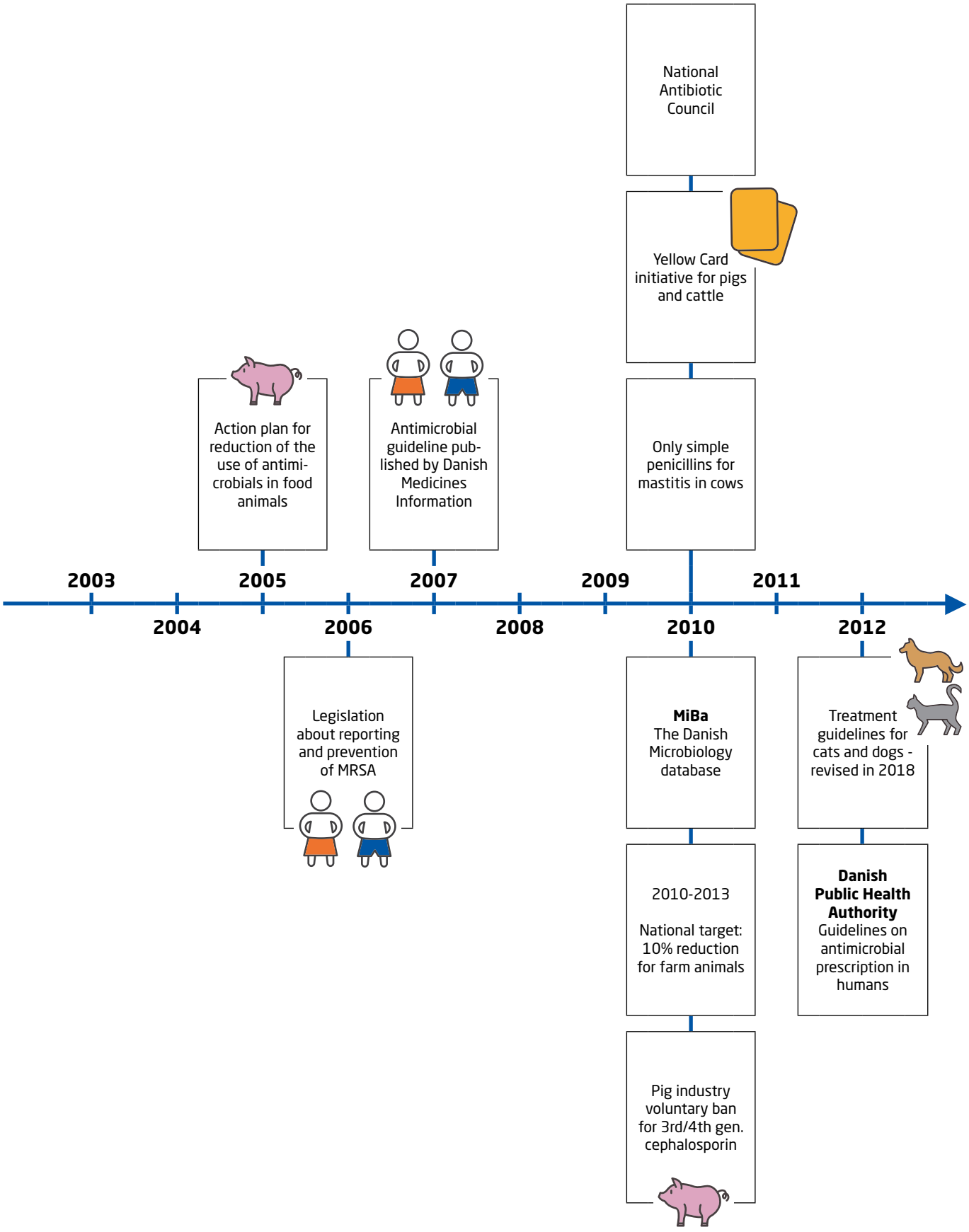
Timeline with initiatives for the prevention and control of AMR and prudent antimicrobial use in animals and public health in Denmark



* Veterinary Advisory Service contracts


2003
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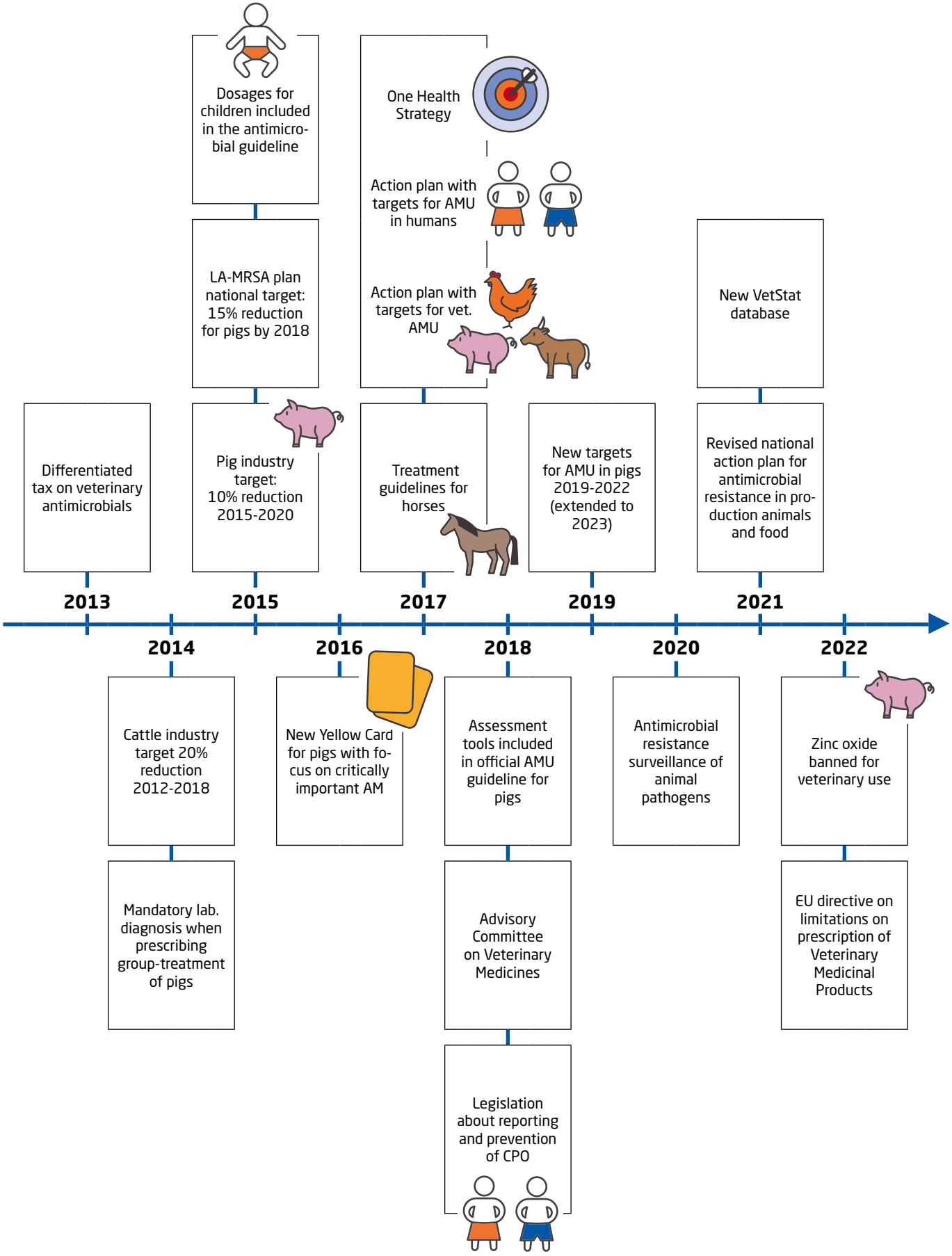
Action plan for reduction of the use of antimicrobials in food animals
Antimicrobial guideline published by Danish Medicines Information
Legislation about reporting and prevention of MRSA
National Antibiotic Council
Yellow Card initiative for pigs and cattle
Only simple penicillins for mastitis in cows
MiBa The Danish Microbiology database
2010-2013
National target: 10% reduction for farm animals
Pig industry voluntary ban for 3rd/4th gen. cephalosporin
Treatment guidelines for cats and dogs - revised in 2018
Danish Public Health Authority Guidelines on antimicrobial prescription in humans

continued ... Timeline of DANMAP



1. Editorial

One Health, collaboration and systems thinking help shift AMR surveillance to action

One Health surveillance for antimicrobial resistance (AMR) has been promoted by national and international organisations for more than a decade and is a central recommendation of the World Health Organizations´ *Global action plan on antimicrobial resistance*. Surveillance systems that integrate information about resistant microorganisms circulating in humans, animals and ecosystems with information on drivers of resistance (e.g. antimicrobial use) are needed to enhance our understanding of the complex epidemiology of AMR and, more importantly, to inform targeted actions. In recent years, we have been reflecting on the structure, operation and impacts of integrated surveillance systems for AMR based on our experience in Canada with the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), and in the context of our research with the CoEval AMR consortium to develop guidance for the evaluation of integrated surveillance systems[1][2]. Our reflections coalesced into two major themes that, we believe, are relevant for people working with or using the information produced by DANMAP.

First, the added value of a One Health approach in surveillance goes beyond the integration and joint analysis of data from different sectors. One Health should be reflected in the way individuals, teams and organisations collaborate and work together across and beyond disciplines. Strong networks that include government, industry, and non-governmental organisation perspectives are essential to mobilise knowledge produced and to generate evidence of impact for actions informed by this knowledge. Indeed, the implementation and maintenance of integrated surveillance programs such as DANMAP in Denmark, and CIPARS in Canada, has created and solidified networks of people and organisations from different disciplines and sectors that are key to using and enhancing integrated surveillance data to change practices and policies. For example, in Canada, integrating and analysing AMR data from chicken meat and humans created evidence that antimicrobial use in poultry meat production may lead to resistant bacteria in chicken meat, which can potentially be transferred to humans. Working with the Canadian poultry industry, in 2013 CIPARS was able to develop and implement on-farm AMR surveillance in the poultry sector that was critical for assessing the effectiveness of industry led changes in antimicrobial use policies and practices and their positive impact on AMR. Unfortunately, this important aspect of One Health surveillance is less often recognised or valued but should be repeatedly highlighted: without investment of time, resources and ongoing commitment from partners to build and maintain these networks, the effectiveness of integrated surveillance cannot be optimised or sustained.

Second, strong collaboration and engagement across sectors create space for inclusion of new and different perspectives in the analysis and interpretation of surveillance data. This helps provide a clearer view of the complex system from which AMR emerges and spreads, which again can lead to enhanced knowledge generation and suggestions for action that would be impossible when working in silos. For example, many systems, including CIPARS and DANMAP have long integrated the collection and analysis of data on antimicrobial use in different sectors, and some programs include further information on reasons for use and management practices. While the data may be collected, routinely integrating this information and the perspectives of those, who provided it, in the interpretation of results happens less often and chances for actions based on these crucial insights are missed. But incorporation of different perspectives and the enhanced knowledge created could position integrated AMR surveillance systems to support rethinking how our food production and health systems could be less dependent on antimicrobials, more sustainable and better able to cope with increasing threats from social and environmental crises.

We recognize that implementing transdisciplinary collaboration and systems thinking to address complex problems such as AMR requires special skills. We believe that teams and organisations working within programs such as DANMAP are pioneers of these approaches and are important models for other teams, organisations, or countries wishing to strengthen the integration of a One Health approach in their AMR surveillance strategy.

One Health surveillance is essential to face the increasing challenge of AMR and has the potential to generate outcomes that go beyond the knowledge produced from integration of data from different sources and sectors. People, teams and networks involved in AMR surveillance systems are as important as the data, as they translate data into action, and enable us to better understand the systemic changes that are required to achieve better health for all, without sacrificing one sector for the short or long-term benefit of another.

Cécile Aenishaenslin (Associate Professor, University of Montreal; CoEvalAMR network lead) and Jane Parmley (Associate Professor, University of Guelph)

[1] Rüegg SR, Antoine-Moussiaux N, Aenishaenslin C, Alban L, Bordier M, Bennani H, et al. Guidance for evaluating integrated surveillance of antimicrobial use and resistance. CABI One Health. 2022;2022:ohcs20220007.

[2] CoEval-AMR. Convergence in evaluation frameworks for integrated surveillance of antimicrobial resistance and antimicrobial use: the CoEval-AMR Network. https://coeval-amr.fp7-risksur.eu/network. Accessed 25 Jun 2020.

Acknowledgements

DANMAP is based on a strong collaboration between several institutions and on the contributions from highly skilled staff from many specialties and professions. Without their knowledge and engagement, there would be no DANMAP surveillance.

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- The staff of the Danish Veterinary and Food Administration's Laboratory, Ringsted for analysing animal and food samples and discussing the interpretation of the results
- The Danish Veterinary and Food Administration, for collecting and transmitting data on antimicrobial resistance in food and animal samples. Furthermore, we would like to thank the staff of the Food and Feed Safety Division for discussing the interpretation of the data
- The Danish Veterinary and Food Administration, for collecting and transmitting data on veterinary consumption of antimicrobial agents from VetStat, financing data collection and providing data for interpretation. Furthermore, we would like to thank the staff of the Animal Welfare and Veterinary Medicine Division for introducing the New Vetstat database and discussing interpretation of the data
- The Danish Agriculture and Food Council for collaboration regarding the estimation of live biomass of production animals
- Statistics Denmark for providing data necessary for the estimation of live biomass of poultry
- The Danish Aquaculture Producer Organisation for providing data necessary for the estimation of live biomass of fish
- Colleagues at the National Food Institute, DTU, for valuable discussions on many topics related to the report

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- The staff of the Neisseria and Streptococcus Typing Unit at SSI for providing data on samples and resistance in beta-haemolytic streptococci, *H. influenzae* and *Neisseria gonorrhoeae*
- The staff of the Foodborne Pathogens Unit at SSI for providing data on resistance in *Campylobacter* and *Salmonella* from human clinical isolates
- The staff of the Staphylococcus Laboratory at SSI for providing data on invasive staphylococcal infections as well as all MRSA
- The staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI for providing data on resistance in the referred *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and vancomycin and linezolid-resistant enterococci
- The staff at the Unit of Mycology at SSI for providing resistance data for human *Candida* and *Aspergillus*
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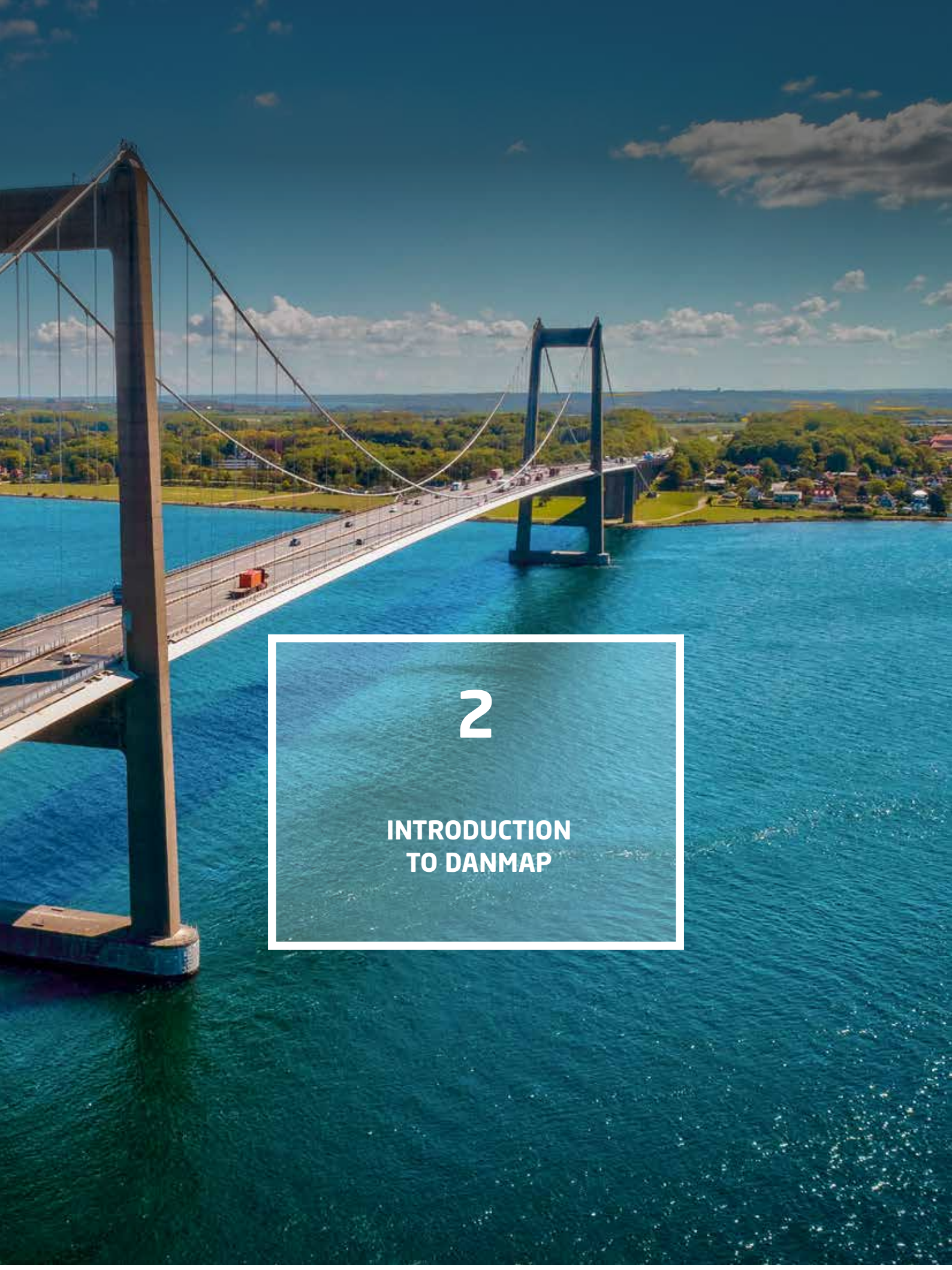
- Colleagues at the Data Integration and Analysis Secretariat at SSI
- The Danish Health Data Authority and the Register of Medicinal Products Statistics for providing data on antimicrobial consumption on healthcare activity
- All Danish hospital pharmacies for providing data on antimicrobials consumed at hospitals through special deliverances



2

INTRODUCTION TO DANMAP

2. Introduction to DANMAP

2.1 The DANMAP surveillance system

DANMAP is a surveillance system with five key objectives:

- To establish the state-of-nation in regards to the use of antimicrobial agents in food-producing animals and humans
- To carry out surveillance of the occurrence of antimicrobial resistance in bacteria isolated from food-producing animals, food of animal origin (meat) and humans
- To identify areas for further research, e.g. antimicrobial resistance transmission or possible associations between antimicrobial consumption and antimicrobial resistance
- To deliver data to veterinarians, medical doctors and other health professionals for the development of antibiotic treatment guidelines
- To act as a knowledge base for authorities, academia and politicians when performing risk assessment and management, thus supporting decision making in the prevention and control of resistant bacterial infections

Since 2021, DANMAP also provides an integrated analysis of resistance in bacteria from humans and food animals.

The monitoring programme was initially developed in 1995 by researchers, based on frequent discussions and exchange of knowledge and results from research. Since then, DANMAP has evolved into a governmentally supported programme.

However, much of the design of the programme, including participation of the human laboratories and referral of strains is based on a voluntary principle.

DANMAP surveillance relies on four equally important components: well-established and well-functioning diagnostic systems, well-designed and representative surveys, reliable registers as well as mutual trust and openness between all collaborators.

A positive effect of the regular meetings and exchange between stakeholders is that these prove helpful in other aspects, for example, by contributing to a common knowledge pool regarding laboratory methods. This ensures and contributes to continuous improvements and harmonisation of the laboratory work. Meetings across sectors and between different stakeholders also contribute to a better mutual understanding, facilitating development and work towards mutual goals.

Surveillance is a complex undertaking and DANMAP encompasses many different surveillance components and covers resistance in different populations and contexts.

Three categories of bacteria are always included in DANMAP:

- Human clinical isolates to reflect the antimicrobial resistance levels in the human population that seeks medical care
- Foodborne zoonotic bacteria along the whole farm-to-patient chain to monitor the levels of antimicrobial resistance in shared pathogens
- Indicator bacteria from healthy food-producing animals to monitor status of antimicrobial resistance in the animal reservoirs

Surveillance resistance in pathogens from sick animals was included in DANMAP in 2022. The National Food Institute at the Technical University of Denmark, DTU and the National AMR reference laboratory at Statens Serum Institut (SSI) are responsible for data interpretation and output communication mainly via the annual DANMAP report. Interpretations are independent of policy, risk management and private industries.

The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of Food, Agriculture and Fisheries. Support from the ministries has also helped build the databases and ensuring the registers, which the current surveillance system relies upon.

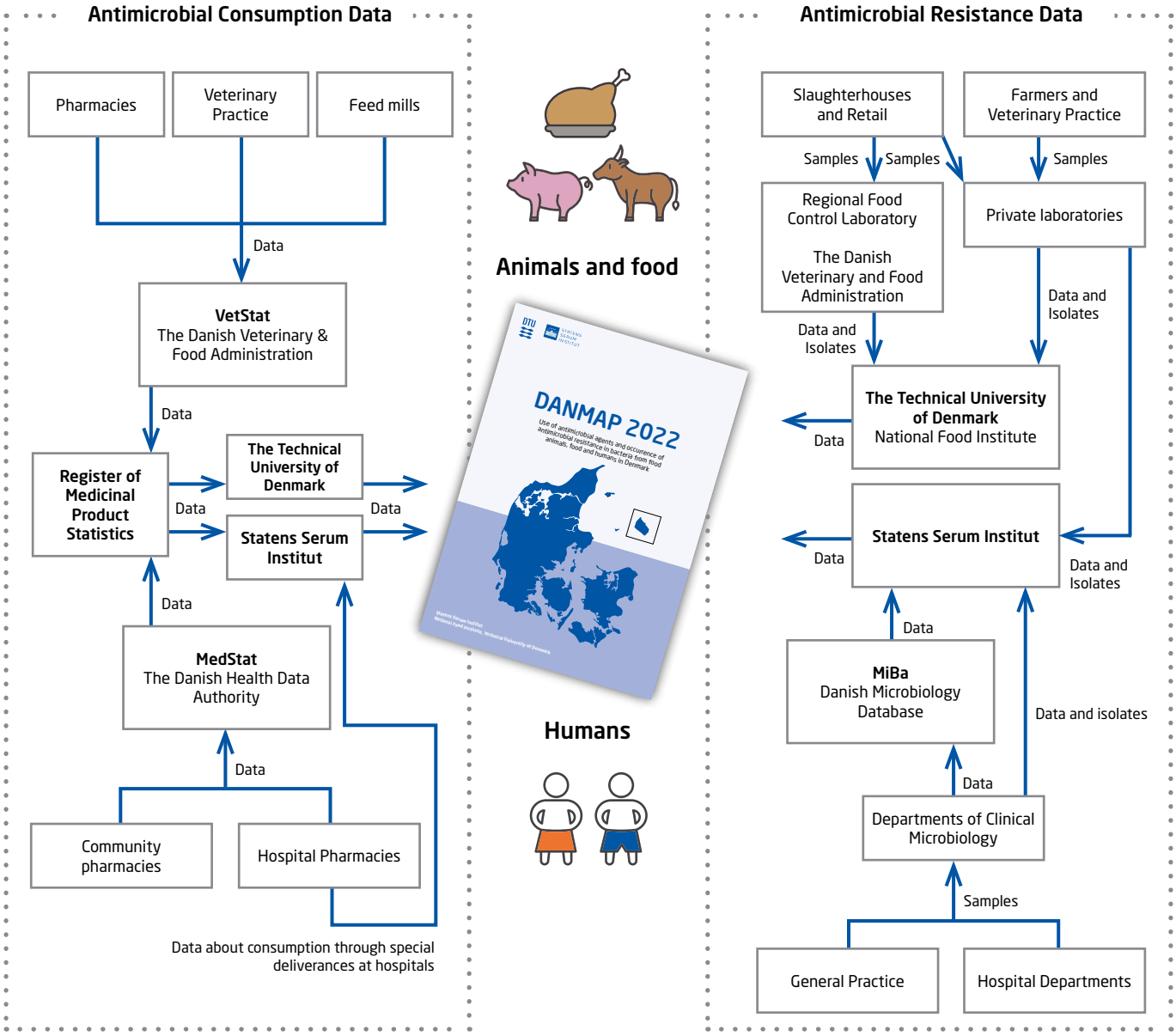
For further information on the development and history of DANMAP, please read chapter 2, "DANMAP – A 20 year perspective" in DANMAP 2015 and Chapter 1, "DANMAP - the beginning" in DANMAP 2020.

Organisation and data flow

Since 1995, a main purpose of DANMAP has been to monitor the entire chain from farm to fork to patient. The organisation and collection of DANMAP data and the interdisciplinary collaboration between sectors and organisations is presented in Figure 2.1.

The introduction of whole genome sequencing (WGS) has been a big step forward for surveillance purposes and in outbreak situations and has become routine standard in many clinical laboratories and most reference laboratories. However, phenotypical testing is still considered relevant, more feasible, cheaper and sometimes faster, especially in a clinical setting. Phenotypical testing also continues to be used in combination with WGS to describe and determine which resistance genes are relevant to look for when using molecular analysis. Furthermore, it complies with EU regulations in food and animal testing.

Figure 2.1 Organisation DANMAP regarding data and data flow DANMAP 2022



Bacterial isolates from food, food animals and humans are submitted to the Regional Food Control Laboratory or occasionally the Technical University of Denmark and Statens Serum Institut, respectively, for further phenotypic and genotypic characterisation (Figure 2.1). The choice of the methods in surveying different bacteria and infections is described in more detail in the different chapters and sections of the report.

Figure 2.2 The five Danish healthcare regions and their respective population distributions. In addition, the ten DCMs are marked by black dots. The grey dot indicates the national reference laboratories (NRL) situated at Statens Serum Institut DANMAP 2022

North Denmark Region

No. of inhabitants	591,740
No. of inhabitants/km^2	75
No. of inhabitants/GP	1,785

Central Denmark Region

No. of inhabitants	1,341,856
No. of inhabitants/km^2	103
No. of inhabitants/GP	1,636

Capital Region of Denmark

No. of inhabitants	1,867,948
No. of inhabitants/km^2	729
No. of inhabitants/GP	1,699

Region Zealand

No. of inhabitants	843,513
No. of inhabitants/km^2	117
No. of inhabitants/GP	1,671

Region of Southern Denmark

No. of inhabitants	1,228,362
No. of inhabitants/km^2	100
No. of inhabitants/GP	1,527

GP = General Practioner
DCM = Department of Clinical Microbiology
NRL = National Reference Laboratories

Data source: Statistics Denmark [www.dst.dk] and the Danish Medical Association [www.laeger.dk]

2.2 Information on demographics and health care system

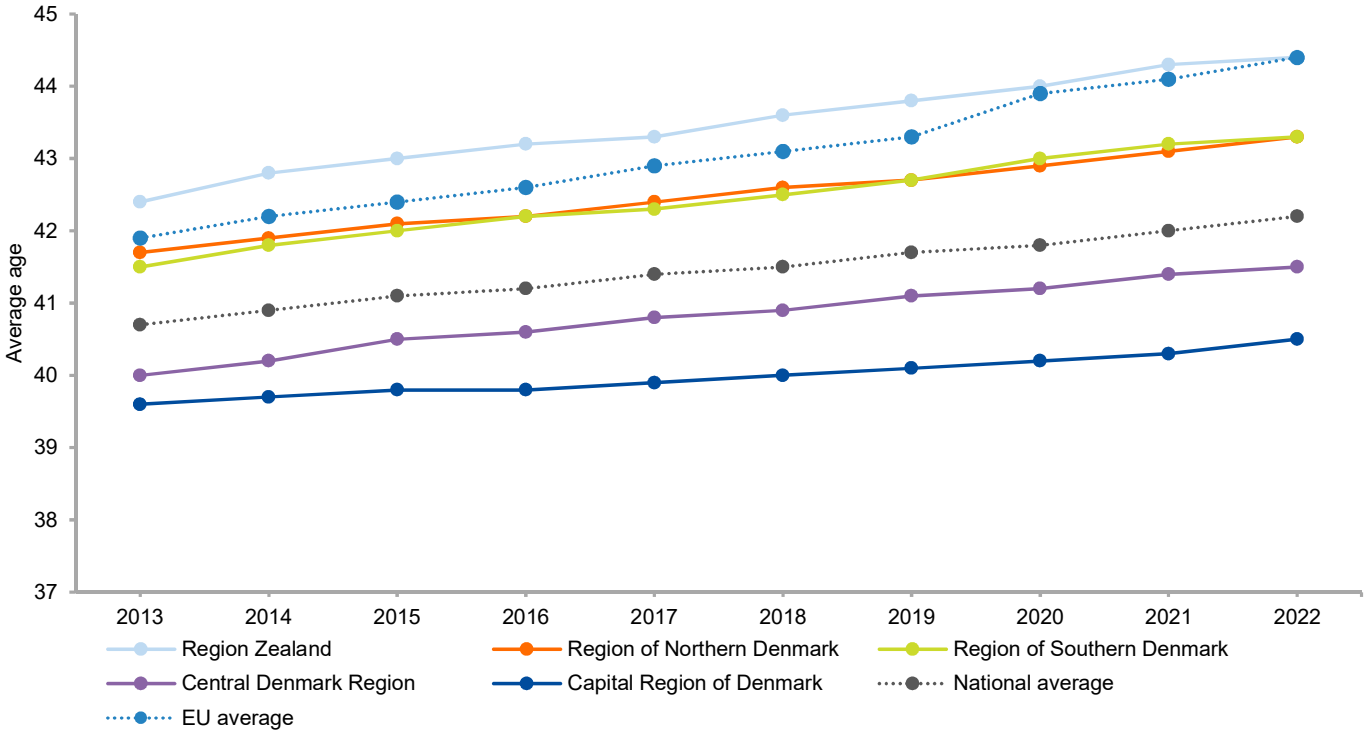
During the past 27 years, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to 5.9 million in 2022 [www.dst.dk]. Simultaneously, the average age has increased gradually. In 2022, the national average age was 44 years. The population and the respective regional distribution, in 2022, is presented in Figure 2.2, while regional differences and changes in age are presented in Figure 2.3.

In Denmark, microbiological analyses are carried out by ten hospital departments of clinical microbiology (DCMs) situated at the main regional hospitals, Figure 2.2. The analyses performed cover all samples from public hospitals and most samples from general practitioners (GPs). In addition, some GPs perform culturing of urinary samples from their patients. In the Capital Region of Denmark one private laboratory also performs additional analyses for the GPs.

The activity in general practice during 2020-2022 differed from 2019. Figure 2.4 shows the number of consultations in general practice per thousand inhabitants from 2013-2022. The number of consultations per 1,000 inhabitants was 2.5% higher in 2022 compared to 2019.

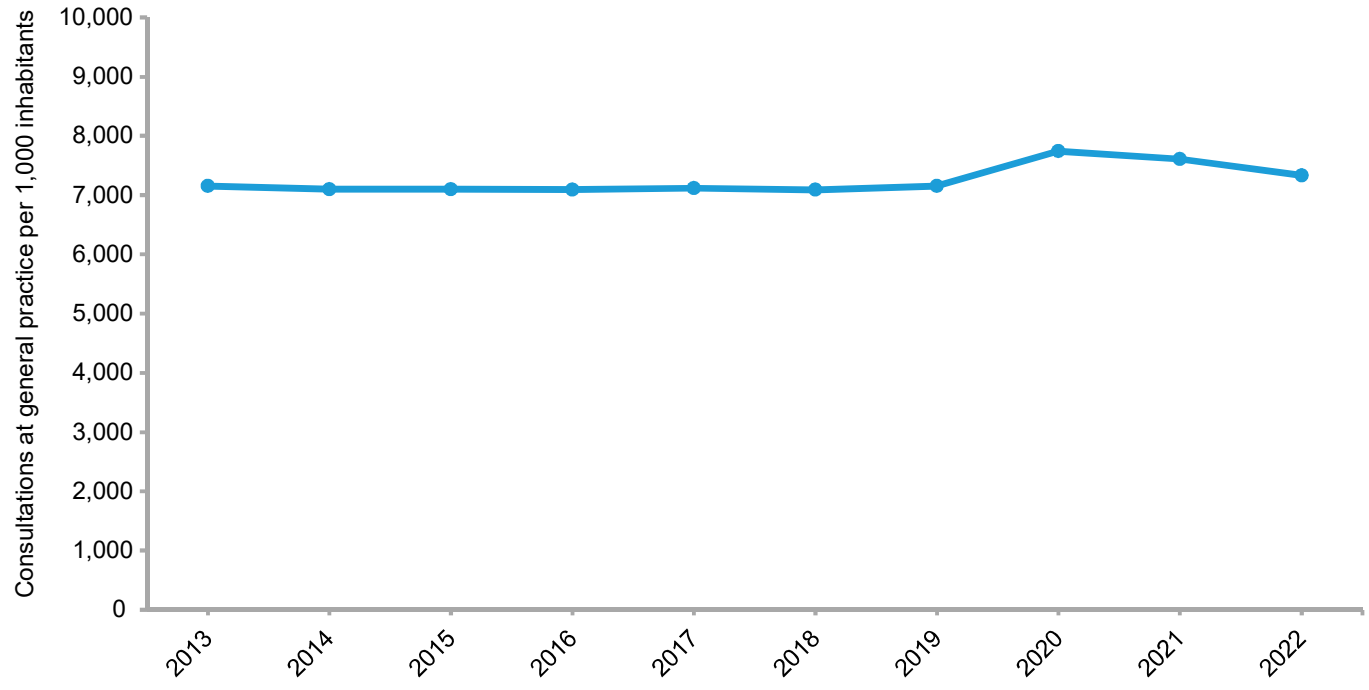
Data on regional and national health care activity at hospitals in 2013 and 2022 are presented in Table 2.1. Denmark has a very high bed occupancy rate at hospitals and can reach maximum capacity during winter time for example due to high influenza activity. In 2022, the number of admissions at Danish somatic hospitals was registered to be 701.482 and the number of bed-days was registered to be 2,976,666. From 2013-2022, the number of bed-days decreased by 20%, the number of admissions decreased by 9% whereas the Danish population grew by 5%.

Figure 2.3 Changes in average age, Denmark and EU, 2013-2022 DANMAP 2022



Data source: Statistics Denmark and Eurostat

Figure 2.4 Number of consultations per 1,000 inhabitants in general practice, Denmark, 2013-2022 DANMAP 2022



Data source: The National Health Insurance Service Registry and Register of Health Insurance Service Providers

Table 2.1 Activity at Danish hospitals, 2013 and 2022 DANMAP 2022

Region	Number of bed-days in somatic hospitals		Number of admission to somatic hospitals		Population	
	2013	2022	2013	2022	2013	2022
Capital Region of Denmark	1,306,920	970,169	255,073	230,629	1,732,068	1,867,948
Zealand Region	514,913	450,438	105,690	103,899	816,359	843,513
Region of Southern Denmark	760,382	606,951	163,450	145,567	1,201,419	1,228,362
Central Denmark Region	744,177	614,411	167,757	148,303	1,272,510	1,341,856
North Denmark Region	384,881	334,698	75,410	73,084	580,272	591,740
Denmark	3,711,273	2,976,666	767,380	701,482	5,602,628	5,873,420

Data: Activity at somatic hospitals
Data source: The National Patient Register

2.3 Information on animal population and food production system

Denmark is an agricultural country, with more than half of its area managed by the agricultural sector. Livestock is of great importance and approximately 25% of the agricultural enterprises are specialised in the production of livestock, mainly pigs, cattle and chicken. The agricultural sector contributes around 24% of the Danish export earnings [Danish Agriculture and Food Council, 2019].

The production of food-producing animals as well as the production of meat and milk are presented in Table 2.2 and 2.3.

2.4 Registered antimicrobial agents

Table 2.4 shows the antimicrobial agents registered to treat bacterial infections in humans and animals. Some of these are listed on the highest priority list of critically important antimicrobial agents for the treatment of bacterial infections in humans, according to definitions made by a working group under the World Health Organization [AGISAR, 6.revision, WHO 2019]. In order to be considered critically important, an antimicrobial must meet two criteria; 1) be the only - or one of a limited number of compounds available to treat serious human disease and 2) be used to treat infections caused by bacteria that are either possibly transmitted from non-human sources, or carry resistance genes from non-human sources.

In the newest revision from 2019, five drug classes were considered critically important and of highest priority: fluoroquinolones, 3rd, 4th and 5th generation cephalosporins, macrolides, glycopeptides and polymyxins. In addition, in Europe carbapenems are not allowed to be used in food production. In Denmark, the use of these drug classes (except macrolides) in food-producing animals has generally been low or reduced through either voluntary or legislative restrictions. See Chapter 4 for more information.

Furthermore, other antimicrobials may also be restricted due to national risk mitigation. For trends and preferred therapeutic choices in the antimicrobial treatment of humans, see Chapter 5.

Growth promoters are no longer used for animals in Denmark and are shown in parentheses in Table 2.4. Most of these influenced Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat have been used to monitor resistance towards former growth promoters.

Table 2.2 Production (1,000 heads) of food animals, Denmark DANMAP 2022

	Pigs		Cattle		Poultry	
Year	Total	Exported [a]	Slaughter cattle	Dairy cows	Broilers	Turkeys [b]
2013	28996	9864	551	582	117315	692
2014	30002	11120	556	563	115497	595
2015	30874	12133	511	561	114238	598
2016	31660	13280	540	572	120685	834
2017	31662	14173	509	570	117602	601
2018	32571	14449	533	575	122268	642
2019	31694	14897	518	567	123976	661
2020	32018	14736	500	567	120508	684
2021	32646	14092	506	564	118431	467
2022	31669	13856	493	557	114698	427

Source: Statistics Denmark (www.dst.dk). Export data for poultry from Statistics Denmark (personal communication)
a) Export of live pigs. These are included in total number of heads
b) Since 2006, more than 99% of the turkeys have been exported for slaughter

Table 2.3 Production (mill kg) of meat, milk and fish, Denmark DANMAP 2022

						Farmed fish [c]	
Year	Pork	Beef	Broiler meat [a]	Turkey meat	Milk [b]	Land based	Marine net ponds
2013	1903	140	177	8	5507	33	15
2014	1944	143	174	9	5592	32	14
2015	1954	135	172	9	5744	36	16
2016	1943	142	182	10	5892	36	12
2017	1896	135	178	7	6088	37	14
2018	1967	142	185	10	6305	38	14
2019	1864	137	187	8	6323	41	14
2020	1952	133	195	8	6394	36	11
2021	2079	134	144	6	6390	37	12
2022	1956	128	200	6	6392	-	-

Source: Statistics Denmark (www.dst.dk). Export data for poultry and average weight after slaughter from Statistics Denmark (personal communication). Production data for farmed fish from the Danish Aquaculture Producer Organisation (personal communication)
a) In 2022, a final slaughtered weight of 1.74 kg per broiler produced and 12.93 kg per turkey produced was estimated
b) conventional and organic
c) The numbers for 2022 are not final. Data are based on accounts statistics for aquaculture. The production of farmed fish includes fish transferred from one production facility to another

Table 2.4 Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark DANMAP 2022

ATC / ATCvet codes [a]	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, tetracycline, tigecycline, eravacyclin
QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, cloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	First-generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin, cefazolin
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DD	Third-generation cephalosporins incl. comb. with beta-lactamase inhibitors	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, ceftazidime/avibactam
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, imipenem and cilastatin
J01DI	Fifth-generation cephalosporins incl. comb. with beta-lactamase inhibitors		Ceftaroline fasamil, ceftolozan/tazobactam, ceftobiprol
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim, sulfamethoxasol/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin, tildiprocin	Erythromycine, roxithromycine, clarithromycine, azithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX [b]	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin, amikacin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin, pradofloxacin	Ciprofloxacin, levofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ [b]	Quinoxalines	(Carbadox, olaquindox)	
J01XA,A07AA / Not in ATCvet [b,c]	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin, dalbavancin
J01XB / QA07AA [b]	Polypeptides (incl. polymyxins)	Colistin, bacitracin	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB [c]	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin, fosfomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet [b]	Oligosaccharides	(Avilamycin)	
Not in ATCvet [b]	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes start with a Q
b) Animal growth promoters used before 1999 are listed in parentheses
c) Intestinal antiinfectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) were, for the first time, included in DANMAP 2014, since their widespread use in the treatment of *Clostridium difficile* infections makes them belong to the most used antibiotics in human infections in Denmark

3

ONE HEALTH AMR

3. One Health AMR

3.1 Introduction

One Health is defined as a unified approach to optimise the health of people, animals and the environment, under which multiple sectors must collaborate at varying levels [www.who.int].

DANMAP was established with the aim of understanding the drivers of AMR in humans and in the livestock industry and their interconnectedness.

DANMAP has always been considered an integrated research and surveillance programme, but with integration taking place at the decision-making and implementation level rather than at the level of data management. Hence, data are handled and stored in separate databases by the animal and human sectors, although interpretation of results is done in cooperation. Moreover, integration happens when discussing resistance findings in common indicators (i.e. indicator *E. coli* and enterococci), using it as a basis for recommendations and treatment guidelines among different participants of the programme. Furthermore, DANMAP supports the development and definition of strategies and action plans to reduce AMR on both sides in a collaborative manner, and by fostering the dialogue between different actors and stakeholders, across sectors.

However, there has always been the wish to get a more in depth understanding of the potential relationship between the veterinary, food-producing and human sector, concerning antimicrobial usage (AMU) and development of antimicrobial resistance (AMR). To be able to foresee if changes in one sector will have a potentially significant impact on the other sector, it requires knowledge of the possible routes of transmission and the size and speed of transfer. This again calls for further harmonised data collection, to define common denominators and units and to be able to perform cross-analyses on data from both the veterinary and human sector.

At the EU level, an attempt to perform cross-analysis has been made since 2015 in the JIACRA reports [JIACRA III, 2016-2018, ECDC, EFSA, EMA; 2021], despite the additional challenge of jointly analysing data collected in different countries. At the national level, even in a country such as Denmark, with a long-established detailed monitoring system based on stable delivery of high quality data, there are a number of challenges in the implementation of integrated data analysis. Data are collected in the animal, food and human sectors often under different premises - following different legislation, with varying sampling strategies and magnitudes.

Here we try to cross-analyse antimicrobial resistance data from monitoring in livestock animals and humans in Denmark. We map the frequency of multilocus sequence types (MLST) and resistance genes of ESBL/AmpC-producing *E. coli* isolates recovered from livestock animals and meat and from humans with bloodstream infections. Such analysis is a visual demonstration of possible relationships between isolates of both origins, and can help identifying strains that may require more targeted genomic analyses to further investigate on possible transmission between human and animal reservoirs and vice-versa.

3.2 Genotypic comparison of ESBL/AmpC-producing *E. coli* from humans, animals and food

There has been decreasing numbers of extended spectrum beta-lactamase-producing *E. coli* (here abbreviated to ESBL Ec) bloodstream infections (BSIs) in humans in Denmark since 2019 (see Chapter 8, Section 8.3.1), and a significant reduction in ESBL Ec has been observed in Danish broilers and broiler meat (see Chapter 7, Section 7.3.1) . Mughini-Gras, et al. [Mughini-Gras, et al. 2019. *Lancet Planet Health* 3(8):e357-e369] found that the primary source of community-acquired ESBL Ec was through human-to-human transmission, although transmission to and from non-human sources was also evident. Other studies [Liu et al 2023. One Health, 16: 100518; Roer, et al. 2019. *J Antimicrob Chemother* 74(3):557- 560; Valcek, et al. 2019. *J Antimicrob Chemother* 74(8):2171- 2175] report possible zoonotic transmissions, underlining the importance of surveying the possibility of zoonotic transfer of resistance from animals to humans.

The objective was to compare the MLST and ESBL/AmpC-genes between humans, food-producing animals and meat to identify any major overlaps between sectors – suggesting a zoonotic link or transmission of resistance genes.

To the data used in the analysis presented in DANMAP 2021, Chapter 3, we added new data from 2022 to comprise a dataset of 1,649 ESBL Ec isolates from humans and animals from 2018 through 2022. The 1,649 human isolates were clinical isolates from infections sent voluntarily to the SSI reference laboratory for antibiotic resistance from the departments of clinical microbiology. The animal isolates (broiler meat: 145, broilers: 90, cattle: 43, beef: 32, pigs: 165, pork: 36, and turkey meat: 81) stem from the mandatory screening programme from healthy animals and meat products. See Chapter 7, Section 7.3.1, and Chapter 8, Section 8.3.1, for more information regarding the data and data collection.

Each isolate has been sequenced as part of the surveillance activities and the multilocus sequence type and ESBL/AmpC-genes were extracted from the sequences. All data handling was done in Python 3.11.0 and R statistical software version 4.2.1 and the plotly package version 5.15.0 was used to make the Sankey diagram. For the purposes of this report, only flows of five or more isolates are shown on the Sankey diagram, which reduced the number of included isolates from 1,649 to 993 isolates.

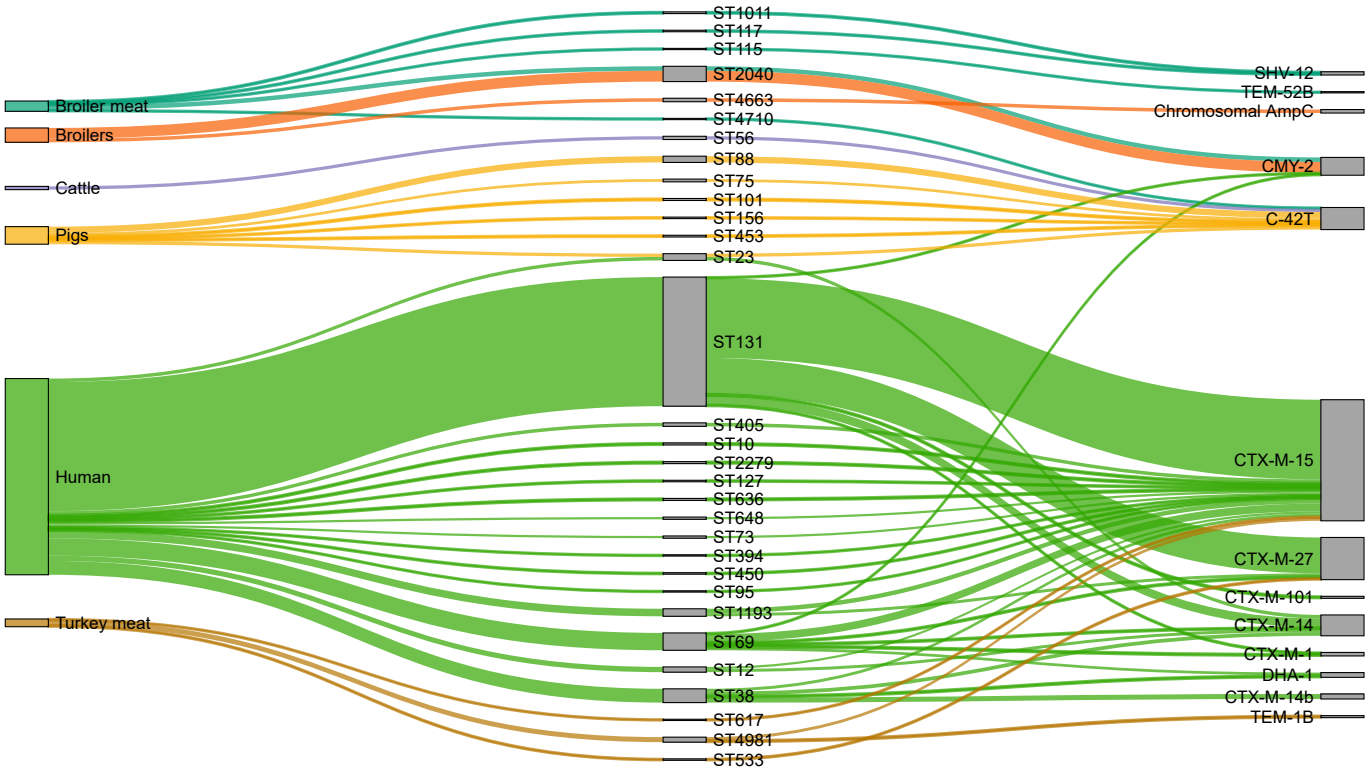
Limited overlap was found in both STs and ESBL/AmpC genes from humans vs. animals and food (Figure 3.1). The few overlaps observed were in accordance with former findings (see DANMAP 2015, Textbox 7.3): ST23 was found in both humans and pigs. Likewise, ST38 was found in both humans and broiler meat. However, for both sequence types the ESBL/AmpC genes detected differed between human and animal strains. The pig isolates from ST23 harboured C-42T mutations, whereas the human isolates harboured CTX-M-14. The broiler meat isolates from ST38 were of the CMY-2-kind, whereas the human isolates carried mainly CTX-M-14 and CTX-M-14b. Only CMY-2, CTX-M-15 and CTX-M-27 were found in both humans and food-producing animals or food, but not in high abundance. ST131 was responsible for roughly 50% of the ESBL-bacteraemia cases in humans, usually accompanied by a CTX-M-15 gene. Interestingly, turkey meat isolates in many cases also carried CTX-M-15, but differed from human isolates on sequence types. ST2040 was found exclusively in broilers or broiler meat and only carried the CMY-2-gene. In general, sequence types seem to associate with species, whereas there is more variance in combinations of sequence types and ESBL/AmpC-genes.

In the 2018 DANMAP report (described in Textbox 7.2), Roer, et al., used whole genome sequencing on a similar, but smaller, dataset to investigate for possible zoonotic links. A possible link was found in ST69/CTX-M-1, but the SNP-distance was not indicative of an outbreak or a direct transmission, but rather of a clonal relationship.

A One Health compartmental analysis over a three-year period from Réunion [Miltgen, et al. 2022. J Antimicrob Chemother 77(5):1254-1262] investigated transmission of ESBL Ec from humans, animals and the environment to human colonization and infection. The study found little evidence of transmission and suggested that focus should be primarily on preventing human-to-human transmission.

Conclusively, it remains challenging to find clear evidence of zoonotic transmission of ESBL Ec, even though the animal and food sectors are potential reservoirs and possibly have a role in the introduction of ESBL Ec into the human sector, as detailed by Mughini-Gras, et al., 2019. Thus, it remains important to monitor the occurrence of ESBL Ec in humans and animals, as part of an integrated antimicrobial resistance surveillance program.

Figure 3.1 A Sankey diagram comprised of 993 ESBL-isolates from humans, animals and food showing the relationship between the isolates' source, sequence type and ESBL/AmpC-gene
DANMAP 2022



The flows between nodes are coded according to source. Only flows of five or more isolates are shown to limit clutter

Conlusion and future perspectives

This One Health chapter presents recent work DANMAP has done towards the integrated analysis of surveillance data for antimicrobial resistance (AMR) from the human and animal/food sectors.

Genomic analysis of ESBL/AmpC-producing *E. coli* isolates from livestock animals, meat and human bloodstream infections suggests limited overlap between the sources with regards to sequence type and ESBL/AmpC-genes. These findings seem to indicate that efforts to prevent zoonotic transmission of AMR *E. coli* are currently successful in Denmark but warrant continued monitoring. Extension of this analysis to other pathogens should be explored. Additionally, more sophisticated analyses are in the works taking a deeper dive into the genomics of ESBL Ec with the likes of source attribution and flank analysis (see Textbox 3.1) to provide a deeper comprehension of how AMR spreads between sectors.

As this year's editorial highlights, the One Health approach has been a pillar of AMR and AMU surveillance in Denmark since the start of the programme and is based on high quality data and strong stakeholder engagement. In order to further strengthen preparedness and detect AMR outbreaks and transmission across sectors, more timely and routine comparison of surveillance data from both the human and animal sectors will be explored. New schemes, such as the surveillance of AMR in pathogenic bacteria from livestock (see Chapter 9), facilitate such initiatives, creating a base for further comparison of data with not only healthy but diseased animals.. Furthermore, it should be investigated how and to what extent it is relevant to monitor the occurence of AMR in the environment as to judge where the environment is an important reservoir for AMR. Likewise, it would be of interest to use these data for risk assessments and forecasts of possible spread between sectors, helping to fill the knowledge gaps about cross-sectoral AMR transmission.

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Textbox 3.1

Flankophile: A bioinformatics pipeline for prokaryotic genomic synteny analysis

Background

Antimicrobial resistance (AMR) is a serious and increasing threat to human health globally. While it is generally accepted that antimicrobial use will select for AMR, the transmission of AMR bacteria and antimicrobial resistance genes (ARGs) also shapes the occurrence of AMR. Thus, investigating, understanding and quantifying the spread of ARGs across geographical borders, between different animal and human hosts, as well as within hospitals is important for designing optimal interventions against the spread of AMR.

However, while several studies have documented the transmission of specific AMR clones globally and between hosts, it has been more difficult to determine transmission of specific ARGs. It is especially challenging to determine the epidemiological importance for human health of ARGs found in uncultivable bacterial species from environmental samples and livestock. Occasionally, the same ARGs have been found in several different reservoirs and bacterial species, but a direct transmission link can be difficult to establish.

Flankophile - a new bioinformatics tool to analyze flanking regions

Analysis of flanking region sequences can be useful when comparing mobile prokaryotic sequences from different bacterial isolates or metagenomes. A new bioinformatics tool, a pipeline called Flankophile [1], can analyze flanking regions and sequence variants. The main feature of Flankophile is that it visualizes flanking region synteny (the genetic code that comes before and after a gene) and sequence variants in publication-ready plots with distance trees, gene annotations and metadata.

The study of acquired ARGs is an obvious use case for Flankophile due to the typically diverse genetic context of the genes. To demonstrate Flankophile, we applied it to a dataset of sequenced 2,006 bacterial clinical isolates from humans (including various species recovered from different infections) and 273 faecal metagenomes from pigs in Denmark, and compared the ARGs found in each host species.

Results

Gene variant results, i.e. considering hits for unique gene variants in the reference database and not closest match hits, showed that only approximately 4% (N=42) of all unique ARG variant sequences found among all samples (N=1,052) were detected in samples from both humans and pigs. Among those ARG variants detected in both hosts, and with long enough flanking region sequences available, flanking region analysis showed multiple examples of ARGs where the entire 3,000 base pair flanking region was identical in samples from both hosts, but also examples of ARGs where no such overlap was observed. Thus, the use of Flankophile provides further resolution and better genomic evidence for zoonotic ARG transmission, by disclosing the possibility of a recent common source of an ARG variant detected in different hosts.

In addition, more than 80% of the unique ARG variants identified, including some frequently observed, were not identical to reference sequences. This suggests that there are possibly many more circulating common gene variants than the ones found in extensive reference databases, such as the ResFinder database. Thus, Flankophile is also well suited to discover and report such new ARG variants.

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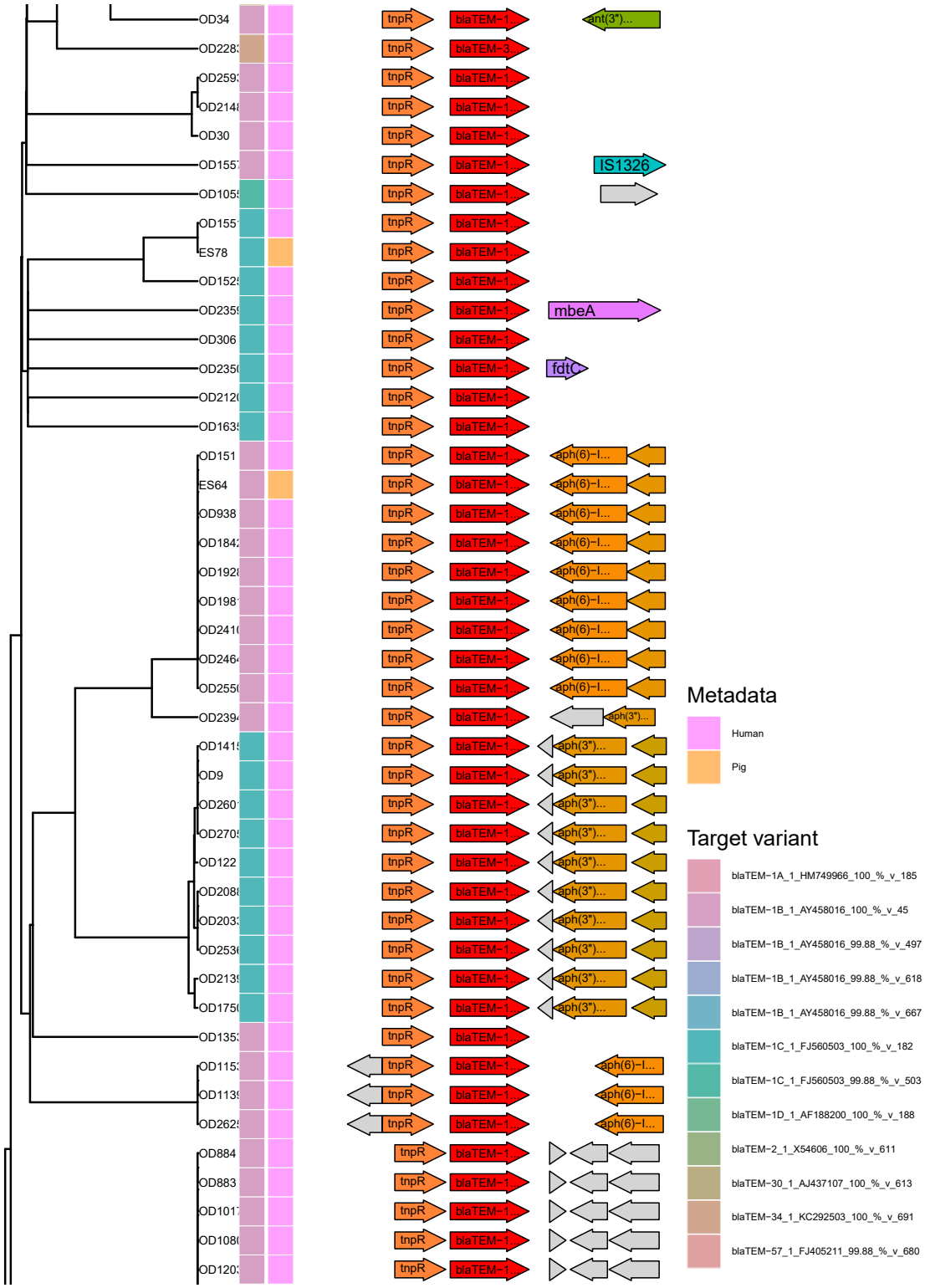
References

[1] Flankophile is available at https://genomicepidemiology.org/services/

continued ... Textbox 3.1

Figure 1 Two β-lactamase antimicrobial resistance genes from the blaTEM family of pig origin (orange) were found to be identical in both gene and flanking region sequences to those of human origin (pink) DANMAP 2022



Example of an output of Flankophile. Detail of a Flankophile plot from the study of a collection of bacterial samples from pigs and humans from Denmark (the plot has been cropped due to size). It is a gene synteny plot of all blaTEM gene hits from the study. From left to right: 1) Distance tree of the blaTEM flanking regions (straight vertical lines indicate that the flanking regions are 100% identical); 2) Color annotation columns representing the target variant (left) and the host species (right); 3) Arrows depicting the gene synteny, with the target sequence in the middle (red)

4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: In 2022, the **total consumption** of antimicrobials in animals amounted to 86.2 tonnes of active compound of products approved for animals. Additionally, 109.7 kg of active compound of products approved for humans were used for **companion animals** or unspecified category.

The **pig sector** consumed 82.8% of all prescribed veterinary antimicrobials, equal to 71.3 tonnes active compound. Calculated in treatment proportions, an estimated 2.7% (26.9 DAPD) of all pigs, on average, received antimicrobial treatment per day in 2022. In sows and piglets and in finishers, the treatment proportions remained at 1.5%, corresponding to 15.4 DAPD and 14.8 DAPD, respectively. The highest treatment proportion was observed in the treatment of weaners: 9.8%, corresponding to 98.1 DAPD. This was an increase compared to 2021 (91.2 DAPD) and could be a result of the ban of the use of prescribed zinc oxide use in pig production by June 2022 (Textbox 4.1). Most notably, there was an increase in neomycin, used to treat post-weaning diarrhoea.

Over time, the antimicrobial classes used in the treatment of pigs have changed notably. The critically important antimicrobials: 3rd and 4th generation cephalosporins, glycopeptides, polymyxins, and fluoroquinolones have been phased out. However, over the last decade, there has been an increase in the consumption of macrolides from 6.4 DAPD to 7.1 DAPD, aminoglycosides from 1.5 DAPD to 4.1 DAPD, and simple penicillins from 2.9 DAPD to 3.0 DAPD. During the same period, the consumption of tetracyclines has decreased from 10.1 DAPD in 2013 to 3.9 DAPD in 2022.

In 2022, antimicrobial consumption in **cattle** amounted to 8.2 tonnes. Approximately two thirds of the consumption were used to treat older cattle (>1 year). Over the past decade, the total antimicrobial consumption has decreased for older cattle (>1 year), from 2.9 DAPD to 2.0 DAPD. During the same period, a decrease in the total consumption from 8.2 DAPD to 6.7 DAPD was observed in young cattle. Also in cattle, the changes in usage of antimicrobial classes are noticeable i.e., there has been an increased consumption of macrolides, tetracyclines and simple penicillins (beta-lactamase sensitive penicillins) for treatment of younger cattle and increased consumption of simple penicillins (beta-lactamase sensitive penicillins) for intramammary treatment.

The antimicrobial consumption in **poultry** was 1,259.5 kg and has only increased by 53.6 kg from 2021 to 2022. In 2022 the consumption of macrolides increased by 214.1 kg active compound compared to 2021, which was likely caused by disease in several flocks in a single farm.

In 2022, cephalosporins were prescribed mainly for **pets and horses** (61.1 kg) or as intramammary treatment for **cattle** (45.6 kg). Furthermore, fluoroquinolones (14.2 kg) were prescribed almost exclusively for horses and pets.

4.1 Introduction

The DANMAP programme began monitoring the national consumption of antimicrobials in humans and animals in 1995. Since the early 1990s, there has been increased political and public focus on the consumption of antimicrobials in the Danish animal production. This has resulted in discontinued usage of antimicrobials for growth promotion combined with several other initiatives, including voluntary bans on the use of 3rd and 4th generation cephalosporins in the pig and cattle production, as well as regulatory legislation regarding therapeutic use.

Figure 4.1 presents the total consumption of antimicrobials in animals and humans since 1990 and 1997, respectively. Increases in, and intensification of, pig production has also had a significant impact on the overall consumption during this time.

The observed decrease in antimicrobial consumption after 1994 was foremost due to the discontinued usage of antimicrobials for growth promotion and most likely also the result of 1) limitation of veterinary practitioners' profit from sales of medicine; 2) implementation of Veterinary Advisory Service contracts (VASCs) with regular visits from the veterinarian to promote preventive veterinary strategies and optimize antimicrobial consumption; and 3) enforcement of the so-called "cascade rule" [Order (DK) 142/1993], limiting the prescription of (cheaper) extemporaneously produced medicines.

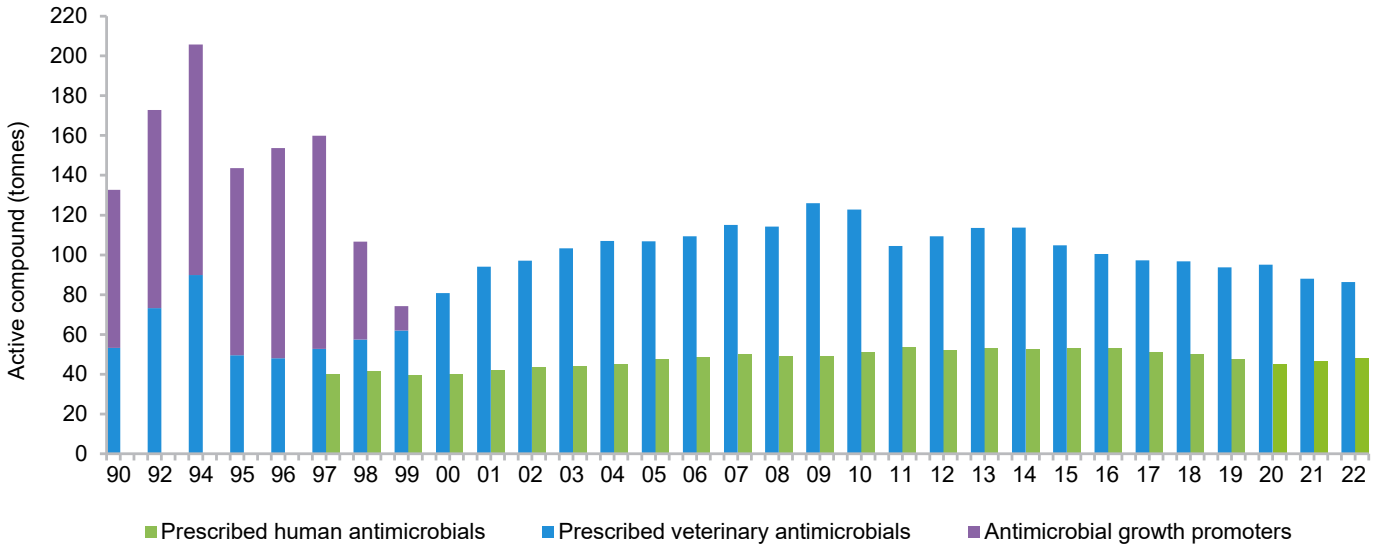
Other important interventions were the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003, and the voluntary ban on the use of cephalosporins in the pig production in 2010, followed by a similar initiative in the dairy cattle production in 2014. Furthermore, the cattle production implemented a ban on use of 3rd and 4th generation cephalosporins for cattle from 2019.

The national action plan against antimicrobial resistance has had several goals throughout time. Initially, a 10% reduction of antimicrobial consumption in production animals by 2014 compared to the 2009 level was set as a national target. In 2015 the national action plan to reduce livestock-associated MRSA called for a 15% reduction in antimicrobial consumption in pigs from 2015 to 2018.

To achieve the action plan goals, the Yellow Card initiative was established in 2010, introducing surveillance at herd level and instating threshold values for antimicrobial consumption in individual herds to enable legal action on pig farmers with high antimicrobial consumption per pig [DANMAP 2010]. As a result, a distinct decrease in antimicrobial consumption was observed from 2010 to 2011.

Effects from other parts of the legislation may be less obvious but are also likely to have affected prescription patterns. As an example, the rules for group medication in pig herds were tightened in 2014 [Order (DK) 534 of 27/05/2014], calling for thorough laboratory diagnoses and frequent veterinary visits before and during prescription of antimicrobials for peroral treatment through water or feed of groups of pigs rather than injection treatment of individual pigs.

Figure 4.1 Antimicrobial consumption for humans and all animal species, tonnes of active compound, Denmark DANMAP 2022



Sources: Human therapeutics: The Danish Medicines Agency. Antimicrobials for animals: Data are based on reports from the pharmaceutical industry of total annual sales (until 2001), from the Federation of Danish pig producers and slaughterhouses (1994-1995), from the Danish Medicines Agency and Danish Plant Directorate (1996–2000), and since 2001 from VetStat. For DANMAP 2022, consumption data were extracted from the VetStat on 22 May 2023 and include all antimicrobials approved for use in animals for the period 2004-2022

In 2016, the Yellow Card initiative was revised, adding on multiplication factors to adjust the consumption of certain antimicrobials. Tetracyclines were multiplied by 1.2, and the factor was increased to 1.5 in 2017. Fluoroquinolones, cephalosporins and colistin (added in 2017) were given the highest multiplication factor of 10 [DANMAP 2017].

In 2017, the Ministry of Environment and Food in Denmark and the Ministry of Health in Denmark presented a new One Health strategy against antimicrobial resistance, setting the framework for reducing the development and occurrence of antimicrobial resistance (AMR).

At the same time, two national action plans to reduce AMR were introduced, setting specific targets to further reduce the antimicrobial consumption for both humans and animals in the coming years. As part of the political agreement on the veterinary strategy 2018-2021 (Veterinærforlig III), an Advisory Committee on Veterinary Medicines was established in 2018.

Also, to reduce the need for disposal of excess antimicrobials, veterinarians and pharmacies were permitted to split packages of veterinary medicine as from 2019 [Order (DK) 1655/2018]. This initiative may also enhance surveillance by reducing the difference between amounts of antimicrobials prescribed and amounts consumed.

Official treatment guidelines for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the appropriate antimicrobial treatment of all common problems in the major production animal species. Since 2005, the Danish Veterinary and Food Administration (DVFA) has updated the guidelines in collaboration with stakeholders and university experts. The guidelines were updated in 2010, when new dynamic evidence-based treatment guidelines for pigs were launched [DANMAP 2010, www.foedevarestyrelsen.dk/], and a revised version was published in April 2018. In June 2022, the use of prescribed zinc oxide in pig production was banned in Denmark (Textbox 4.1).

In 2012, to promote prudent use of antimicrobials in dogs and cats the Danish Veterinary Association (DVA) published treatment guidelines developed by clinical specialists and experts from the Faculty of Health and Medical Sciences at the University of Copenhagen and the National Food Institute, Technical University of Denmark. Revised treatment guidelines for dogs and cats were published in 2018. Similarly, DVA published treatment guidelines for use of antimicrobials in horses in 2017.

Order 2019/6 on veterinary medicinal products has applied since 28 January 2022. There is a particular focus on reducing the risk of antimicrobial resistance [Order (DK) 6/2019] (Textbox 4.2).

4.1.1 Data sources

In Denmark, antimicrobials are available by prescription only, and data on antimicrobial consumption have been collected since 1990.

Since 2001, data on all medicines prescribed for consumption in animals, including vaccines, antimicrobial growth promoters, and coccidiostatica have been recorded in the national database VetStat. Since 2010, the VetStat database has been hosted and maintained by DVFA. In June 2021, DVFA launched an updated platform for VetStat. The 2022 data presented in this report were extracted from this new VetStat on 22 May 2023. The data were extracted, analysed, and interpreted for DANMAP by the National Food Institute, Technical University of Denmark.

4.1.2 Methods

Metrics of antimicrobial consumption are numerous, each with its own advantages and limitations. Therefore, the selection of metrics used for monitoring must depend on the monitoring objective and the information available.

The total amount of antimicrobial consumption is measured in kg active compound and is used in Section 4.2 for the purpose of an overall crude comparison of consumption in the veterinary and human sectors (Figure 4.1).

Since 2012, the metrics "defined animal daily dose" (DADD) and "proportion of population in treatment per day" (DAPD) have been added to monitor trends in antimicrobial consumption. These metrics are defined below, and for additional information on methodology, please see Chapter 10, Section 10.2.

The **Defined animal daily dose (DADD)** is the average maintenance dose per day for the main indication of a drug in the appropriate animal species. DADD is not defined at product level but for each antimicrobial, administration route, and animal species as mg active compound per kg live animal. DADDs were defined specifically for use in DANMAP based on current knowledge, and may vary from the prescribed daily dose, or the recommended dosage, in the summaries of product characteristics (SPC) or in the VetStat database.

The **Proportion of population in treatment per day (DAPD)** is used to describe trends in antimicrobial consumption in animals where possible. DAPD is equal to DADD per 1,000 animals per day, where "animals" are represented by their live biomass and adjusted for lifespan. The estimated live biomass is expressed as the number of standard animals with an estimated average weight on a given day. This may also be referred to as the "standard animals at risk". This metric allows for comparison of antimicrobial consumption between species regardless of large differences in body mass and lifespan.

DAPD, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial. For example, 10 DAPD means that an estimated 1.0% of the population, on average, receives a certain treatment on a given day this is repeating what is in Materials and Methods and does not belong in this chapter.

In DANMAP 2022, the treatment proportions DAPDs were calculated for pigs and cattle.

4.2 Total antimicrobial consumption in animals

Together with the introduction of the new VetStat database in 2021, the criteria for allocation antimicrobial consumption to the different animal species and age groups were revised i.e., consumption is allocated to the species and age group combinations from the categories defined in VetStat [Order (DK) 2542/2021]. This affected the calculated amounts per species while the overall trends of antimicrobial consumption remain the same.

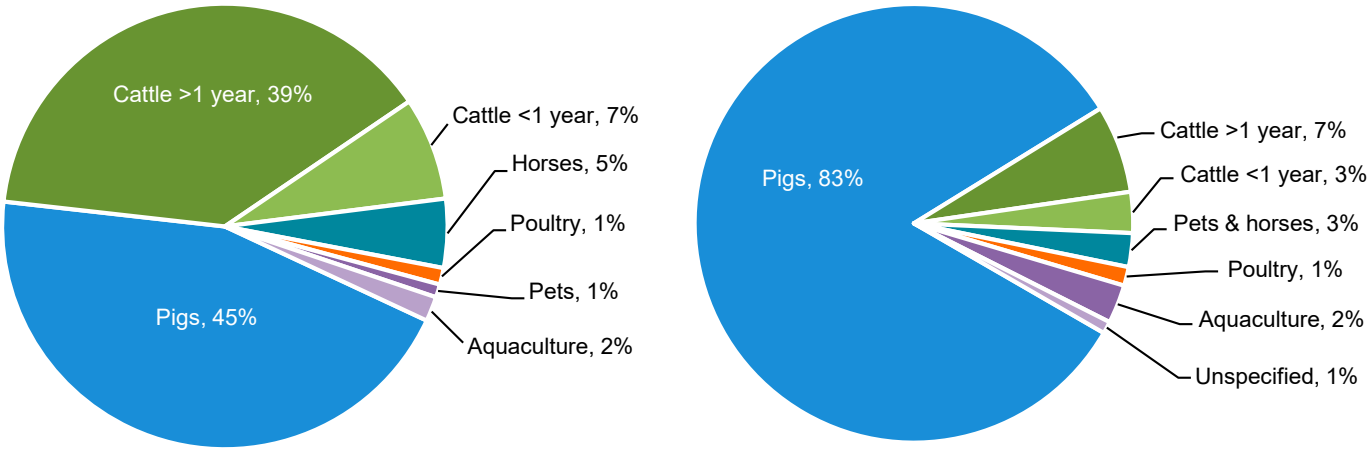
The total consumption of antimicrobials in all animals amounted to 86.2 tonnes active compound, representing a 2.1% (-1878.8 kg) decrease compared to 2021 (Figure 4.1). Like in previous years, the 2022 consumption in pigs, cattle and poultry comprised approximately 82.8%, 9.5%, and 1.5%, respectively, of the total antimicrobial consumption in animals (Figure 4.2).

The pig production is the main driver of consumption of antimicrobials in animals in Denmark, due to the magnitude of the production. Cattle and pigs comprise almost equal proportions of the total live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobials compared with growing animals such as slaughter pigs.

Historically, the overall consumption of kg active compound of antimicrobials was 58.1% lower in 2022 compared to 1994. A major part of this reduction can be explained by the discontinued consumption of growth promoters from 1994 to 1999.

Between 2000 (start of VetStat) and 2009, the amount of kg active compound of antimicrobials used in animals increased by 61.7% (Figure 4.1). During this period, the number of pigs produced also increased, as did the proportion of exported live pigs at approximately 30 kg. Since then, the proportion of these pigs has continued to increase, while there has been an overall gradual decrease in the consumption of antimicrobials in animals.

Figure 4.2 Distribution of live biomass and antimicrobial consumption in main animal species, tonnes, Denmark DANMAP 2022



The live biomass is estimated from census data (pigs, cattle, and companion animals) and production data (poultry, and aquaculture). The live biomass estimates for poultry (turkeys and broilers), aquaculture, horses and pets are based on 2012 data and may well be underestimated. The estimation procedures are described in Chapter 10, Section 10.2

Table 4.1 Antimicrobial consumption by animal species and age group, kg active compound, Denmark DANMAP 2022

	Aminoglycosides	Amphenicols	Cephalosporins [a]	Fluoroquinolones	Lincosamides	Macrolides	Other antimicrobials [b]	Other quinolones	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	2021	2022
Pigs	15159.0	578.8	-	0.1	2294.7	11586.1	-	-	11368.7	7862.3	7043.3	7043.3	10807.7	72344.8	71355.9
Sows, piglets, gilts and boars	2124.6	259.8	-	0.1	410.2	470.6	-	-	5892.5	2426.7	678.3	678.3	1113.7	19517.7	17083.0
Weaners, =<30kg	12958.3	297.9	-	-	1318.8	7780.7	-	-	1588.6	4797.1	2395.5	2395.5	7036.9	35571.6	39023.4
Finishers and polts	76.1	21.2	-	-	565.8	3334.8	-	-	3887.5	638.5	3969.5	3969.5	2657.1	17255.5	15249.5
Cattle	869.7	907.3	45.6	-	3.7	209.5	0.1	-	4300.6	544.2	-	-	950.8	9413.6	8177.9
Intramammaries	27.9	-	45.6	-	3.0	-	0.0	-	250.8	123.5	-	-	-	486.3	450.8
Cows, bulls, heifers and steers >24 months	205.0	11.6	0.0	-	0.5	61.8	0.1	-	3426.7	317.8	-	-	570.4	5522.7	4853.4
Calves <12 months	540.7	882.5	-	-	0.2	146.0	0.0	-	486.2	97.2	-	-	362.6	2834.2	2600.8
Young cattle btw 12 and 24 months	96.1	13.2	-	-	-	1.6	0.0	-	136.9	5.7	-	-	17.9	570.3	272.8
Poultry	49.0	-	-	-	15.0	382.7	-	-	217.1	129.3	19.0	19.0	437.2	1205.9	1259.5
Poultry	-	-	-	-	-	-	-	-	-	-	-	-	-	528.7	-
Broilers	27.3	-	-	-	11.6	291.0	-	-	4.9	42.5	-	-	267.7	344.9	652.7
Layer hens	0.7	-	-	-	0.3	49.0	-	-	96.5	21.7	18.8	18.8	16.5	66.6	204.6
Turkeys	5.9	-	-	-	3.0	-	-	-	111.2	57.6	-	-	117.5	243.1	295.2
Other poultry	15.1	-	-	-	0.1	42.8	-	-	4.6	7.5	0.3	0.3	35.5	22.6	107.0
Other production animals	0.5	143.9	0.0	-	0.0	0.2	0.0	366.5	0.9	1.2	-	-	5.3	1787.6	2460.0
Aquaculture	-	143.9	-	-	0.0	-	0.0	366.5	-	-	-	-	0.6	1771.2	2451.8
Fur animals	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	0.6
Other	0.5	0.1	0.0	-	0.0	0.2	0.0	-	0.9	1.2	-	-	4.7	15.5	7.6
Companion animals	2.9	0.4	61.1	12.5	66.8	0.2	76.2	-	14.0	469.2	0.1	0.1	41.5	2519.7	2143.3
Horses	0.3	0.0	0.0	0.0	0.0	-	0.2	-	4.5	0.2	-	-	7.3	113.1	148.5
Pets	1.7	0.0	22.3	3.9	16.5	0.2	24.3	-	9.4	79.5	0.1	0.1	22.3	484.8	345.7
Unspecfied	1.0	0.4	38.8	8.6	50.3	-	51.7	-	0.0	389.5	-	-	11.9	1921.8	1649.1
Unknown [c]	83.8	1.9	1.1	1.1	7.4	55.2	1.0	3.1	448.6	41.8	3.2	3.2	95.2	773.7	769.9
Total	16165.0	1632.3	107.8	13.7	2387.7	12233.9	77.2	369.5	16349.8	9047.9	7065.6	7065.6	12337.7	88045.3	86166.5
Products approved for human consumption															
Horses	-	0.0	-	0.0	-	0.6	0.3	-	-	0.0	-	-	-	-	0.9
Pets	0.0	0.0	-	0.1	0.0	0.9	3.0	-	1.6	2.3	-	-	1.7	-	10.3
Unspecfied	-	0.0	-	0.4	-	1.3	7.9	-	8.0	5.5	-	-	2.3	-	25.8
Unknown [c]	0.1	0.1	3.8	-	-	0.2	1.5	-	53.3	13.6	-	-	-	-	72.6
Total	16165.1	1632.5	111.7	14.2	2387.7	12236.8	89.9	369.5	16412.7	9069.3	7065.6	7065.6	12341.7		86276.2

Data for 2022 were extracted from VetStat on 22 May 2023 for veterinary approved products, and on 17 August 2023 for human approved products
Combination products are split into active compounds
a) In 2022, 3rd and 4th generation cephalosporins were only used in pets (1.6 kg)
b) Including other antiinfectives, dermatologicals, ontological, opthamologicals, polymyxin, quinolones, and sulfonamides, plain
c) Including data with no information on animal species/age group, or mismatch between animal species and age group

As part of the EU project Alternatives to Veterinary Antimicrobials (AVANT) coordinated by the University of Copenhagen, Faecal microbiota transplantation (FMT) and other alternatives are being evaluated for whether they could be effective in the treatment of pigs with diarrhoea, and hence contribute to reducing antimicrobial consumption in pigs (Textbox 4.3).

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

Most of the antimicrobials were consumed within the pig production in 2022. The total consumption in pigs was 71.3 tonnes of active compound, which was 988.9 kg less than in 2021 (Table 4.1).

The national MRSA action plan aimed to reduce the antimicrobial consumption in pigs by 15% in 2018 compared to 2014. This goal was reached in 2019, where the achieved reduction was 16%. A revised action plan with new targets were agreed upon in 2019 i.e., antimicrobial consumption in the pig production should decrease by 2% each year from 2019-2022 compared to the consumption level in 2018 (74.7 tonnes). To achieve this target, the antimicrobial consumption in 2022 should have been 70.3 tonnes of active compound, thus 1.1 tonnes of active compound lower than the observed for 2022.

The **treatment proportion** (DAPD) of the total population reflects the trends in selection pressure within the population. DAPD is much higher in weaners than in finishers and sows. The DAPDs in the pig population overall and by age group are presented in Figures 4.3 a. and 4.4. The distribution of parental and peroral administration for overall population and by age group are shown in Figures 4.3 b.- c.

Historically, DAPD increased from 2004 to 2009, followed by a clear decrease in 2010 and 2011 with introduction of the Yellow Card initiative. Since 2013, an overall slightly decreasing trend in treatment proportion has been observed (Figure 4.3 a).

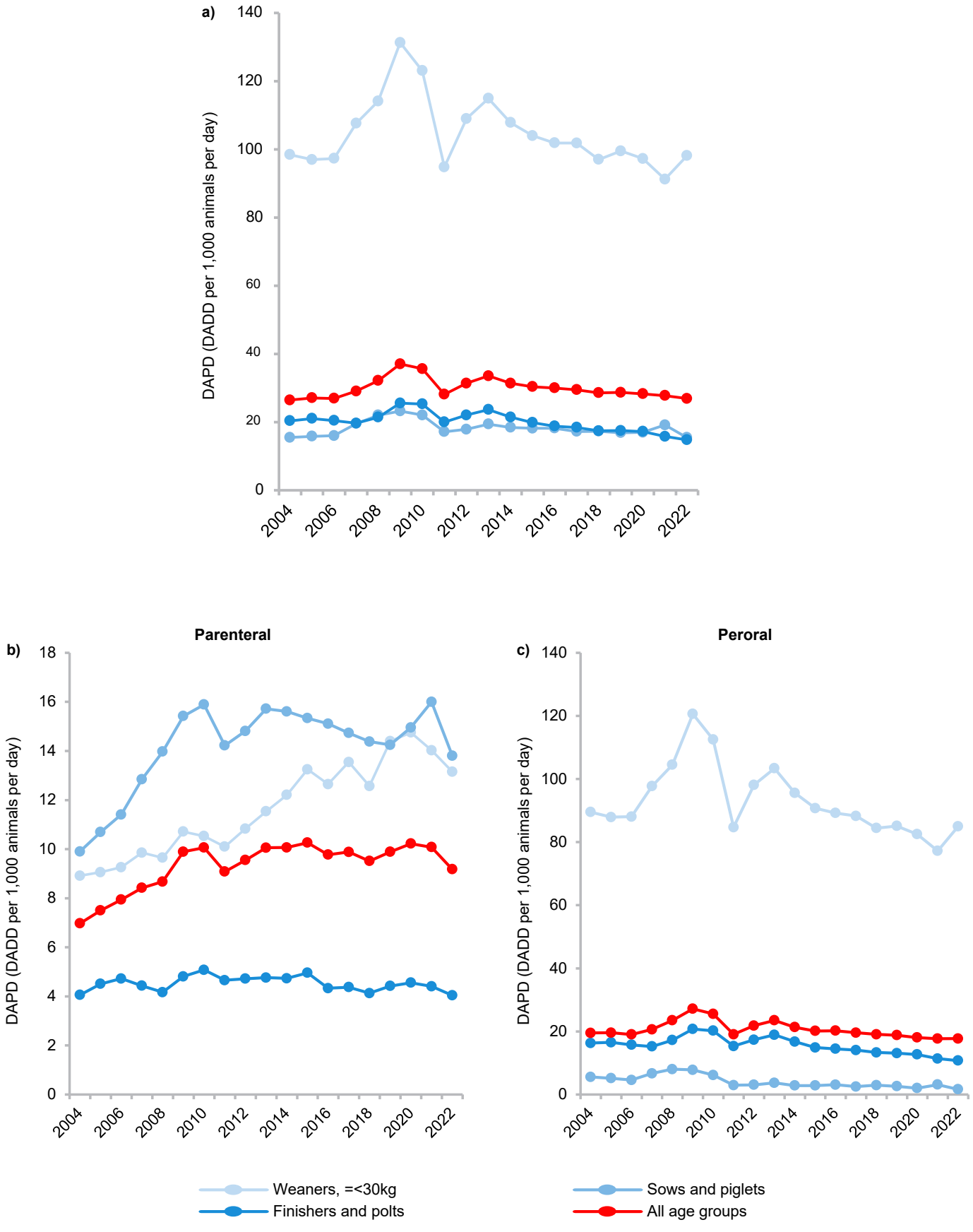
While there was a decrease in the antimicrobial consumption in pigs when inspecting crude consumption data (Table 4.1), the changes in the overall treatment proportion are more subtle and vary between age groups and antimicrobial classes. When comparing 2022 to 2021, DAPD decreased slightly in sows and finishers, but in weaners a considerable increase was observed (Figure 4.3 a.). Thus, on a given day in 2022, approximately 1.5% of sows, piglets and finishers, as well as 9.8% of weaners were treated with antimicrobials. The main prescription indication of antimicrobial consumption was for diarrhoea in weaners (Table 4.2).

Tetracyclines have been some of the most consumed antimicrobials in the Danish pig production, especially for oral treatment of gastrointestinal disease in weaners and finishers. The overall use of tetracyclines has decreased since 2013, and in both 2021 and 2022 the treatment proportion was at the lowest level registered in the last 18 years, with the most marked changes following the recent adjustments to the Yellow Card initiative (Figure 4.4).

The proportion of weaners treated with tetracycline on any given day has decreased from approximately 4.3% (42.9 DAPD) in 2013 to 1.7% (17.1 DAPD) in 2022. In contrast, the consumption of aminoglycosides, mainly neomycin, has increased by 67,6% from 15.4 DAPD in 2021 to 25.9 DAPD in 2022 and to some extent also lincosamides from 2.9 DAPD in 2021 to 3.9 DAPD in 2022 (Figure 4.4).

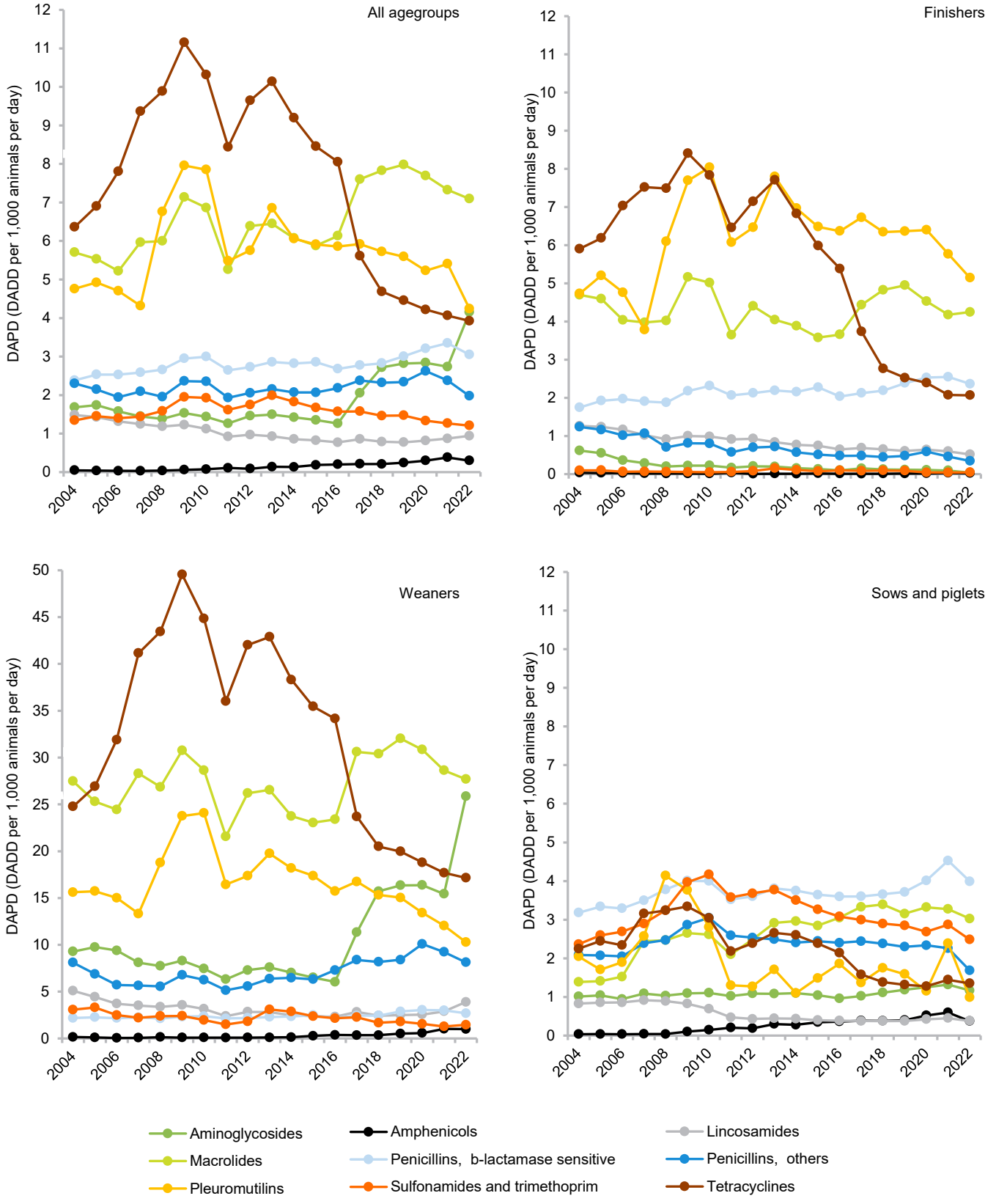
In 2022, no consumption of the critical important antimicrobials 3rd and 4th generation cephalosporins was registered in pigs (Table 4.1).

Figure 4.3 a Total antimicrobial consumption in the pig production, DAPD, Denmark. b. and c. Total antimicrobial consumption in the pig production at administration level, DAPD, Denmark
DANMAP 2022



"Sows and piglets" include treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group. DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group of the total population (in tonnes)

Figure 4.4 Antimicrobial consumption in the total pig production and in each age group at antimicrobial class level, DAPD, Denmark
DANMAP 2022



DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes)
The age group “sows and piglets” includes treatment in boars, where boars constitute 4-5% of the estimated live biomass for the age group

Table 4.2 Prescription indications for parentally and perorally administration of antimicrobials at antimicrobial class level in pigs, DAPD, Denmark DANMAP 2022

	Sows, piglets, gilts and boars							Weaners, =<30kg							Finishers and polts						
	Gastrointestinal disorders	Joints, limbs, hooves, central nervous system, skin	Metabolism, digestion, circulation	Reproduction, urogenital system	Respiratory disorders	Udder	Unknown	Gastrointestinal disorders	Joints, limbs, hooves, central nervous system, skin	Metabolism, digestion, circulation	Reproduction, urogenital system	Respiratory disorders	Udder	Unknown	Gastrointestinal disorders	Joints, limbs, hooves, central nervous system, skin	Metabolism, digestion, circulation	Reproduction, urogenital system	Respiratory disorders	Udder	Unknown
Parenteral																					
Total	**1.03**	**6.32**	**0.07**	**2.10**	**2.60**	**1.68**	**0.00**	**4.73**	**5.66**	**0.02**	**0.00**	**2.73**	**-**	**0.00**	**0.87**	**2.89**	**0.00**	**0.01**	**0.26**	**0.00**	**0.00**
Aminoglycosides	0.07	0.63	0.00	0.01	0.02	0.00	-	0.12	1.20	0.00	0.00	0.03	-	-	0.00	0.01	-	0.00	0.00	-	0.00
Amphenicols	0.10	0.12	0.01	0.02	0.12	0.01	-	0.12	0.07	0.00	-	0.22	-	0.00	0.00	0.00	-	-	0.01	-	-
Fluoroquinolones	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lincosamides	0.03	0.29	0.00	0.00	0.02	0.00	-	0.06	0.05	0.00	-	0.01	-	-	0.00	0.50	0.00	0.00	0.01	-	-
Macrolides	0.14	0.32	0.00	0.05	2.30	0.15	0.00	0.53	0.10	0.00	-	2.13	-	-	0.22	0.01	-	0.00	0.03	-	0.00
Penicillins, b-lactamase sensitive	0.02	2.65	0.00	0.74	0.03	0.56	0.00	0.00	2.53	0.01	0.00	0.14	-	0.00	0.00	2.16	0.00	0.01	0.19	0.00	0.00
Penicillins, others	0.04	1.30	0.01	0.10	0.04	0.05	0.00	0.08	1.53	0.00	0.00	0.07	-	-	0.00	0.08	-	0.00	0.01	-	-
Pleuromutilins	0.01	0.11	0.04	0.01	0.03	0.00	0.00	0.11	0.01	0.00	-	0.03	-	-	0.12	0.06	0.00	0.00	0.01	-	-
Sulfonamides and trimethoprim	0.49	0.06	0.00	1.07	0.00	0.86	-	1.18	0.06	0.00	0.00	0.00	-	-	0.01	0.01	-	-	0.00	-	-
Tetracyclines	0.13	0.85	0.00	0.10	0.05	0.05	0.00	2.53	0.10	-	-	0.11	-	-	0.52	0.05	0.00	0.00	0.01	-	0.00
Peroral																					
Total	**0.58**	**0.17**	**0.01**	**0.04**	**0.84**	**0.00**	**-**	**65.82**	**5.00**	**0.06**	**0.00**	**14.07**	**0.00**	**0.01**	**9.68**	**0.44**	**0.00**	**0.00**	**0.60**	**-**	**0.00**
Aminoglycosides	0.43	0.00	-	0.00	-	0.00	-	24.42	0.02	0.01	-	0.05	0.00	0.00	0.02	-	-	-	-	-	-
Amphenicols	0.00	0.00	0.00	-	0.00	-	-	0.05	0.10	-	-	0.42	-	-	0.00	-	-	-	0.01	-	-
Fluoroquinolones	0.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lincosamides	0.03	0.00	-	0.00	-	-	-	3.73	0.03	-	-	0.00	0.00	0.00	0.01	-	-	-	-	-	-
Macrolides	0.01	0.00	-	-	0.04	-	-	19.72	0.23	0.01	0.00	4.98	-	0.00	3.90	0.02	-	-	0.06	-	-
Penicillins, others	0.05	0.05	0.00	0.01	0.03	-	-	1.07	3.66	0.04	-	1.67	-	-	0.02	0.12	0.00	-	0.12	-	-
Pleuromutilins	0.03	0.10	0.00	0.01	0.65	-	-	9.33	0.44	0.00	-	0.37	-	-	4.59	0.20	0.00	-	0.15	-	0.00
Sulfonamides and trimethoprim	0.00	0.00	-	-	0.00	-	-	0.07	0.10	-	-	0.05	-	-	0.02	-	-	-	0.00	-	-
Tetracyclines	0.02	0.01	0.00	0.02	0.11	-	-	7.45	0.42	0.01	-	6.52	-	0.01	1.12	0.09	-	0.00	0.26	-	-

Data for 2022 were extracted from VetStat on 22 May 2023
Combination products are split into active compounds

4.3.2 Antimicrobial consumption in cattle

Legislation-supported thresholds for antimicrobial consumption in cattle have been in place since 2011. In 2022, approximately 8.2 tonnes were recorded for use in cattle, of which approximately 450.8 kg of active compound were used for intramammary therapeutic or dry-cow treatment (Table 4.1).

About 33.6% of the antimicrobial consumption for systemic treatment was used for young cattle (<12 months), and the rest were used to treat adult cattle (>12 months) (Table 4.1). The production of veal, beef and milk has remained relatively stable over the past 5 years (Chapter 2, Table 2.3).

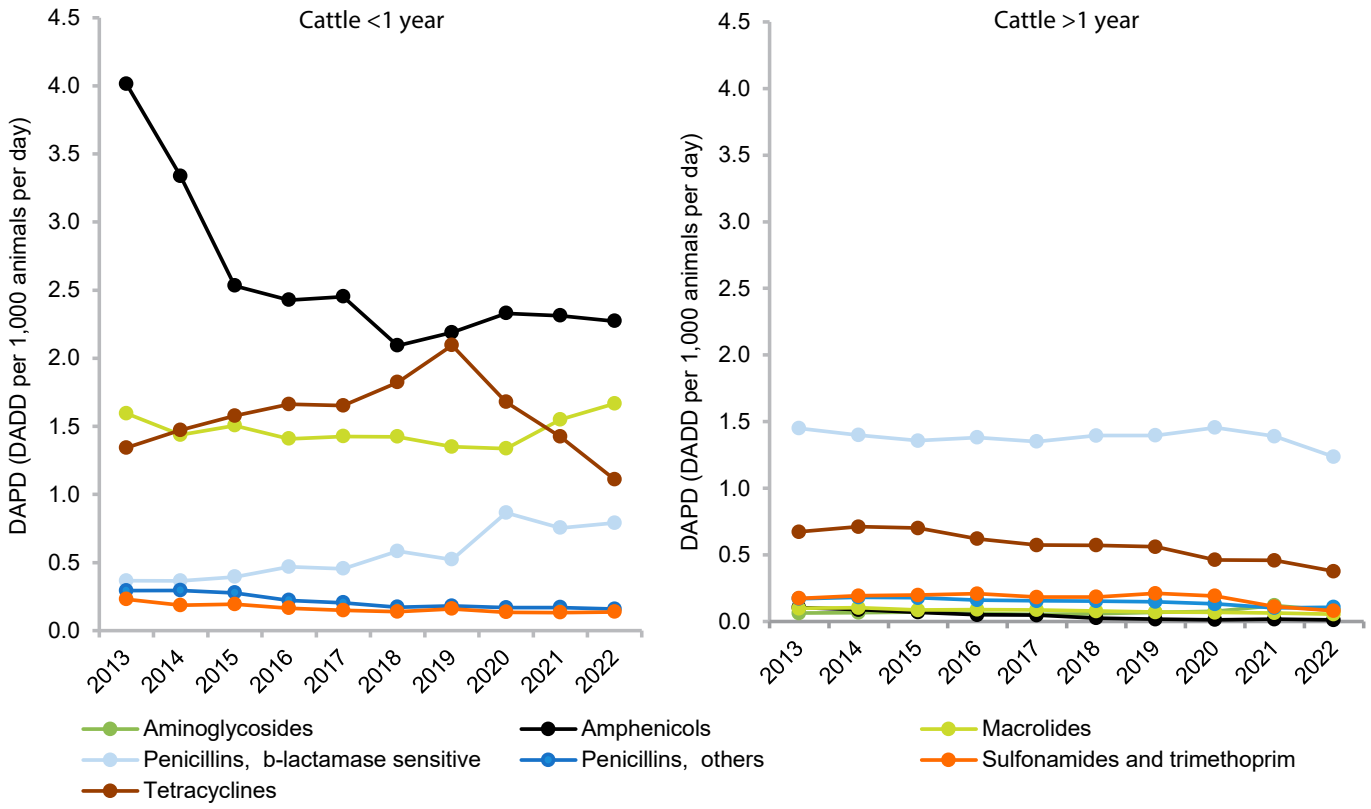
Measured in kg active compound, in adult cattle, the consumption was 15.2% lower in 2022 than in 2021. Moreover, there has been a gradual decrease in the overall use of antimicrobials for systemic treatment in adult cattle over the past decade. The consumption was 23.4% lower in 2022 compared to 2018 and 30.5% lower than in 2013.

However, measured as treatment proportions, the use in adult cattle has been between 2.3 and 3.2 DAPD from 2013 to 2021. In 2022, the treatment proportion was 2.0 DAPD, compared to 2.3 DAPD in 2021 and 2.9 DAPD in 2013.

The main indication for treatment in adult cattle was mastitis (udder), and simple penicillins (beta-lactamase sensitive) accounted for 47.5% of the antimicrobial consumption in this age group (Figures 4.5 and 4.6).

The antimicrobial consumption in calves and young cattle increased until 2012, followed by a slight decrease in the following years with approximately 8.2 DAPD used in 2013, 6.9 DAPD used in 2018 and 6.7 DAPD used in 2022. Measured in kg active compound, there has been an 8.2% reduction from 2021 to 2022. The main indication for systemic treatment in calves is respiratory disease followed by joint/limb and gastrointestinal infections (Table 4.3).

Figure 4.5 Antimicrobial consumption in cattle production by age groups at antimicrobial class level, DAPD, Denmark DANMAP 2022



DAPDs are calculated as the number of standard doses for one kg animal divided by the estimated live biomass in the age group or the total population (in tonnes)
Intramammary applications are not included (doses needed for calculating DAPD not available)
The DAPDs of amphenicols in cattle <1 year differ from previous reports, due to missing data in the old VetStat

Table 4.3 Prescription indications for parentally and perorally administration of antimicrobials at antimicrobial class level in cattle, DAPD, Denmark DANMAP 2022

	Cattle <1 year							Cattle >1 year						
	Gastrointestinal disorders	Joints, limbs, hooves, central nervous system, skin	Metabolism, digestion, circulation	Reproduction, urogenital	Respiratory disorders	Udder	Unknown	Gastrointestinal disorders	Joints, limbs, hooves, central nervous system, skin	Metabolism, digestion, circulation	Reproduction, urogenital	Respiratory disorders	Udder	Unknown
Parenteral														
Total	0.20	0.80	0.01	0.02	4.90	0.02	0.00	0.02	0.43	0.02	0.36	0.11	1.01	0.00
Aminoglycosides	0.00	0.10	0.00	0.00	0.05	0.00	0.00	0.00	0.04	0.00	0.01	0.00	0.02	0.00
Amphenicols	0.00	0.04	0.00	0.00	2.21	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	-
Lincosamides	0.00	0.00	-	-	0.00	-	-	0.00	0.00	-	0.00	0.00	0.00	-
Macrolides	0.02	0.11	0.00	0.00	1.42	0.00	0.00	0.00	0.03	0.00	0.00	0.02	0.01	-
Penicillins, b-lactamase sensitive	0.01	0.36	0.00	0.01	0.40	0.01	0.00	0.01	0.19	0.01	0.09	0.01	0.93	0.00
Penicillins, others	0.03	0.04	0.00	0.00	0.08	0.00	0.00	0.00	0.03	0.00	0.03	0.04	0.01	0.00
Sulfonamides and trimethoprim	0.12	0.00	0.00	0.00	0.01	0.00	-	0.01	0.01	0.00	0.04	0.00	0.02	0.00
Tetracyclines	0.01	0.15	0.00	0.00	0.73	0.00	-	0.00	0.12	0.01	0.19	0.03	0.02	0.00
Peroral														
Total	0.43	0.02	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
Aminoglycosides	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00	0.00	0.00	-
Amphenicols	-	0.02	-	0.00	-	-	-	0.00	0.00	0.00	0.00	-	-	-
Macrolides	0.00	-	-	-	0.11	-	-	-	-	-	-	-	0.00	-
Penicillins, others	-	-	-	-	-	-	-	-	-	-	0.00	-	-	-
Tetracyclines	0.01	-	0.00	-	0.20	-	0.00	0.00	-	-	-	0.00	-	-

Data for 2022 were extracted from VetStat on 22 May 2023
Combination products are split into active compounds

In calves and young cattle, treatment (DAPD) with amphenicols (florfenicol) has decreased steadily since 2020, although amphenicols are still the most frequently prescribed (33.8%), followed by macrolides and tetracyclines, 24.8% and 16.5%, respectively.

The use of fluoroquinolones in cattle has been close to zero for the last decade. Fluoroquinolones are only prescribed in food-producing animals as a last-line drug, based on microbiological analysis and susceptibility testing in an accredited laboratory. Use of fluoroquinolones in food-producing animals is also notifiable to the DVFA. No fluoroquinolones were registered for consumption in cattle in 2022.

In 2014, the cattle production began to phase out the use of 3rd and 4th generation cephalosporins used for systemic treatment, resulting in a significant drop in 2015. In 2019, the cattle production implemented a ban on use of 3rd and 4th generation cephalosporins in all cattle, and no use has been registered since 2020.

By the year of 2020 the board of Danish dairy and beef producers strategy for good udder health aimed at a 20% reduction in the use of antimicrobials for treatment of mastitis and other cattle diseases compared to 2012, as well as lowering of geometric mean bulk tank cell counts to 150,000.The dairy industry also aims to promote the use of simple penicillins (beta-lactamase sensitive penicillins) when dry-cow therapy or mastitis treatment is required.

The board of Danish dairy and beef producers renewed it's strategy for disease prevention in calves and cows, including good udder health for 2021-2023. The goals are, for the given time period, a 10% reduction in use of antimicrobials for treatment of cattle <1 year old and a reduction of 3% in cattle >1 year old on average annually. Moreover, they aim to reduce the proportion of milk producers with a cell count >200,000 from 60% to 30%.

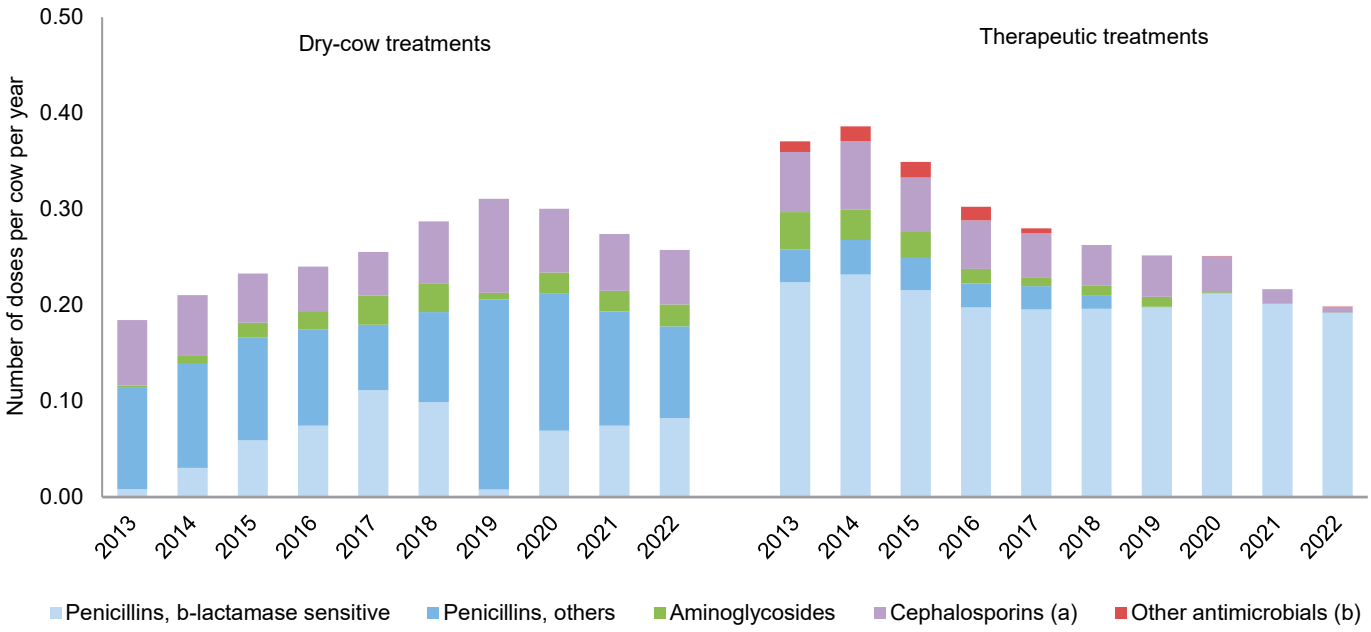
In 2022, the overall antimicrobial consumption in cattle was 25% lower than in 2013 and the bulk tank milk counts were at 185.400 in February 2022.

Most antimicrobials administered parenterally in cattle are used in dairy cows, primarily to treat mastitis (Table 4.3). The consumption of intramammary treatment, measured as doses per cow per year, is shown in Figure 4.6. The consumption of simple penicillins (beta-lactamase sensitive penicillins) has increased, whereas the consumption of 1st generation cephalosporins has decreased.

In 2019, there was a remarkable shift in the dry-cow treatments and the use of the beta-lactamase sensitive penicillins for this purpose almost ceased, while the use of the other penicillins, especially cloxacillin, increased. This shift was caused by a product shortage, where the only beta-lactamase sensitive penicillins for dry-cow treatment was unavailable for longer periods of 2019, and other penicillins especially products containing cloxacillin, had to be used instead [Personal communication; Michael Farre, Danish Agriculture and Food Council]. In 2020 and onwards, it again shifted to the use of only beta-lactamase sensitive penicillins (Figure 4.6).

For therapeutic treatments, beta-lactamase sensitive penicillins remained the most used antimicrobial class.

Figure 4.6 Consumption of antimicrobials for intramammary application in cattle, treatments per cow per year, Denmark DANMAP 2022



For intramammary treatment, the consumption has been estimated as the number of doses
Combination products are split into active compounds
a) 1st generation cephalosporins only
b) Includes lincomycin for dry-cow treatments. For therapeutic treatment, mainly sulfonamides-trimethoprim, but also lincomycin and bacitracin

4.3.3 Antimicrobial consumption in poultry

The poultry production comprises broiler production, egg layers, and turkey production. In addition, there is a small production of ducks, geese, and game birds. Conventional broiler farms have a very high level of biosecurity, and the antimicrobial consumption in broiler production is generally low compared with other species. Accordingly, disease outbreaks in just a few farms can markedly affect the national statistics on antimicrobial usage in the poultry sector (Table 4.4).

Previously, VetStat did not allow easy differentiation of antimicrobial use in different types of poultry production. However, this has been amended in the new VetStat. From June 2021 antimicrobial use has been reported in more detail, subsequently in a few years it will be possible to follow trends in antimicrobial usage in the different types of poultry production.

In 2022, the total antimicrobial usage has increased by 53.6 kg active compound compared to 2021 (Table 4.4). While the consumption of penicillins, tetracyclines and others, has decreased by 104.7 and 186.3 kg active compound respectively, the macrolide consumption has increased by 214.1 kg active compound. The increase is very likely caused by disease in several flocks in a single farm [personal communication, Susanne Kabel, Danish Agriculture and Food Council]. For the past decade, cephalosporins have not been used in the poultry production, and the use of fluoroquinolones stopped in 2021. Colistin has not been used since 2016.

Table 4.4 Consumption of antimicrobials in poultry, kg active compound, Denmark DANMAP 2022

	Aminoglycosides	Amphenicols	Fluoroquinolones	Lincosamides	Macrolides	Other antibacterials [a]	Penicillins, b-lactamase sensitive	Penicillins, others	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2013	36.0	9.0	0.0	17.7	293.0	1.4	171.9	220.1	4.1	61.6	488.5	1303.3
2014	21.4	8.5	0.1	10.5	399.5	2.3	133.3	373.8	0.4	82.6	604.0	1636.4
2015	258.5	4.4	1.0	129.1	133.3	9.5	204.4	566.0	0.5	446.0	816.6	2569.2
2016	60.2	4.8	0.0	23.8	175.6	8.0	264.6	257.6	0.4	111.0	764.6	1670.6
2017	64.9	5.1	0.0	31.7	244.9	1.0	355.6	334.8	0.5	84.6	487.5	1610.4
2018	50.6	-	0.0	25.3	195.0	-	357.8	242.6	0.8	36.6	521.1	1429.7
2019	54.8	0.2	0.0	27.4	274.8	-	368.4	234.3	0.6	64.2	694.3	1719.1
2020	58.2	-	0.0	29.0	156.9	-	334.1	237.3	0.2	54.6	1587.9	2458.3
2021	53.9	-	-	25.2	168.6	-	112.5	188.9	0.4	33.0	623.5	1205.9
2022	49.0	-	-	15.0	382.7	-	217.1	129.3	19.0	10.2	437.2	1259.5

Data for 2022 were extracted from VetStat on 22 May 2023
VetStat does not differentiate between consumption in the different sectors of poultry production
Combination drugs are divided into active compounds
a) Other antibacterials also include other quinolones and polymyxins

4.3.4 Antimicrobial consumption in aquaculture, and companion animals

Aquaculture

Antimicrobial consumption in aquaculture is mainly driven by the summer air temperatures and hours of summer sunlight because bacterial diseases are more likely to occur when water temperatures are high [Villumsen and Bojesen, 2022. Microbiol Spectr. 10(5):e0175222]. Although the aquaculture production continues to focus on developing improved vaccination strategies to reduce the risk of bacterial diseases that may require treatment with antimicrobials, the antimicrobial consumption varies significantly from year to year. In 2022 the antimicrobial consumption increased by 5% compared to the average consumption in the previous five years. The increase was solely due to increased usage of combination products of sulfonamides and trimethoprim (Table 4.5).

In 2022, mainly three antimicrobial classes were used to treat bacterial infections in aquaculture: 79.2% of sulfonamides and trimethoprim, 14.9% of other quinolones (oxolinic acid), and 5.9% of amphenicols (florfenicol) (Table 4.5).

Table 4.5 Consumption of antimicrobials in aquaculture, kg active compound, Denmark DANMAP 2022

	Amphenicols	Other antibacterials[a]	Other quinolones	Penicillins, others	Sulfonamides and trimethoprim	Tetracyclines	Total
2013	180.5	0.2	961.1	10.1	2278.6	1.8	3432.3
2014	297.1	-	1706.3	9.8	3132.1	-	5145.2
2015	311.1	-	1019.5	5.2	1655.0	0.7	2991.5
2016	313.9	0.0	900.1	13.6	1085.9	0.4	2313.9
2017	350.3	0.1	652.3	35.1	679.3	0.1	1717.2
2018	323.5	0.0	949.3	51.6	2292.6	0.5	3617.5
2019	292.6	-	456.5	43.9	1720.9	22.0	2535.9
2020	341.2	0.0	574.9	27.1	1030.2	1.0	1974.3
2021	295.4	0.2	392.3	19.5	1088.9	0.8	1797.2
2022	143.9	0.0	366.5	-	1940.8	0.6	2451.8

Data for 2022 were extracted from VetStat on 22 May 2023
Combination products are split into active compounds
a) Other antibacterials also includes lincosamides

Companion animals - horses and pets

The information available on antimicrobial consumption in companion animals is not as accurate as for production animals, since VetStat allows registration of antimicrobials for companion animals without defining animal species. Table 4.6 shows the antimicrobial consumption registered for companion animals in three categories: horses, pets, and "unspecified". In addition, the Table 4.6 includes the category "unknown", which are products where the animal species was not registered.

The total amount of antimicrobials estimated for consumption in companion animals in 2022 was 2180.4 kg (Table 4.6, Figure 4.7). Since human approved antimicrobial products were not included in DANMAP 2021 a comparison to the previous year will be inaccurate. In total, 109.7 kg active compound of products approved for humans was used for companion animals in 2022 (Table 4.7).

As in previous years, a substantial amount of sulfonamide/trimethoprim registered as used for pets is oral paste, a product normally used for horses. Thus, a substantial amount of sulfonamide/trimethoprim included in Table 4.6 is likely to have been used for horses (165.5 kg in 2022 and 270.8 kg in 2021).

A large proportion of antimicrobials for dogs and cats are prescribed for the treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, repeated use of critically important antimicrobials may pose a risk to the owners, and the use of these antimicrobials is therefore monitored carefully. Since the treatment guidelines by DVA were published in 2012 (revised in 2018), the use of cephalosporins has been reduced from 272.7 kg in 2012 to 61.1 kg of active compound in 2022 (Table 4.6 and 4.7).

In 2022, the consumption of fluoroquinolones in companion animals, mainly dogs and cats, was 14.2 kg active compound and represented the majority (91.6%) of fluoroquinolones used in all animals (Table 4.1, 4.6 and 4.7). Similarly, the pets accounted for 55.7% (61.1 kg) of all the cephalosporins consumed in animals (Table 4.1, 4.6 and 4.7). In 2022, the consumption of 3rd generation cephalosporins were registered in the unspecified category, 1.6 kg active compound in total. 4th generation cephalosporins were not used in 2022.

Table 4.6 Estimated consumption of antimicrobials for horses, pets and unspecified animals, kg active compound, Denmark

DANMAP 2022

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other antibacterials [a]	Penicillins, b-lactamase sensitive	Penicillins, others	Sulfonamides and trimethoprim	Tetracyclines	Total
Horses												
2013	1.4	1.8	0.2	0.0	-	-	0.0	8.2	0.1	86.8	5.4	104.0
2014	1.4	0.3	0.4	0.0	-	0.1	0.0	9.3	0.2	98.0	6.7	116.5
2015	2.8	0.4	0.4	0.0	0.0	0.1	0.0	6.9	0.1	114.4	4.8	129.7
2016	0.8	0.4	0.1	0.0	-	-	0.0	5.1	0.0	108.0	5.2	119.7
2017	0.9	0.3	0.1	0.0	-	-	0.0	5.3	0.1	106.4	3.0	116.1
2018	0.7	0.0	0.2	.	-	0.1	0.0	5.7	0.0	100.6	3.8	111.1
2019	0.9	-	0.1	0.0	-	0.0	0.0	4.9	0.0	94.2	3.8	104.0
2020	0.5	-	0.0	0.0	-	-	0.0	4.2	0.0	111.5	3.5	119.7
2021	0.2	-	0.0	0.0	0.0	0.0	0.1	5.2	0.1	105.5	2.0	113.1
2022	0.3	0.0	0.0	0.0	0.0	-	0.2	4.5	0.2	136.0	7.3	148.5
Pets												
2013	3.6	0.3	75.1	4.8	16.6	3.2	7.3	7.9	114.0	252.3	19.4	504.6
2014	5.6	0.6	81.3	5.0	19.0	5.0	7.0	12.1	122.3	261.0	13.3	532.3
2015	4.8	3.6	61.8	5.6	21.8	3.3	8.6	13.2	123.4	226.2	20.4	492.7
2016	3.4	3.4	55.3	5.4	21.8	2.3	7.6	9.8	131.2	269.1	21.5	530.7
2017	3.8	0.7	41.7	5.2	18.4	1.7	8.4	9.2	125.8	272.4	19.3	506.6
2018	3.9	0.3	35.9	4.9	17.5	1.7	14.8	10.0	113.7	253.2	21.1	477.1
2019	3.7	0.3	32.3	4.5	17.2	7.4	15.6	10.4	108.4	236.8	14.8	451.4
2020	4.3	0.6	30.7	5.1	19.1	3.8	18.1	12.9	103.4	262.3	17.7	478.0
2021	3.2	0.7	28.0	4.7	19.2	2.2	20.9	11.4	100.1	270.8	23.7	484.8
2022	1.7	0.0	22.3	3.9	16.5	0.2	24.5	9.4	79.5	165.5	22.3	345.7
Unspecfied												
2013	18.4	0.0	155.4	8.6	47.0	0.1	26.2	1.0	416.3	843.6	17.4	1534.0
2014	18.3	0.0	131.8	8.2	50.0	-	26.7	2.3	419.6	967.6	20.2	1644.7
2015	14.4	0.3	95.6	8.6	45.7	0.0	25.1	1.4	413.7	944.8	15.9	1565.6
2016	14.9	0.4	81.6	9.6	47.6	0.3	26.3	2.2	456.1	1014.5	16.2	1669.7
2017	15.1	0.2	69.1	9.2	48.5	0.0	28.2	1.8	458.6	1071.1	14.6	1716.4
2018	13.8	1.3	61.4	9.7	44.2	-	34.8	1.7	443.2	1135.5	12.9	1758.6
2019	14.3	0.2	60.6	9.9	46.9	0.1	36.8	1.6	435.4	1139.2	15.5	1760.6
2020	10.0	0.4	56.9	10.7	49.8	-	40.0	2.7	440.9	1221.3	15.6	1848.4
2021	10.7	0.4	49.4	10.1	54.6	-	47.3	0.8	451.6	1282.5	14.4	1921.8
2022	1.0	0.4	38.8	8.6	50.3	-	51.7	0.0	389.5	1096.9	11.9	1649.1
Unknown												
2013	187.0	-57.7	8.3	3.2	13.6	41.9	44.9	551.3	199.4	292.3	174.6	1458.9
2014	176.8	-95.0	5.9	1.5	7.9	44.8	66.9	511.4	187.9	322.9	164.9	1395.8
2015	219.2	129.9	5.5	1.9	23.1	50.0	22.0	529.2	162.7	340.5	168.4	1652.3
2016	175.9	202.2	2.7	1.7	6.4	19.5	14.0	506.2	211.9	274.4	124.5	1539.4
2017	237.1	117.2	3.6	0.9	9.0	52.7	9.2	573.6	122.8	279.1	156.0	1561.4
2018	266.9	-44.2	3.6	1.3	9.0	20.8	5.9	564.1	149.3	176.1	105.3	1258.1
2019	336.2	-11.4	-8.3	1.4	9.8	33.1	5.0	537.8	140.2	109.3	147.8	1300.9
2020	296.1	1.9	1.5	1.5	11.2	17.3	12.5	539.0	151.3	16.4	141.1	1189.7
2021	117.2	-4.7	0.9	1.7	8.1	4.8	6.8	371.6	111.4	20.8	135.0	773.7
2022	83.8	1.9	1.1	1.1	7.4	55.2	4.2	448.6	41.8	26.6	95.2	766.8

Data for 2022 were extracted from VetStat 22 May 2023
Combination products are split into active compounds
The estimates include all veterinary approved antimicrobials, for use in horses, pets, as well as products without a specified animal species (unknown)
a) Other antibacterials also include other otologicals, pleuromutilins, polymyxins and sulfonamides, plain

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Table 4.7 Estimated consumption in 2022 of veterinary and human approved antimicrobials for horses, pets and unspecified animals, kg active compound, Denmark DANMAP 2022

	Veterinary-approved	Human-approved	Total
Horses			
Aminoglycosides	0.3	-	0.3
Amphenicols	0.0	0.0	0.0
Cephalosporins	0.0	-	0.0
Fluoroquinolones	0.0	0.0	0.0
Lincosamides	0.0	-	0.0
Macrolides	-	0.6	0.6
Other antibacterials[a)]	0.2	0.3	0.4
Penicillins, b-lactamase sensitive	4.5	-	4.5
Penicillins, others	0.2	0.0	0.3
Sulfonamides and trimethoprim	136.0	-	136.0
Tetracyclines	7.3	-	7.3
Pets			
Aminoglycosides	1.7	0.0	1.7
Amphenicols	0.0	0.0	0.0
Cephalosporins	22.3	-	22.3
Fluoroquinolones	3.9	0.1	4.0
Lincosamides	16.5	0.0	16.5
Macrolides	0.2	0.9	1.1
Other antibacterials[a)]	24.5	3.0	27.5
Penicillins, b-lactamase sensitive	9.4	1.6	11.0
Penicillins, others	79.5	2.3	81.8
Sulfonamides and trimethoprim	165.5	0.7	166.2
Tetracyclines	22.3	1.7	24.0
Unspecified			
Aminoglycosides	1.0	-	1.0
Amphenicols	0.4	0.0	0.4
Cephalosporins	38.8	-	38.8
Fluoroquinolones	8.6	0.4	9.0
Lincosamides	50.3	-	50.3
Macrolides	-	1.3	1.3
Other antibacterials[a)]	51.7	7.9	59.6
Penicillins, b-lactamase sensitive	-	8.0	8.0
Penicillins, others	389.5	5.5	395.0
Sulfonamides and trimethoprim	1096.9	0.5	1097.3
Tetracyclines	11.9	2.3	14.2
Unknown			
Aminoglycosides	83.7	0.1	83.8
Amphenicols	1.6	0.1	1.8
Cephalosporins	1.1	3.8	4.9
Fluoroquinolones	0.1	-	0.1
Lincosamides	7.4	-	7.4
Macrolides	55.2	0.2	55.3
Other antibacterials[a)]	3.9	1.5	5.4
Penicillins, b-lactamase sensitive	446.9	53.3	500.2
Penicillins, others	40.5	13.6	54.1
Sulfonamides and trimethoprim	26.9	-	26.9
Tetracyclines	94.5	-	94.5
Total	2906.2	109.7	3015.8

Data for 2022 were extracted from VetStat on 22 May 2023 for veterinary approved products, and on 17 August 2023 for human approved products
Combination products are split into active compounds
The estimates include all veterinary and human approved antimicrobials, for use in horses, pets, as well as products typically used for companion animals, but without a specified animal species (unspecified)
a) Other antibacterials include other antibacterials, other otologicals, pleuromutilins, polymyxins and sulfonamides (plain)

Textbox 4.1

No more high dose zinc oxide in veterinary medicinal products

Following a review of the safety and effectiveness of veterinary medicinal products containing zinc oxide to be administered orally to food-producing species, in the spring of 2017, the European Medicines Agency (EMA) concluded that the benefits of zinc oxide for the prevention of diarrhoea in pigs did not outweigh the risks for the environment. Based on the review and recommendations from EMA, the European Commission issued a decision on the 26th of June, 2017, to withdraw all existing marketing authorisations. Member States could defer the withdrawal of the marketing authorisations for up to five years from that date.

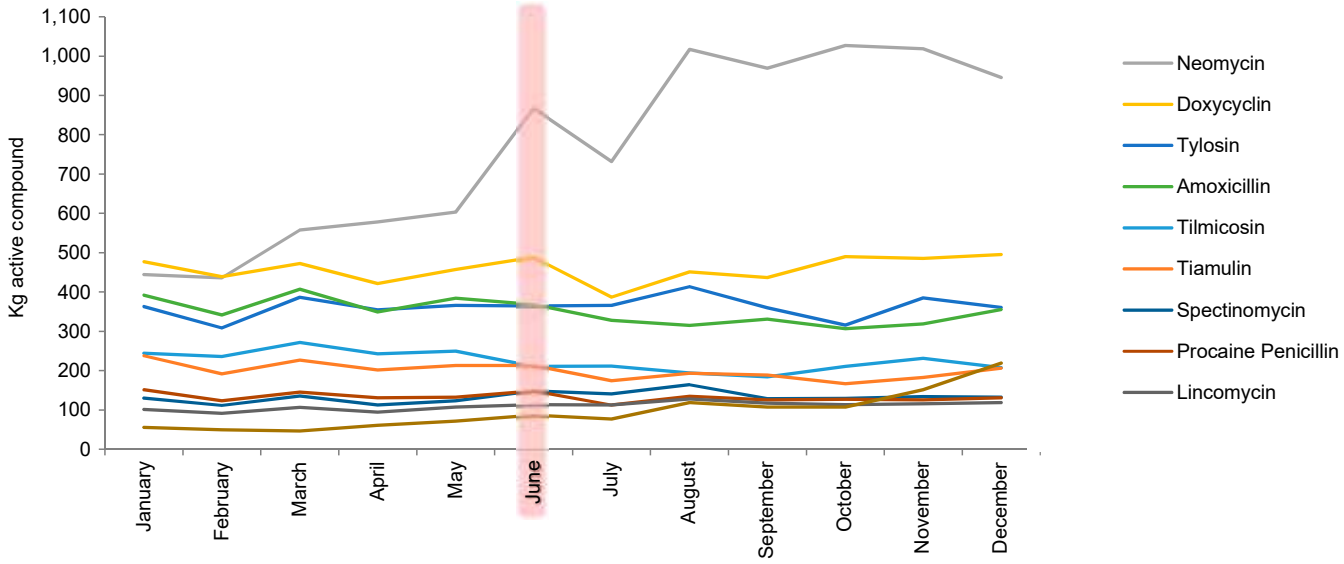
The Danish Medicines Agency implemented this decision on the 26th of June 2022. Thus, it has not been possible to use veterinary medicinal products (VMPs) containing zinc oxide for food-producing animals since then. It is still possible to add zinc oxide to the feed as a feed additive. However, this is at much lower doses than that found in the VMPs.

Zinc oxide as a VMP has primarily been used to control post-weaning diarrhoea in pigs. In the years leading up to the removal of VMPs containing zinc oxide, the Danish pig industry worked hard to find suitable alternatives in the post-weaning period. Despite these efforts, it has not been possible to find a solution that can replace the zinc oxide completely and at the same price. However, much good knowledge has been gained and many possible strategies to control post-weaning diarrhoea have been developed.

Despite this, there has been an increase in the antibiotic use for pigs in the post-weaning period since June 2022. Most notably, there has been an increase in the use of Neomycin (Figure 1) which is used to treat post-weaning diarrhoea in pigs. It should however be noted, that one dose of Neomycin contains more mg active compound than alternative treatment options and the rise in kg active compound will therefore seem larger. However, there is still a considerable increase in treatments for pigs in the post-weaning period.

The Danish Veterinary and Food Administration continues to follow the antibiotic use closely to see whether this rise in consumption is just a temporary trend or if new initiatives to reduce the antibiotic consumption in pigs are needed. The Danish Agriculture and Food Council and the Danish Veterinary and Food Administration are working together to find solutions for the farmers who find it difficult to get through the post-weaning period without VMPs containing zinc oxide.

Figure 1 The development in tthe use of the ten most used antibiotics in weaned pigs (age group 56), 2022 DANMAP 2022



Changes in the 10 most commonly used active compounds for pigs in age group 56 (weaned pigs up to 30 kg). Medicinal zinc oxide was phased out in June 2022

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Textbox 4.2

New EU legislation on veterinary medicinal products

Order 2019/6 on veterinary medicinal products has applied since 28 January 2022. The Order aims to reduce the administrative burden, enhance the internal market and increase the availability of veterinary medicinal products, while guaranteeing the highest level of public and animal health and environmental protection. There is a particular focus on reducing the risk of antimicrobial resistance (AMR).

In Denmark, the responsibility for the legislation on veterinary medicinal products (VMPs) is shared between the Danish Veterinary and Food Administration (DVFA) and the Danish Medicines Agency (DMA).

The DVFA is responsible for the regulatory framework on the use of VMPs.

The DMA is responsible for the regulatory framework for the placing on the market, manufacturing, import, export, supply, distribution and pharmacovigilance.

Provisions on the prescription and use of VMPs
The provisions on the prescription and use of VMPs appear from Articles 105 – 118. Provisions of particular relevance for the use of antimicrobials are:

- Article 105(6) which states that the quantity of the medicinal products prescribed shall be limited to the amount required for the treatment or therapy concerned. As regards antimicrobial medicinal products for metaphylaxis or prophylaxis, they shall be prescribed only for a limited duration to cover the period of risk.

- Article 106(1) according to which VMPs must be used in accordance with the marketing authorisations. This means that the instructions given in the marketing authorisation (SPC), including the dose and duration of treatment, must be followed.

- Article 107 provides restrictions on the use of antimicrobial medicinal products.
  Antimicrobials must not be used routinely nor be used to compensate for e.g. poor hygiene.

  Restrictions are set for prophylaxis and metaphylaxis:
  - Prophylaxis only allowed in exceptional cases and only for an individual animal or a restricted number of animals when the risk of infection is very high.
  - Antibiotics for prophylaxis allowed to an individual animal only when the risk of infection is very high.
  - Antimicrobials for metaphylaxis only allowed when the risk of spread of an infection is high and no appropriate alternatives are available.

Antimicrobials listed in Order 2022/1255 are reserved for treatment of certain infections in humans. For many years, Denmark has had a fairly detailed legislation on the use of VMPs. This legislation has been continued with minor adjustments to ensure compliance with the Regulation.

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Textbox 4.3

Faecal microbiota transplantation for prevention of diarrhoea in pigs

Background

Faecal microbiota transplantation (FMT) is a medical procedure in which faecal material from a healthy donor is transplanted into the gastrointestinal tract of a recipient to restore a healthy microbial balance in the gut. The procedure is used in human medicine to treat certain gastrointestinal disorders associated with an imbalance of the gut microbiota, particularly recurrent *Clostridioides difficile* infection [1]. In veterinary medicine, transplants of faeces or ruminal fluid have been used in horses and cows to restore the gastrointestinal microbiome after antibiotic treatment. In pigs there is experimental evidence that transplants of intact faeces or faecal filtrates can be used to colonize the gut immediately after birth [2-3]. As part of the EU project AVANT (https://avant-project.eu/), which is coordinated by the University of Copenhagen, FMT and other alternatives to antibiotics are being evaluated as a tool to reduce antimicrobial use in animals. AVANT focuses on diarrhoea in pigs because this is the animal species that accounts for most antimicrobials use in animals (see chapter 4, Figure 4.2) and diarrhoea is the main indication for antimicrobial use in pigs [4]. Alternatives to antimicrobials are an attractive solution to reduce antimicrobial use in the management of this disease as well as to address the lack of effective therapies following the zinc ban, the restrictions in the use of colistin and the rise in neomycin resistance in the causative pathogen, enterotoxigenic Escherichia coli (see Text Box 9.2). In another project led by the University of Copenhagen (financed by the Independent Research Foundation Denmark and SEGES-Innovation), we established an experimental paradigm for faecal transplants in neonatal pigs and then applied this for an experiment under faming conditions. Here, we report the methodology and the most significant data from this experiment. Samples from this experiment are now subject to further analysis in AVANT to understand the microbial changes occurring in the gut microbiota of treated animals.

Methods

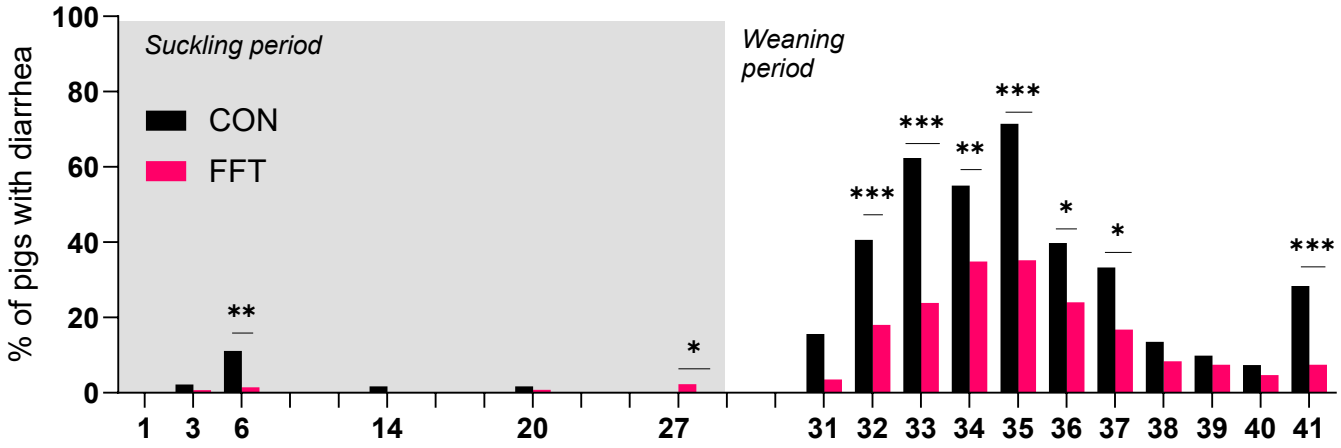
After thorough screening to exclude the presence of common pathogens, faeces from a healthy sow were diluted in a sterile buffer, centrifugated and filtrated using 0.45 µm membrane filters. The resulting filtrate was administered orally to 150 newborn piglets (FFT, 15 pigs from each of 10 litters) on the same farm as the donor sows to minimize the risk of pathogen transmission between farms. An equal number of piglets were treated with sterile buffer as the control group. On day 28, just before weaning, two piglets from each litter (40 piglets in total) were euthanized to collect intestinal tissue and content. The remaining piglets were weaned in a separate facility and transitioned to solid feed until the end of the study (41 days). A faecal scoring system was used to identify diarrhoeic piglets and the daily occurrence of diarrhoea and death were recorded in each group throughout the study.

Results and discussion

A significant lower occurrence of diarrhoea (Figure 1) and death (Figure 2) was observed in the weaning period compared to the control group. It should however be noted that considerable proportions of piglets in the treatment group suffered from diarrhoea and were euthanized or died during the post-weaning period (4% in the FFT group versus 16% in the control group), suggesting that although promising, FFT was unable to completely prevent post-weaning diarrhoea. Noteworthy, the experimental farm was selected based on its history of recurring post-weaning diarrhoea, and whether effects would be reproducible in other farms remains unknown at this stage. As it stands now, this intervention could help reduce the need for antimicrobials, but is unlikely to eliminate the use of antimicrobials to treat this disease when implemented in intensive pig farms. Further research is needed to optimize dosage, mode of administration, and donor selection, as well as to determine the individual effects of various components of filtrated transplants, including small bacteria, bacteriophages, and metabolites. During the latter part of the project, the AVANT consortium will evaluate how the use of antimicrobials in pig production would be reduced by the implementation of this and other alternatives to antimicrobials on a European scale.

Figure 1 Daily occurrence (%) of piglets displaying symptoms of diarrhoea in the treatment (FFT) and control (CON) groups

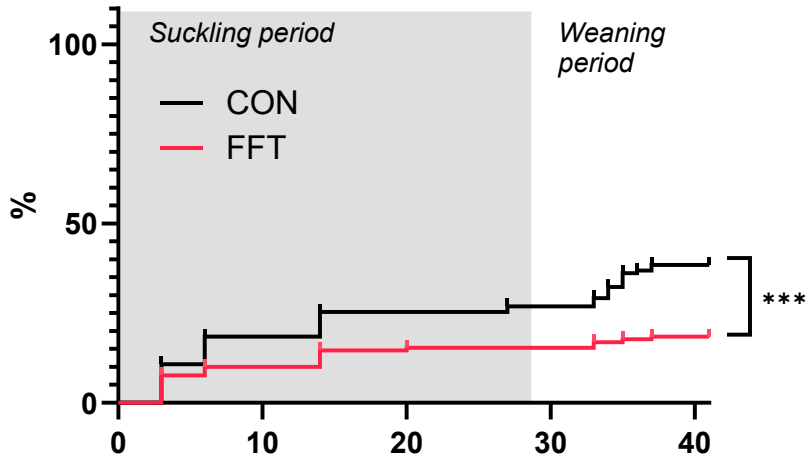
DANMAP 2022



Significant differences between groups are indicate as *p <0.05; **, p <0.01; ***, p <0.001

Figure 2 Mortality rate (%) in the treatment (FFT) and control (CON) groups

DANMAP 2022



Significant differences between groups are indicate as *p <0.05; **, p <0.01; ***, p <0.001

continued ... Textbox 4.3

Based on these results, the FMT protocol described in this study was selected by the AVANT consortium as one of the two interventions to be tested by a large clinical trial in Denmark, France and the Netherlands. However, the French and Danish national competent authorities denied the trial permission with reference to Regulation EC 767/2009, which prohibits to feed production animals with any material of faecal origin. Furthermore, this intervention cannot be considered a veterinary medicinal product as its content cannot be standardized and the active substance(s) are not characterised. A possible solution to this regulatory hurdle requires adaptation of feed legislation or new legislation to accommodate the development and commercialization of on-farm interventions that do not fall into the category of feed additives or veterinary medicines, while ensuring safety standards and efficacy. Accordingly, the AVANT consortium engaged with the European Commission and other stakeholders to initiate a legislative pathway to facilitate future clinical trials and commercialization of FMT approaches to prevent disease and reduce the use of antimicrobials in livestock.

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5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights

Total antimicrobial consumption in Denmark was 15.50 DID in 2022, 15% lower than 10 years ago in 2013 (18.44 DID) and minus 1.7% compared to consumption in 2019 (15.70 DID), underlining that consumption has resurged since the COVID-19 related marked decreases in 2020 and 2021.

In primary health care, total antimicrobial consumption was 13.59 DID in 2022, 1.3% lower than the 13.77 DID in 2019 and 16% lower than in 2013 (16.19 DID). Penicillins constituted 73% of the consumption and penicillins with extended spectrum and beta-lactamase sensitive penicillins were the two most used groups of antimicrobials (accounting for 24% each of total consumption in primary health care).

Antimicrobials prescribed for respiratory tract infections dropped sharply with the emergence of COVID-19 in 2020. The implemented societal restrictions prevented also the spread of viral respiratory infections. In 2022, the usual winter peak in antimicrobial consumption reached a higher level than observed in 2018-2019. This was probably due to a surge in respiratory infections altogether, including an early RSV epidemic in the autumn that overlapped with an early influenza season.

Antimicrobials prescribed to children also demonstrated marked decreases during the pandemic, regaining higher levels in 2022 but maintaining an observed downward trend for the past decade. Among the 0-4 year olds, consumption in 2022 was 188 treated patients per 1000 inhabitants, a 36% decrease compared to 358 treated patients per 1000 inhabitants in 2013. For the 5-9 year olds, 122 patients per 1000 inhabitants were treated in 2022 compared to 195 patients per 1000 inhabitants in 2013 (-37%).

Elderly inhabitants living at care homes during 2022 received 88% more antimicrobials than elderly inhabitants living in their own homes (1,833 prescriptions per 1000 inhabitants at long term care facilities compared to 976 prescriptions per 1000 inhabitants in their own homes). Urinary tract infections were the main cause of the observed difference in the treatment frequency. However, consumption for elderly inhabitants living at care homes has decreased by 30% from 2016 to 2022, while consumption for elderly living in their own homes has decreased by 18%.

Consumption in hospital care measured in DID (i.e. not accounting for hospital activity) was 1.86 DID in 2022, 4% lower than both in 2019 (1.93 DID) and in 2013 (1.90 DID). When measuring in DDD per 100 bed-days (DBD), the consumption in 2022 (127.89 DBD) was 7% higher than in 2019 (119.82 DBD) and 24% higher than in 2013 (103.51 DBD).

Product shortages are of increasing concern in antimicrobial supply. In 2022, penicillin/beta-lactamase inhibitor combinations decreased sharply in July and August 2022 due to product shortages. However, prescribers had access to the antimicrobials via special deliveries, why the overall consumption level was not affected.

5.1 Introduction

In Denmark, antimicrobials are available by prescription from medical doctors, veterinarians or dentists. Sale is restricted to licensed pharmacies who have exclusive right to sell prescription-only medicines, and no over-the-counter sale takes place. All consumption of medicinal products for humans is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority (Figure 2.1). This includes sales data from all public and private healthcare providers. Antimicrobial sales data have been submitted from the primary care sector since 1994 and from the hospital sector since 1997.

Registration of medicines consumption in the primary care sector covers sales from pharmacies to individuals and private clinics. Sales data contain an identifier of the prescriber and the patient's age, gender and address in addition to information about the ATC code, formulation, package size and number of packages sold. Since 2004, the Register of Medicinal Product Statistics also receives information on the indication for prescribing. This allows a very detailed and near-complete surveillance of all systemic antimicrobials used in Denmark in the primary health care.

For the hospital sector, antimicrobial consumption data from all public somatic hospitals with acute care function (referred to as somatic hospitals) are included in the report. Data from psychiatric hospitals, private hospitals and hospices are excluded, since they only account for a minor share of the consumption and no reliable denominator for measuring antimicrobial consumption in these facilities is available.

In this chapter, the term 'antimicrobials' covers all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code J01. In addition, since 2014 metronidazole (ATC code P01AB01) and for hospitals vancomycin (ATC code A07AA09) have been included. Consumption of tuberculostica, antifungal drugs and antivirals are not included in this chapter.

Changes in consumption of antimicrobials often mirror initiatives promoting prudent use of antibiotics and changes in health care organization. In 2012, the National Antibiotic Council was established following decisions on a national AMR strategy from 2010. The task of the National Antibiotic Council was to propose, promote and oversee actions for better management and prevention of antimicrobial resistance including fostering research in the area. In the following years, many different initiatives regarding prudent use of antibiotics were undertaken with particular focus on better diagnostics guiding antibiotic prescribing by general practisioners and working with antibiotic stewardship at hospitals. The former led to the establishment of the Danish Research Center for General Medicine while the latter was supported by the establishment of a network based on experiences from the Learning and Quality Teams at the bigger regional hospitals.

As many other European countries Denmark has also worked with annual antibiotic awareness campaigns since 2013, - except for in the pandemic years 2020-2022 - many of which can be found at www.antibiotikaellerej.dk.

Reorganization of the Danish healthcare system has led to functions being reassigned from hospital ambulatory care to smaller health units, rehabilitation centers and general practitioners. The resulting changes in activity across the healthcare sector may affect the consumption of antimicrobials. Finally, the COVID-19 pandemic and other infection waves had impact on activity in society, the healthcare system, and thus the spread and treatment of infectious diseases. These changes need to be considered when interpreting antimicrobial consumption surveillance data.

5.2 Total antimicrobial consumption in the Danish healthcare system

During the first five years of surveillance from 1996 to 2000, the consumption of systemic antimicrobials in Denmark showed no significant changes and consumption was estimated to be at 13 to 14 Defined Daily Doses per 1,000 inhabitants per day (DID). These first five years of reporting are not fully comparable to later years due to changes in reporting and in data systems. Between 2001 and 2011, consumption of antimicrobials increased steadily and peaked at a total of 18.95 DID in 2011 (not shown). From 2011 to 2021, consumption has decreased markedly (Figure 5.1).

In 2022, total consumption of antimicrobials was 15.50 DID (including all public and private healthcare facilities), which is 5% higher than the consumption in 2021 (14.72 DID) and 15% lower than the consumption 10 years ago in 2013 (18.14 DID) (Figure 5.1). In 2022, the primary care sector accounted for 13.59 DID (88%), the somatic hospital sector for 1.86 DID, whereas psychiatry, private hospitals and unspecified use accounted for 0.06 DID (not shown). The total consumption in 2021 corresponded to 48,215 kg active compound consumed.

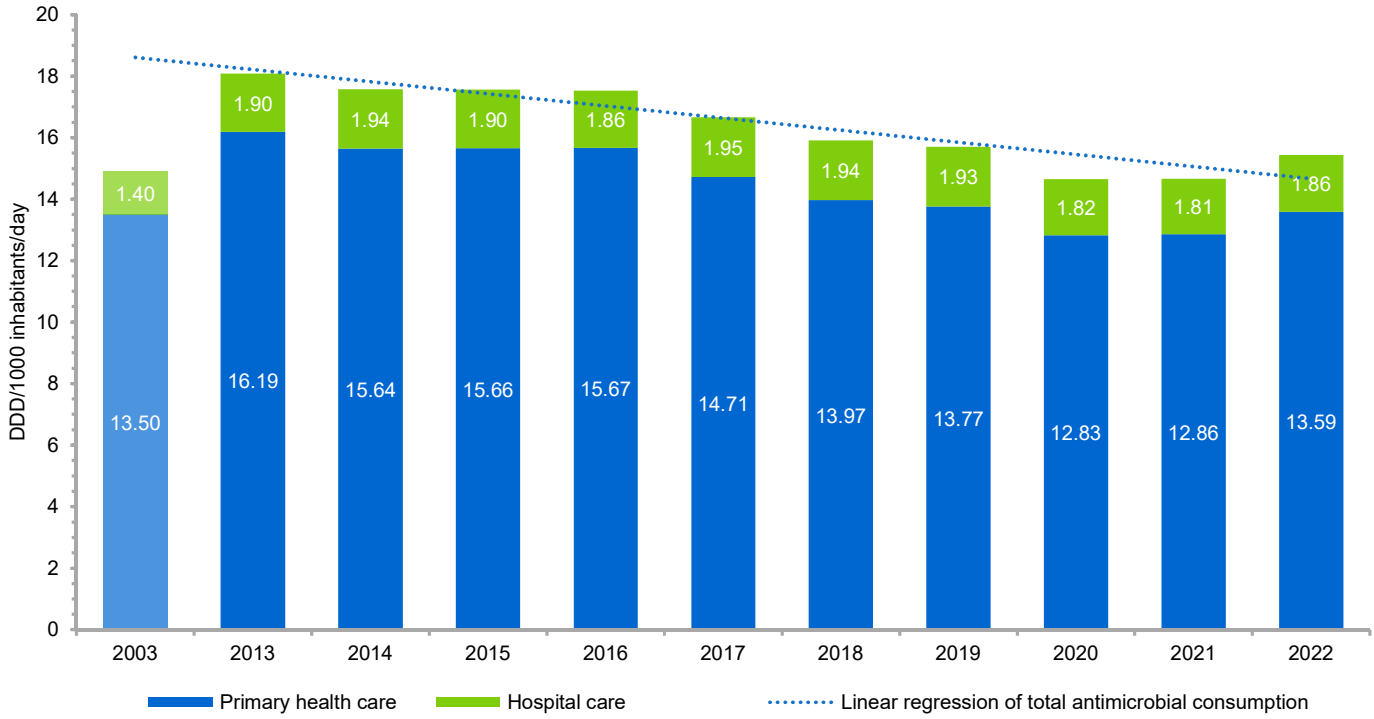
The decrease in total antimicrobial consumption since 2013 in Denmark has mainly been driven by reduced prescribing in primary health care. Measured in DID and not adjusted for hospital activity, antimicrobial consumption at hospitals fluctuated over the years; moving between the lowest levels of 1,86 DID in 2016 to highest levels of 1,95 DID in 2017. The notably lower levels of 1,82 DID in 2020 and 1,81 DID in 2021 are considered exceptions due to the COVID-19 pandemic. The hospital share of the total antimicrobial consumption increased from 10% in 2013 to 12% in 2022.

The main antimicrobial drug classes and their consumption in primary health care and at somatic hospitals are presented in Figure 5.2. Most notable are high use of beta-lactams in both health care sectors and low to none use of cephalosporins/aminoglycosides and of carbapenems in primary health care.

Consumption of antimicrobials in primary health care and somatic hospitals in the five Danish health regions is presented in Figure 5.3. The consumption decreased in all five regions in the primary sector since 2017. In 2022, the consumption was higher than in 2021 in all regions (4-6% increase). Region Zealand showed the highest total consumptions of 16.13 DID in 2022, whereas Central Region of Denmark had the lowest total consumption of 13.85 DID.

Figure 5.1 Total consumption of systemic antimicrobial agents in humans, DDD per 1,000 inhabitants per day, Denmark, 2003 and 2013-2022

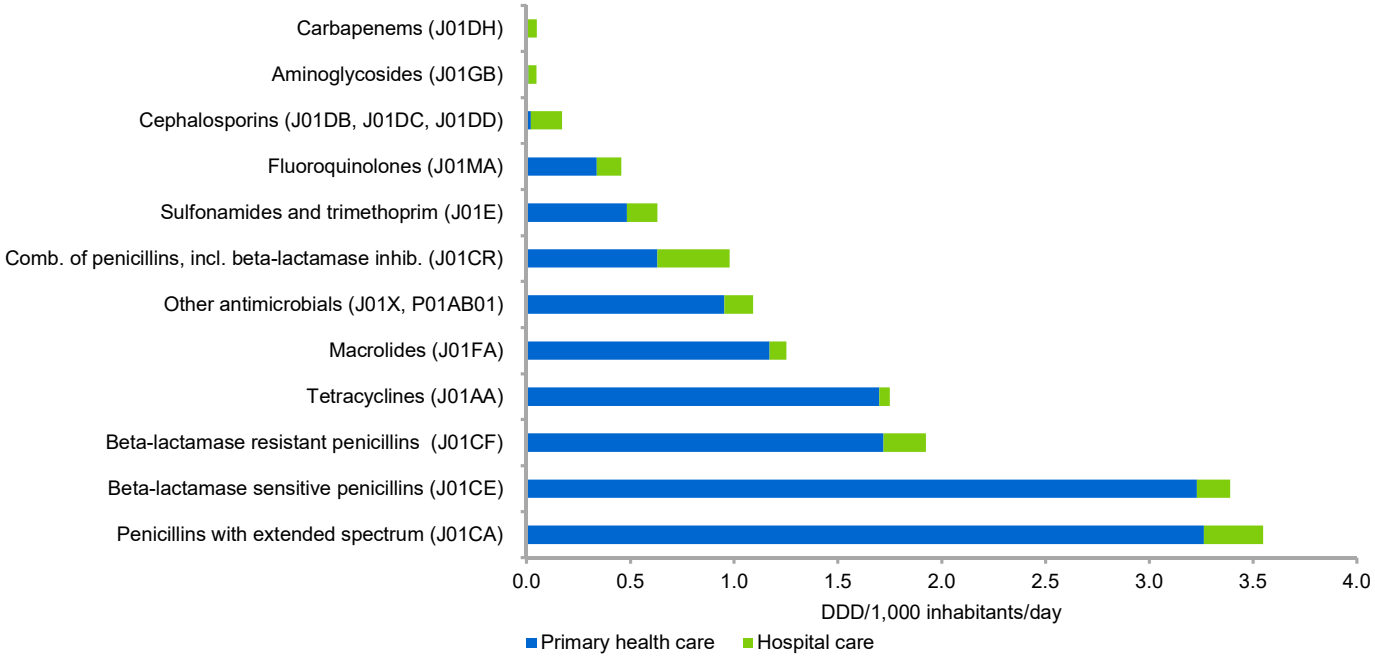
DANMAP 2022



Data: Total sale of antimicrobials in Denmark
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.2 Distribution of main antimicrobial classes used for humans in primary and hospital care, DDD per 1,000 inhabitants per day, Denmark, 2022

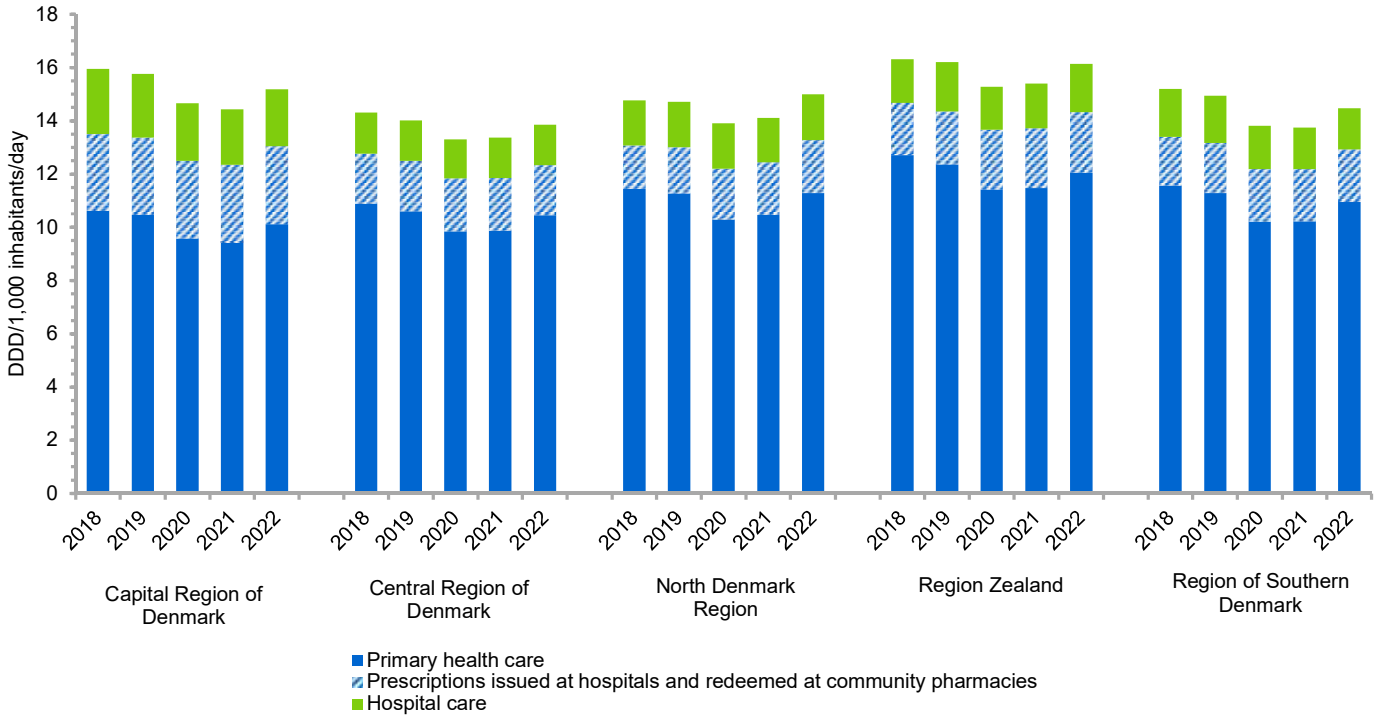
DANMAP 2022



Data: Registered sale of antimicrobials to individuals and antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

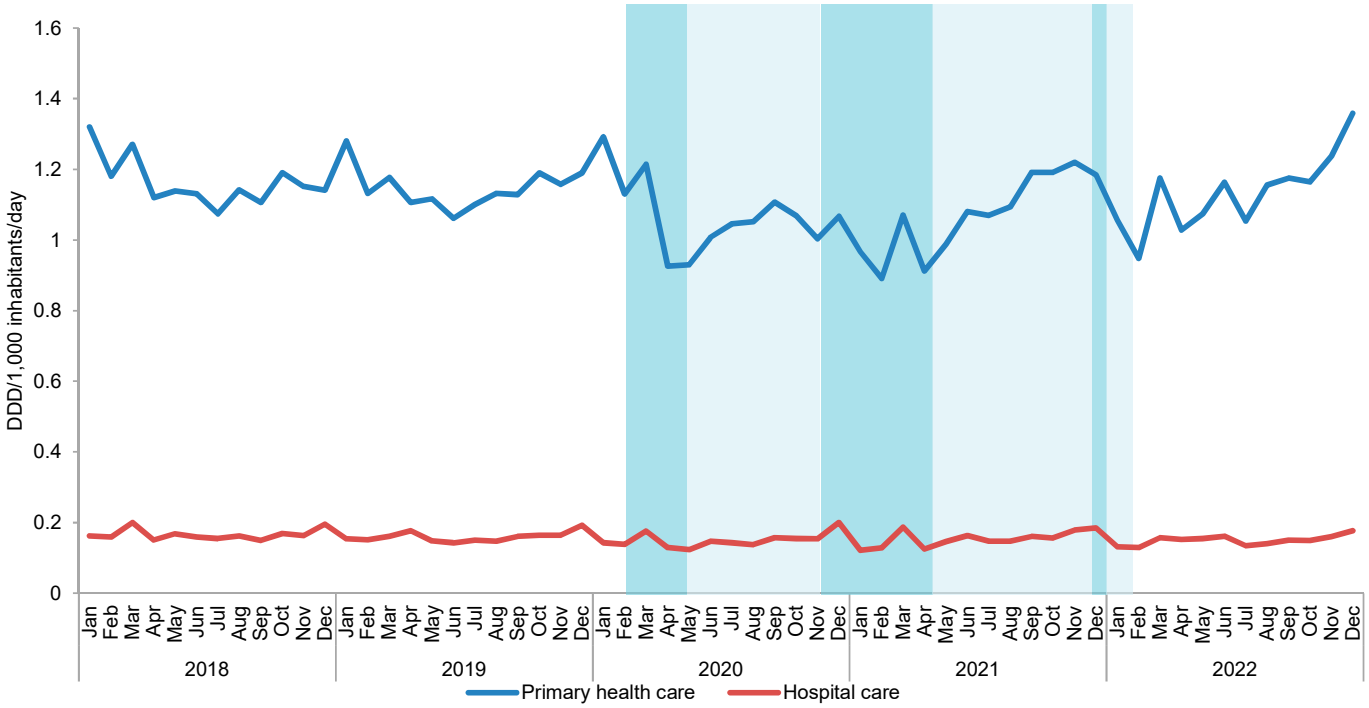
Figure 5.3 Consumption of systemic antimicrobial agents in primary health care and at somatic hospitals, DDD per 1,000 inhabitants per day, by Danish region, 2018-2022

DANMAP 2022



Data: Registered sale of antimicrobials to individuals and antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.4 Total consumption of systemic antimicrobial agents in humans per month in primary health care and at hospitals, DDD per 1,000 inhabitants per day, Denmark, 2018-2022 DANMAP 2022



COVID-19 restrictions in place
Fewer restrictions in place
Data: Total sale of antimicrobials in Denmark
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Monthly consumption data from 2018 to 2022 show the effect of the COVID-19 pandemic on antimicrobial consumption through changed healthcare delivery, infection rates and social life (Figure 5.4): Total antimicrobial consumption in primary care measured in DID was lower from April 2020 until May 2021 when compared to previous years but increased with the lifting of restrictions in the summer of 2021. Towards the end of 2022, antimicrobial consumption peaked at 1,36 DID in primary health care, which can be associated to increased treatment of sore throat and upper respiratory infection.

Detailed analysis of antimicrobial consumption data from primary health care and hospital care can be found in Section 5.3 and Section 5.4. For information on population size and hospital activity, see Figure 2.2 and Table 2.1 in Chapter 2 'Introduction'. A comparison of antimicrobial consumption in the human and the animal sector is shown in Figure 4.1 in Chapter 4 'Antimicrobial consumption in animals'.

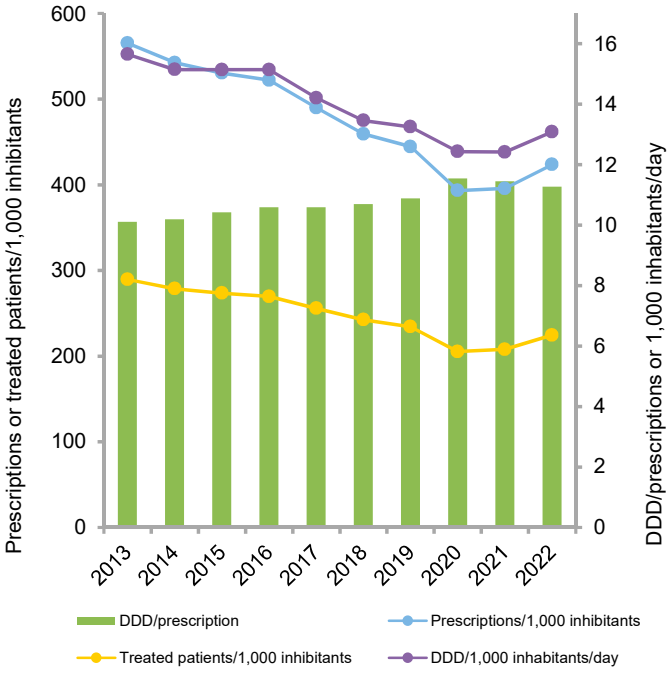
5.3 Antimicrobial consumption in primary health care

In the following sections, the consumption of antimicrobials in primary health care is described by the units DDD per 1,000 inhabitants per day, number of prescriptions per 1,000 inhabitants and number of treated patients per 1,000 inhabitants. The estimates are thus based on sales to individuals and do not include the approximately 4% of antimicrobials, mainly penicillins, sold to clinics and doctors on call. The antimicrobial consumption in 2022 is mainly compared to 2013 (10-year trend) and to 2019 to avoid the unusual COVID-19 years of 2020 and 2021.

5.3.1 Overall antimicrobial consumption in primary health care

Comparison of consumption trends over time by different indicators showed decreased consumption from 2013-2020, no change from 2020-2021 and increased consumption from 2021-2022 (Figure 5.5). In 2022, the average DDD/prescription was 11.3, 4% higher than in 2019 (10.9 DDD/prescription) and 12% higher than 2013 (10.1 DDD per prescription). The total number of prescriptions was 424 per 1,000 inhabitants in 2022, a 25% reduction from the 565 prescriptions per 1,000 inhabitants in 2013.

Figure 5.5 Consumption of systemic antimicrobial agents in primary health care, Denmark, 2013-2022 DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

In 2022 the total number of patients treated was 225 per 1,000 inhabitants (Table 5.3). In comparison, the number was 290 treated patients per 1,000 inhabitants in 2013. Thus, the number of treated patients and prescriptions has decreased over the decade, probably due to raising awareness among prescribers and the public. However, doses per prescription have increased, partly due to switch to antibiotics that contribute with more DDDs per treatment, e.g. the switch to pivmecillinam as drug of choice in the treatment of urinary tract infections and the switch to tetracycline as drug of choice in the treatment of chlamydia.

5.3.2 Consumption of antimicrobial groups

In compliance with treatment guideline, beta-lactamase sensitive penicillins were the most used antimicrobials in primary health care in Denmark for decades. However, this changed in 2020 where the consumption decreased 17% compared to 2019 due to COVID-19 restrictions. Since then, in 2020 and 2021 penicillins with extended spectrum were the most used antimicrobials. In 2022, beta-lactamase sensitive penicillins and penicillins with extended spectrum accounted both for 24% of total consumption in primary health care. Altogether the four penicillin groups (penicillins with extended spectrum; beta-lactamase sensitive penicillins; beta-lactamase resistant penicillins; combinations of penicillins, including beta-lactamase inhibitors) accounted for 8.84 DID (65%) of antimicrobials consumed in primary health care in 2022. Tetracyclines accounted for 1.70 DID (13%) and macrolides for 1.17 DID (9%). Fluoroquinolones accounted for 0.34 DID (2.5%), which is 8% lower than in 2019 (0.37 DID).

In 2013, the four groups of penicillins accounted for 9.89 DID, corresponding to 61% of the total consumption. Beta-lactamase sensitive penicillins accounted for 29%, penicillins with extended spectrum for 19%, beta-lactamase resistant penicillins for 8%, and combinations of penicillins, including beta-lactamase inhibitors accounted for 5%. Macrolides accounted for 12%. Other beta-lactams such as cephalosporins, monobactams and carbapenems were either used at extremely low level or restricted to hospital use only. For most other antimicrobial groups, the proportion of total consumption did not change notably.

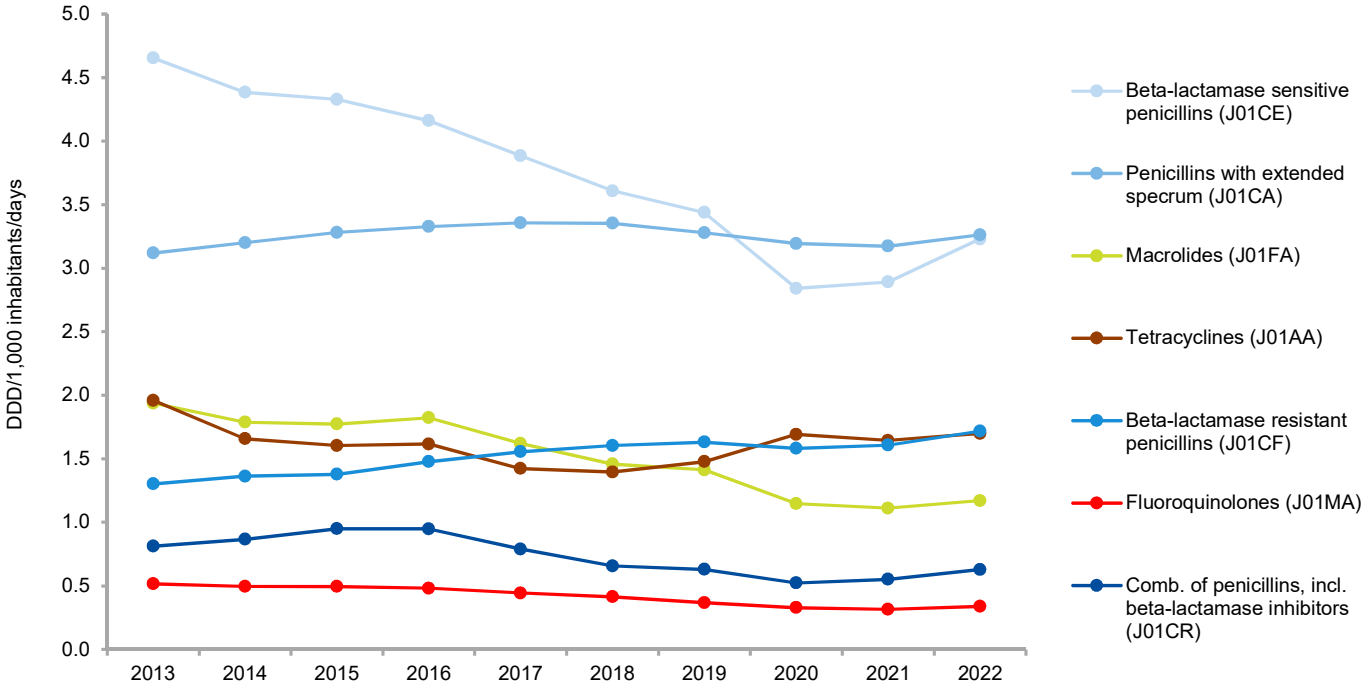
Penicillins

From 2013 to 2022, consumption of beta-lactamase sensitive penicillins decreased by 31% (from 4.65 DID in 2013), while beta-lactamase resistant penicillins increased by 32% (1.30 DID in 2013) (Figure 5.6). Consumption of penicillins with extended spectrum increased during the first years of the decade, but has since levelled off. Combination penicillins increased continuously from their introduction to the Danish market in 2009 until 2015 (0.95 DID), showed no changes in 2016 and since then declined until 2020. In 2021 and 2022, the consumption increased again and was in 2022 at the same level as in 2019 (0.63 DID).

The increases described for the penicillins with extended spectrum are primarily due to increases in the consumption of pivmecillinam, which accounted for 75% of this antimicrobial class in 2022 (not shown). Over the decade pivmecillinam increased by 15% from 2.13 DID in 2013 to 2.45 DID in 2022. In the same time period pivampicillin decreased by 74% from 0.25 DID to 0.07 DID and amoxicillin increased by 4% from 0.72 DID to 0.74 DID (not shown). Consumption of amoxicillin fluctuated within the decade, decreasing from 2011 to 2016 (0.61 DID), increasing from 2016-2019 by 12%, decreasing from 2019-2020 by 11% and increasing from 2020-2022 by 21% (not shown). Increases in the use of pivmecillinam were related to changed recommendations for the treatment of urinary tract infections, while the decreased use of pivampicillin followed increased resistance towards ampicillin in *E. coli* (see Section 8.2.1.) and use of amoxicillin followed recommendations on a more prudent use in children.

Figure 5.6 Consumption of leading antimicrobial groups for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2013-2022

DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care, DDD per 1,000 inhabitants per day, Denmark, 2003 and 2013-2022

DANMAP 2022

ATC group	Therapeutic group	Year										
		2003	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
J01AA	Tetracyclines	1.08	1.96	1.66	1.60	1.62	1.42	1.40	1.48	1.69	1.64	1.70
J01CA	Penicillins with extended spectrum	2.18	3.12	3.20	3.28	3.33	3.36	3.35	3.28	3.19	3.17	3.26
J01CE	Beta-lactamase sensitive penicillins	5.12	4.65	4.38	4.33	4.16	3.88	3.61	3.44	2.84	2.89	3.23
J01CF	Beta-lactamase resistant penicillins	0.86	1.30	1.36	1.38	1.48	1.56	1.60	1.63	1.58	1.61	1.72
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.03	0.81	0.87	0.95	0.95	0.79	0.66	0.63	0.52	0.55	0.63
J01D	Cephalosporins and other betalactam antibiotics	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02
J01EA	Trimethoprim and derivates	0.38	0.53	0.55	0.56	0.56	0.56	0.53	0.45	0.43	0.42	0.39
J01EB	Short-acting sulfonamides	0.36	0.22	0.21	0.18	0.16	0.15	0.14	0.13	0.11	0.09	0.09
J01EE	Combination of sulfonamides and trimethoprim, including derivates	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
J01FA	Macrolides	2.15	1.94	1.79	1.77	1.82	1.62	1.46	1.41	1.15	1.11	1.17
J01FF	Lincosamides	0.01	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.07	0.07	0.07
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluroquinolones	0.24	0.52	0.50	0.49	0.48	0.44	0.41	0.37	0.33	0.32	0.34
J01XC	Steroid antibacterials (combination fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00
J01XE	Nitrofuran derivates (nitrofurantoin)	0.42	0.49	0.48	0.45	0.43	0.26	0.15	0.27	0.27	0.28	0.27
J01XX	Other antibacterials (metheamine >99%)	0.32	0.24	0.24	0.25	0.27	0.28	0.29	0.32	0.34	0.39	0.42
J01XD and P01AB01	Nitroimidazole derivates (metronidazole)	0.18	0.28	0.28	0.28	0.28	0.25	0.24	0.24	0.23	0.24	0.24
J01 and P01AB01	Antibacterial agents for systemic use (total)	13.43	16.19	15.64	15.66	15.67	14.71	13.97	13.77	12.83	12.86	13.59

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.2 Number of prescriptions per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2003 and 2013-2022

DANMAP 2022

ATC group	Therapeutic group	Year										
		2003	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
J01AA	Tetracyclines	18.58	22.89	20.00	17.90	17.18	15.89	14.63	15.11	20.19	18.25	18.71
J01CA	Penicillins with extended spectrum	98.79	114.30	113.83	113.53	113.16	114.37	114.31	112.19	105.93	107.97	112.19
J01CE	Beta-lactamase sensitive penicillins	226.86	180.54	170.70	163.09	157.13	148.52	136.81	128.77	104.07	107.28	122.88
J01CF	Beta-lactamase resistant penicillins	37.47	41.25	41.04	40.81	41.87	41.87	43.35	43.16	42.87	43.17	45.66
J01CR	Combinations of penicillins, including betalctamase inhibitors	1.64	28.01	29.02	30.73	31.13	27.09	23.71	23.07	19.14	20.36	23.45
J01E	Sulphonamides and trimethoprim	53.98	43.53	41.51	38.39	36.41	34.29	31.74	28.14	25.59	23.07	21.26
J01FA	Macrolides	87.58	74.51	68.01	68.00	68.85	60.00	52.64	50.71	33.66	33.80	36.94
J01MA	Fluoroquinolones	11.70	20.65	19.67	19.50	18.74	17.37	15.97	13.99	12.07	11.41	11.96
J01X	Other antibacterials (methenamine >99%)	13.95	17.41	16.73	16.28	15.82	10.18	6.76	10.29	10.62	10.70	10.72
P01AB01	Nitroimidazole derivatives (metronidazole)	13.93	19.26	19.06	19.15	18.63	17.26	16.31	15.78	15.62	16.00	16.17
J01 and P01AB01	Antibacterial agents for systemic use (total)	565.60	565.26	542.53	530.56	522.19	490.08	459.39	444.53	393.34	395.76	423.71

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.3 Number of treated patients per 1,000 inhabitants for leading antimicrobial agents in primary health care, Denmark, 2003 and 2013-2022

DANMAP 2022

ATC group	Therapeutic group	Year										
		2003	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
J01AA	Tetracyclines	11.35	13.86	12.20	11.32	11.04	10.35	9.69	10.10	14.43	12.99	13.64
J01CA	Penicillins with extended spectrum	68.76	76.10	75.32	74.87	74.05	74.04	73.56	71.97	67.14	68.60	71.45
J01CE	Beta-lactamase sensitive penicillins	172.55	142.19	134.79	130.06	125.69	119.32	110.90	104.70	84.93	87.69	100.09
J01CF	Beta-lactamase resistant penicillins	26.38	29.07	29.24	28.85	29.70	29.96	31.10	31.06	30.52	30.89	32.93
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	1.10	19.71	20.52	22.03	22.17	19.89	17.73	17.33	14.43	15.50	17.90
J01E	Sulphonamides and trimethoprim	36.46	26.16	24.65	22.45	21.17	19.87	18.42	16.63	15.04	13.66	12.67
J01FA	Macrolides	64.13	56.16	51.38	51.75	53.21	46.01	40.11	38.45	25.13	24.97	27.16
J01MA	Fluoroquinolones	8.89	16.04	15.30	15.04	14.37	13.36	12.26	10.74	9.01	8.52	9.10
J01X	Other antibacterials (methenamine >99%)	6.95	7.48	7.16	7.35	7.47	5.01	3.62	5.66	5.80	5.95	5.91
P01AB01	Nitroimidazole derivatives (metronidazole)	11.94	16.51	16.31	16.47	16.03	14.84	14.05	13.57	13.36	13.77	13.94
J01 and P01AB01	Antibacterial agents for systemic use (total)	305.03	289.54	278.62	273.49	269.72	255.72	242.55	234.34	205.27	207.85	224.57

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Tetracyclines and macrolides

In 2022, tetracycline consumption in primary health care was 1.70 DID, corresponding to 13% of the total consumption, whereas macrolides accounted for 1.17 DID (9%) (Figure 5.6). During the last decade, the consumption of tetracyclines decreased from 1.96 DID in 2013 (13%). Compared to 2019, the consumption of tetracyclines was 15% higher in 2022. Macrolides decreased from 1.94 DID in 2013 (-40%) and from 1.41 DID in 2019 (-17%).

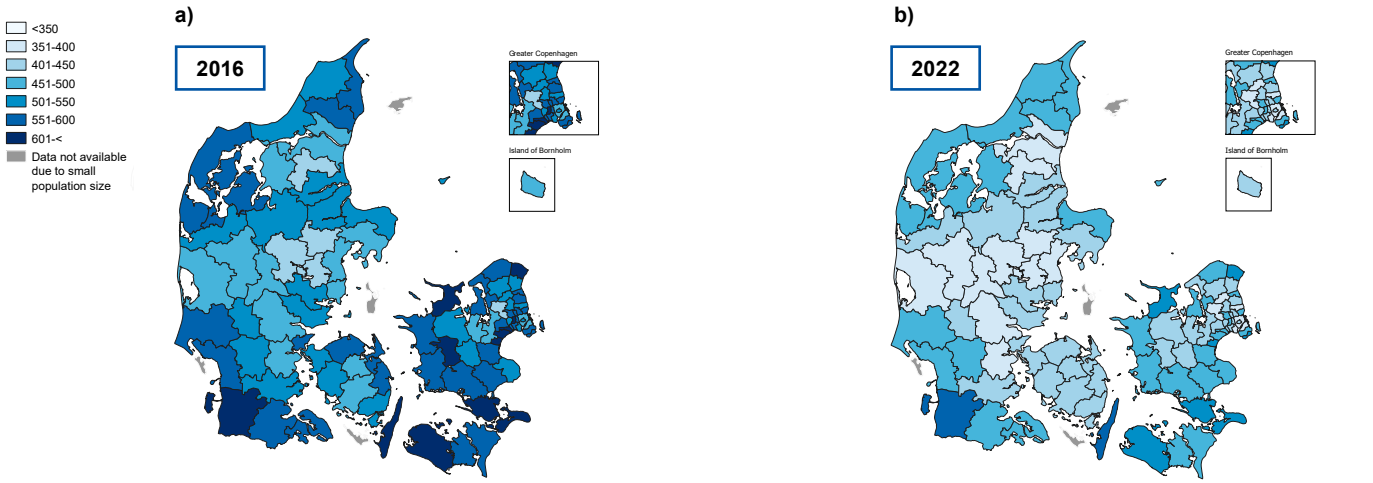
These changes in tetracycline and macrolide consumption may reflect compliance with the new guideline for chlamydia treatment issued by the Danish Dermatological Society in 2019. The guideline recommends doxycycline as first-line treatment instead of the previously recommended azithromycin. The treatment recommendation was changed due to concerns in Denmark about increasing azithromycin-resistance in *Mycoplasma genitalium*, a frequent co-infection in patients with chlamydial infections.

5.3.3 Antimicrobial consumption by prescriber

Interregional differences in the levels of prescribing have been described in DANMAP since 2017 (Table 5.4). In general, the Danish population is relatively homogenous and health care is of standardized quality, which, combined with several initiatives to educate GPs in appropriate prescribing, diminishes potential differences in prescribing trends. However, observed variations in prescribing may owe to differences in population density (distance to nearest general practitioner), differences in age and comorbidity of the population (younger populations in bigger cities and in the capital region) as well as behavioral differences between urban and rural populations.

Figure 5.7 shows the number of prescriptions per 1,000 inhabitants at municipality level in 2016 and 2022, respectively. In 2022, the consumption ranged from 351 to 582 prescriptions per 1,000 inhabitants. In 2016, the range was 434-727 prescriptions per 1,000 inhabitants. Of note is that prescribers in all municipalities reduced their prescribing activities in the shown period. Demographic differences might impact the range of prescribing. Distribution of elderly inhabitants above 60 years in the municipalities follows almost the distribution of prescriptions per 1,000 inhabitants with higher prescription rates in municipalities with bigger population of elderly inhabitants above 60 years (data not shown).

Figure 5.7 Number of prescriptions from primary health care per 1,000 inhabitants in Danish municipalities in a) 2016 and b) 2022
DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.4 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark, 2018-2022 DANMAP 2022

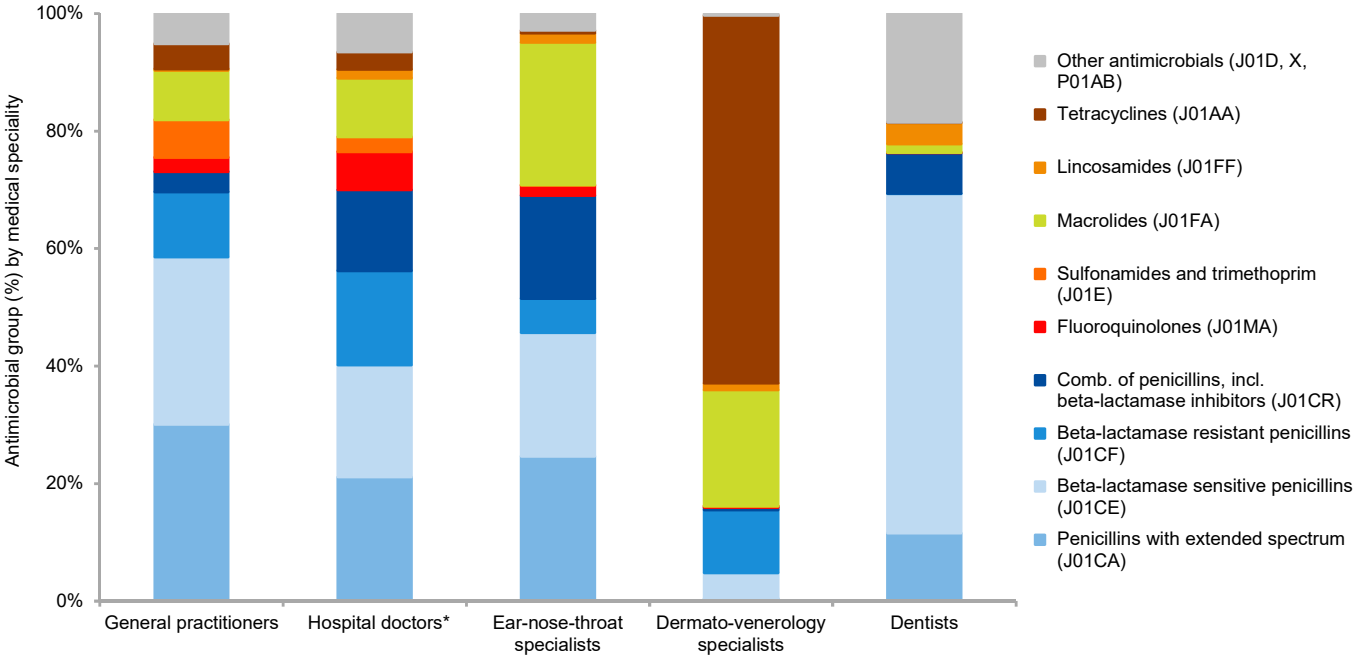
Region	Indicator	Year				
		2018	2019	2020	2021	2022
Capital Region	DDD/1,000 inhabitants/day	13.49	13.37	12.49	12.34	13.05
	Prescriptions/1,000 inhabitants	453	441	382	378	410
Region Zealand	DDD/1,000 inhabitants/day	14.68	14.36	13.66	13.72	14.32
	Prescriptions/1,000 inhabitants	501	482	436	440	466
Region of Southern Denmark	DDD/1,000 inhabitants/day	13.39	13.16	12.17	12.18	12.92
	Prescriptions/1,000 inhabitants	470	455	401	405	434
Central Denmark Region	DDD/1,000 inhabitants/day	12.76	12.49	11.84	11.84	12.33
	Prescriptions/1,000 inhabitants	431	417	374	380	402
North Denmark Region	DDD/1,000 inhabitants/day	13.07	13.00	12.21	12.43	13.28
	Prescriptions/1,000 inhabitants	452	436	390	400	432
Denmark (total)	DDD/1,000 inhabitants/day	13.46	13.25	12.43	12.42	13.08
	Prescriptions/1,000 inhabitants	459	445	393	396	424

Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Prescribing trends in primary health care also clearly differ by prescriber's specialty. Table 5.5 shows an overview of number of prescriptions issued by different specialists, including hospital doctors issuing prescriptions for patients at hospitals, which then are redeemed at a community pharmacy. Compared to 2019, the number of prescriptions per 1,000 inhabitants in 2022 was 8% lower for general practitioners and 15% lower for dermato-venerology-specialists. On the other hand, the number of prescriptions per 1,000 inhabitants was 20% higher for dentists in 2022 compared to 2019. For other specialties, minor changes were observed (<5%). Numbers of DDD per prescription also differ by prescriber's specialty (Table 5.5). Dermato-venerology-specialists prescribed the highest number of DDDs per prescription in 2022 (35 DDD/prescription, 12% higher than 2018). Number of DDDs/prescription increased by 5% and 8% for general practitioners and hospital doctors since 2018. These changes can among other be due to changes in treatment guidelines recommending antimicrobials that contribute with more DDDs per treated infection.

Figure 5.8 shows the main antimicrobial groups prescribed by medical specialty in primary health care in 2022. Also here, prescriptions from "hospital doctors" cover prescriptions issued to patients in ambulatory care and upon discharge from hospital and redeemed at the community pharmacy. In 2022, 63% of antimicrobial prescriptions from dermato-venerology-specialists were tetracyclines, which are indicated for treatment of severe acne and sexually transmitted chlamydia/mycoplasma infections. Majority of prescriptions by dentists were narrow-spectrum beta-lactamase sensitive penicillins (58%) reflecting adherence to the recommended first-line treatment for common dental infections in primary health care.

Figure 5.8 Antimicrobial groups prescribed by main medical specialities, primary health care, Denmark, 2022 DANMAP 2022



* Hospital doctors issuing prescriptions for patients in ambulatory care or upon discharge from hospital
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table 5.5 Number of prescriptions per 1,000 inhabitants by main medical specialities, Denmark, 2018-2022 DANMAP 2022

Prescriber		Year				
		2018	2019	2020	2021	2022
General practitioners	Prescriptions per 1,000 inhabitants	341.8	327.1	280.4	279.0	300.9
	DDD per prescription	10.3	10.4	11.1	11.0	10.8
Hospital doctors*	Prescriptions per 1,000 inhabitants	63.0	63.2	64.6	63.5	62.0
	DDD per prescription	12.6	12.8	13.1	13.2	13.6
Ear-nose-throat specialists	Prescriptions per 1,000 inhabitants	8.4	7.8	6.1	6.9	8.1
	DDD per prescription	8.1	8.1	8.9	8.3	8.1
Dermato-venerology specialists	Prescriptions per 1,000 inhabitants	5.2	5.4	5.3	5.0	4.6
	DDD per prescription	31.3	33.4	33.9	35.5	35.0
Dentists	Prescriptions per 1,000 inhabitants	27.8	28.8	25.6	28.9	34.4
	DDD per prescription	7.8	7.9	7.9	7.7	7.7

* Hospital doctors issuing prescriptions for patients in ambulatory care or upon discharge from hospital, redeemed at community pharmacies
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system

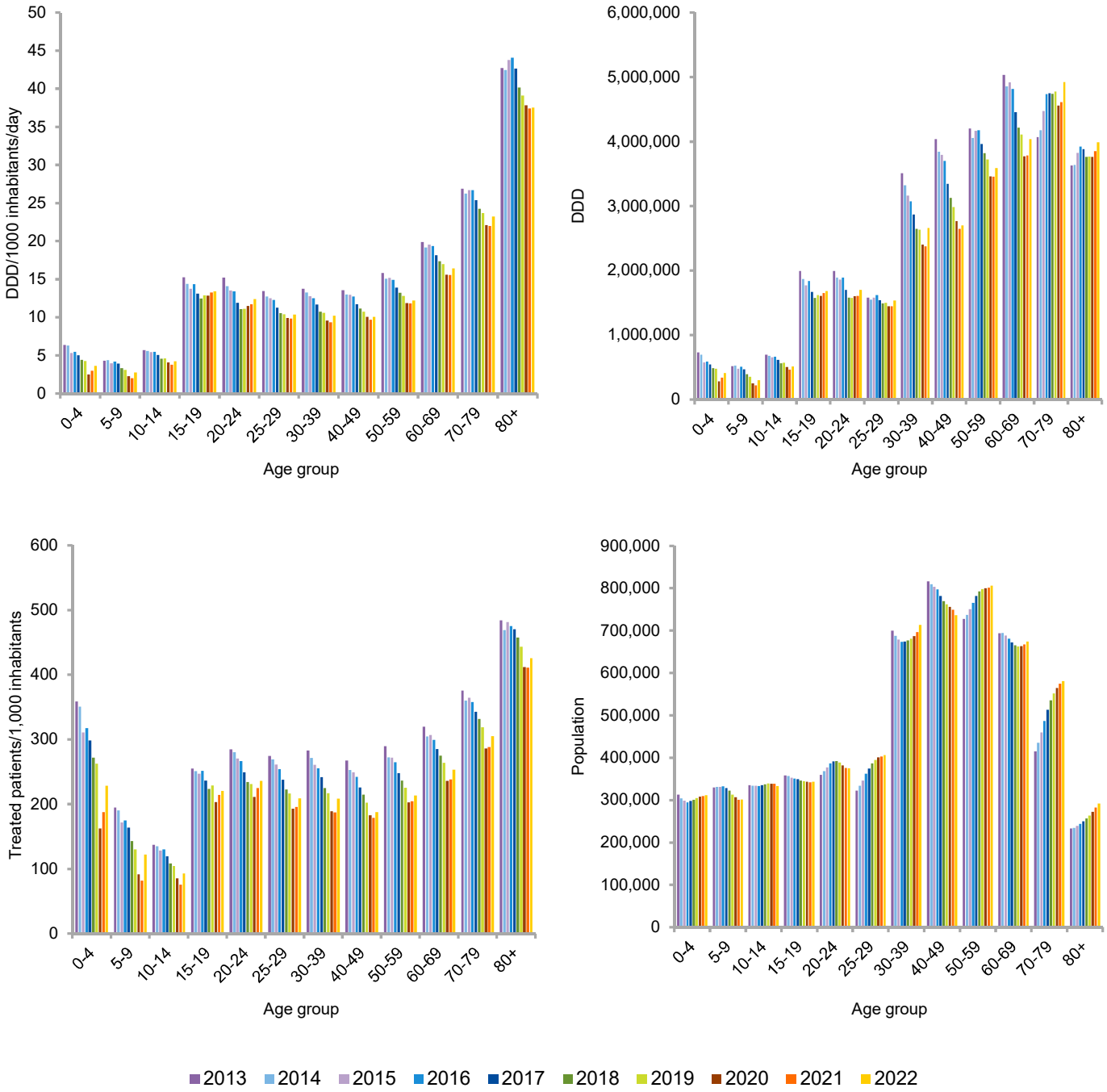
General practitioners have access to their own prescribing data through ordiprax+, an online dashboard with personal log-in which visualises prescribing data and enables comparisons with other practices on regional level (DANMAP 2020 Textbox 5.2).

5.3.4 Antimicrobial consumption by patient case mix

Antimicrobial consumption is highly affected by patient case mix. The need for antimicrobials is different throughout life and for the two genders. Antimicrobial consumption is also affected by other sociodemographic factors (Textbox 5.2).

Figure 5.9a-c presents consumption of antimicrobials by age group based on different denominators: Figure 5.9a presents consumption in DDD per 1,000 inhabitants per day, Figure 5.9b in crude DDD, i.e. not corrected for population size. Figure 5.9c presents the number of patients treated per 1,000 inhabitants. Figure 5.10d presents population size by age group. All figures show data from 2013 to 2022. Children and adolescents are presented in five-year age groups, while adults are clustered in 10-year age groups.

Figure 5.9 Consumption of systemic antimicrobial agents in primary health care by age group, measured in a) DDD per 1,000 inhabitants per day, b) DDD, c) treated patients per 1,000 inhabitants and d) population size, Denmark, 2013-2022 DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and Statistics Denmark

In 2022, 225 patients per 1,000 inhabitants were treated with antimicrobials, receiving 424 prescriptions per 1,000 inhabitants. In 2019, the corresponding numbers were approximately 4% higher, 234 treated patients and 445 prescriptions per 1,000 inhabitants. Since 2013, the consumption decreased from 290 treated patients per 1,000 inhabitants and 565 prescriptions per 1,000 inhabitants (reduction by 22% and 25% from 2013 to 2022, respectively).

Estimates of antimicrobial consumption for children using DDD need to be interpreted with caution since the DDD is defined as "maintenance dose per day for its main indication in adults". The maintenance dose per day for children may differ from the one for adults due to different pharmacodynamics and -kinetics. Furthermore, infants and young children in the same age group might be treated with different doses based on body weight. Therefore, other units of measurement might be more suitable to monitor consumption in children, e.g. number of treated patients per 1,000 inhabitants and number of prescriptions per 1,000 inhabitants.

Consumption in the 0-4 year olds. Consumption of antimicrobial agents in the youngest age group decreased by 36% from 2013 (358 treated patients per 1,000 inhabitants) to 2022 (229 treated patients per 1,000 inhabitants). After the significant decrease observed from 2019 to 2020 (-38%), the consumption remained 13% lower in 2022 than in 2019 (262 treated patients per 1,000 inhabitants) (Figure 5.11a). The antimicrobials used also changed during the last decade, but penicillins with extended spectrum and beta-lactamase sensitive penicillins remained the main antimicrobial agents used to treat children between 0-4 years in 2022 (117 and 119 patients per 1,000 inhabitants, respectively) (Figure 5.10a).

Consumption in the 5-9 year olds. In 2022, 122 patients per 1,000 inhabitants of 5-9 years were treated with antimicrobial agents (Figure 5.9c). This is 37% lower than 2013 (195 patients per 1,000 inhabitants) and 6% lower than 2019 (130 patients per 1,000 inhabitants). The distribution of the antimicrobials used to treat 5-9 year olds did not change markedly over the last decade (Figure 5.10b), and beta-lactamase sensitive penicillins remained the main antimicrobial agent used (81 patients per 1,000 inhabitants, 66% of total consumption in 2022).

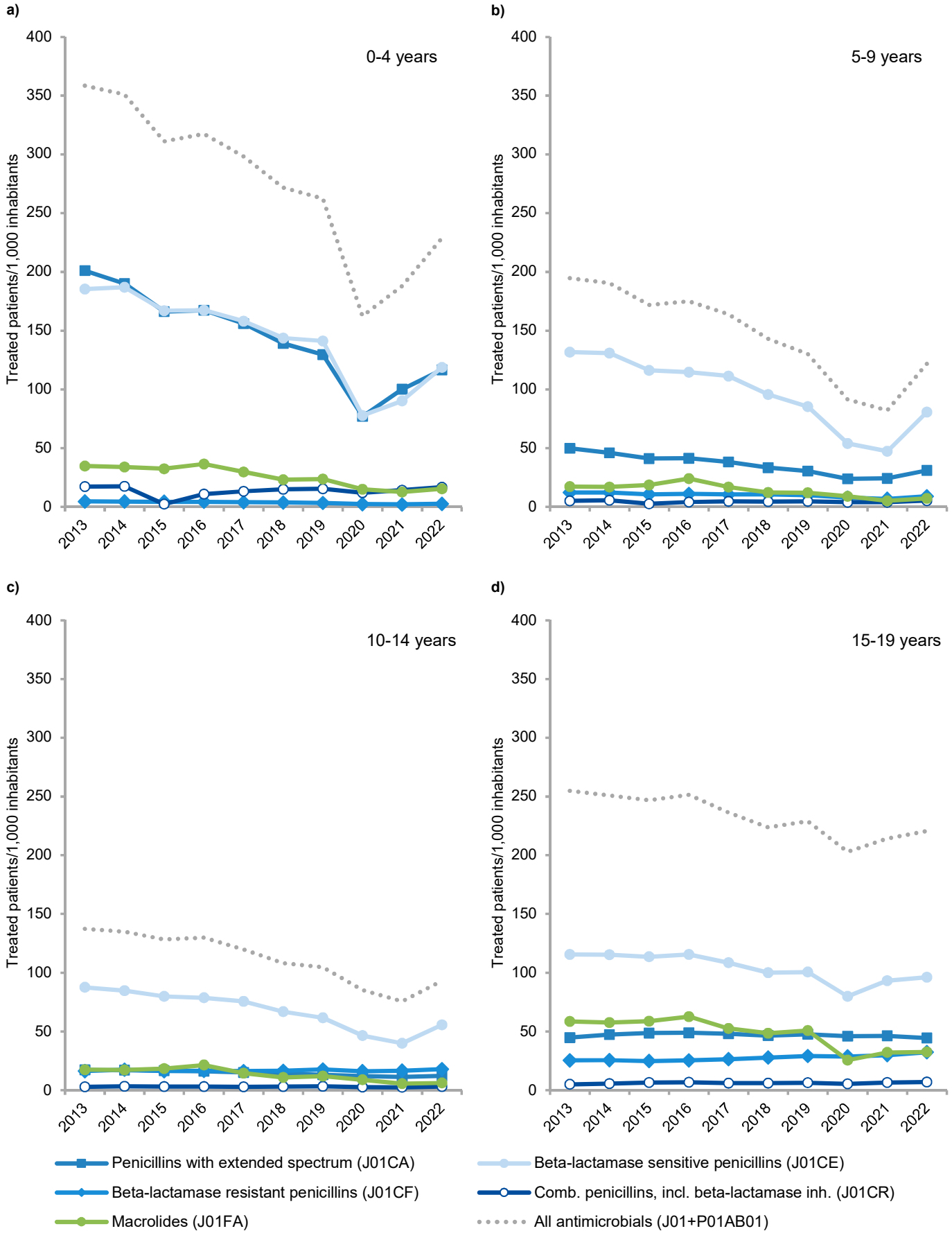
Consumption in the 10-14 year olds. In 2022, the total consumption of antimicrobial agents (93 patients per 1,000 inhabitants) was 32% lower than a decade ago (137 patients per 1,000 inhabitants) and 11% lower than 2019 (105 patients per 1,000 inhabitants) (Figure 5.10c). Consumption data shows the lowest level of consumption in 2021 (76 patients per 1,000 inhabitants). Hereafter the consumption increased 23% to the level observed in 2022. Beta-lactamase sensitive penicillins remained the main antimicrobial agent (60%) even with continuous reduction in consumption the last decade (Figure 5.10c).

Consumption in the 15-19 year olds. Consumption of antimicrobial agents in older teenagers was 13% lower in 2022 than in 2013 and 4% lower than in 2019 (Figure 5.9a-c). In 2022, 221 patients per 1,000 inhabitants were treated with antimicrobial agents, whereas 229 patients per 1,000 inhabitants were treated in 2019. The observed decrease the last decade, was driven by a 44% reduction of macrolides and a 17% reduction of beta-lactamase sensitive penicillins (Figure 5.10d).

Macrolides play an important role in the treatment of bacterial respiratory tract infections in children and adolescents. Macrolides were also used as first-line treatment for chlamydia infections until the change in guidance (already described in DANMAP2020) which may be the reason for the relatively high consumption of macrolides in the 15-19 year olds. However, penicillins are the most used antimicrobial agents for children and adolescents, constituting between 44% and 66% of all antimicrobials prescribed depending on age group (Figure 5.10).

Figure 5.10 Consumption of five main antimicrobial agents for children/adolescents by age group, a) 0-4 years, b) 5-9 years, c) 10-14 years and d) 15-19 years, Denmark, 2013-2022

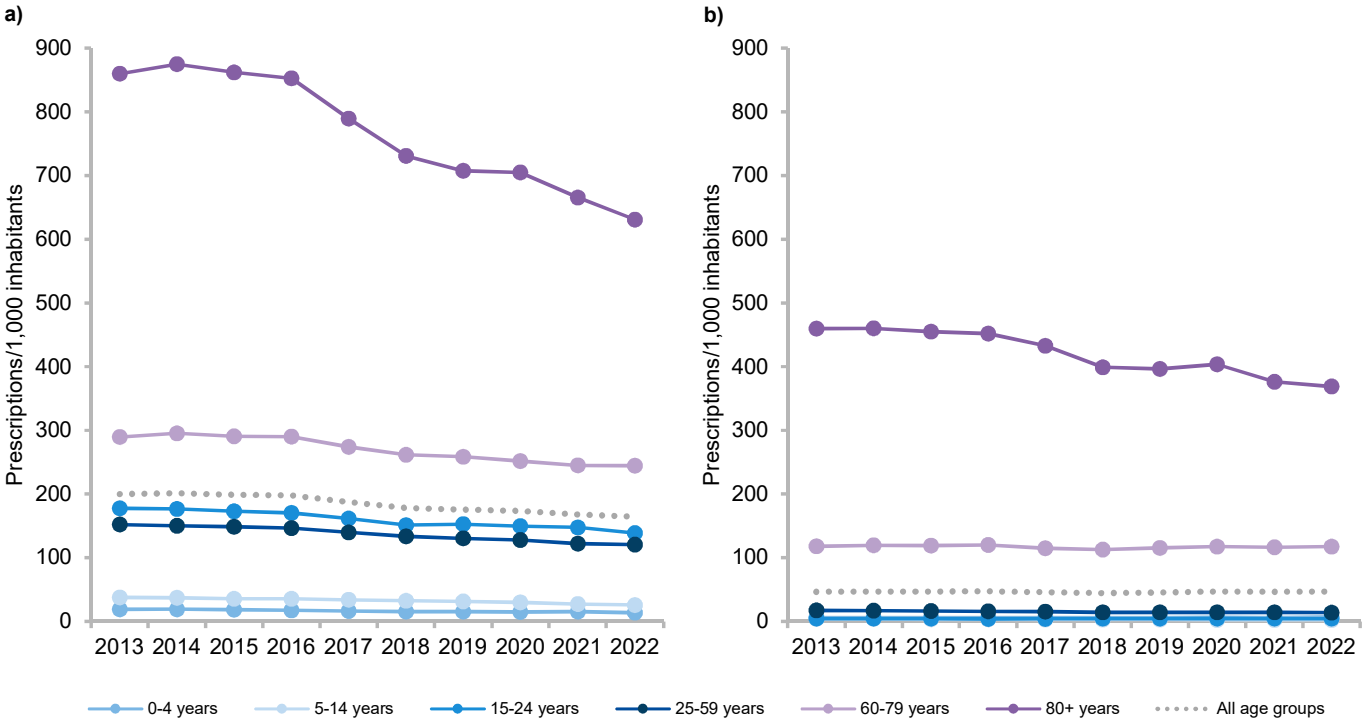
DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Differences in antimicrobial consumption between genders are well known. From 2013 to 2022, the number of treated females (all age groups) decreased by 22% from 339 to 265 per 1,000 inhabitants per year and the number of treated males decreased by 23% from 240 to 183 per 1,000 inhabitants per year. In general, females receive more treatment – a trend driven by higher incidence of urinary tract infections and different healthcare-seeking behavior. Thus, the consumption of pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin, all indicated for treatment of urinary tract infections, is approximately three times higher for females than for males (Figure 5.11). The reduction in consumption of these antimicrobials was primary driven by fewer prescriptions for elderly women (80+ years), who are the most frequently treated (631 prescriptions per 1,000 females above 80 years). Also for antimicrobials used to treat respiratory tract infections (penicillins and macrolides) the differences in consumption between genders are substantial (Figure 5.12). These differences may be due to different healthcare seeking behavior more than higher incidence of infection.

Figure 5.11 Consumption of antimicrobials primarily used for treatment of urinary tract infections* in primary health care for a) females and b) males, prescriptions per 1,000 inhabitants, Denmark, 2013-2022 DANMAP 2022



* Pivmecillinam, sulfonamides, trimethoprim and nitrofurantoin
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

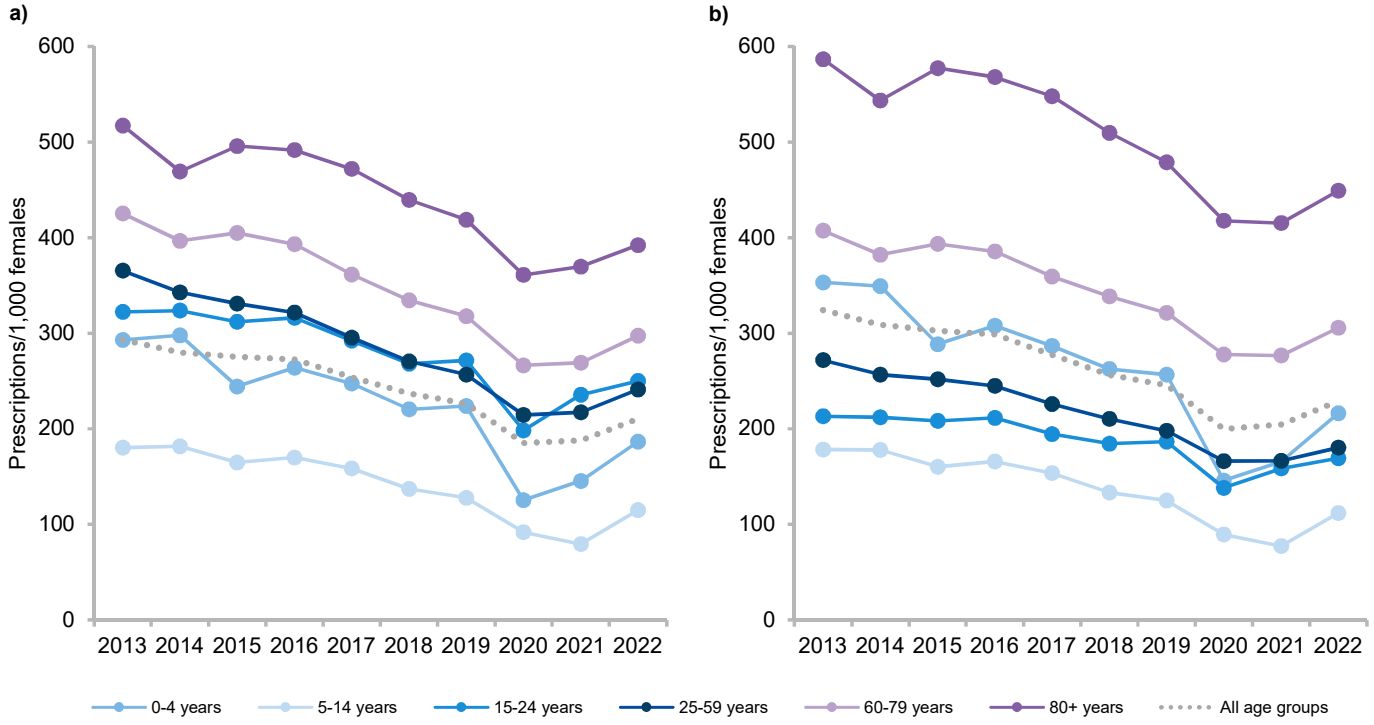
5.3.5 Antimicrobial consumption for treatment of respiratory tract infections

One of the main indications provided by prescribers in primary health care for treatment with antimicrobials is upper and/or lower respiratory tract infections. In 2020, consumption of antimicrobials prescribed for treatment of respiratory tract infections started slightly lower compared to previous years, and in addition was followed by a sharp drop in consumption from April 2020 to July 2021. This coincided with a sharp decrease in number of laboratory confirmed influenza and RSV infections, most likely due to the societal restriction implemented in March 2020 due to the COVID-19 pandemic (Figure 5.13). However, from August 2021 the consumption went back to levels similar to the corresponding pre-pandemic months in 2019, again coinciding with the Respiratory Syncytial Virus (RSV) summer epidemic in 2021. In 2022, the winter peak in antimicrobial consumption reached a higher level than observed in 2018-2019. This coincided with an early RSV and influenza season as well as an outbreak of Group A streptococci, as also observed in other European countries.

Figure 5.13 shows the first influenza A, influenza B and RSV positive PCR test per person per season for 2018 to 2022. The influenza season starts in week 40 and ends in week 39 in the following year. The RSV season starts in week 21 and ends in week 20 in the following year. Laboratory confirmed infections could originate from both primary health care and hospital care.

Figure 5.12 Consumption of antimicrobials primarily used for respiratory infections* in primary health care for a) females and b) males, prescriptions per 1,000 inhabitants, Denmark, 2013-2022

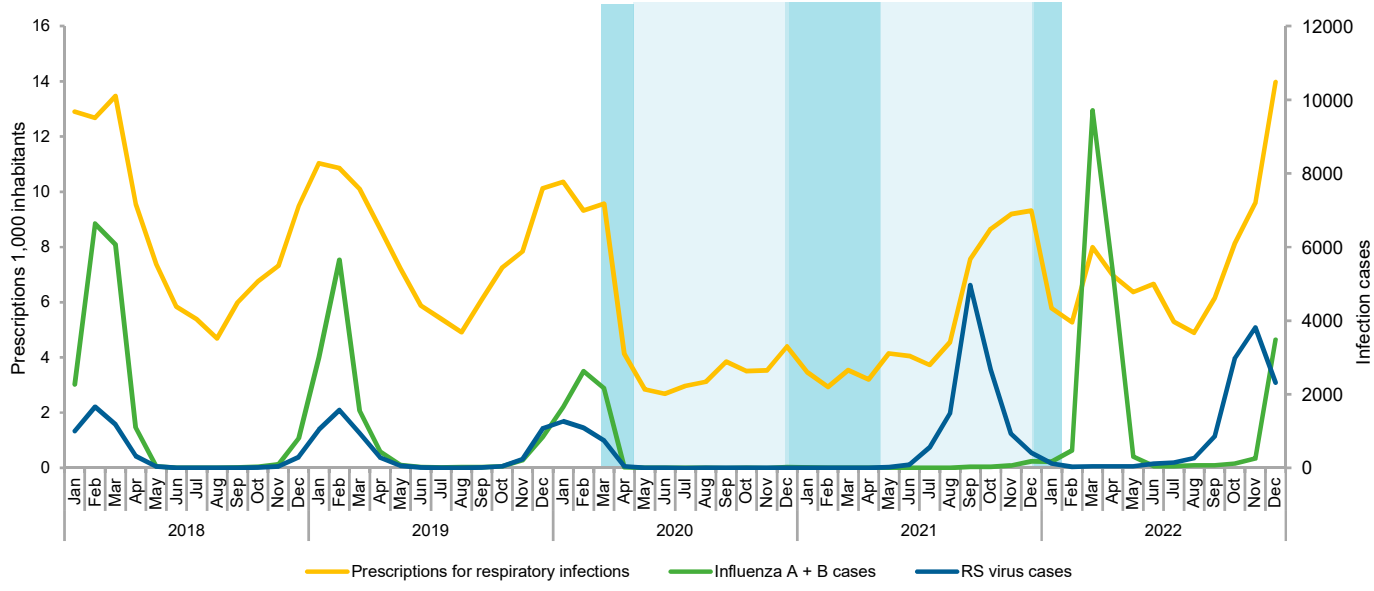
DANMAP 2022



* Penicillins (beta-lactamase sensitive, beta-lactamase resistant and combination penicillins) and macrolides
Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics and 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.13 Monthly consumption of systemic antimicrobials for treatment of respiratory tract infections in primary health care, prescriptions per 1,000 inhabitants, and monthly number of individuals with laboratory confirmed influenza A and/or B and RSV, Denmark, 2018-2022

DANMAP 2022



COVID-19 restrictions in place
Fewer restrictions in place
Data: Registered sale of antimicrobials to individuals and labboratory confirmed Invluenza A, B and Respiratory Syncytial Virus (RSV)
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and Danish Microbiology Database (MiBa)

5.3.6 Antimicrobial consumption for elderly inhabitants

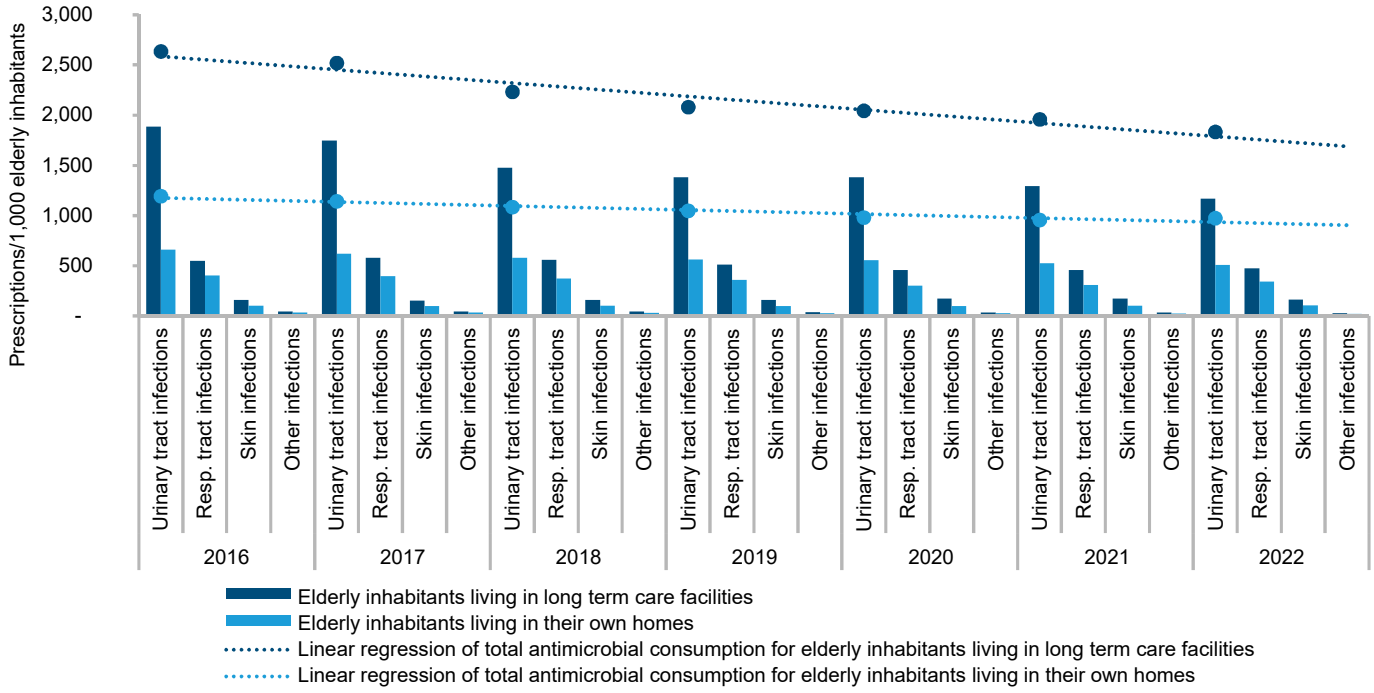
One of the recent surveillance approaches added to the DANMAP program is surveillance of antimicrobial consumption in elderly inhabitants aged 65 years and above. Close surveillance of antimicrobial consumption in this population is necessary as it is one of the most fragile populations in society. Surveillance contributes to high quality treatment of infections and thereby prevents emergence of antimicrobial resistant pathogens.

The surveillance is based on the Danish Care Home Register and Danish Civil Registry. By combining these registries, it is possible to divide elderly inhabitants into two populations; elderly inhabitants living in their own homes and elderly inhabitants living at long term care facilities. For more details, see Chapter 10.

Figure 5.14 shows antimicrobial consumption for elderly inhabitants aged 65 years and above. Elderly inhabitants living at care homes received 88% more antimicrobials than elderly inhabitants living in their own homes in 2022. The figure also compares treatment of specific infections in the two populations as it is well known that treatment of urinary tract infections is the main cause of the difference observed in the treatment frequency of the two populations of elderly inhabitants.

These differences in treatment of elderly inhabitants are observed despite a continuous decrease in the antimicrobial consumption for elderly inhabitants living at long term care facilities (Figure 5.14). From 2016 to 2022, the consumption of antimicrobials decreased by 30% (from 2,636 prescriptions/1,000 inhabitants to 1,833 prescriptions/1,000 inhabitants). In the same period, consumption of antimicrobials for elderly living in their own homes decreased by 18% (from 1,195 prescriptions/1,000 inhabitants to 976 prescriptions/1,000 inhabitants).

Figure 5.14 Consumption of antimicrobials (J01 and P01AB01) in primary health care for elderly inhabitants living in long term care facilities and for elderly inhabitants living in their own homes, Denmark, 2016-2022 DANMAP 2022



Data: Registered sale of antimicrobials to individuals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system, Care Home Register and Danish Civil Registry

5.4 Antimicrobial consumption at hospitals

Sales of systemic antimicrobials (ATC code J01, P01AB01 and A07AA09) from Danish hospital pharmacies to hospitals in 2022, excluding private hospitals and psychiatric departments (approximately 2-3% of the total hospital consumption), are shown in Table 5.6. Antimicrobial consumption data are presented as DDD per 100 occupied bed-days (DBD) to account for hospital activity. Information on consumption at patient level is currently not available to DANMAP for the hospital sector. This information is expected to become available to DANMAP through the "Hospital Medicine Register" in coming years. The antimicrobial consumption in 2022 is mainly compared to 2013 (10-year trend) and to 2019 to avoid the unusual COVID-19 years of 2020 and 2021.

Changes in hospital activity, for example due to earlier discharge of patients, i.e. decreased numbers of bed-days, and increasing ambulatory care functions in the community as well as in care homes, need to be considered when interpreting antimicrobial consumption trends in hospitals (see Table 2.1 in Chapter 2 'Introduction').

5.4.1 Antimicrobial consumption at public somatic hospitals accounting for hospital activity

In 2022, the consumption of antimicrobial agents at somatic hospitals was 127.89 DBD. This is 7% higher than in 2019 (119.82 DBD) and 24% higher than a decade ago (103.51 DBD in 2013) (Table 5.6).

Table 5.6 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 bed-days, Denmark, 2013-2022

DANMAP 2022

ATC group	Therapeutic group	Year									
		2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
J01AA	Tetracyclines	1.71	1.78	2.00	2.43	2.19	2.78	3.67	3.13	3.25	3.52
J01CA	Penicillins with extended spectrum	15.00	14.72	15.63	16.76	16.89	18.01	18.73	20.33	20.49	20.48
J01CE	Beta-lactamase sensitive penicillins	10.95	10.07	10.05	10.62	10.89	12.18	11.42	11.51	10.73	11.46
J01CF	Beta-lactamase resistant penicillins	10.22	10.05	10.26	10.82	10.70	12.25	13.08	14.09	14.15	14.66
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	12.71	13.81	16.21	17.43	14.91	19.29	20.15	22.23	23.63	24.97
J01DB	First-generation cephalosporins	0.13	0.07	0.05	0.05	0.04	0.04	0.03	0.04	0.03	0.03
J01DC	Second-generation cephalosporins	14.29	12.29	11.21	10.69	11.80	10.54	9.46	9.31	8.83	9.12
J01DD	Third-generation cephalosporins	1.26	1.08	1.15	1.19	1.42	1.41	1.39	1.38	1.38	1.52
J01DF	Monobactams	0.17	0.07	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02
J01DH	Carbapenems	3.25	3.57	3.22	3.12	3.07	3.28	3.46	3.76	3.61	3.52
J01EA	Trimethoprim and derivatives	0.44	0.51	0.44	0.43	0.44	0.51	0.47	0.52	0.49	0.46
J01EB	Short-acting sulfonamides	0.19	0.15	0.13	0.12	0.11	0.12	0.10	0.07	0.07	0.06
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	5.12	5.23	5.77	6.21	5.98	7.02	7.77	8.42	9.28	9.58
J01FA	Macrolides	3.81	3.94	4.81	5.44	6.10	7.34	7.84	7.07	5.64	5.75
J01FF	Lincosamides	0.74	0.70	0.63	0.72	0.69	0.89	0.86	0.83	0.79	0.81
J01GB	Aminoglycosides	2.51	2.21	2.39	2.26	2.38	2.51	2.84	2.95	2.79	2.75
J01MA	Fluoroquinolones	10.04	9.33	9.18	8.67	7.70	8.20	7.90	8.12	8.37	8.51
J01XA	Glycopeptides	1.53	1.25	1.28	1.26	1.40	1.48	1.56	1.73	1.74	1.69
J01XB	Polymyxins	0.31	0.24	0.21	0.22	0.21	0.27	0.26	0.28	0.27	0.28
J01XC	Steroid antibacterials (fusidic acid)	0.26	0.25	0.18	0.13	0.07	0.07	0.07	0.06	0.07	0.05
J01XD	Imidazole derivatives	4.76	4.78	4.66	5.22	4.97	5.07	4.79	4.93	4.56	4.43
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.39	0.34	0.30	0.27	0.27	0.31	0.33	0.40	0.36	0.36
J01XX05	Methenamine	0.08	0.06	0.10	0.09	0.08	0.12	0.09	0.10	0.13	0.13
J01XX08	Linezolid	0.41	0.37	0.48	0.42	0.40	0.61	0.62	0.57	0.58	0.65
J01XX09	Daptomycin	0.03	0.06	0.04	0.06	0.09	0.17	0.08	0.11	0.14	0.13
P01AB01	Nitroimidazole derivatives (metronidazole)	2.61	2.14	2.22	2.52	2.18	2.28	2.23	2.30	2.22	2.17
A07AA09	Intestinal antiinfectives (vancomycin)	0.58	0.56	0.52	0.56	0.56	0.58	0.64	0.77	0.67	0.78
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin	103.51	99.64	103.16	107.74	105.52	117.34	119.82	125.03	124.29	127.89

Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

The four penicillin groups (penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins and combinations of penicillins, including beta-lactamase inhibitors) accounted for 71.56 DBD, corresponding to 56% of the total consumption of antimicrobials at somatic hospitals in Denmark in 2022. The consumption of combinations of penicillins, including beta-lactamase inhibitors, continued to increase (96% higher than in 2013 and 24% higher than in 2019) and accounted for 24.97 DBD, making it the largest group consumed in 2022 (20%). Penicillins with extended spectrum also increased markedly over the last decade (37% higher in 2022 compared to 2013) and were the second largest group consumed at Danish hospitals with 20.48 DBD (16%). Beta-lactamase sensitive penicillins accounted for 11.46 DBD (9%) and beta-lactamase resistant penicillins for 14.66 DBD (11%).

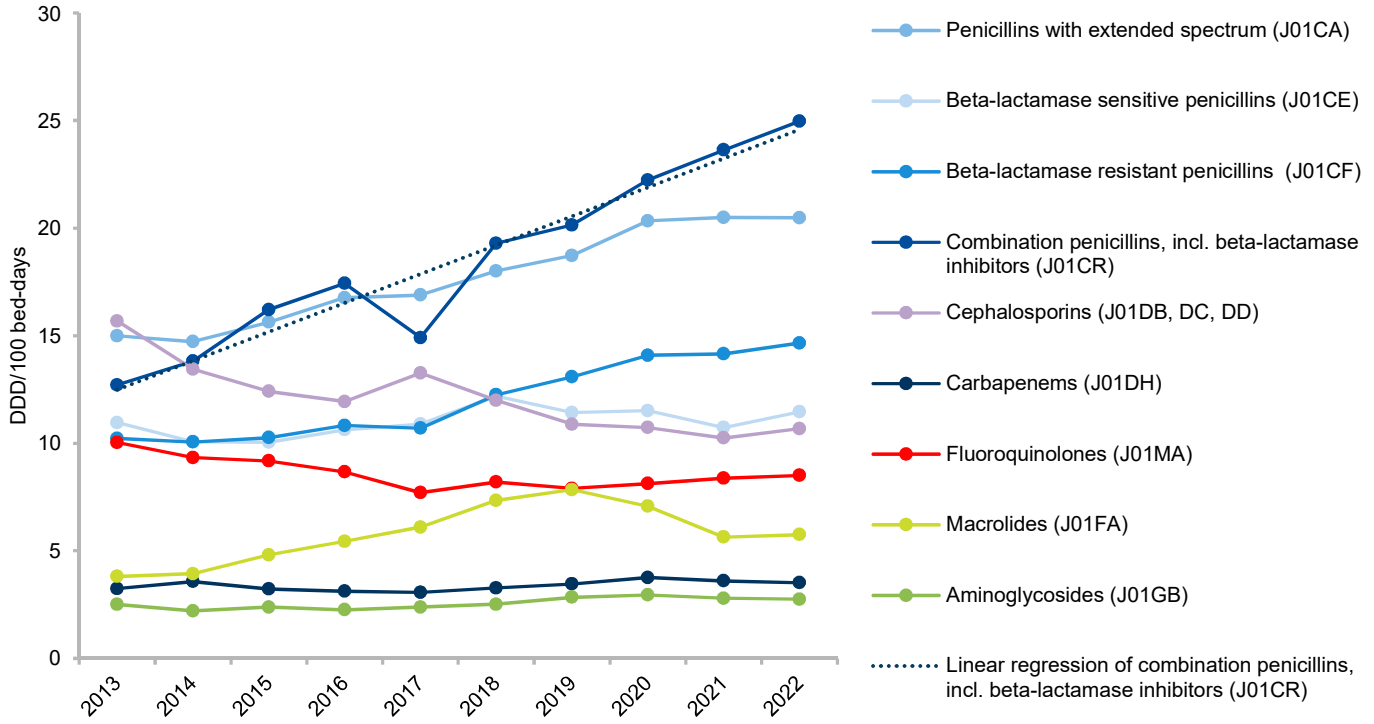
Tetracycline consumption increased during the past decade from 1.71 DBD in 2013 to 3.67 in 2019 but has fallen slightly since to 3.52 DBD in 2022. Consumption of combinations of sulfonamides and trimethoprim increased from 5.12 DBD in 2013 to 9.58 DBD in 2022, a total increase of 87% over the decade. Macrolide consumption continuously increased between 2013 and 2019 but has since decreased to 5.75 DBD in 2022. Consumption of carbapenems increased over the last decade from 3.25 DBD in 2013 to 3.52 DBD in 2022 (Table 5.6, Figures 5.15 and 5.16).

Linezolid consumption has increased to 0.65 DBD in 2022 which is the highest level observed the last decade. Over the past decade, the consumption of linezolid increased by 56% (0.41 DBD in 2013). Consumption of daptomycin peaked in 2018 (0.17 DBD), decreased in 2019 (0.08 DBD) and increased again up to 0.13 DBD in 2022 (Table 5.7). Although the overall consumption of both antimicrobials is low, these changes are of concern since both are reserved for treatment of serious infections caused by vancomycin-resistant enterococci (VRE) or methicillin-resistant *Staphylococcus aureus* (Section 8.3.3 and 8.3.4, Chapter 8 'Resistance in human pathogens').

The consumption of antimicrobials at hospitals can also be measured in relation to the number of patients being admitted, i.e. DDD per 100 admissions (DAD) (Table 5.7). Consumption estimated in DAD showed similar trends compared to trends measured in DBD between 2013 and 2022.

Figure 5.15 Consumption at somatic hospitals by leading groups of antimicrobial agents, DDD per 100 bed-days, Denmark, 2013-2022

DANMAP 2022



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Table 5.7 Consumption of antimicrobial agents for systemic use in somatic hospitals, DDD per 100 admissions, Denmark, 2013-2022

DANMAP 2022

ATC group	Therapeutic group	Year									
		2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
J01AA	Tetracyclines	8.28	9.01	9.87	11.36	10.97	12.54	16.22	13.43	13.91	14.93
J01CA	Penicillins with extended spectrum	72.52	74.28	76.91	78.38	84.75	81.27	82.82	87.14	87.84	86.89
J01CE	Beta-lactamase sensitive penicillins	52.97	50.83	49.44	49.68	54.67	54.99	50.52	49.35	45.99	48.64
J01CF	Beta-lactamase resistant penicillins	49.44	50.74	50.48	50.60	53.69	55.29	57.86	60.38	60.66	62.20
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	61.48	69.71	79.77	81.51	74.81	87.06	89.10	95.29	101.30	105.94
J01DB	First-generation cephalosporins	0.63	0.34	0.24	0.23	0.22	0.20	0.14	0.16	0.15	0.14
J01DC	Second-generation cephalosporins	69.13	62.04	55.18	50.00	59.20	47.59	41.86	39.91	37.85	38.71
J01DD	Third-generation cephalosporins	6.09	5.45	5.65	5.57	7.13	6.34	6.14	5.92	5.91	6.45
J01DF	Monobactams	0.80	0.35	0.15	0.06	0.04	0.03	0.05	0.04	0.03	0.06
J01DH	Carbapenems	15.69	18.03	15.85	14.61	15.41	14.79	15.29	16.11	15.45	14.93
J01EA	Trimethoprim and derivatives	2.13	2.55	2.16	2.03	2.22	2.31	2.06	2.23	2.09	1.94
J01EB	Short-acting sulfonamides	0.91	0.78	0.65	0.55	0.55	0.53	0.45	0.32	0.31	0.25
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	24.76	26.39	28.41	29.02	30.01	31.66	34.36	36.09	39.79	40.67
J01FA	Macrolides	18.42	19.87	23.68	25.44	30.60	33.13	34.68	30.31	24.17	24.41
J01FF	Lincosamides	3.60	3.53	3.10	3.38	3.46	4.03	3.82	3.57	3.39	3.45
J01GB	Aminoglycosides	12.12	11.15	11.75	10.55	11.96	11.34	12.58	12.63	11.98	11.67
J01MA	Fluoroquinolones	48.54	47.09	45.17	40.53	38.66	36.99	34.93	34.83	35.90	36.10
J01XA	Glycopeptides	7.40	6.29	6.29	5.88	7.03	6.70	6.88	7.42	7.46	7.19
J01XB	Polymyxins	1.51	1.22	1.05	1.05	1.03	1.20	1.14	1.18	1.17	1.19
J01XC	Steroid antibacterials (fusidic acid)	1.25	1.25	0.89	0.62	0.36	0.33	0.29	0.26	0.29	0.22
J01XD	Imidazole derivatives	23.04	24.14	22.95	24.43	24.93	22.88	21.18	21.14	19.56	18.79
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.91	1.72	1.46	1.28	1.36	1.42	1.45	1.73	1.53	1.55
J01XX05	Methenamine	0.41	0.30	0.48	0.43	0.38	0.55	0.41	0.45	0.56	0.57
J01XX08	Linezolid	2.00	1.85	2.38	1.97	1.99	2.76	2.74	2.43	2.49	2.74
J01XX09	Daptomycin	0.13	0.30	0.21	0.27	0.44	0.75	0.33	0.48	0.61	0.54
P01AB01	Nitroimidazole derivatives (metronidazole)	12.65	10.78	10.92	11.80	10.92	10.28	9.88	9.87	9.51	9.19
A07AA09	Intestinal antiinfectives (vancomycin)	2.78	2.84	2.55	2.63	2.79	2.62	2.81	3.30	2.88	3.33
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin	500.61	502.82	507.66	503.83	529.58	529.58	529.99	535.97	532.78	542.67

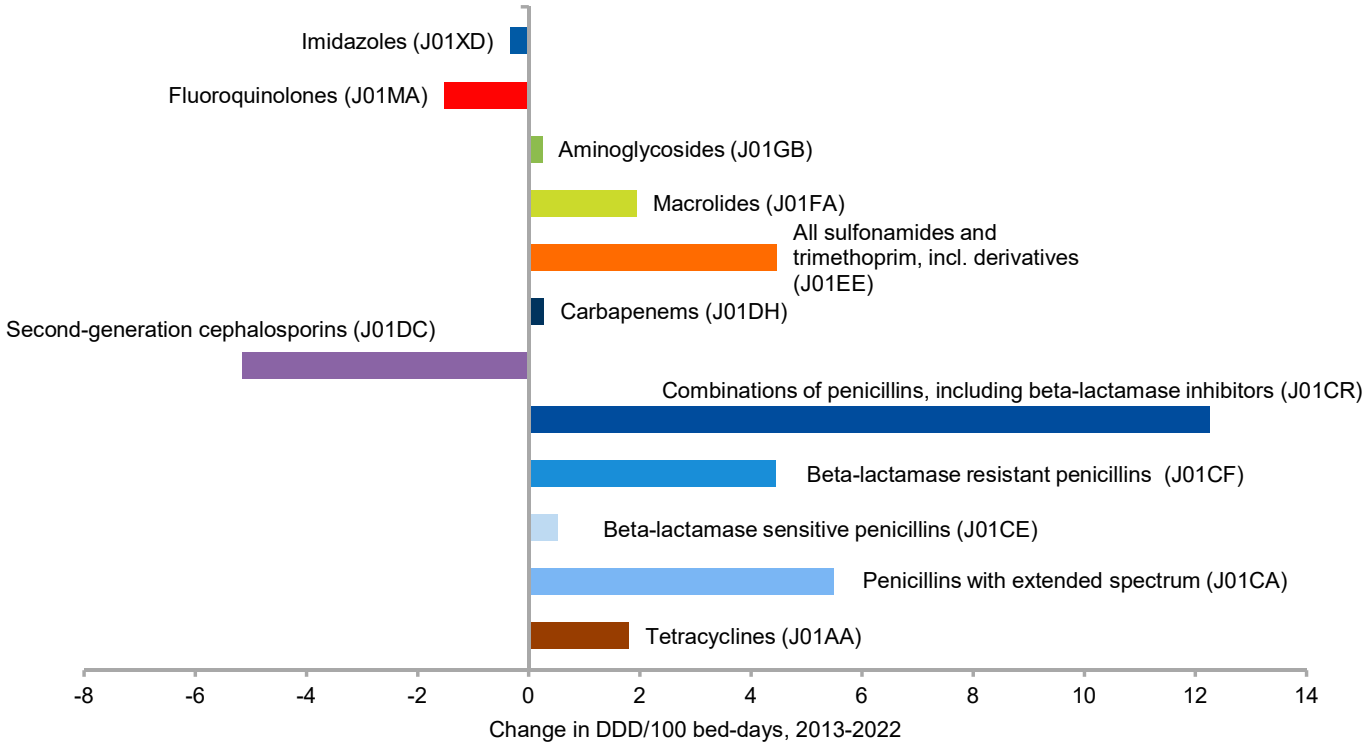
Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2022 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Monthly consumption of the main antimicrobial groups for treatment of critically ill patients at hospitals as well as the monthly number of bed-days from 2018 to 2022 are shown in Figures 5.17. Consumption of carbapenems (ertapenem, imipenem, meropenem) and penicillin/beta-lactamase inhibitor combinations (amoxicillin/clavulanic acid, piperacillin/tazobactam) per bed-day were high during the COVID-19-related lockdowns in Denmark in 2020 and 2021. This reflects most likely changes in hospital activity and in case mix in hospitals during these periods. In 2022, penicillin/beta-lactamase inhibitor combinations decreased sharply in July and August 2022 due to product shortages. However, prescribers had access to penicillin/beta-lactamase inhibitor combinations via special deliveries. Approximately 70,000 DDD penicillin/beta-lactamase inhibitor combinations were purchased through special delivery in 2022, whereas in 2019-2021 the number was approximately 4,000 DDD.

Piperacillin-tazobactam in combination with gentamicin is the recommended antibiotic treatment for septic patients with no known complications. From 2013 to 2022 piperacillin-tazobacam use increased from 6.55 DBD to 16.59 DBD (153%).

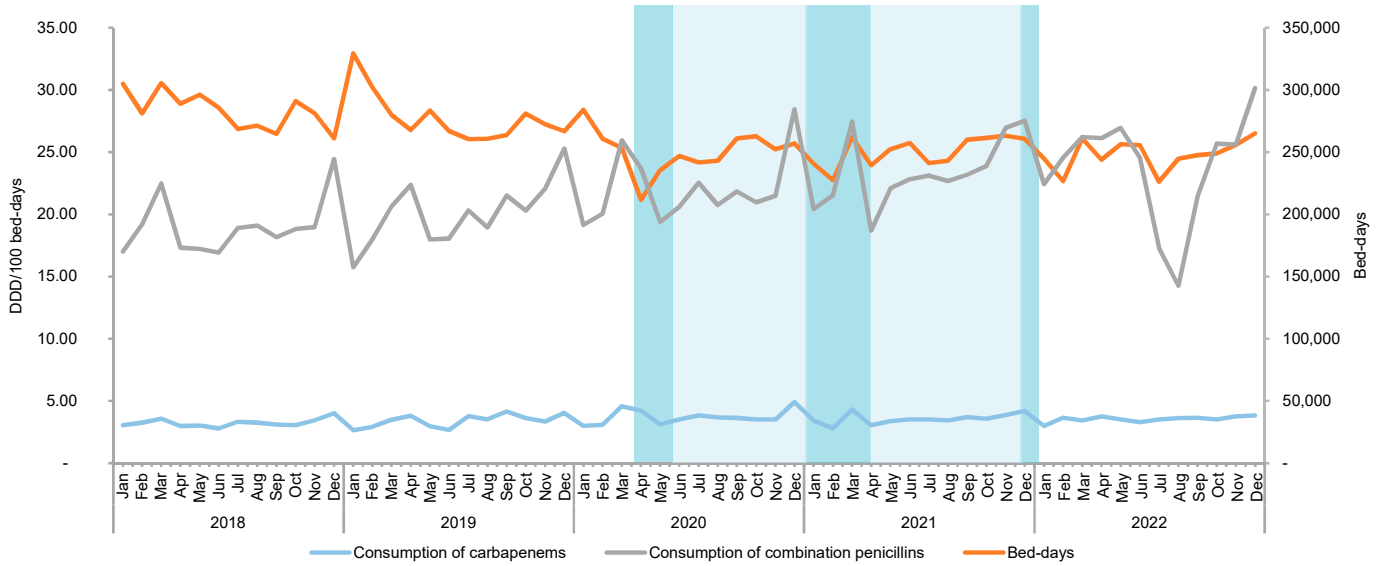
Figure 5.16 Changes in consumption of leading groups of antimicrobial agents at somatic hospitals, DDD per 100 bed-days, Denmark, 2013-2022

DANMAP 2022



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.17 Consumption of key antimicrobials used for treatment of seriously ill patients in hospital, DDD per 100 bed-days, Denmark, 2018-2022 DANMAP 2022



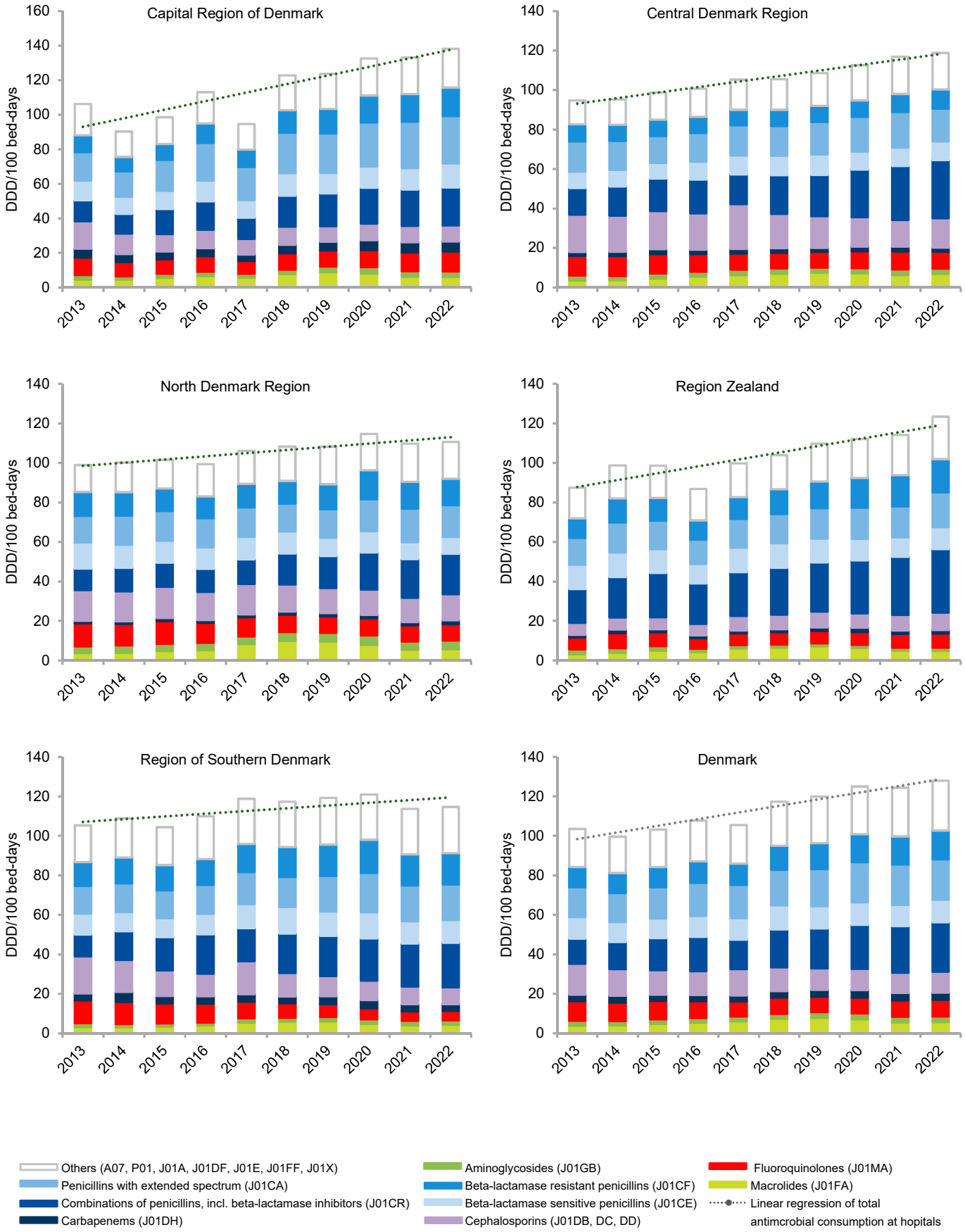
COVID-19 restrictions in place
Fewer restrictions in place
Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

5.4.2 Antimicrobial consumption at regional level at public somatic hospitals

Trends in hospital consumption at regional level measured in DDD per 100 bed-days are presented in Figure 5.18. The Capital Region of Denmark shows the highest level of consumption when compared to the other regions in 2022. It is also notable that consumption increased for each region the last decade when measured in DBD (Figure 5.18) but remains almost unchanged over the same period when measured in DID (Figure 5.19). This reflects that hospital activity changes during the years and more antimicrobials were used in relation to hospital patients' bed-days.

Figure 5.18 Consumption of antimicrobial agents for systemic use at hospitals in the five health regions, DDD per 100 bed-days, Denmark, 2013-2022

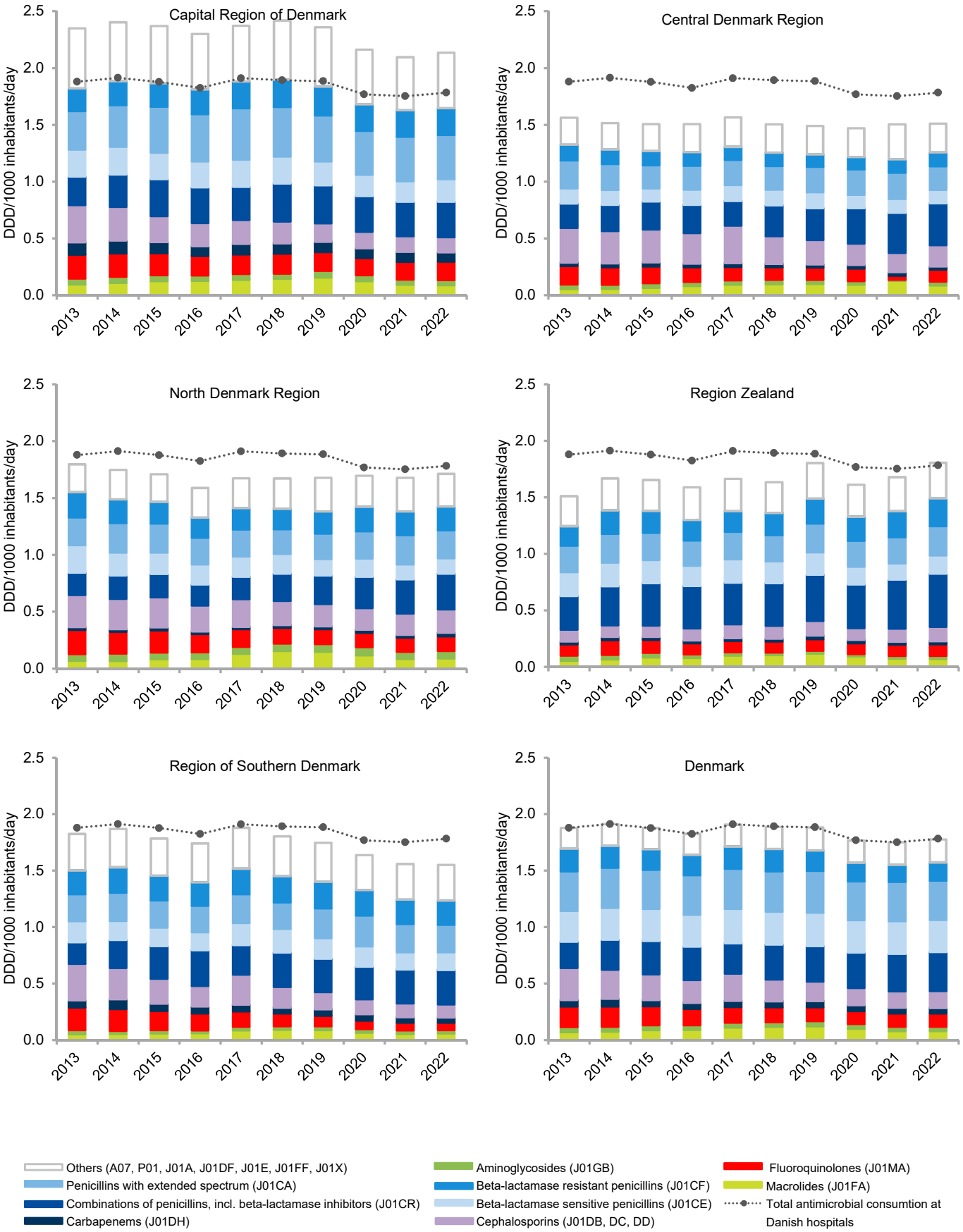
DANMAP 2022



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

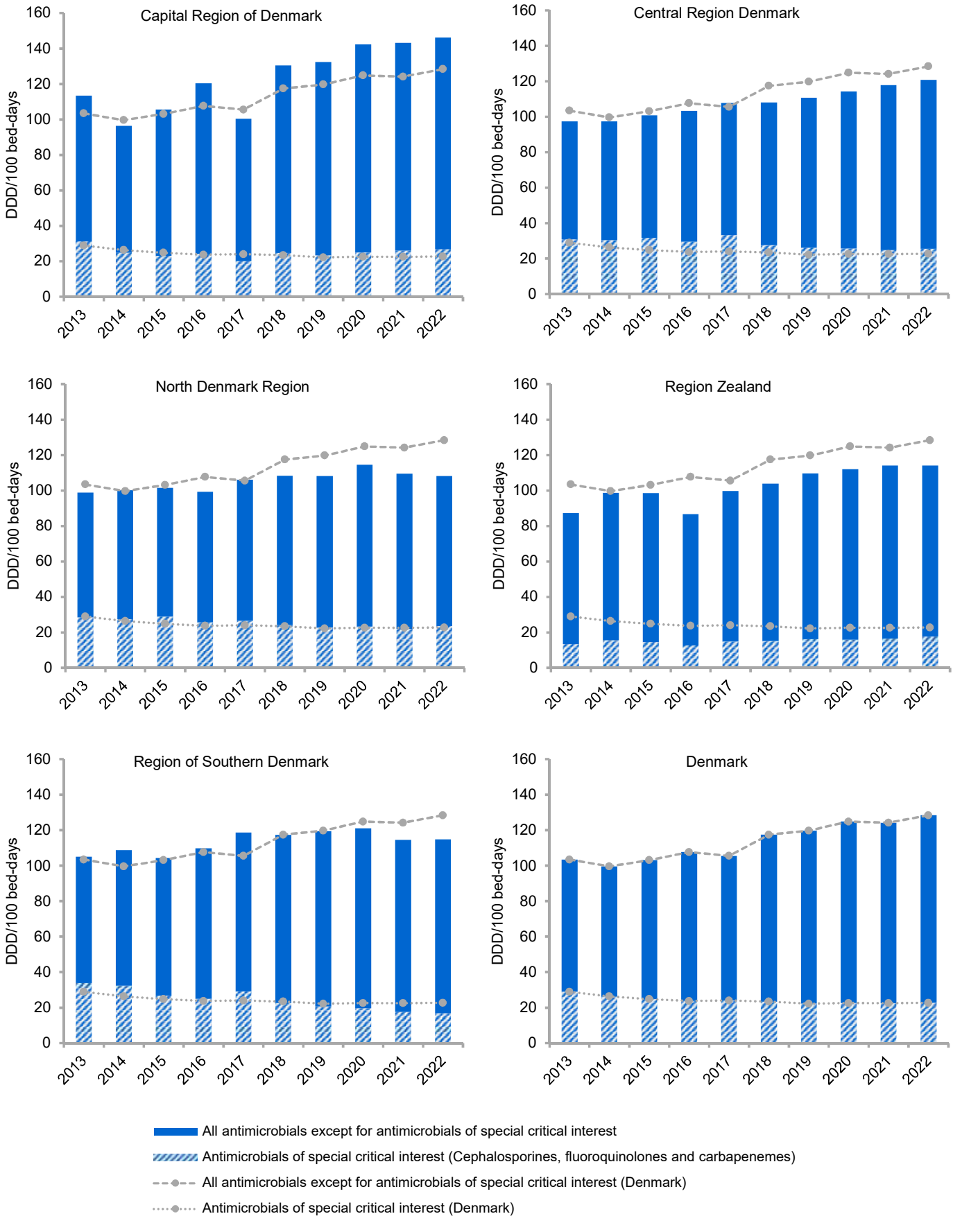
Figure 5.19 Consumption of antimicrobial agents for systemic use at hospitals in the five health regions, DDD per 1,000 inhabitants per day, Denmark, 2013-2022

DANMAP 2022



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

Figure 5.20 Consumption of antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) and all other antimicrobials in the five health regions, DDD per 100 bed-days, Denmark, 2013-2022 DANMAP 2022



Data: Antimicrobial consumption at somatic hospitals
Data source: Register of Medicinal Product Statistics, 2023 edition of the Anatomical Therapeutic Chemical (ATC) classification system and The National Patient Register

5.4.4 Changes in the consumption of antimicrobials of special critical interest

In Denmark, cephalosporins, fluoroquinolones and carbapenems have been defined as antimicrobials of special critical interest due to their resistance potential and their reserved use for treatment of severe infections. In 2022, the antimicrobials of special critical interest constituted 18% of the total consumption at somatic hospitals compared to 28% in 2013 (Table 5.6, Figure 5.20).

In 2022, cephalosporins accounted for 8.4%, fluoroquinolones for 6.7% and carbapenems for 2.8% of the total antimicrobial consumption in somatic hospitals in Denmark. The consumption trends for antimicrobials of special critical interest and all other antimicrobials are presented at regional and national level from 2013 to 2022 in Figure 5.20.

We would like to acknowledge Maja Laursen from the National Health Data Authority in Denmark for data on antimicrobial consumption and activity hospital care. We would also like to acknowledge all hospital pharmacies in Denmark for data on consumption of special delivery antimicrobials at the hospitals.

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Textbox 5.1

Comparison of antibiotic prescribing for elderly in long-term care facilities and elderly living at home

Background

Elderly people of 75 years and above are the age group that receive most antibiotics in Denmark. Urinary tract infection is the main indication. Antibiotic prescribing for this population was studied by comparing the use among elderly living in long-term care facilities and elderly living at home. Special attention was paid to the difference in prescribing antibiotics for urinary tract infection.

Methods

The study was observational and registry-based, and included all elderly Danish residents aged ≥ 75 years in 2016. Total antibiotic prescription rates were examined by including all antibacterial agents for systemic use (ATC J01). Prescription rates for urinary tract infection included pivmecillinam (ATC J01CA08), sulfamethizole (ATC J01EB02), trimethoprim (ATC J01EA01), nitrofurantoin (ATC J01XE01) and amoxicillin (ATC J01CA04). Antibiotic prescribing data were retrieved from the Danish National Prescription Database and residence status from the Nursing Home Register (AEPI-registry). Confounders were chosen a priori based on empirical evidence and on a directed acyclic graph. The chosen confounders were age, sex and comorbidity. Comorbidity was assessed via the Charlson Comorbidity Index[1] using data from the National Patient Register. Linear regression models were used to examine the difference in antibiotic prescription rates. The main outcomes were number of prescriptions per individual per year and DDD per individual per year.

Results

Out of the 416,627 elderly individuals aged ≥75 years included in the study population, 23,863 resided in a long-term care facility (5,7%) (Table 1). A slight difference in age between the two groups was noted, however this was insignificant. Regression models showed that elderly living in long-term care facilities received 1.7 [CI 1.7-1.7] prescriptions/individual/year more than elderly living at home. For urinary tract infection the difference between elderly living in long-term care facilities and elderly living at home was 1.2 [CI 1.2-1.3] prescriptions/individual/year (Table 2).

Table 1 Baseline characteristics of the study population aged ≥75 years according to residence status in 2016 DANMAP 2022

	Living in LTCF	Living at home
Individuals aged ≥75 years	23.863	392.764
Mean age (years ± SD)	86.2 ± 6.1	81.0 ± 5.1
Women (%)	72.4	57.3
Mean number of antibiotic prescriptions ± SD	2.9 ± 4.9	1.0 ± 2.1
Mean number of UTI antibiotic prescriptions ± SD	1.9 ± 4.3	0.4 ± 1.5
Mean number of antibiotic DDD ± SD	43.9 ± 118.7	11.3 ± 35.1
Mean number of UTI antibiotic DDD ± SD	29.7 ± 93.3	5.1 ± 25.3
Mean CCI ± SD	2.2 ± 2.1	1.8 ± 2.0

SD: standard deviation; UTI: urinary tract infection; DDD: defined daily dose; CCI: Charlson Comorbidity Index, LTCF: Long term care facility
Prescriptions/DDD are per individual per year
[1] Charlson Comorbidity Index is a weighted index that classifies individuals' prognostic comorbidity. The index takes the seriousness and the number of comorbid diseases into account

Table 2 Results of unadjusted and adjusted linear regression of antibiotic prescription rate comparing elderly living in LTCF to elderly living at home DANMAP 2022

	Crude parameter estimate [CI95%]	Adjusted* parameter estimate [CI95%]
Antibiotic prescriptions	2.0 [2.0-2.0]	1.7 [1.7-1.7]
UTI prescriptions	1.5 [1.4-1.5]	1.2 [1.2-1.3]
Antibiotic DDD	32.5 [32.2-32.9]	28.9 [28.5-29.3]
UTI antibiotic DDD	22.8 [22.5-23.1]	20.2 [20.0-20.5]

* Adjusted for age, sex and comorbidity, the latter assessed via the Charlson Comorbidity Index, LTCF: Long term care facility
Prescriptions/DDD are per individual per year

The unadjusted and adjusted analyses resulted in similar prescription rates, indicating that the relationship between long-term care facility residency status and prescription rate was not affected by the potential confounders: age, sex and comorbidity (Table 2).

Conclusion

A higher antibiotic prescription rate for elderly living in long-term care facilities could not be explained by higher morbidity in this group of elderly. The registry-based methodology limits the ability to assess the appropriateness of the antibiotic prescribing, why further investigation is needed to understand the underlying causes of the differences in prescription rates.

Link to published paper: https://pubmed.ncbi.nlm.nih.gov/35587537/.

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Textbox 5.2

Sociodemographic characterisation of antibiotic heavy users in the Danish elderly population

Background

Elderly people (≥65 years) have the highest use of antibiotics and studies have shown an overuse within this population (1-3). Sociodemographic inequality is a well-known problem in health care, but it is not known whether sociodemographic factors also influence antibiotic use among Danish elderly people. The aim of this study was to investigate whether sociodemographic factors were associated with an excess use of antibiotics (i.e., being an antibiotic heavy user) in general practice among elderly people in Denmark.

Methods

The study was based on national register data on antibiotic (ATC J01 and P01AB01) prescriptions redeemed by patients of 65 years or older in 2017. Only prescriptions issued by general practitioners were included. A linear regression model was applied to predict an individual's antibiotic use based on age, sex and morbidity level. Morbidity level was assessed by using data on hospitalization from the Danish National Patient Registry. Information on sociodemographic characteristics was collected from various registries, e.g. the educational registry.

A positive difference between the actual antibiotic use and the predicted antibiotic use was interpreted as excess use. Heavy users were defined as the 10% of the study population with the highest excess use. Stratified by sex, heavy users were compared to non-heavy users both in univariable and multivariable analyses using a logistic regression method. The relative importance of statistically significant sociodemographic factors was also examined.

Figure 1 Distribution of antibiotic excess use in the study population, Denmark, 2017 DANMAP 2022

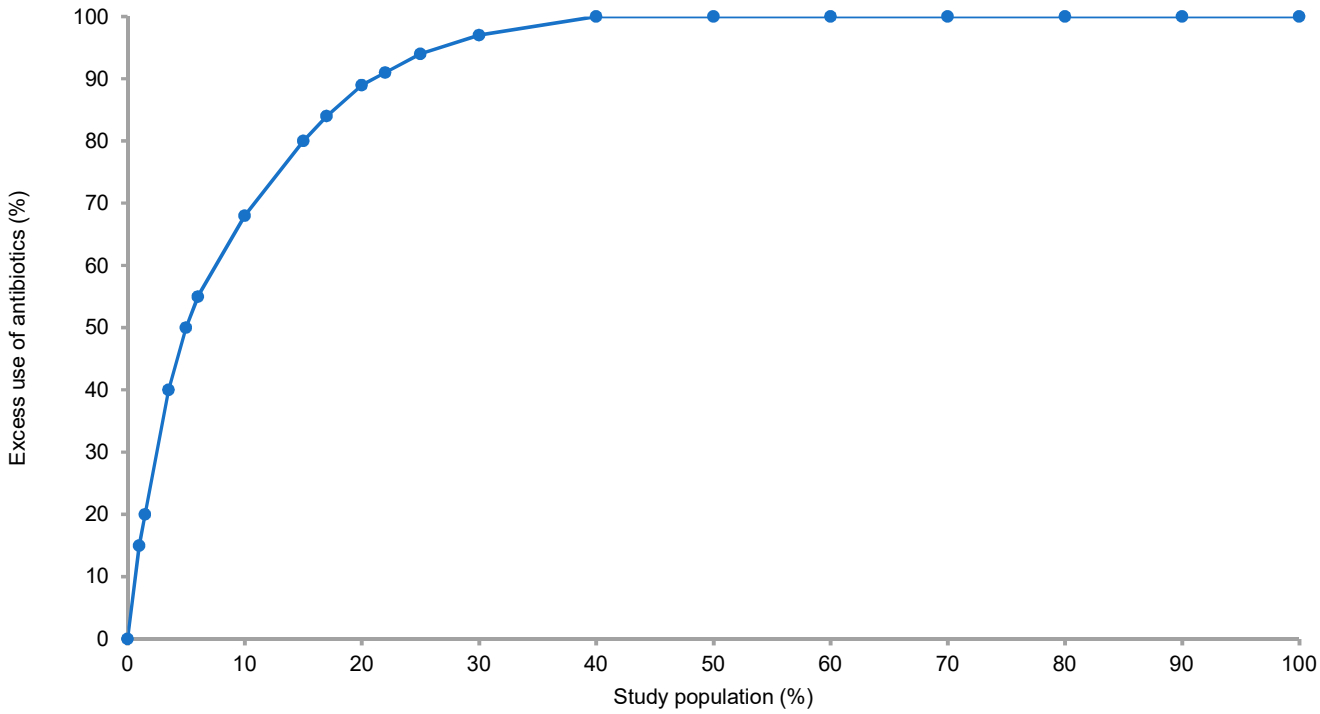
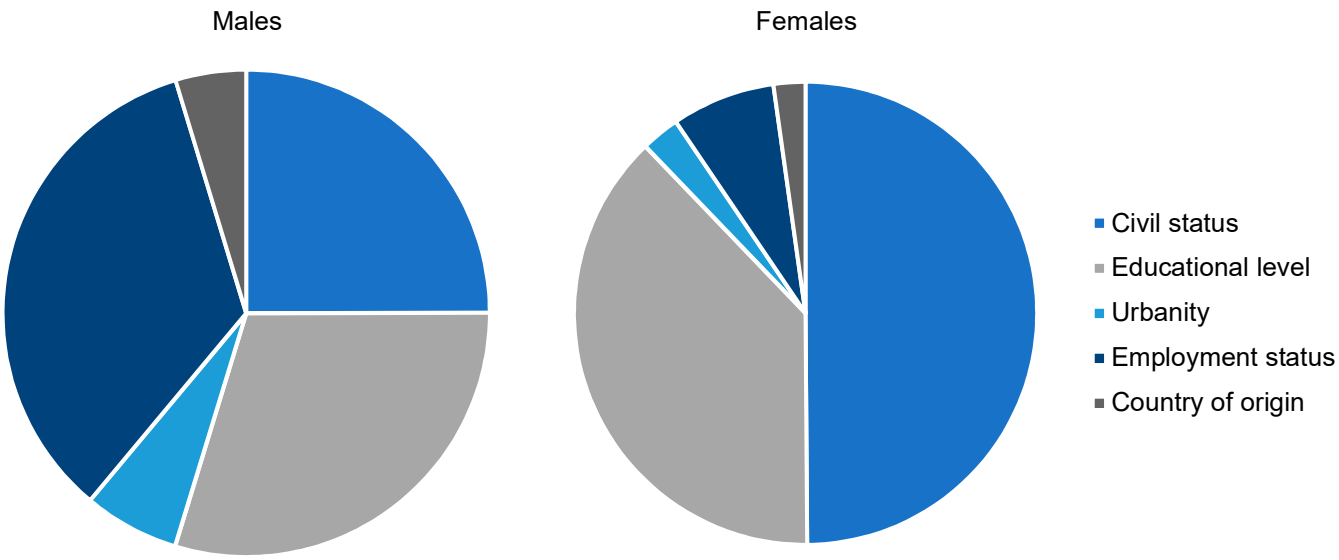


Figure 2 Distribution (%) of sociodemographic factors contributing to the explained variance of excess antibiotic use, Denmark, 2017
DANMAP 2022



Results

The study included 251,733 individuals, 95,544 males and 156,189 females, who redeemed one or more antibiotic prescriptions in 2017. The analyses showed that increasing educational level significantly lowered the risk of antibiotic heavy use. On the other hand, not being married (single, divorced and widowed) increased the risk of antibiotic heavy use, as did being born outside Scandinavia (Denmark, Norway and Sweden). The results were similar for both sexes. The linear regression model also showed that the defined 10% heavy users were responsible for 68% of all excess use of antibiotics in general practice in Denmark (Figure 1). The relative importance analysis showed that civil status and educational level contributed considerably to the explained variance for both sexes (Figure 2). For males, employment status was important as well.

Conclusion

The study showed that heavy users were responsible for about 2/3 of all excess antibiotic use among elderly people. Furthermore, an association between several sociodemographic characteristics and antibiotic heavy use was found. The relative importance analysis showed that for males and females, civil status and educational level were important, and for males, employment status was important as well. The study indicate that the risk of heavy use is substantially affected by socioeconomic characteristics and future interventions to reduce overuse of antibiotics among elderly should target individuals at highest risk.

Sociodemographic characterisation of antibiotic heavy users in the Danish elderly population. Jensen, Maria L V; Aabenhus, Rune M; Holzknecht, Barbara J; Bjerrum, Lars; Siersma, Volkert; Cordoba, Gloria; Jensen, Jette N. ISSN: 1403-4948, 1651-1905; DOI: 10.1177/14034948221119638; PMID: 36076357 Scandinavian journal of public health., 2022, p.14034948221119638.

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References

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Textbox 5.3

Incidence of multiresistant bacteria and consumption of antimicrobial agents in Greenland

Background
Greenland has a population of 56.562 inhabitants (January 2022, StatBank Greenland) and Nuuk is the capital with 19.261 inhabitants (January 2022, StatBank Greenland). Greenland has its own Ministry of Health and the country is divided into five health regions. There are five smaller hospitals, one national hospital and 11 health care centres in the five health regions. The national and largest hospital Dronning Ingrids Hospital (182 beds), is situated in Nuuk. Around 15-16,000 persons are admitted to hospital once or several times a year. Patients with specific or serious diseases which cannot be treated at Dronning Ingrids Hospital (DHI) are transferred to Denmark or Iceland, e.g. haemodialysis, cancer treatment, brain surgery etc.

Resistant bacteria
From 2000 to 2022, 129 patients were diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 195 patients with extended spectrum beta-lactamase (ESBL)-producing *Enterobacterales*, four patients with vancomycin-resistant enterococci (VRE), and 217 patients with *Clostridium difficile* infection.

MRSA
In the latest years there has been a huge increase in incidence of MRSA with several large outbreaks throughout the country. The largest outbreak was seen during 2021 in Ilulissat involving 21 persons at two long term care facilities (LTCFs). Most of the affected persons were old residents, however two health care workers were also colonized. Several residents had chronic wounds which made treatment of carrier state impossible and this was a great challenge concerning prevention of further transmission in the LTCFs. The second largest outbreak involved 12 persons in Tasiilaq at the East coast of Greenland (described in details in DANMAP 2017). Most infections or colonizations with MRSA are seen in the community with transmission in families.

VRE
In spite of ongoing VRE outbreaks in Denmark, so far only four patients have been diagnosed with VRE in Greenland. Three patients were colonized with VRE in the rectum and one patient had pleurisy – in all four cases VRE occurred after hospitalization in Denmark. No transmission was seen in the wards.

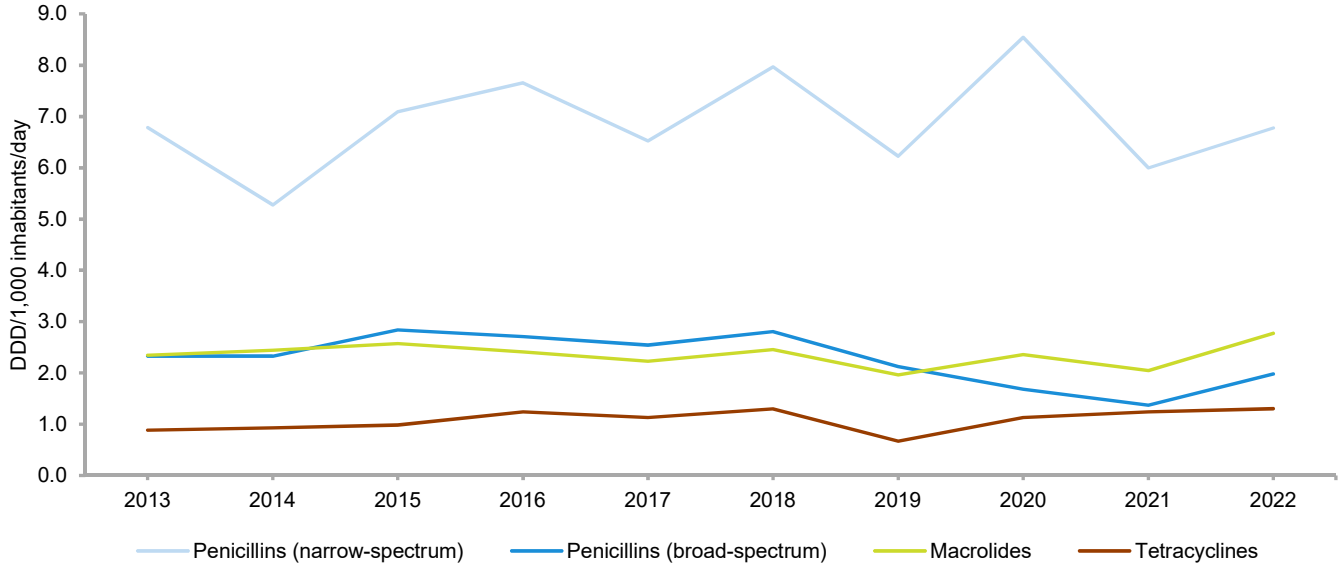
CPO
In recent years, an increase in incidence of carbapenemase-producing organisms (CPO) in Denmark has been observed but until now, no CPO has been reported in Greenland.

Other resistant bacteria
Most of the other resistant bacteria observed were imported from Denmark or abroad, but in some cases, especially in patients with ESBL-producing *Enterobacterales*, treatment with broad-spectrum antimicrobial agents in Greenland has probably selected for these bacteria. From 2012 to 2013, there were outbreaks with *C. difficile* type 027 in the hospitals, and transmission within the country occurred. But due to a great infection prevention and control effort from the hospital staff, these outbreaks were quickly stopped. Of the 23 new *C. difficile* patients in 2022, two of them were of the 027 type.

Consumption of antimicrobial agents
All antimicrobial agents in Greenland are purchased and distributed from the National Pharmacy. Figure 1a and b show the total purchase of selected antimicrobial agents in DDD per 1,000 inhabitants per day (DID) from 2013 to 2022. Throughout the last 10 years the largest consumption of antimicrobial agents is seen in the group of narrow-spectrum penicillins (with fluctuations from year to year). There has been a decline in broad-spectrum penicillins from 2018 to 2021 (50%) with an increase (43%) from 2021 to 2022. Macrolides have been quite stable throughout the years but an increase (40%) is seen from 2021 to 2022. A larger decrease in fluoroquinolones from 2014 to 2017 (60%) was alternated by some fluctuations/increases but in 2022 it is still half of the consumption in 2014. Cephalosporins have been decreasing since 2017 with a low and stable consumption since 2019. A remarkable increase has been seen since 2015 in piperacillin-tazobactam with an increase (50%) from to 2021 to 2022. Meropenem still has a low and stable consumption.

Figure 1a Consumption of narrow- and broad-spectrum penicillins, macrolides and tetracyclines in humans in Greenland, DDD/1,000 inhabitants/day, 2013–2022

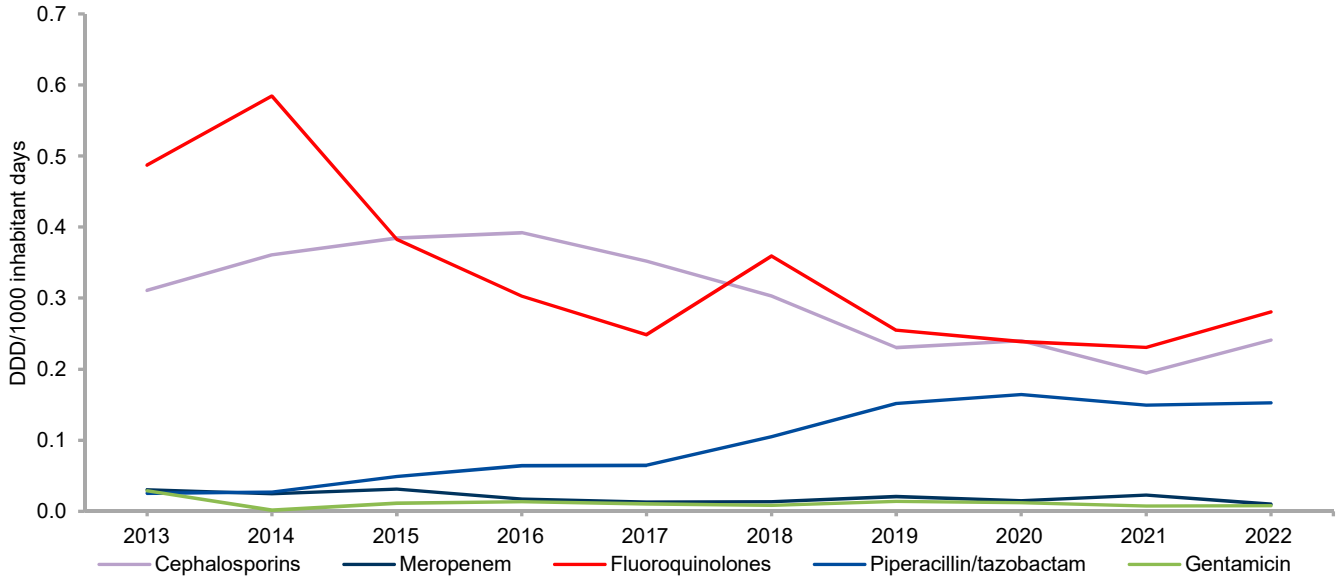
DANMAP 2022



Narrow-spectrum penicillins include benzylpenicillin, phenoxymethylpenicillin and dicloxacillin and broad-spectrum penicillins include ampicillin, pivampicillin, amoxicillin and amoxicillin with enzyme inhibitor

Figure 1b Consumption of cephalosporins, meropenem, fluoroquinolones, piperacillin/tazobactam and gentamicin in humans in Greenland, DDD/1,000 inhabitants/day, 2013-2022

DANMAP 2022



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Conclusion

The consumption data for antimicrobial agents are based on purchases and fluctuations are therefore seen from year to year. It is however noteworthy that the increased focus on prescription of antibiotics in the recent years has resulted in remarkable decreases in purchases of cephalosporins, fluoroquinolones and meropenem, and continued increase in purchase of piperacillin-tazobactam.

Continued focus on the use of broad-spectrum antimicrobial agents, on the incidence of multiresistant bacteria, and on compliance to guidelines for infection prevention and control is still important in Greenland in the future.

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Textbox 5.4

Shortage of antibiotics at Community Pharmacies in Denmark

The Association of Danish Pharmacies is the employer and professional organization of community pharmacies in Denmark. The association's Executive Board has the overall responsibility for the association's activities covering member services and promoting community pharmacy professional health services as an integrated part of the health care sector.

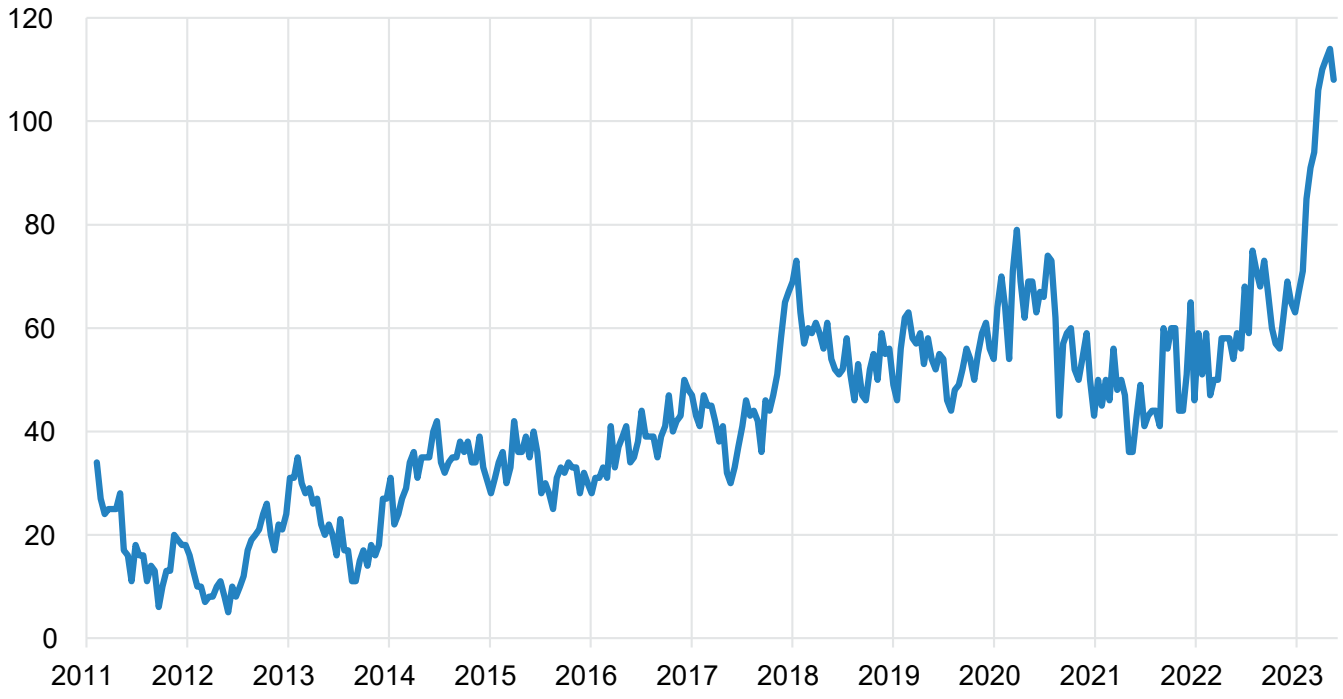
In Denmark, legislation obliges pharmacies to offer patients the cheapest, generic product of the prescribed medicine - also known as generic substitution. The legislation requires these substitutional products to have the same active component, formulation, strength, and same or smaller package size to ensure the same pharmaco-dynamics and -kinetics as the originally prescribed product. Antibiotics are allocated in dispensing group 'B' which means that package size is allowed to differ by up to 25% from the prescribed package size.

In case of supply shortages, the restrictions in the substitution legislation can be a challenge. If the pharmacy only can offer an alternative with different formulation, strength or larger package size, the patient will need a new prescription to get the medicine. The same applies in case of extreme price jumps of the prescribed medicine, where a cheaper alternative with a different formulation, strength or larger package size will demand a new prescription. In special circumstances and to limited degree the Danish Medicines Agency can authorize sale or dispensing of medicine, which is not on the market in Denmark. This 'compassionate use permit' requires an application from the prescriber and is given on an individual patient case and for a specified prescribed treatment only and cannot be extended to cover prolonged periods or several patients.

Increased shortages of antibiotics in Denmark

The number of antibiotics (J01) in shortage in Denmark almost doubled in the first half of 2023. As shown in Figure 1, the number of antibiotic packages in shortage at all pharmaceutical wholesalers in Denmark increased gradually from around 20-40 packages in 2011-2017 to around 60 packages in 2018-2022. In the first half of 2023 the number reached 110 antibiotic packages in shortage. However, most of the shortages did not reach the patients, as generic substitutes were available.

Figure 1 Number of antibiotic packages (J01) in shortage, Denmark, 2011-2023 DANMAP 2022

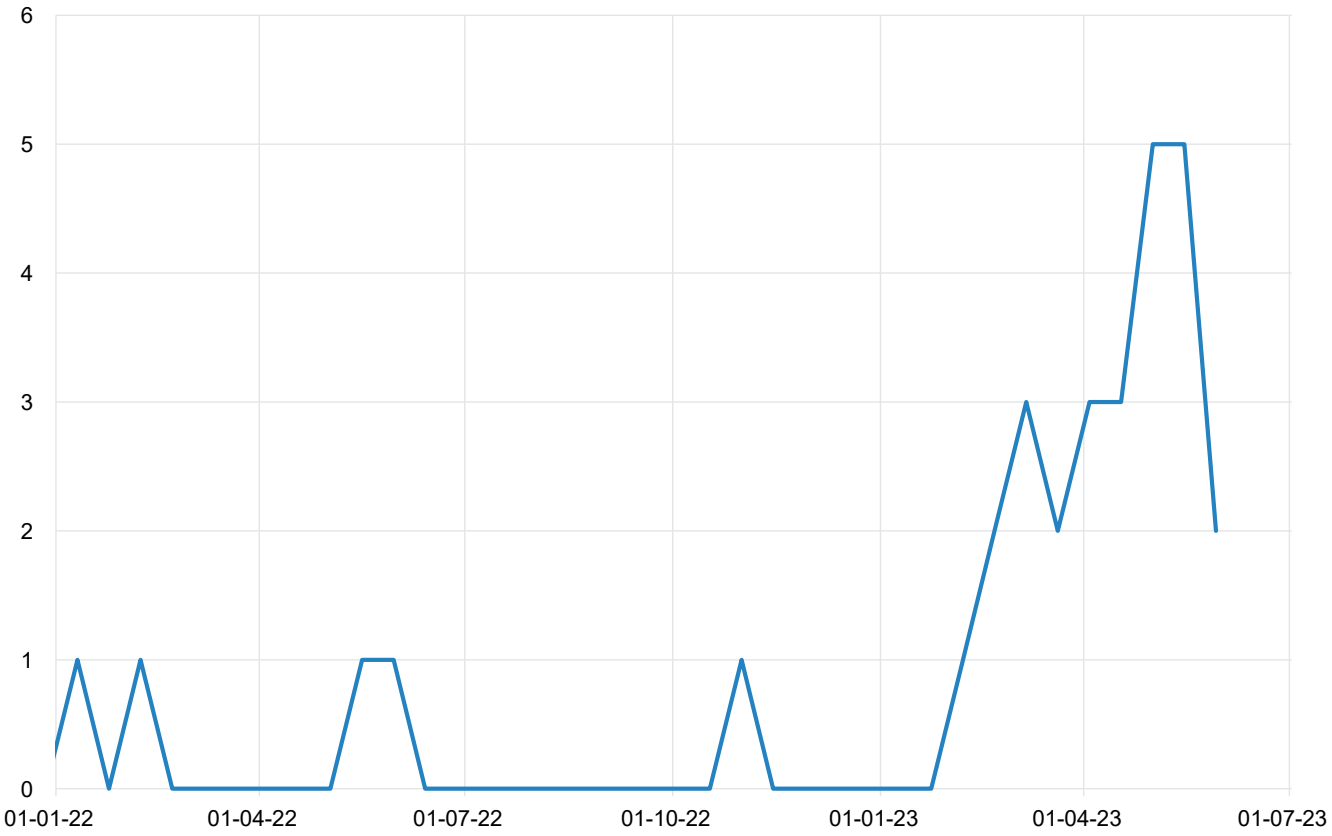


Source: The Danish Medicines Agency and the Association of Danish Pharmacies

continued ... Textbox 5.4

In 2022, shortages covered all packages in one generic substitution group only a few times and for limited time (Figure 2). However, in the first half of 2023 the shortages several times covered 2 to 5 entire generic substitution groups, making it increasingly burdensome for patients, doctors, and pharmacies to find an adequate treatment.

Figure 2 Number of entire generic substitution groups of antibiotics (J01) in shortage, Denmark, 2022-2023 DANMAP 2022



Source: The Danish Medicines Agency and the Association of Danish Pharmacies

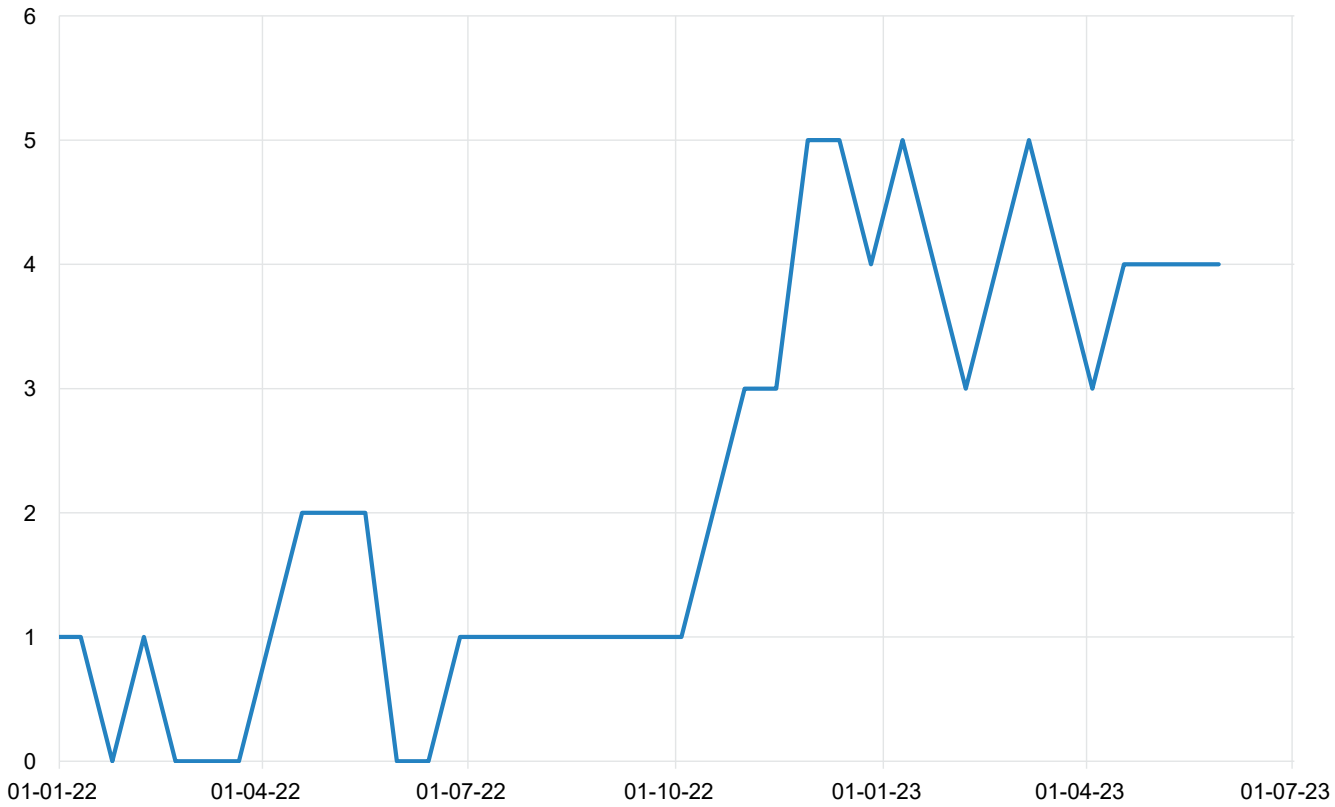
At the same time, the shortage of antibiotics with no marketed generic substitute increased from 0-2 packages in shortage in the first 3 quarters of 2022 to 3-5 packages in the 4th quarter of 2022 and in the first half of 2023 (Figure 3).

The Danish pharmacies have two main pharmaceutical supply wholesalers. If one wholesaler is out of stock, the pharmacy is required to order the medicine from the other wholesaler. If a pharmacy is out of stock of a certain medicine and the medicine is in shortage at both wholesalers, the pharmacy can check electronically if other pharmacies still have the medicine in stock and refer patients to these pharmacies. Furthermore, the pharmacy app 'Apoteket' refers patients to the nearest 25 pharmacies with the prescribed medicine in stock. It is not possible to trade pharmaceuticals between pharmacies.

Each day, pharmacies secure patients access to – and safe and efficient use of – the right pharmaceutical treatment. However, in recent years this access is increasingly challenged by medicine shortages. These are partly due to globalization and concentration of pharmaceutical production facilities and the aftermaths from the covid-19 pandemic and the Ukrainian war.

Due to the increase in medicine shortages, it is now possible for a regional administration to apply for compassionate use permits regarding certain pharmaceuticals for all doctors and other practitioners in the region. This reduces the administrative pressure on the medicines agency and speeds up the process of receiving such a permit, but it still takes time and the individual patient still needs a new prescription for the authorized medicine. Therefore, the compassionate use permit is not a quick-fix-solution for the patient in cases of supply shortages at the pharmacy.

Figure 3 Number of antibiotic packages (J01) in shortage where no generic substitute is marketed, Denmark, 2022-2023 DANMAP 2022



Note: Excl. injection and infusion medicine.
Source: The Danish Medicines Agency and the Association of Danish Pharmacies

Table 1 Examples of generic substitution groups of antibiotics (J01) in full shortage, Denmark, May 2023 DANMAP 2022

ATC Code	Pharmaceuticals	Strength	Package size
J01CA04	Amoxicillin	500 mg	10 tablets
J01CE02	Phenoxymethylpenicillin	50 mg/ml	200 ml
J01CR02	Amoxicillin & beta lactamase inhibitor	50 mg/ml + 12,5 mg/ml	100 ml
J01FA06	Roxithromycin	150 mg	20 tablets
J01FA06	Roxithromycin	300 mg	7 tablets

continued ... Textbox 5.4

Since 2019, pharmacies in the United Kingdom have been able to solve more medicine shortages by adhering to 'Serious Shortage Protocols' which contain information from the health authorities on authorized alternative pharmaceutical options when no generic product is available. The protocol allows the pharmacy to deviate from the standard rules and hand out a suitable alternative to the lacking drug in specific circumstances, for a specific period and in clearly defined clinical situations.

In Denmark, all pharmacies have a prescribing pharmacist, who is trained and authorized to re-prescribe certain pharmaceuticals. Expanded substitution through a Danish Serious Shortage Protocol in case of medicine shortages would result in:

- Better access to treatment
- Improved continuity in treatment
- Fewer contacts to the doctor for new prescriptions.

Thus, in the case where no generic substitute is available, a 'Serious Shortage Protocol´ could ease the burden for patients, doctors and pharmacies and increase timely access to adequate treatment.

Overall, most medicine shortages are solved swiftly by the pharmacies through generic substitution. But every single day pharmacies meet patients who cannot get their medicine due to medicine shortages. And pharmacy staff must be the 'bad guys' telling patients they cannot get the medicine from the shelf without a new prescription – even though the pharmacy staff would be able to help several of the patients and relieve doctors from the task of changing prescriptions, if legislation provided a wider range of substitution alternatives at pharmacies. This could relieve patients, practitioners and pharmacy staff from some of the burden of increasing medicine shortages.

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Textbox 5.5

Research Units for General Practice in Denmark

In Denmark, about 75% of antibiotics for human use are prescribed in general practice - mostly for infections related to the respiratory- and urinary tract systems. Also eye, skin, gastro-intestinal and sexually transmitted infections, among others, are treated with antibiotics in general practice.

Many patients present with viral, self-limiting infections; however, some do have serious, bacterial infections - in need of antibiotic treatment.

Research on antibiotic use

It can be challenging to find the "needle in the haystack" and no doubt both under- and overtreatment with antibiotics occur in general practice. For years, research on the management of infectious diseases and antibiotic use have been prioritised at the research units for general practice in Denmark. Professor Lars Bjerrum was one of the first researchers within general practice, to focus on rational use of antibiotics. In 2007, he coordinated a large European-funded project entitled the HAPPY AUDIT. The project used the Audit Project Odense method to collect data on the management of acute respiratory tract infections in six different European countries.

History of the research units

In Denmark, four research units for general practice exist. The first unit was established in Copenhagen in 1978; then Aarhus followed in 1992, Odense in 1993, and Aalborg in 2015. Recently, satellite research units in Esbjerg and Køge have been established. All units have an interdisciplinary academic environment. The research units are funded by the Danish Research Foundation of General Practice, established by the Danish Organisation of General Practitioners (PLO) and the Danish Regions. Most projects conducted at the research units are funded by external sources. All research units work closely with the universities - and joint organisations (center for general practice) have been established in some of the respective cities.

Research with and for general practice

The research units for general practice have a proud tradition for conducting projects in close collaboration with general practice. Often, projects are performed across the four research units, and sometimes including external partners, such as for example specialists in clinical microbiology or infectious diseases. Also, the Danish College of General Practice (DSAM) and the Danish Organisation for General Practice (PLO) are involved in some projects.

New projects in pipeline

The table below summarises ongoing/soon to start projects at the four research units for general practice in Denmark, focusing on management of infections and/or antibiotic use in general practice.

Project title	Aim	Research group
Acute respiratory tract infections		
When are you cured? Defining a cut-off point using the Acute Respiratory Tract Infection Questionnaire	Define cut-off points to determine when patients can be considered cured from their acute respiratory tract infection.	Eskild Johansen Volkert Siersma Malene Plejdrup Hansen Rune Munck Aabenhus
The optimal antibiotic treatment duration for community-acquired pneumonia in adults diagnosed in general practice in Denmark: an open-label, pragmatic, randomised controlled trial.	Identify the optimal treatment duration with phenoxymethylpenicillin for community-acquired pneumonia in adult patients diagnosed in Danish general practice.	Eskild Johansen Henrik Nielsen David Gillespie Rune Aabenhus Malene Plejdrup Hansen
The effect of focused lung ultrasonography on antibiotic prescribing in patients with acute lower respiratory tract infections in Danish general practice – a pragmatic randomised controlled trial.	Determine if adding focused lung ultrasonography to usual care of patients presenting with symptoms of an acute lower respiratory tract infection in general practice reduces the general practitioner's antibiotic prescribing at index consultation.	Julie Jepsen Strøm Camilla Aakjær Martin Bach Jensen Janus Laust Thomsen Christian Borgbjerg Laursen Malene Plejdrup Hansen
Patient decision aids for acute respiratory tract infections; how to use in Danish general practice?	Investigate the feasibility of implementing the use of four patient decision aids for acute respiratory tract infections in Danish general practice.	Lotti Eggers-Kaas Anna Mygind Dorte Ejg Jarbøl Malene Plejdrup Hansen

continued ... Textbox 5.5

Project title	Aim	Research group
Urinary tract infections		
Optimisation of antibiotic use in nursing homes through cross-sectorial collaboration: a registry study of safe and effective practice.	Investigate the long-term impacts of a complex intervention on patient safety, systemic antibiotic consumption, and health services usage among nursing home residents, and identify the nursing home and resident level factors that influence these impacts over the same period.	Sif Helene Arnold Anne Holm Maria Louise V. Mandrup Lars Bjerrum Jette Nygaard Jensen (Project group not fully established)
Quality improvement		
Do quality clusters in general practice improve antibiotic prescribing?	Investigate whether engagement in quality clusters improves antibiotic prescribing in a general practice setting. High-quality register data on redeemed antibiotics prescriptions are linked with survey data on whether, when, and how the clusters engaged with antibiotics as a quality improvement topic.	Maria Bundgaard Dorte Ejg Jarbøl Eskild Klausen Fredslund Jens Søndergaard Marius Brostrøm Kousgaard Sonja Wehberg Line Bjørnskov Pedersen
Point-of-care testing		
A cluster-randomised trial of point-of-care PCR diagnostics of respiratory tract infections in general practice.	The scientific evaluation of the study comprises 3 parts: 1. Effectiveness study 2. Economic evaluation 3. Process evaluation	Kirubakaran Balasubramaniam Jens Søndergaard Jesper Bo Nielsen Trine Thilsing Sonja Wehberg Dorte Jarbøl Jesper Hallas Tina Lein Rasmussen Line Planck Kongstad Rikke Sand Andersen Elisabeth Hvidt Line Simonsen And others
Out-of-hours primary care		
Use of C-reactive protein testing and help-seeking behaviour in out-of-hours primary care.	Investigate whether patients contact out-of-hours primary care more frequently if they have previously encountered a general practitioner with high use of C-reactive protein testing.	Jesper Lykkegaard Jonas Kanstrup Olsen Malene Plejdrup Hansen Claus Høstrup Vestergaard Linda Huibers
Antibiotic use in out-of-hours primary care: the influence of video consultations.	Investigate how use of video consultations in Danish out-of-hours primary care influences the antibiotic prescribing pattern.	Mette Amalie Nebjerg Malene Plejdrup Hansen Linda Huibers (Project group not fully established)
European-funded projects		
HAPPY PATIENT (2021-2023)	Evaluate the impact of a multifaceted intervention for rational use of antibiotics to treat community-acquired infections in four different settings; general practice, out-of-hours, nursing homes and pharmacies. More information available at: https://happypatient.eu/	Jesper Lykkegaard Jonas Kanstrup Olsen Anders Munck Jens Søndergaard Jette Nygaard Jensen Lars Bjerrum Malene Plejdrup Hansen (Only Danish partners mentioned)
IMAGINE (2023-2025)	Systematise and enhance efforts to prevent infections, mainly urinary tract infections, and reduce antibiotic inappropriateness by implementing a multifaceted intervention targeting healthcare professionals in nursing homes. More information available at: https://imagineproject.eu/	Jesper Lykkegaard Jonas Kanstrup Olsen Anders Munck Jens Søndergaard Jette Nygaard Jensen Anna Marie Theut Valeria Antsupova Athina Chalkidou Lars Bjerrum Malene Plejdrup Hansen (Only Danish partners mentioned)

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6

RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Highlights: In 2022, compared to 2021, the number of *Campylobacter jejuni* (285) and *Salmonella* Typhimurium (190) isolates from humans tested for antimicrobial susceptibility decreased, although the number of outbreaks increased, from three to ten for *C. jejuni* and from three to six for *S.* Typhimurium. The number of tested travel-associated isolates also increased in 2022 for both bacteria, from 29 to 52 for *C. jejuni* and from 13 to 30 for *S.* Typhimurium. The number of *Salmonella* Typhimurium isolates recovered from Danish pork increased in 2022, from 39 to 48.

As in previous years, the occurrence of resistance in ***C. jejuni*** isolated from humans was higher than in isolates recovered from broilers and cattle. Among human infections, resistance was higher in travel-associated compared to domestically-acquired cases.

Compared to 2021, the percentage of fully-sensitive *C. jejuni* decreased in isolates from broilers (from 73% to 59%) and increased in isolates from cattle (from 68% to 76%).

In 2022, resistance to erythromycin, chloramphenicol and gentamicin was not detected in any of the monitored *Campylobacter* isolates. Like in 2021, ertapenem resistance was observed in 1% and 12% of isolates from domestic- and travel-related cases, respectively (Table 6.1), and in 2% of isolates from broilers.

Fluoroquinolone (ciprofloxacin) resistance remained common in *C. jejuni* isolates obtained from human cases (58%), broilers (38%) and cattle (22%). Resistance towards tetracycline was common in *C. jejuni* from humans and broilers with 29% and 24% of resistant isolates, respectively, whereas only 5% of isolates from cattle were tetracycline-resistant. Contrary to the decrease observed in the latest years, ciprofloxacin resistance and combined resistance to ciprofloxacin and tetracycline increased in *C. jejuni* isolates from broilers.

C. coli from broilers were commonly resistant to ciprofloxacin (39%) and tetracycline (45%). The percentage of fully-sensitive isolates (38%) was lower than for *C. jejuni*, and 23% *C. coli* exhibited resistance to ertapenem.

The level of azithromycin resistance in ***Salmonella*** **Typhimurium, including the monophasic variants**, was less than 1% in human isolates, and 4% in isolates from Danish pork. Ciprofloxacin resistance was observed in 4% of the isolates from domestically-acquired infections and in 20% of travel-associated cases. Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases. Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pork since 2007.

Resistance to the critically important 3rd generation cephalosporins and carbapenems is rare in *S.* Typhimurium. In 2022, third-generation cephalosporin resistance was observed in 1% of the human isolates and no carbapenem resistance was observed. In line with the previous years, resistance to 3rd generation cephalosporins and carbapenems was not observed in *S.* Typhimurium isolates from Danish pork.

6.1 Introduction

6.1.1 Resistance in zoonotic bacteria

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact or indirectly by ingestion of contaminated food or water, or contact with contaminated environment. *Campylobacter* and *Salmonella* are a common cause of zoonotic gastrointestinal infections and are therefore considered a key component of zoonotic monitoring programmes. A description of the trends and sources of these zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2022 [www.food.dtu.dk]. Surveillance of antimicrobial resistance (AMR) in *Campylobacter* and *Salmonella* from food-producing animals, meat and humans has been part of the DANMAP programme since 1995. Phenotypic antimicrobial resistance is monitored in isolates from human clinical cases, broilers, cattle, pigs and corresponding meat. Additionally, whole genome sequencing (WGS) is an integrated part of the surveillance of human *Campylobacter* and *Salmonella* infections in Denmark, and is performed on a selection of *Campylobacter* and *Salmonella* isolates recovered from food-producing animals. Hence, monitoring of genotypic antimicrobial resistance in these bacteria may soon become an integral part of DANMAP (see Textbox 6.1).

In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea in human patients unless there is prolonged duration of disease or the patient is severely ill. If treatment is required, macrolides (azithromycin) are recommended for treatment of *Campylobacter* infections. For *Salmonella* infections, no specific recommendations regarding antibiotic treatment exist for the primary sector. For infections treated in hospitals, intravenous ceftriaxone is recommended for septic patients and per oral azithromycin for less severe cases. For prolonged or recurrent infections with *Salmonella*, combination therapy can be used, with ciprofloxacin or sulfamethoxazole and trimethoprim added. The Register of Medicinal Product Statistics at the Danish Health Data Authority does receive information on the indication for prescribing an antibiotic, but not against which pathogen it was prescribed.

Macrolides are often used to treat infections in food-producing animals in Denmark, especially in pigs. Fluoroquinolones are not used in any production animals, whereas there is a limited use of 2nd generation cephalosporins and no use of 3rd and 4th generation cephalosporins in cattle. The use of antimicrobials in the Danish poultry sector is low and limited to only a few antimicrobial classes, primarily tetracyclines (see Chapter 4, Table 4.1).

In humans, monitoring of antimicrobial resistance is performed on clinical isolates of *Salmonella*. For *Campylobacter jejuni* a geographically stratified selection of isolates is subjected to susceptibility testing. The testing is performed in accordance with the ECDC recommendations (see Chapter 10, Section 10.9). Travel histories of the patients are collected, when possible.

Campylobacter isolates were obtained from healthy animals at slaughter (caecal samples from broilers and cattle), while *Salmonella* isolates were obtained from pig carcasses at slaughter. *C. jejuni* is reported for broilers and cattle, and *C. coli* is reported for broilers (see Chapter 10, Table 10.1 for further details). Since 2021, the antimicrobial susceptibility testing of *Campylobacter* and *Salmonella* from animals and meat has been done in accordance with the Commission Implementing Decision 2020/1729/EU of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (see Chapter 10 for further details).

6.2 *Campylobacter*

A total of 285 human *C. jejuni* isolates were susceptibility tested. The isolates represented 233 domestically-acquired infections and 52 travel-associated infections and included 33 outbreak related isolates from ten different outbreaks. A large number of sequence types (ST) were identified among the susceptibility tested strains with ST21 (41), ST50 (23) and ST52 (21) as the predominant STs.

All *C. jejuni* isolates recovered from broilers (170), most *C. jejuni* recovered from cattle (102), and all *C. coli* isolates recovered from broilers (56) were susceptibility tested.

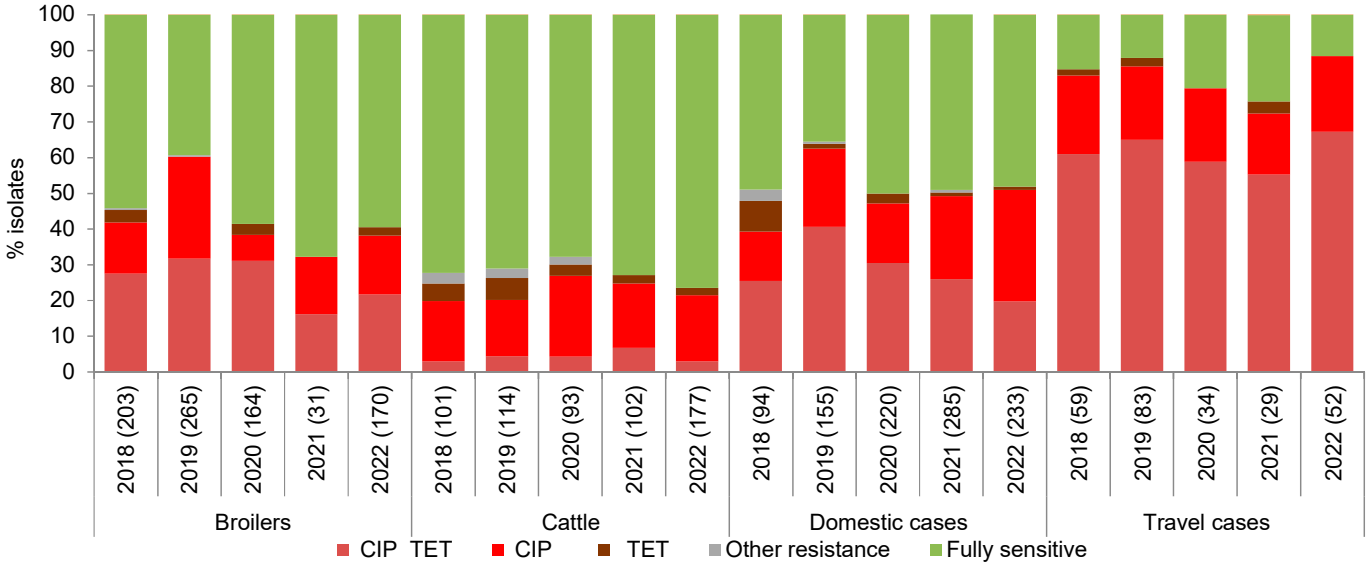
6.2.1 Resistance in *Campylobacter jejuni*

The levels of resistance in *C. jejuni* isolates from humans, and from Danish broilers and cattle at slaughter are presented in Table 6.1. Resistance was common for ciprofloxacin and tetracycline in isolates from humans, broilers, and cattle, whereas no resistance to chloramphenicol, erythromycin and gentamicin was observed in 2022. Ertapenem resistance has been monitored since 2021 and the levels of ertapenem resistance in human isolates were in line with the findings of the previous year. A total of 3% of the isolates, with 1% and 12% of isolates from domestic- and travel-related cases, respectively, showed ertapenem resistance. Unlike 2021, in 2022 ertapenem resistance was also found in 2% of *C. jejuni* isolates from broilers (Table 6.1).

In 2022, 48% from domestically-acquired human cases, 59% of *C. jejuni* from broilers, and 76% from cattle were sensitive to all antimicrobials tested. The percentage of fully-sensitive *C. jejuni* isolates from domestically-acquired human infections was in line with the previous years and likewise the resistance levels were comparatively higher in infections from travel-related cases.

Unlike the previous year, the percentage of fully-sensitive *C. jejuni* isolates from broilers decreased in 2022, while the percentage of full-sensitivity among isolates from cattle has remained relatively constant in the past five years (Figure 6.1).

Figure 6.1 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2022



The number of isolates included each year is shown in parentheses. A human isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. CIP: all isolates with ciprofloxacin resistance but not tetracycline resistance, TET: all isolates with tetracycline resistance but not ciprofloxacin resistance, CIP TET: all isolates with both ciprofloxacin and tetracycline resistance, Other resistance: all isolates with neither ciprofloxacin- nor tetracycline resistance, Fully-sensitive: all isolates susceptible to all antimicrobial agents included in the test panel. CIP TET, CIP and TET isolates may be also resistant to other antimicrobials in the test panel (see Table 6.1)

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2022

	Broilers	Cattle	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Chloramphenicol	0	0	0	0	0
Ciprofloxacin	38	22	51	88	58
Ertapenem	2	0	1	12	3
Erythromycin	0	0	0	0	0
Gentamicin	0	0	0	0	0
Tetracycline	24	5	21	67	29
Fully sensitive (%)	59	76	48	12	41
Number of isolates	170	102	233	52	285

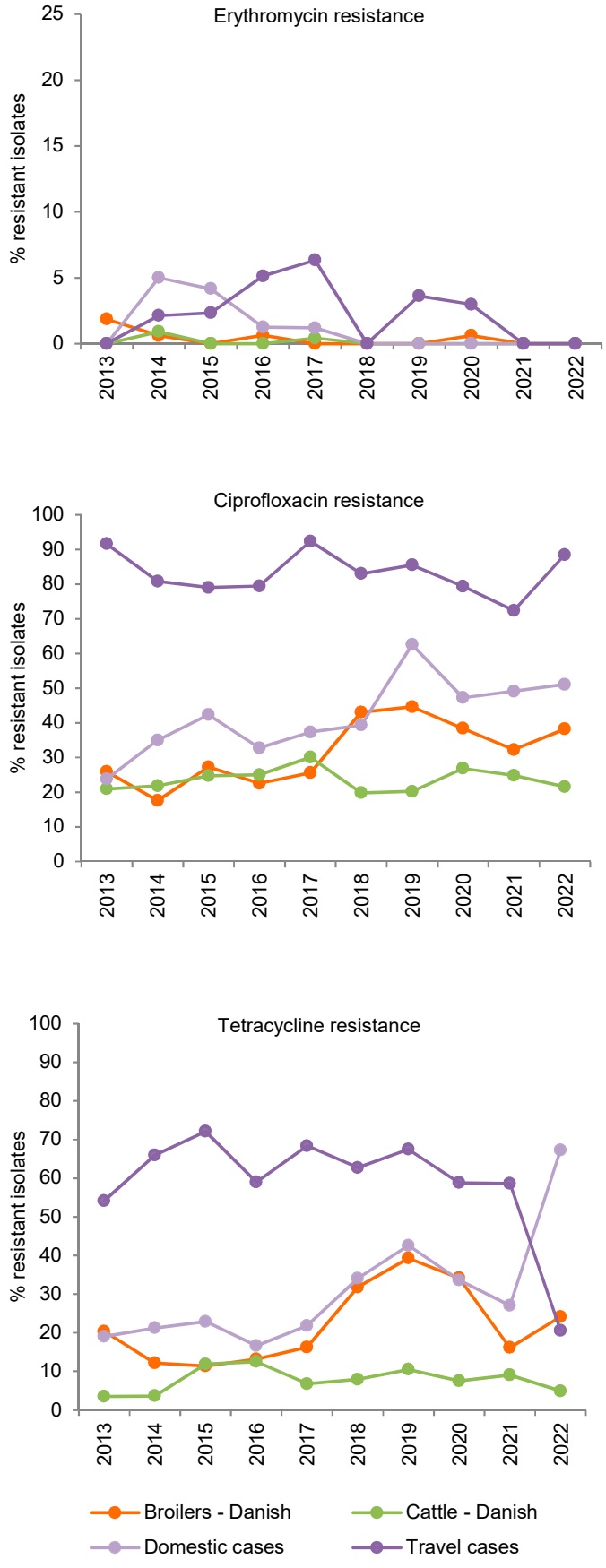
An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease
Fully-sensitive: all isolates susceptible to all antimicrobial agents included in the test panel

In 2022, the percentage of *C. jejuni* isolates from broilers with resistance to ciprofloxacin but not tetracycline was the same as in the previous year (16%), while the percentage of isolates with combined resistance to ciprofloxacin and tetracycline increased (from 16% to 22%). In cattle, *C. jejuni* showed a decrease in the combined resistance to ciprofloxacin and tetracycline (from 7% to 3%), compared to 2021. Resistance to tetracycline but not ciprofloxacin remained rare in isolates from humans and animals in 2022, as did resistance to antimicrobials other than ciprofloxacin and/or tetracycline (Figure 6.1).

As in previous years, the occurrence of resistance to ciprofloxacin and tetracycline in 2022 was higher in travel-associated isolates (88% and 67%, respectively) than in isolates from domestically-acquired infections (51% and 21%, respectively), and it was higher in human clinical isolates than in isolates from broilers and cattle (Figure 6.2).

Over the last decade, and until 2019, ciprofloxacin resistance has overall increased in *C. jejuni* from Danish broilers. This trend shifted to a decrease in 2020 and 2021 (note that the number of isolates was comparably lower in 2021 (N=31), which contributed to a higher uncertainty in the estimated occurrence of resistance in that year). However, in 2022 the occurrence of ciprofloxacin resistance increased again to the level of 2020 (38%) (Figure 6.2). As previously observed, the shift in the trend of resistance to ciprofloxacin coincided with the shifts in resistance to tetracycline (Figure 6.2) and combined resistance to both antibiotics (Figure 6.1). Fluoroquinolones are not used in food-producing animals in Denmark, suggesting that the development and spread of ciprofloxacin resistance in *C. jejuni* in broilers is driven by other mechanisms than direct antimicrobial use.

Figure 6.2 Erythromycin, ciprofloxacin and tetracycline resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2022



An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Macrolide resistance in *Campylobacter* is monitored using erythromycin. Erythromycin resistance was not observed in any *C. jejuni* isolates in 2022. Similarly, gentamicin resistance has been low or absent among *C. jejuni* from human isolates and has not been observed among *C. jejuni* from broilers and cattle in the last 10 years of monitoring. As in 2021, resistance to chloramphenicol was not observed among any of the tested *C. jejuni* isolates from humans or animals (Table 6.1).

Like in 2021, ertapenem resistance was observed in 1% and 12% of isolates from domestic- and travel-related cases, respectively, and in 2022 also in 2% of isolates from broilers (Table 6.1).

6.2.2 Resistance in *Campylobacter coli*

Erythromycin-, gentamicin- and chloramphenicol- resistance were not observed in *C. coli* isolates from broilers in 2022, similarly to what was observed in *C. jejuni* isolates. Resistance to ciprofloxacin in *C. coli* was also at a similar level as the one observed in *C. jejuni* (39%), while the occurrence of tetracycline resistance was comparatively higher in *C. coli* (45%). Resistance to ertapenem was detected in 23% of the *C. coli* isolates, also comparatively higher than the occurrence in *C. jejuni*.

6.2.3 Perspectives

Data from the two years of monitoring of ertapenem resistance in *C. jejuni* and *C. coli* from food-producing animals in many EU Member States, including Denmark, have shown possible different resistance wild-type distributions between the two *Campylobacter* species, as well as between different animal populations. This observation has been discussed between the European Food Safety Authority (EFSA), the European Centre for Disease Control (ECDC), the European Reference Laboratory for Antimicrobial Resistance (EURL-AR), the European Committee on Antimicrobial Susceptibility Testing (EUCAST), and many AMR academic experts.

It has been concluded that several points regarding ertapenem resistance in *Campylobacter* need to be clarified. A collaborative project has thus been initiated between EURL-AR, EFSA, ECDC and EUCAST in order to assess if the present epidemiological cut-off value (ECOFF) for interpretation is set correctly, if ertapenem is the best carbapenem to be included in the antimicrobial test panel, the effect of using different recommended test media, if there are differences in the wild-type resistance distribution between *Campylobacter* species and animal populations, if there are emerging clones with ertapenem resistance and if a resistance mechanism can be identified being responsible for the observed results [EURL-AR, personal communication].

6.3 *Salmonella*

DANMAP focuses on resistance in *Salmonella* Typhimurium and the related monophasic variants, as these serotypes are present in clinical human isolates and in isolates from food-producing animals, especially in pigs. Clonal dissemination plays an important role for the occurrence of antimicrobial resistance among *S.* Typhimurium. The global dissemination of genomic

islands conferring resistance to ampicillin, sulfamethoxazole and tetracycline (the ASuT multidrug-resistance profile) among *S.* Typhimurium continues to contribute to a high level of multidrug-resistance among isolates from humans and animals. In Denmark, *S.* Typhimurium from humans and food-producing animals, and in human isolates the monophasic variants, often carry ASuT resistance. The public health relevance of ASuT multidrug-resistance is of less direct importance than resistance to antimicrobials more commonly used in human medicine for salmonellosis treatment, such as macrolides, fluoroquinolones and cephalosporins. In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formula S. 4, [5],12:i:-, unless otherwise stated.

6.3.1 Resistance in *S.* Typhimurium

In 2022, a total of 190 human isolates of *S.* Typhimurium were susceptibility tested, including 96 *S.* Typhimurium and 94 monophasic variants. The monophasic isolates were dominated by sequence type (ST) 34 (91 isolates) and *S.* Typhimurium variants were dominated by ST19 (53) and ST36 (35). Forty-two isolates were associated with six outbreaks. The largest outbreak encompassed 14 isolates, and 20 and 22 of the outbreak related isolates were di- and monophasic, respectively. Thirty isolates were from travel-associated cases.

Forty-eight isolates from Danish pork were recovered and susceptibility tested in 2022, including 15 *S.* Typhimurium isolates and 33 monophasic variants.

The resistance data for *S.* Typhimurium for a panel of 15 antimicrobials are presented in Table 6.2 human isolates and for domestic pork. The resistance from human isolates is also presented separately for *S.* Typhimurium and monophasic variants.

Ampicillin-, sulfamethoxazole- and tetracycline resistance are common both in isolates from humans and meat. As in previous years, occurrence of resistance continued to be overall higher in isolates from pork than in isolates from humans. Accordingly, the level of fully-sensitive human isolates was higher, 39%, compared to 10% in isolates from Danish pork. Notably, the percentage of fully-sensitive isolates has decreased in *S.* Typhimurium from both humans and pork, compared to 2021 (Figure 6.3).

Figure 6.3 presents the relative distribution in percent of AMR profiles for *S.* Typhimurium from human domestically-acquired and travel-related cases and from domestic pork. The majority of ASuT-resistant isolates from humans were monophasic ST34, which encompassed 52 out of the 53 isolates that exclusively exhibited resistance towards ASuT.

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from domestic pork and humans, Denmark DANMAP 2022

	Pork	Human					
	Danish	Domestically acquired	Travel abroad reported	Unknown origin	Total	*S.* Typhimurium	*S.* Typhimurium monophasic
Antimicrobial agent	%	%	%	%	%	%	%
Amikacin	0	2	7	0	3	1	4
Ampicillin	79	51	53	35	50	15	86
Azithromycin	4	0	3	0	1	1	0
Cefotaxime	0	1	3	0	1	0	2
Ceftazidime	0	1	3	0	1	0	2
Chloramphenicol	13	6	13	5	7	10	4
Ciprofloxacin	0	4	20	0	6	8	3
Colistin	0	1	7	0	2	2	1
Gentamicin	8	1	3	0	1	0	2
Meropenem	0	0	0	0	0	0	0
Nalidixic acid	0	4	13	0	5	6	3
Sulfamethoxazole	81	54	47	35	51	19	84
Tetracycline	56	55	50	25	51	15	88
Tigecycline	2	1	0	0	1	2	0
Trimethoprim	25	11	7	5	9	4	15
Fully sensitive (%)	10	37	33	65	39	74	4
Number of isolates	48	140	30	20	190	96	94

Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formula s. 4,[5],12:i:-. Isolates of Danish pork were recovered from carcass swabs collected at slaughter. An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. An isolate is considered fully-sensitive if susceptible to all antimicrobial agents included in the test panel (Chapter 10, Table 10.3)

Figure 6.3 Relative distribution (%) of multidrug-resistant, resistant and fully-sensitive *S.* Typhimurium from pigs, domestic pork and human cases, Denmark

DANMAP 2022

Number of isolates included each year is presented in parentheses. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formula S. 4,[5],12:i:-. An isolate is considered fully-sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to three or more of all antimicrobial classes included in the test panel (See Chapter 10, Table 10.3). ASuT are multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline. Most ASuT human isolates correspond to monophasic variants of *S.* Typhimurium.
a) No data; b) Distribution not shown due to low number of isolates (N<15)

Most of the *S.* Typhimurium isolates recovered from domestic pork were resistant to several antimicrobials, and 56% were multidrug-resistant (48% of which were ASuT). Only 10% of the Danish pork isolates were fully-sensitive to all tested antimicrobials in 2022, which represents a decrease by 5% compared to 2021. Over the past five years, the resistance patterns among *S.* Typhimurium from Danish pork have fluctuated, and no obvious trends have been detected. However, since 2020 the occurrence of ASuT resistance has gradually decreased, at the exchange of an increase in occurrence of other multidrug-resistance and other resistance profiles (Figure 6.3).

Fluoroquinolones may be used for treatment of human *Salmonella* infections and resistance is monitored using ciprofloxacin. Ciprofloxacin resistance was observed in 4% of the isolates from domestically-acquired infections and in 20% of the isolates from travel-associated cases. Historically, ciprofloxacin resistance has predominantly been observed in isolates from travel-associated cases. During the last ten years, ciprofloxacin resistance in *S.* Typhimurium from Danish pork has been rare. In 2022, ciprofloxacin resistance was not found among isolates from pork (Figure 6.4).

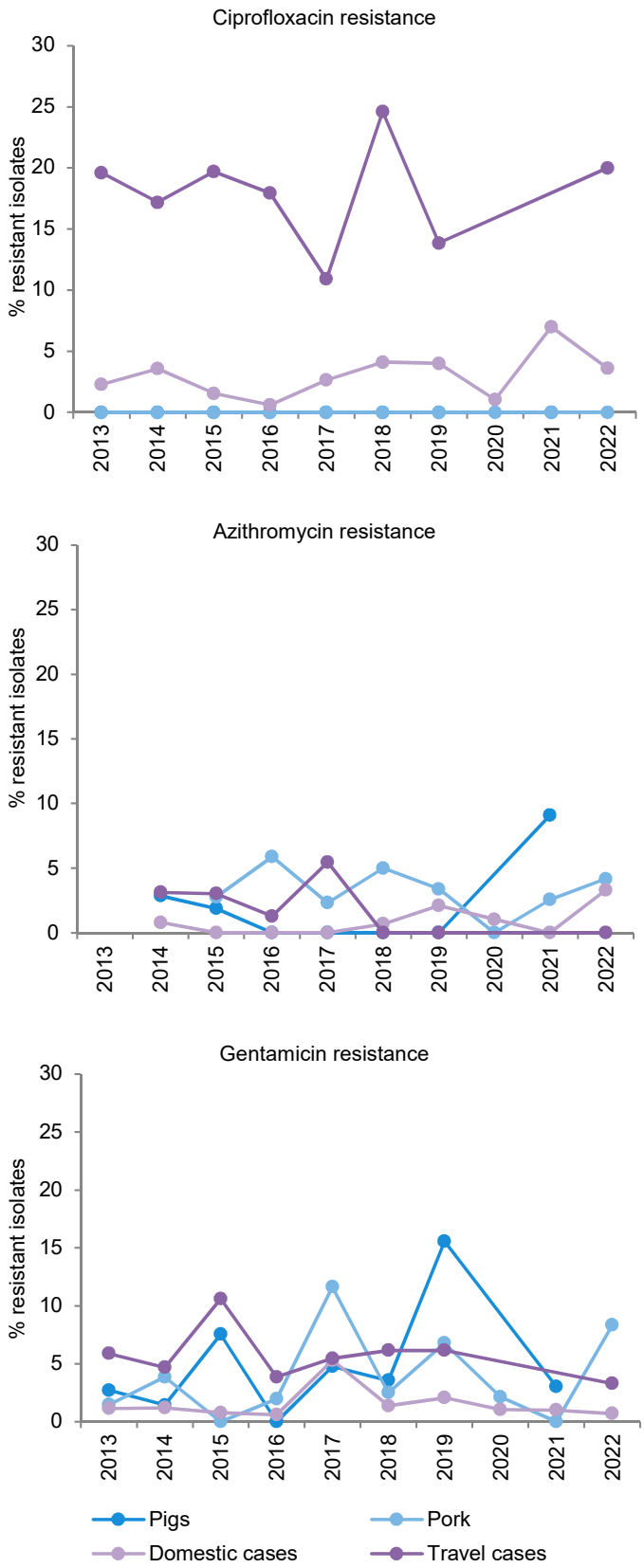
Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin, which is used for treatment of human *Salmonella* infections in Denmark. Resistance to azithromycin in *S.* Typhimurium has been low in human isolates and in 2022 it was only found in one isolate (1%). In 2021, azithromycin resistance was detected in a higher than usual percentage of isolates from pigs. In 2022, *S.* Typhimurium data was not available from pigs, and the results from domestic pork showed an occurrence of 4%, similar to what has been observed in previous years (Figure 6.4).

The levels of gentamicin resistance have been low and stable over the last years, and in 2022 two human isolates (1%) were resistant towards gentamicin. After a decrease in 2020 and 2021 in the occurrence of resistance to gentamicin in isolates from pork, in 2022 it increased to a level (8%) similar to 2019 (Figure 6.4).

Among human isolates, the level of resistance towards 3rd generation cephalosporins was low, and the combination of cefotaxime and ceftazidime resistance was only found in two isolates. Meropenem resistance was not observed among human isolates. As in the previous years, none of the *S.* Typhimurium isolates from domestic pork were resistant to 3rd generation cephalosporins or to meropenem (Table 6.2).

Resistance to tigecycline and colistin in *S.* Typhimurium are rare in Denmark. In 2022, two human isolates were recorded as colistin-resistant, and as in the previous years, no colistin resistance was found among pork isolates. Resistance towards tigecycline was observed in two human isolates and in 2% of the pork isolates. Resistance to amikacin, the antimicrobial that was introduced in the test panel in 2021, was observed in 3% of the human *S.* Typhimurium isolates in 2022 and thus at the same level as in 2021. All isolates from pork were sensitive towards amikacin (Table 6.2).

Figure 6.4 Ciprofloxacin, azithromycin and gentamycin resistance (%) among *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2022



Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formula s. 4,[5],12:i:-. An isolate is categorised as domestically-acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Due to the low number of isolates (N<15), travel-associated cases are not shown separately for 2020 and 2021. No data available for pigs in 2020 and 2022

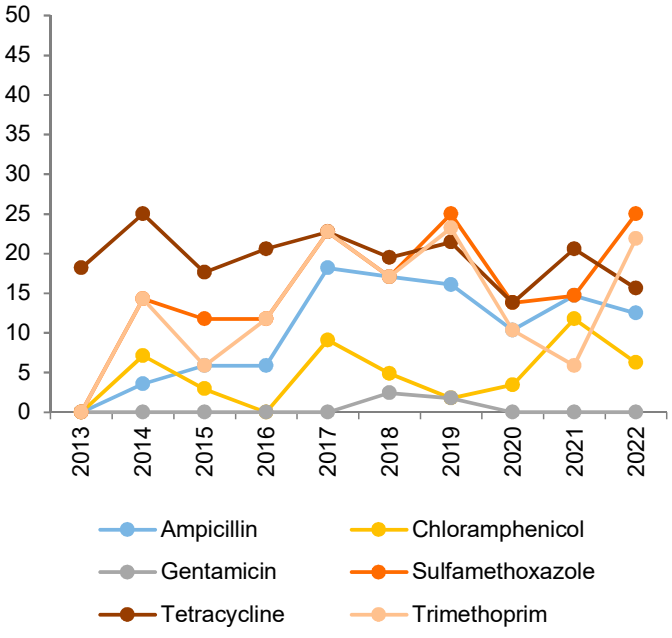
The occurrence of resistance to tetracycline in isolates from Danish pork seems to follow a decreasing trend, having decreased from 77% in 2020 to 67% in 2021 and 56% in 2022. The steady reduction in the use of tetracycline in pig production observed since 2014 (see Chapter 4, Table 4.1) may be one of the explanations for this observation.

6.3.2 Resistance in other *Salmonella* serotypes

Among samples from domestic pork, next to *S.* Typhimurium, the most common serotype detected was *S.* Derby, which was recovered from 32 samples in 2022.

The occurrence of resistance in *S.* Derby is generally lower than in *S.* Typhimurium, and in 2022, 69% of the *S.* Derby isolates from pork were susceptible to all tested antimicrobials, which was similar to the level observed in 2021. In 2022, compared to the previous year, resistance to sulfamethoxazole and trimethoprim increased, while resistance to ampicillin, tetracycline and chloramphenicol decreased (Figure 6.5). Resistance to critically important antimicrobials remained rare in 2022 in *S.* Derby isolates from domestic pork, with only one isolate resistant to azithromycin, and one isolate resistant to tigecycline. *S.* Derby isolates from domestic pork were not resistant to amikacin, 3rd and 4th generation cephalosporins, colistin, gentamicin, meropenem or fluoroquinolones (ciprofloxacin and nalidixic acid).

Figure 6.5 Resistance (%) among *Salmonella* Derby from domestic pork, Denmark DANMAP 2022



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Textbox 6.1

Detection of resistance genes and point mutations in *Salmonella* and *Campylobacter* using whole genome sequencing

Whole genome sequencing (WGS) is an integrated part of the surveillance of human *Campylobacter* and *Salmonella* infections in Denmark. The genome sequences are used to elucidate the genetic relationship between clones that prevail in Denmark in order to identify potential outbreaks, and the data is further used to identify genes and/or point mutations (genetic resistance markers) that confer antimicrobial resistance.

Resistance genotypes are established by WGS. DNA is extracted from the isolates, sequenced using the Illumina platform, and the quality of the sequence data is reviewed using the BIFROST QC pipeline (https://github.com/ssi-dk/bifrost). The genomes are assembled using SKESA (https://github.com/ncbi/SKESA) and the assemblies are analyzed with AMRFinderPlus (https://github.com/ncbi/amr/wiki), Software version: 3.11.18, Database version: 2023-08-08.2, with settings coverage 0.5, identity 0.9, for the in-silico detection of acquired resistance genes and point mutations.

Resistance genes and point mutations are widespread in Danish human clinical isolates in both *Campylobacter* and *Salmonella*. Many genetic resistance markers confer well defined phenotypic resistance and in this textbox, unless otherwise stated, the presence of the specified genetic markers usually confers phenotypic resistance.

The prevalence of genetic resistance markers for selected classes of antimicrobials are presented in Figure 1 for 649 clinical isolates of *C. jejuni* and 58 isolates of *C. coli*. Genetic resistance markers are common in both species but the frequency of resistance markers in *C. coli* is generally higher than the frequency in *C. jejuni*.

Genes conferring resistance towards tetracycline are present in 29% of *C. jejuni* and 55% of *C. coli* isolates. Fluoroquinolone resistance is usually associated with point mutations in the gyrA gene and 58% of *C. jejuni* and 69% of *C. coli* carry genetic markers that typically confer fluoroquinolone resistance. Aminoglycoside resistance markers are frequently identified in *C. coli* (36%) and less frequently in *C. jejuni* (6%), but the majority of the identified genetic aminoglycoside resistance markers are not conferring resistance towards gentamicin. Beta-lactam resistance markers are common in both species, but the clinical significance of many of the resistance genes remains to be resolved. This also applies to the genetic basis for ertapenem resistance. Resistance markers for macrolide resistance are also common in both species, and can be mediated by both genes and point mutations. However, the presence of the genetic macrolide markers rarely confers resistance towards erythromycin. Chloramphenicol resistance markers are rarely identified in both species.

The prevalence of genetic resistance markers for selected classes of antimicrobials are presented in Figure 2 for 782 clinical isolates of *Salmonella* from 2022. The isolates represent 101 monophasic *S.* Typhimurium, 101 *S.* Typhimurium, 246 *S.* Enteritidis and 334 isolates belonging to other serotypes.

Genetic resistance markers are common in all serotypes. It is prominent that the levels of genetic resistance markers for resistance to aminoglycosides, beta-lactams resistance, sulfonamides and tetracyclines are particularly high in monophasic *S.* Typhimurium reflecting phenotypic resistance towards ampicillin, streptomycin, sulfonamide, and tetracycline.

A number of different genetic resistance markers for gentamicin were found in seven isolates (1%). Genes conferring resistance towards neomycin, *aph(3')-Ia*, were found in 2% of all isolates and in 11% of monophasic *S.* Typimurium isolates.

Among the genetic resistance markers for beta-lactam antimicrobials, *blaTEM-1* (ampicillin resistance) was identified in 14% of the isolates and it was harbored by 82% of the monophasic *S.* Typhimurium variants. Genes associated with extended spectrum beta-lactamase production were found in three isolates and genes conferring resistance towards carbapenems were not identified.

Genetic resistance markers for azithromycin resistance were found in five isolates (<1%) and included both isolates with genes *mph(A)* and isolates with point mutations in the *acrB* gene.

continued ... Textbox 6.1

Figure 1 Prevalence of resistance genes and/or point mutations conferring resistance to selected antimicrobial classes in 649 clinical human isolates of *C. jejuni* and in 58 isolates of *C. coli*, Denmark, 2022 DANMAP 2022

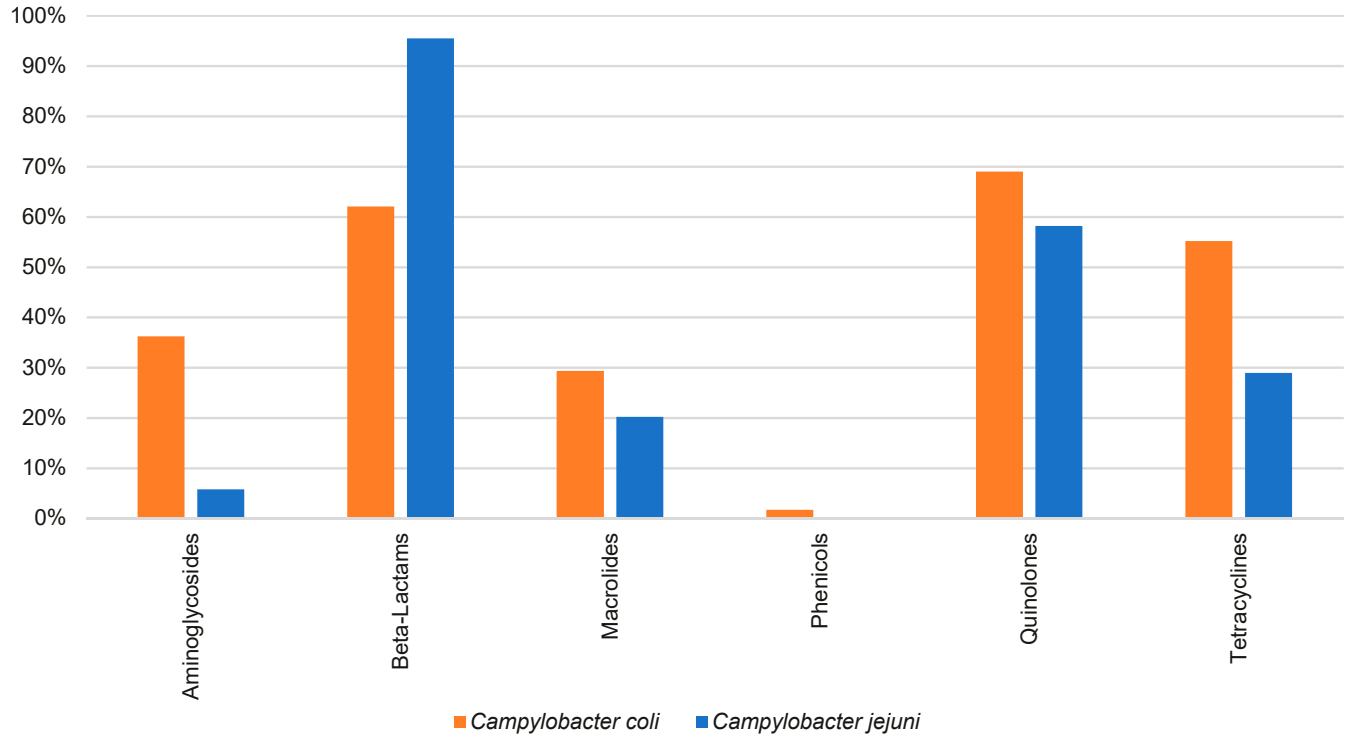
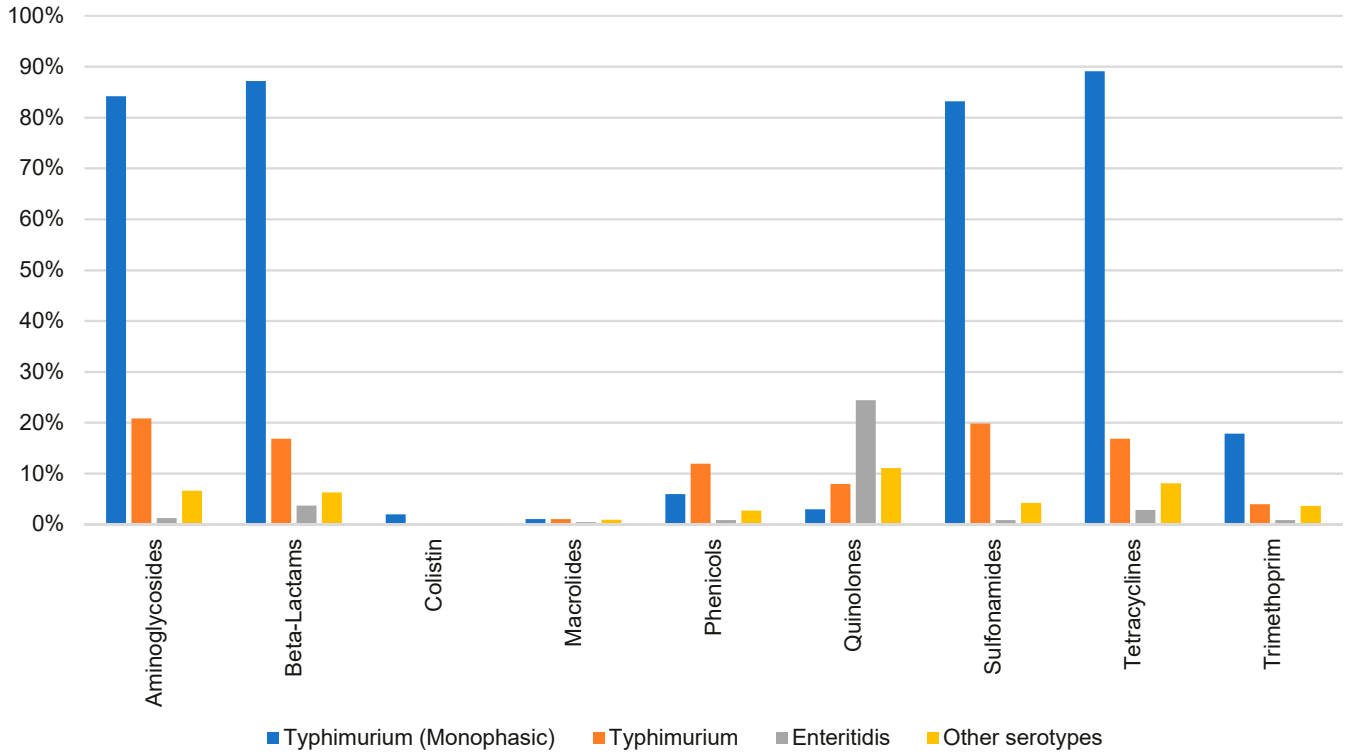


Figure 2 Prevalence of resistance genes and/or point mutation conferring resistance to selected antimicrobial classes in 782 clinical human isolates of *Salmonella*, Denmark, 2022 DANMAP 2022



Salmonella isolates included 101 monophasic *S*. Typhimurium, 101 *S*. Typhimurium, 246 *S*. Enteritidis, and 334 isolates belonging to other serotypes

Resistance markers conferring resistance towards fluoroquinolones were found in 14% of the isolates in total and in 24% of *S.* Enteritidis isolates. Resistance towards fluoroquinolones was mediated by point mutations in 10% of the isolates and by *qnr* genes in 4% of the isolates.

Perspectives

WGS is a valuable tool for surveillance of AMR in *Campylobacter* and *Salmonella* in human clinical isolates. In many cases it is possible to predict the phenotypic susceptibility of isolates based on WGS. More WGS-based data will be presented in the coming DANMAP reports, both for isolates recovered from humans and from healthy and sick animals. This will eventually allow to monitor the spread of genetic resistance markers, and combinations of markers along the farm to fork chain.

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7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: Over the last 5-year monitoring period, there have been no statistically significant trends in the prevalence of fully sensitive **indicator *E. coli*** isolates from broilers, cattle or pigs. Nonetheless, in the last two monitoring years, there was a steady increase in fully sensitive *E. coli* from broilers and a steady decrease in fully sensitive *E. coli* from cattle.

As in previous years, no amikacin, colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Resistance to ciprofloxacin continued to be low in cattle and pigs and increased in broilers, reaching the maximum prevalence of the last 10 years (18%). Similarly to 2021, in 2022 azithromycin resistance was detected in a small number of isolates from pigs (3%), while the occurrence of chloramphenicol resistance decreased (8%).

The relative occurrence of multidrug-resistant indicator *E. coli* compared to the previous year decreased in broilers and pigs, and increased in cattle. Combined resistance to ampicillin, sulfamethoxazole, and tetracycline (ASuT) continued to be the most common multidrug-resistance profile. However, the relative occurrence of other multidrug-resistance profiles has increased in isolates from broilers and cattle in the past 5 years.

Importantly, as in previous years, samples from broilers and from broiler meat examined for **carbapenemase-producing (CP) *E. coli*** (including OXA-48) were found negative. Similarly, CP-producing *E. coli* was not detected in turkey meat in 2022.

The occurrence of **beta-lactamase-producing *E. coli***, obtained through selective procedures, continued the decreasing trend observed since 2018 in broilers and broiler meat. In 2022, imported turkey meat presented an occurrence of 52%. Antimicrobial susceptibility testing of ESBL/AmpC-producing *E. coli* from broilers showed an increase in resistance to fourth generation cephalosporins (cefepime), and a single isolate was found resistant to ertapenem. Resistance to ciprofloxacin was abundantly found in isolates from imported broiler meat (100%) and imported turkey meat (83%). Azithromycin resistance was also found in single isolates from those samples and isolates from imported turkey meat were also somewhat resistant to colistin (7%), ertapenem (2%) and gentamicin (10%).

Whole genome sequencing of beta-lactamase-producing *E. coli* revealed ESBL, AmpC and ESBL+AmpC genotypes. All AmpC genotypes encoded upregulated AmpC promotor C-42T mutations. The plasmid-mediated CMY-2 gene was observed in ESBL+AmpC genotypes. Among the ESBL genotypes, 14 different ESBL genes were detected, with most variation in isolates from imported turkey meat. The latter presented a high frequency of CTX-M-15 and 46% had more than one ESBL encoding gene.

In 2022, 39% of ***E. faecalis*** and 52% of ***E. faecium*** isolated from broilers were fully sensitive. None of the enterococci isolates showed resistance to ampicillin, chloramphenicol, daptomycin, gentamicin, linezolid, teicoplanin, tigecycline or vancomycin. Combined resistance to tetracycline and erythromycin continued to be among the most common resistance profiles.

7.1 Introduction

Escherichia coli and *Enterococcus* are included in the DANMAP programme to monitor the occurrence of antimicrobial resistance in different reservoirs throughout the food chain for the following reasons: i) they are present as commensals in the gut microbiota of healthy animals and humans, ii) they can acquire antimicrobial resistance both via mutations in chromosomal genes and horizontal transfer of antimicrobial resistance genes, and iii) they have the potential to cause infections in both animals and humans, and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

E. coli exhibiting resistance to 3rd generation cephalosporins via the production of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) is among the fastest spreading antimicrobial resistance mechanisms in both humans and food-producing animals worldwide. Some studies have suggested a zoonotic transmission of ESBL/AmpC-producing *E. coli* [Liu et al 2023. One Health, 16: 100518; Roer et al 2019. J Antimicrob Chemother, 74(3):557; Mughini-Gras et al 2019. Lancet Planet Health, 3(8): e357-e369; Liu et al 2018. mBio, 9(4): e00470-18], while others found no evidence of transmission between animals and the general human population [Dorado-Garcia et al 2018, J Antimicrob Chemother, 73: 339-347; Findlay et al 2020, Applied and Environmental Microbiology, 87(1): e01842-20]. The zoonotic nature of ESBL/AmpC-producing *E. coli* isolated in Denmark from humans, animals and meat is addressed in Chapter 3.

Carbapenemase-producing *Enterobacteriaceae* (CPE) pose a great threat to human health, as carbapenems are last-line antimicrobial drugs for the treatment of infections caused by multidrug-resistant Gram-negative bacteria. In recent years, CPE have been increasingly detected in food-producing animals in the EU, which raises the concern that animals might become a CPE reservoir in the future [EFSA/ ECDC 2023, EFSA Journal 2023; 21(3):7867].

Isolation and antimicrobial susceptibility testing of indicator *E. coli*, indicator enterococci and extended-spectrum cephalosporinase (ESC)- and carbapenemase (CP)-producing *E. coli* are performed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2020/1729/EU]. In 2022, isolates were obtained from randomly selected caecal samples collected from healthy broilers, cattle, and pigs at slaughter. Additionally, for the specific monitoring of ESC- and CP-producing *E. coli*, fresh meat from broilers and turkeys was collected at retail. Details on sampling, analysis, susceptibility testing and interpretations are presented in Chapter 10.

7.2 Indicator *Escherichia coli*

Indicator *E. coli* isolates were obtained from 96% of caecal samples from broilers (195/204), 94% of samples from pigs (176/188) and 96% of samples from cattle (112/117). These isolates were obtained with a non-selective isolation procedure. Results obtained by selective procedures for specific monitoring of ESC- and CP-producing *E. coli* are presented in Section 7.3.

Indicator *Escherichia coli* from broilers, cattle and pigs

There has been no statistically significant increasing or decreasing trend in the annual prevalence of fully sensitive *E. coli* isolates from broilers, cattle or pigs during the past five years of monitoring (Figure 7.2) (p-values of 0.09 for broilers, 0.16 for cattle and 0.33 for pigs). Nevertheless, the percentage of broiler isolates susceptible to all antimicrobials in the test panel has increased in the past two years, from 58% in 2020 to 67% in 2022. In contrast, the percentage of cattle isolates fully sensitive has decreased from 91% in 2020 to 84% in 2022 (Table 7.1).

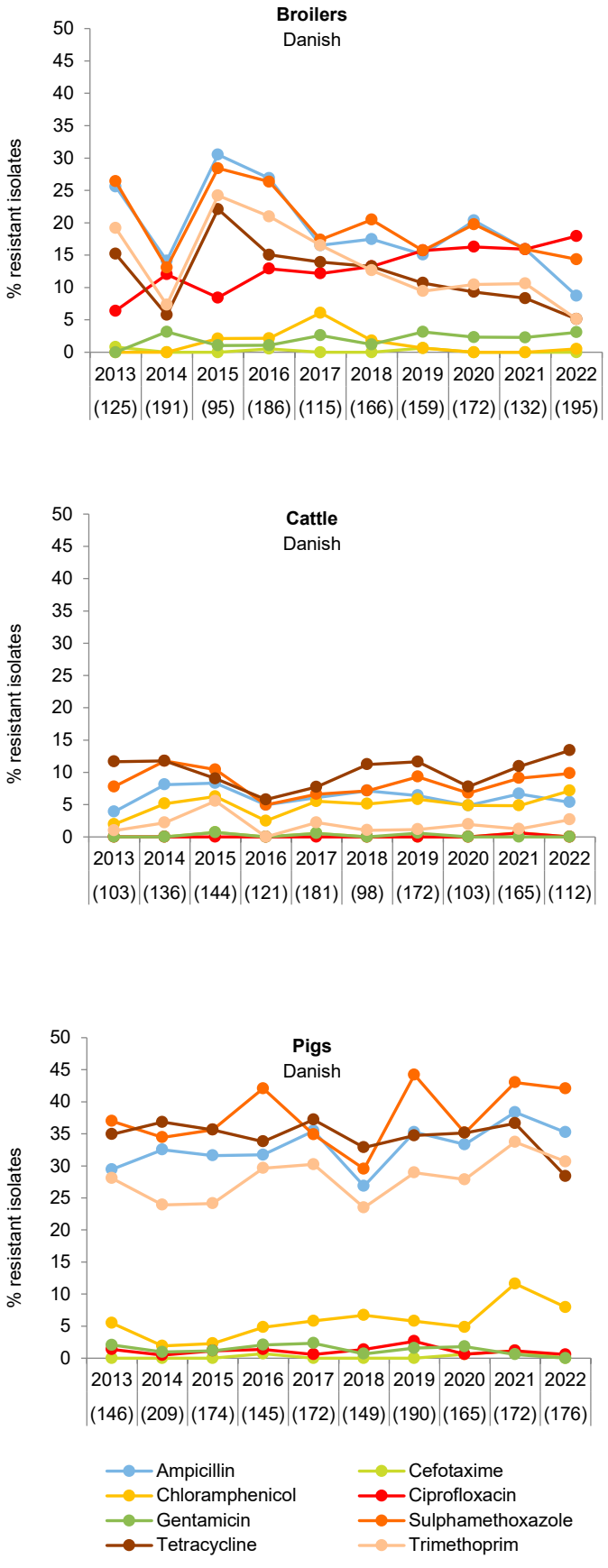
Compared to 2021, the occurrence of resistance to most antimicrobials in the test panel suffered mostly minor fluctuations (1 to 2%). A few exceptions were found in isolates from broilers, where resistance to ampicillin, tetracycline and trimethoprim decreased by 7%, 3% and 6%, respectively, while resistance to (fluoro)quinolones increased (by 4% for nalidixic acid and 2% for ciprofloxacin). In isolates from pigs, resistance to ampicillin, sulfamethoxazole, tetracycline and trimethoprim also decreased (by 9% for tetracycline and by 3% for the other three substances), as well as resistance to chloramphenicol, which decreased by 4%. These decreases contradict the previously observed increase in 2021 among isolates from pigs. In contrast, the percentage of *E. coli* from cattle resistant to ampicillin, sulfamethoxazole, tetracycline, trimethoprim and chloramphenicol all increased in 2022, although only by magnitudes of 1% to 2% (Figure 7.1).

Table 7.1 Resistance (%) in *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022

	Broilers	Cattle	Pigs
Antimicrobial agent	Danish %	Danish %	Danish %
Amikacin	0	0	0
Ampicillin	9	5	35
Azithromycin	0	0	3
Cefotaxime	0	0	0
Ceftazidime	0	0	0
Chloramphenicol	<1	7	8
Ciprofloxacin	18	0	<1
Colistin	0	0	0
Gentamicin	3	0	0
Meropenem	0	0	0
Nalidixic acid	18	0	0
Sulfamethoxazole	14	10	42
Tetracycline	5	13	28
Tigecycline	0	0	0
Trimethoprim	5	3	31
Fully sensitive (%)	67	84	49
Number of isolates	195	112	176

An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Materials and methods, Table 10.3)

Figure 7.1 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022



The number of isolates included each year is shown in parentheses

As in previous years, no isolates resistant to amikacin, colistin, meropenem or tigecycline were detected. Additionally, in 2022, resistance to 3rd generation cephalosporins was not detected in indicator *E. coli* using non-selective methods. Azithromycin resistance was, similarly to 2021, detected in 3% of the isolates from pigs (Table 7.1).

Resistance to (fluoro)quinolones continues to be low (<1%) or not observed in *E. coli* from cattle and pigs, but it increased to 18% in 2022 among broiler isolates, which gives continuity to an ongoing increasing trend (Figure 7.1). While this trend is not significant over the past 5-years (p-value=0.131), a significant increasing trend was determined for the past 10-years of monitoring (p-value=0.0004).

Compared to the two previous years, the occurrence of resistant and multidrug-resistant (MDR) *E. coli* isolates continued to decrease in broilers and to increase in cattle. In pigs, resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT) appears to be relatively stable, and annual oscillations in the occurrence of other MDR profiles occur, with no apparent ongoing increasing or decreasing trend (Figure 7.2). ASuT resistance was again the predominant MDR profile in isolates from pigs, and occurred in half of the MDR isolates from cattle and in 20% (2 out of 8 isolates) of the MDR isolates from broilers.

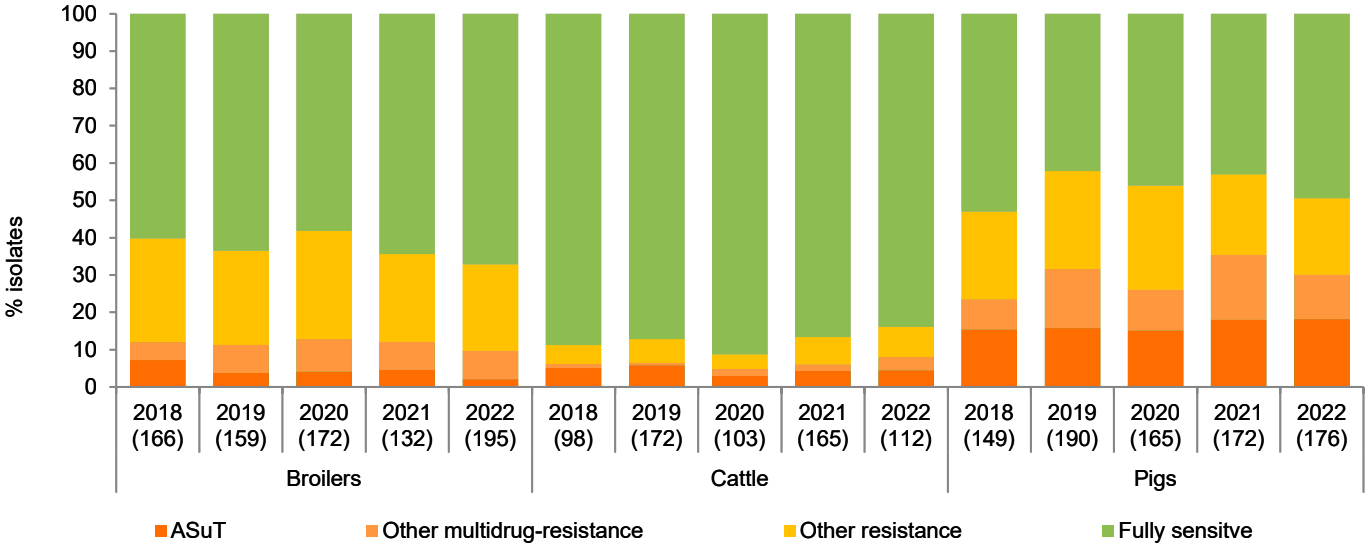
Among indicator *E. coli* isolated with a non-selective procedure, no presumptive ESBL-producing isolates were found (Table 7.1).

7.2.2 Perspectives

At the EU level, full sensitivity in indicator *E. coli* isolated from pigs and cattle has not changed significantly between 2015 and 2021. Significant decreasing trends in antimicrobial resistance have been observed for some countries in isolates from pigs and from cattle. Also at the EU level, full sensitivity in indicator *E. coli* from broilers has significantly increased between 2014 and 2020, with several countries showing this significant trend at the individual level. No significant increasing or decreasing trends in full sensitivity were reported for Denmark, individually, in the European Summary Report [EFSA/ECDC 2023, EFSA Journal 2023; 21(3):7867].

Accordingly, and as in previous years, in DANMAP 2022 no significant increasing or decreasing trends were observed in the occurrence of fully sensitive indicator *E. coli* recovered from broilers, cattle or pigs in the last 5-year monitoring period (in DANMAP 2022, between 2018 and 2022). It is possible that different trends are observed when different monitoring periods are included in the analysis, different statistical methods are applied or data are aggregated at different levels (annually, monthly, etc). In DANMAP 2022, an alternative approach was applied to perform a trend analysis of the occurrence of resistance in indicator *E. coli* (see Textbox 7.1).

Figure 7.2 Resistance (%) among *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2022



The number of isolates included each year is shown in parentheses. An isolate is considered fully sensitive if susceptible to all antimicrobial agents in the test panel, and multidrug-resistant if resistant to three or more of the 12 antimicrobial classes included in the test panel (Materials and methods, Table 10.3). ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline

Furthermore, at the EU level, a clear negative association has been determined between the probability of full sensitivity in indicator *E. coli* and the overall consumption of antimicrobials by food-producing animals [EFSA/ECDC/EMA 2021, JIACRA III, DOI 10.2900/056892]. That analysis was based on monitoring data of total antimicrobial use and the percentage of full sensitivity in isolates from broilers, turkeys, pigs, and veal calves, from more than 20 countries, with a large variation in consumption and resistance levels among them. In the future, the association between antimicrobial use and full sensitivity of indicator *E. coli* should be assessed separately for broilers, pigs and cattle, and specifically for Denmark.

7.3 ESBL/AmpC- and carbapenemase-producing *E. coli*

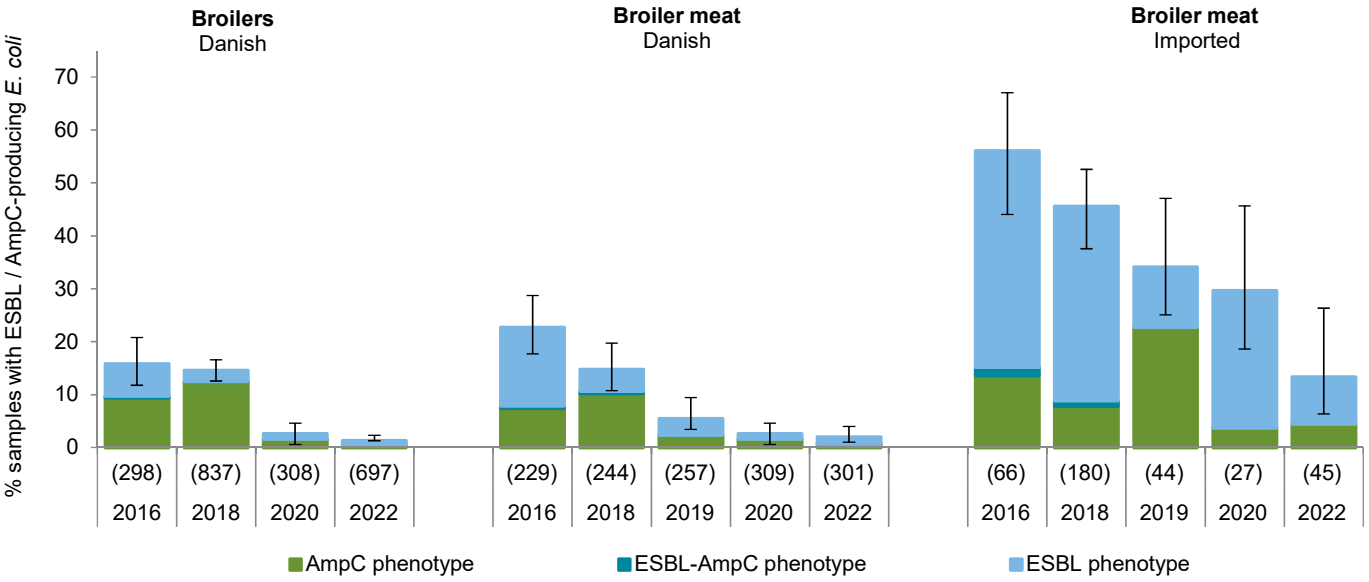
In 2022, caecal samples collected from broilers at slaughter, and from packages of fresh, chilled broiler- and turkey meat collected from Danish wholesale and retail outlets were monitored for the presence of extended-spectrum beta-lactamase (ESBL)-, cephalosporinase (AmpC)-, and carbapenemase (CP)-producing *E. coli*. In accordance with the harmonised EU monitoring, packages of meat were collected at retail without pre-selecting by country of origin, hence including in principle both imported and domestically-produced products. Of the samples randomly collected at retail, 13% of broiler meat and 100% of turkey meat were imported products. According to the new Decision 2020/1729/EU, monitoring of turkey meat for ESBL-, AmpC-, and CP-producing *E. coli* is mandatory in even years.

Using selective procedures, ESBL/AmpC-producing isolates were obtained from broilers (9/697 (1%) flocks), broiler meat (Danish: 6/301 (2%) samples, imported: 6/45 (13%) samples), and imported turkey meat (59/113 (52%) samples). The selective procedures for detection of CP-producing *E. coli* (including oxacillinase-producing OXA-48-like enzymes), recovered no isolates (Table 7.2).

7.3.1 ESBL/AmpC-producing *E. coli* from broilers, broiler meat and turkey meat

Following selective enrichment, *E. coli* resistant to 3rd generation cephalosporins (cefotaxime and/or ceftazidime) were obtained from 1% (CI 95%: 1-2%) of samples from broilers, 2% (CI 95%: 1-4%) of samples from Danish broiler meat, 13% (CI 95%: 6-26%) of samples from imported broiler meat (from three different countries) (Figure 7.3), and 52% (CI 95%: 43-61%) of samples from imported turkey meat (from two different countries, 98% from a single country). In 2022, the prevalence of ESBL/AmpC-producing *E. coli* continued to decrease in isolates from broilers and broiler meat. Furthermore, as observed in the last four monitoring years, the occurrence of ESBL/AmpC-producing *E. coli* continues to be higher in imported broiler meat compared to Danish broiler meat (Figure 7.3). In 2022, the relative frequency of ESBL-producing and AmpC-producing phenotypes remained mostly unchanged in comparison to 2020, except among isolates from imported broiler meat, which showed an increase in the relative occurrence of the AmpC-producing phenotype (from 13% to 33%). In isolates recovered from imported turkey meat, the ESBL-producing phenotype was clearly predominant (90%) (Table 7.2).

Figure 7.3 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark 2016-2022 DANMAP 2022



Number of samples tested per year is presented in parentheses. Classification of ESBL and AmpC phenotypes is based on the MIC results (Materials and Methods, Section 10.7.2). Confidence intervals for total proportion of samples positive for a phenotype calculated as 95% binomial proportion Wilson intervals

All the recovered ESBL/AmpC-producing isolates from broilers and Danish broiler meat were resistant to both 3rd generation cephalosporins (cefotaxime and ceftazidime) and ampicillin. Isolates from imported broiler- and turkey meat were also 100% resistant to cefotaxime and to ampicillin, but 83% and 95% resistant to ceftazidime, respectively. In contrast, resistance to 4th generation cephalosporins (cefepime) was found at a higher prevalence in imported broiler meat (83%) and imported turkey meat (95%), when compared to Danish broilers and broiler meat (67%) (Table 7.2). Compared to 2020, the levels of resistance to cefepime increased by 17% in isolates from domestic broilers and broiler meat, and decreased by 17% in isolates from imported broiler meat.

During the same period, the observed resistance to fluoroquinolones (ciprofloxacin) has markedly decreased in ESBL/AmpC-producing *E. coli* from Danish broilers (88% to 44%) and from Danish broiler meat (63% to 17%). In contrast, resistance markedly increased in isolates from imported broiler meat (50% to 100%). In 2022, ciprofloxacin resistance was also observed in 83% of the isolates from imported turkey meat. As in 2020, no resistance to colistin, meropenem or imipenem was observed in the specific monitoring of ESBL/AmpC-producing *E. coli* from broilers and broiler meat in 2022. However, ertapenem resistance was observed in one out of 11 isolates (11%) from broilers and one out of 59 isolates from imported turkey meat (2%), while colistin-resistant ESBL/AmpC-producing *E. coli* were observed in 7% of the turkey meat samples. Azithromycin resistance was observed in one out of six isolates from imported broiler meat (17%) and in a single isolate from imported turkey meat (2%), while gentamicin resistance was only detected among isolates from imported turkey meat (10%). As in previous years, resistance to tigecycline and temocillin was not observed among the isolates collected in 2022 (Table 7.2).

The genetic basis for ESBL- and AmpC enzymes was detected in most isolates recovered by selective enrichment. The detected enzymes corresponded to the phenotypes derived from the susceptibility testing for the majority of the isolates. In 5 isolates (2 from broiler meat and 3 from broilers), whole genome sequencing revealed both ESBL and AmpC encoding genes, even though the susceptibility testing showed a AmpC-producing phenotype (Tables 7.2 and 7.3). Three isolates from turkey meat showed an ESBL and AmpC producing phenotype however, only ESBL-encoding genes were detected.

Among the AmpC-producing isolates recovered in 2022, resistance was mainly conferred by upregulated AmpC promotor C-42T mutations (one isolate from broilers, one from Danish broiler meat and three from imported turkey meat). The CMY-2 plasmid-mediated AmpC enzyme was detected in a single AmpC-producing *E. coli* from imported broiler meat (Table 7.3).

Among all ESBL-producing isolates, 14 different ESBL genes were detected, of which 9 occurred as the only encoding gene (CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-27, CTX-M-32, CTX-M-55, SHV-12, TEM-1B and TEM-52B). Overall, the most commonly observed ESBL encoding genes across isolates from broilers, broiler meat and turkey meat were CTX-M-1 and TEM-1B. Notably, the encoding gene CTX-M-15 was highly frequent in isolates from imported turkey meat only (Table 7.3). Furthermore, in isolates from imported turkey meat, 13 out of the

total 14 different detected ESBL genes were observed, and 46% of the isolates had more than one ESBL encoding gene, with two isolates harbouring three different genes (SHV-12/TEM-1B/TEM-1D and SHV-12/TEM-1B/TEM-135) (Table 7.3).

Among isolates that harboured both ESBL and AmpC encoding genes, upregulated AmpC promotor C-42T mutation was detected in two isolates from broilers. ESBL- and AmpC-producing isolates harbouring the AmpC plasmid-mediated CMY-2 were observed in single isolates from Danish broilers and broiler meat, and in two isolates from imported broiler meat. All ESBL and AmpC genotypes detected in 2022 were due to the presence of the ESBL encoding gene TEM-1B. No ESBL and AmpC genotypes were detected in isolates from imported turkey meat (Table 7.3).

In total, 35 MLSTs were observed among all ESBL/AmpC-producing *E. coli* isolates. The most common MLSTs were ST1001 in Danish broiler meat, ST6448 in imported broiler meat, ST4663 in broilers, and ST4981 in imported turkey meat. The isolates that harboured the CMY-2 genotype were attributed to ST1001 (Danish broiler meat), ST2473 (broilers), ST155 and ST1112 (imported broiler meat).

7.3.2 Perspectives

An obvious reduction in the ESBL/AmpC-producing *E. coli* in Danish broilers and broiler meat has occurred since 2018 (Figure 7.3). This reduction is likely the result of the requirement that imported breeding and production animals must be tested and found negative for ESBL/AmpC-producing *E. coli* before they are allowed into the country.

Table 7.2 Resistance (%) and beta-lactam resistance phenotype distribution in ESBL/AmpC-producing *Escherichia coli* recovered by selective enrichment from animals and meat, Denmark DANMAP 2022

	Broilers	Broiler meat		Turkey meat
Antimicrobial agent	Danish %	Danish %	Import %	Import %
Amikacin	0	0	0	0
Ampicillin	100	100	100	100
Azithromycin	0	0	17	2
Cefepime	67	67	83	95
Cefotaxime	100	100	100	100
Cefotaxime/clavulanic acid	44	33	33	5
Cefoxitin	44	33	33	10
Ceftazidime	100	100	83	95
Ceftazidime/clavulanic acid	44	33	33	5
Chloramphenicol	0	17	50	38
Ciprofloxacin	44	17	100	83
Colistin	0	0	0	7
Ertapenem	11	0	0	2
Gentamicin	0	0	0	10
Imipenem	0	0	0	0
Meropenem	0	0	0	0
Nalidixic acid	44	17	83	58
Sulfamethoxazole	0	17	100	58
Temocillin	0	0	0	0
Tetracycline	11	17	83	58
Tigecycline	0	0	0	0
Trimethoprim	11	0	83	43
Number of AmpC phenotypes	4	2	2	3
Number of ESBL phenotypes	5	4	4	53
Number of ESBL+AmpC phenotypes	0	0	0	3
Number of isolates (%)	9 (1%)	6 (2%)	6 (13%)	59 (52%)
Number of samples	697	307	45	113

Classification of ESBL-, AmpC- and AmpC+ESBL-producing phenotypes is based on the MIC results (Chapter 10, secrion 10.7.2). AmpC, ESBL and AmpC+ESBL indicate the number of isolates expressing each specific phenotype

At the EU-level, there is a large variation in the prevalence of ESC-producing *E. coli* recovered from animals and meat. In 2020, prevalence ranged between 0.3% and 97% in broilers and broiler meat and between 0% and 70.4% in fattening turkeys [EFSA/ECDC 2022. EFSA Journal 2022;20(3):7209]. With the prevalence levels observed in 2022 (1% to 2%), Denmark continues to be among the countries with a lower occurrence of beta-lactamase-producing *E. coli* in domestic broilers and meat thereof. The enzymes of the ESBL and AmpC-producing *E. coli* in broilers and broiler meat seem to be consistent with the enzymes detected in previous years, but their attribution to different MLSTs suggests the occurrence of horizontal gene transfer.

This was the first year of mandatory monitoring of ESBL/AmpC-producing *E. coli* in turkey meat, according to Decision 2020/1729/EU. Results have shown a relatively high occurrence of ESBL/AmpC-producing *E. coli* in imported turkey meat when compared to imported broiler meat. Additionally, there was a large variety of ESBL enzymes detected in isolates from turkey meat, among which the most frequently detected (CTX-M-15) is also often encountered in isolates from bloodstream infections in humans (see Chapter 3). The public health relevance of these findings can be best assessed after some years of continuous harmonized monitoring.

The zoonotic transmission of beta-lactamase-producing *E. coli* continues to be investigated, with studies presenting different conclusions. In Chapter 3, ESBL/AmpC-producing *E. coli* isolates collected in Denmark between 2018 and 2022 from animals and meat and from human bloodstream infections were compared to address the likelihood of transmission between animals/meat and humans in Denmark.

Still, no carbapenemase-producing *E. coli* were detected in the 1162 samples tested in 2022. In 2020 and 2021, in the EU-monitoring of CP-producing *E. coli*, 14 and 29 isolates were detected with phenotypic and genotypic methods, respectively. Those included in total only four isolates from broilers and one from fattening turkeys [EFSA/ECDC 2022. EFSA Journal 2022;20(3):7209]. However, 2022 was the first year of mandatory monitoring of CP-producing *E. coli* in poultry, according to Decision 2020/1729/EU. Thus, it is possible that a higher number of isolates will be reported at EU-level in the forthcoming EU Summary Report.

Table 7.3 Number of ESBL and AmpC enzymes detected in beta-lactamase-producing *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark DANMAP 2022

	Broilers	Broiler meat		Turkey meat
Enzymes	Danish	Danish	Import	Import
CTX-M-1	2	1		3
CTX-M-14				2
CTX-M-15				28
CTX-M-27				6
CTX-M-32				2
CTX-M-55			2	5
CTX-M-65			1	1
OXA-1				2
SHV-12		1		6
TEM-135				3
TEM-176				1
TEM-1B	3	1	4	21
TEM-1D				1
TEM-52B	1	2		
CMY-2	1	1	2	
Chromossomal AmpC (C-42T)	3	1		3
Number of AmpC genotypes	1	1	1	3
Number of ESBL genotypes (two or more enzymes)	3 (0)	4 (0)	4 (2)	54 (25*)
Number of AmpC+ESBL genotypes	3	1	1	0
Not available	2	0	0	2
Number of ESC isolates (%)	9 (1%)	6 (2%)	6 (13%)	59 (52%)
Number of samples	697	307	45	113

Number (%) positive samples are isolates recovered by selective enrichment methods for specific monitoring of beta-lactamase-producing *E. coli*. ESBL/AmpC enzymes were determined by whole genome sequencing of the recovered isolates (Chapter 10, Section 10.6). Not available refers to isolates without available WGS results. * Two of the 25 isolates from turkey meat with more than one ESBL enzyme had three different enzymes

7.4 Indicator Enterococci

Enterococci were obtained from 331 (99%) of the 336 faecal samples taken from broiler flocks at slaughter, and antimicrobial susceptibility testing was performed on 28 *E. faecalis* and 305 *E. faecium* isolates.

7.4.1 *E. faecalis* and *E. faecium* from broilers

Overall, 39% of the *E. faecalis* and 52% of the *E. faecium* isolates from broilers were susceptible to all antimicrobials in the test panel (Table 7.4).

As in 2020, the previous year of monitoring in broilers, no enterococci isolates showed resistance to ampicillin, linezolid, gentamicin, teicoplanin, tigecycline or vancomycin. Additionally, in 2022, no isolates showed resistance to chloramphenicol or daptomycin. Resistance to tetracycline and erythromycin continued to be the most common.

Among *E. faecalis* isolates, compared to 2020, the occurrence of erythromycin resistance increased from 38% to 43%, while the occurrence of tetracycline resistance decreased from 62% to 50%. Resistance of *E. faecium* isolates to ciprofloxacin, erythromycin and tetracycline was at the same level as in 2020 (Table 7.4 and Figure 7.4).

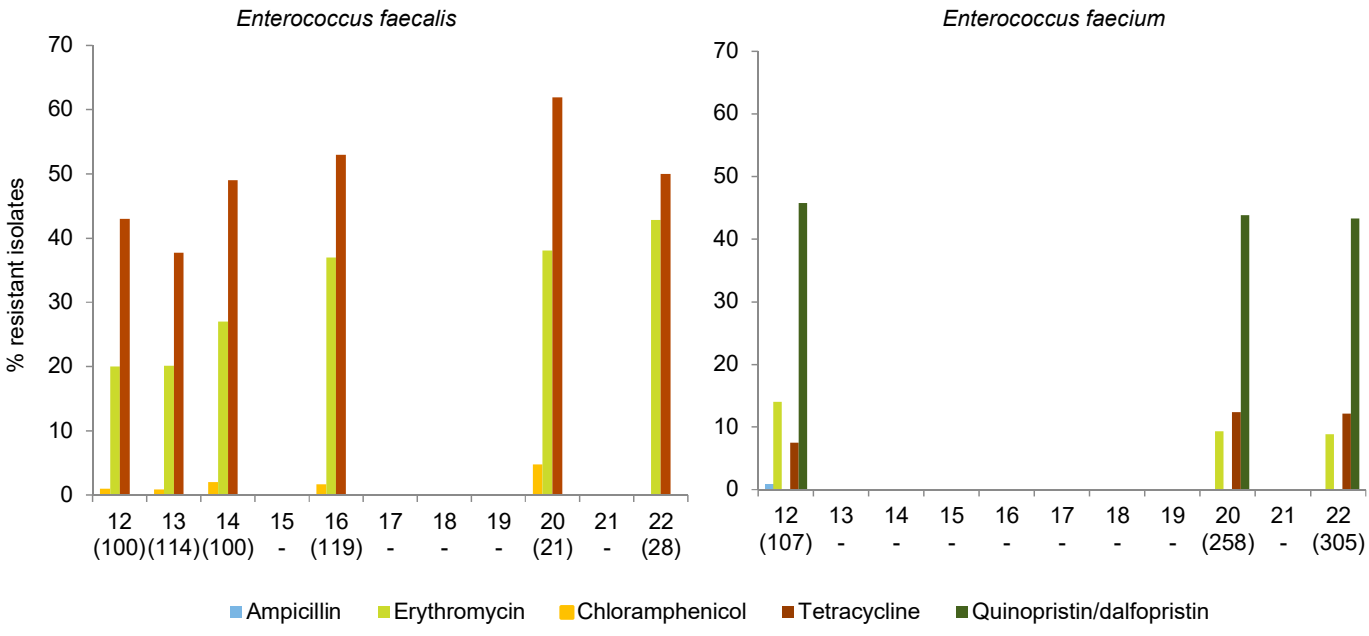
Among the resistant *E. faecalis* (N=17), three different resistance profiles were observed. Combined resistance to tetracycline and erythromycin was the most common (9 isolates) however, isolates resistant to only tetracycline (N=5) or erythromycin (N=3) were also observed. Among the resistant *E. faecium* isolates (N=147) four different resistance profiles were observed, with 102 isolates showing resistance to a single antimicrobial, 33 showing resistance to two substances, and 12 showing multidrug-resistance. The most common profile was resistance to quinopristin-dalfopristin (N=92), followed by combined resistance to quinopristin-dalfopristin and tetracycline (N=16), and by multidrug-resistance to tetracycline, erythromycin and quinopristin/dalfopristin (N=11). One *E. faecium* isolate was resistant to all four antimicrobials.

Table 7.4 Resistance (%) in Enterococci isolates from broilers, Denmark DANMAP 2022

Antimicrobial agent	*Enterococcus faecalis* %	*Enterococcus faecium* %
Ampicillin	0	0
Chloramphenicol	0	0
Ciprofloxacin	0	3
Daptomycin	0	0
Erythromycin	43	9
Gentamicin	0	0
Linezolid	0	0
Quinupristin/dalfopristin	-	43
Teicoplanin	0	0
Tetracycline	50	12
Tigecycline	0	0
Vancomycin	0	0
Fully sensitive (%)	39	52
Number of isolates	28	305

E. faecalis are assumed inherently resistant to streptogramins (Quinupristin/dalfopristin)

Figure 7.4 Resistance (%) among Enterococci isolates from broilers, Denmark 2012-2022 DANMAP 2022



Number of isolates included each year is presented in parentheses

7.4.2 Perspectives

Enterococci are commensal gut bacteria in both animals and humans, and can occasionally cause human disease. In Denmark, the majority of human infections are caused by *E. faecalis* and *E. faecium*, and a 5.8% increase in invasive infections caused by these two species was observed between 2013 and 2022 [Chapter 8, Section 8.2.5].

In 2022, *E. faecalis* and *E. faecium* isolates recovered from broilers exhibited no resistance to ampicillin, gentamicin or vancomycin, antimicrobials often used to treat complicated infections in humans caused by enterococci. Furthermore, isolates showed no resistance to linezolid or daptomycin, which are used to treat multidrug-resistant vancomycin-resistant enterococci infections (Table 7.4).

Monitoring of enterococci in animals has not been constant in DANMAP. Nevertheless, compared to previous monitoring years, occurrence of resistance in *E. faecium* in broilers seems relatively constant, while resistance in *E. faecalis* has increased since 2012 (Figure 7.4).

Compared to *E. faecalis*, *E. faecium* recovered from broilers exhibited lower occurrence of resistance. Among enterococci recovered from humans, the occurrence of resistance is generally higher in *E. faecium* than in *E. faecalis* [Chapter 8, Section 8.2.5].

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Textbox 7.1

Antimicrobial resistance trends in indicator *E. coli* in Danish pigs, broilers, and cattle 2014 to 2022

Background

Since 2014, the monitoring of antimicrobial resistance (AMR) in indicator *E. coli* isolated from food-producing animals (pigs, broilers, and cattle) has been mandatory by EU legislation.

Although surveillance is essential to follow antimicrobial resistance trends over time and to determine the effects of interventions such as reductions in antimicrobial use, the evaluation and optimization of AMR trend analysis are still needed [1]. To better understand the data collected through DANMAP on indicator *E. coli*, we investigated temporal trends and points in time when these trends show significant changes.

Materials and Methods

We aggregated data collected through DANMAP on monthly phenotypic resistance of indicator *E. coli* for the period 2014 – 2022 by food-producing animal species (broilers, pigs, and cattle). Each isolate was investigated for full sensitivity and for multidrug-resistance (as defined in DANMAP). We analysed the monthly variation in the proportion of fully sensitive and multidrug-resistant isolates for broilers, pigs, and cattle separately. Using linear weighted moving averages, we imputed missing values to ensure a continuous time series. After, we performed a seasonal trend decomposition using the locally weighted regression (LOESS) method to separate the time series into its trend, seasonal, and irregular components. Finally, we did a change-point analysis using the pruned exact linear time (PELT) method to detect any point in time at which a statistically significant change in the proportion of fully sensitive or multidrug-resistant indicator *E. coli* occurred.

Results and discussion

Data on antimicrobial susceptibility testing collected between 2014 and 2022 were available for 1,411, 1,552, and 1,232 indicator *E. coli* isolates from broilers, pigs, and cattle, respectively. The estimated monthly proportion of fully sensitive and multidrug-resistant isolates for each food-producing animal species is shown in Figures 1-3. Results show that the proportion of fully sensitive indicator *E. coli* is highest on average within the cattle population, whereas pigs show the lowest proportion. The trend lines (Figures 1–3) show a visual increase in the proportion of fully sensitive indicator *E. coli* isolates in broilers and pigs starting in the first quarter of 2016 and in the second quarter of the same year, respectively. This increase in *E. coli* from pigs can be associated with the implementation, in 2016, of the new Yellow Card for pigs with a focus on reducing the use of critically important antimicrobials. In contrast, a decreasing trend can be observed for cattle from July 2020. This coincided with an increase in the use of b-lactamase sensitive penicillins and amphenicols observed in calves in 2020 [2]. Variations in the proportion of fully sensitive isolates are accompanied by an inverse parallel trend in the proportion of multidrug-resistance for broilers. For pigs and cattle, these combined trend fluctuations are not so clear. Note however that proportions of isolates with resistance to only one or two substances were not considered.

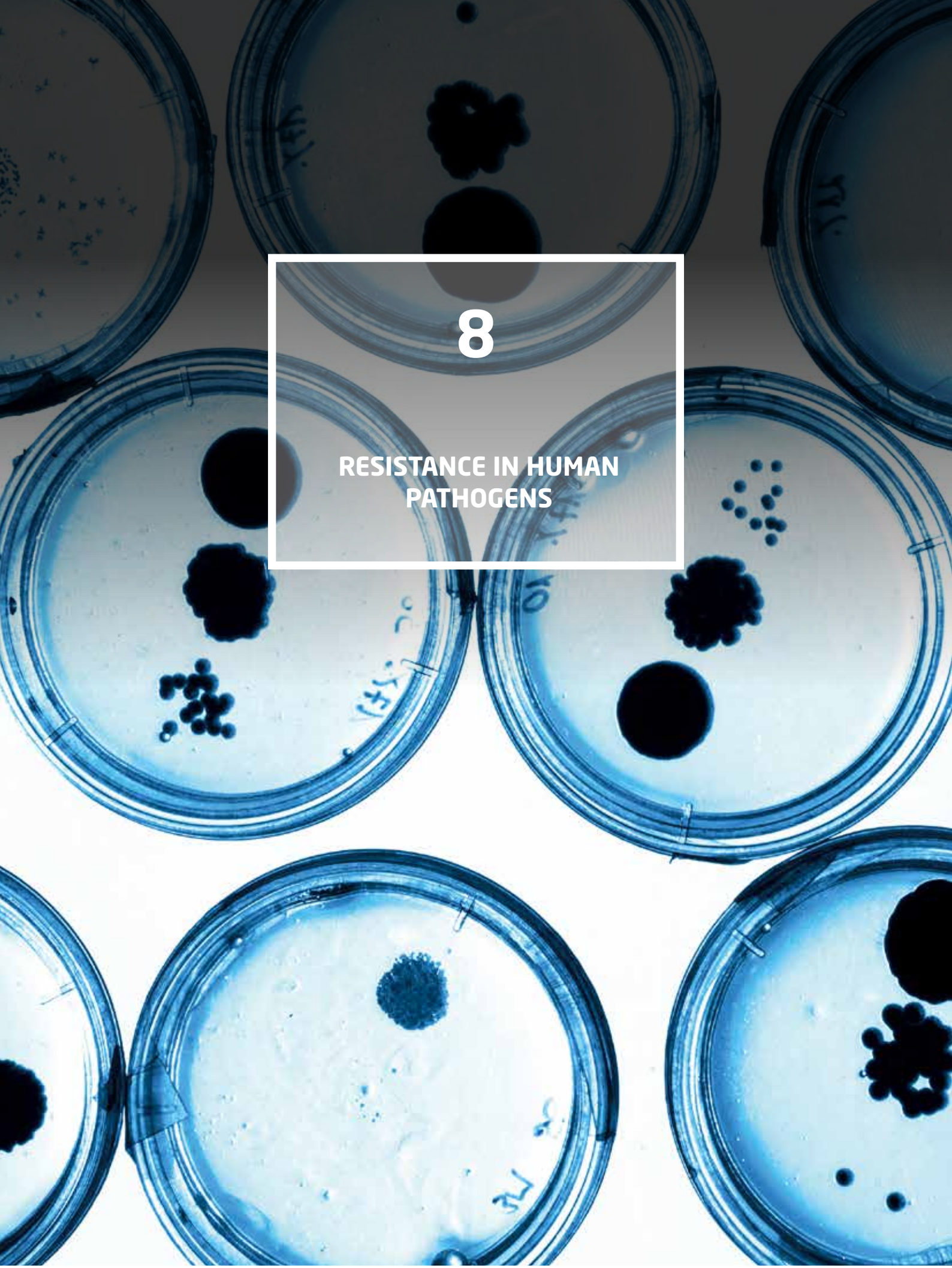
Although we can visually detect these fluctuations in the proportion of fully sensitive and multidrug-resistant indicator *E. coli*, our results show no statistically significant changes over the time period studied. Still, in the future, these data can be explored with different aggregation levels, namely by antimicrobial class, and using a larger time period, which can possibly show different results. Furthermore, a multivariate time series analysis can be implemented where the associations between both phenotypic and genotypic AMR and AMU can be explored.

continued ... Textbox 7.1

Figure 1 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from broilers (n = 1,411), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022

Figure 2 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from pigs (n = 1,552), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022

Figure 3 Monthly proportion of fully sensitive and multidrug-resistant indicator *E. coli* isolated from cattle (n = 1,232), Denmark 2014-2022. The grey lines identify the time series trend calculated using the locally weighted regression (LOESS) method DANMAP 2022



8

RESISTANCE IN HUMAN PATHOGENS

8. Resistance in human pathogens

Highlights

Invasive bacterial infections. The number of invasive infections reached a plateau at 12,373 for the monitored species after increasing steadily for the past ten years (8,954 cases in 2013, a 38% increase). The notable exception are invasive infections with *Streptococcus pneumoniae*, showing an overall decrease over the last decade (789 cases in 2013, 538 cases in 2022), and two significant drops in 2020 and 2021 (363 and 333 total cases, respectively). *Escherichia coli* remained the most common cause responsible for about half of all cases, followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Following declining rates of resistance against most antibiotics in almost all monitored bacterial species during the COVID-19 pandemic, resistance rates are now either stable or increasing but generally remain low.

Escherichia coli. After a substantial decline in the number of ESBL- and pAmpC-producing *E. coli* in bloodstream infections from 2020 to 2021 (352 and 254 cases, respectively), the number of cases resurged to 336 in 2022, a 32% increase compared to the previous year. CTX-M-15 remained the most common ESBL enzyme present in 52% of cases. Resistance rates to gentamicin were declining over the decade (6.5% in 2013, 4.5% in 2022), and resistance to carbapenems remained below 1%.

Klebsiella pneumoniae. Specific note should be taken for piperacillin-tazobactam resistance rates in *K. pneumoniae* as these have been increasing over the past ten years, nearing 10% in both invasive infections (6.2% in 2013, 9.2% in 2022) and urinary tract infections (7.2% in 2013 and 9.9% in 2022) in hospitalized patients.

Combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin remained low in both *E. coli* and *K. pneumoniae* from invasive infections (1.3% and 1.0%, respectively).

Carbapenemase-Producing Organisms/Enterobacterales (CPO/CPE). The number of CPO continued to increase throughout 2022 with 355 affected patients compared to 242 in 2021. This is, in part, due to an influx of patients from Ukraine, but also due to an outbreak caused by a contaminated pharmaceutical product. The number of outbreaks at Danish hospitals remained stable, but for some of the known outbreaks the number of cases continued to grow.

Enterococci. While there has been a decrease in the number of invasive infections with enterococci, the number of vancomycin-resistant enterococci (VRE) continues to increase. In 2022, 845 VRE/vancomycin variable enterococci (VVE) were identified compared to 742 in 2021. This calls for intensified efforts in infection prevention and control.

Staphylococcus aureus. Following a decrease in the number of methicillin-resistant *S. aureus* (MRSA) cases during the COVID-19 pandemic, the number increased by 10% to 2,996 cases from 2021 to 2022. MRSA-screening accounted for 49% of cases, whereas infections accounted for 51%. Thirty-nine MRSA outbreaks were registered at hospitals, nursing homes and other institutions for a total of 143 cases, of which 70 were infections. Fifty bacteraemias were caused by MRSA with seven being livestock-associated MRSA.

8.1 Introduction

The Danish national surveillance programme of antimicrobial resistance in human clinical bacteria collects data on routine diagnostics from all of Denmark. It is an active catchment system collecting results from all clinical and screening samples from patients. Data coverage is high; microbiology data from all hospitals and the majority of general practitioners feed into the system, hereby covering a close to complete proportion of microbiological analyses performed in Denmark.

In addition, the programme includes phenotypic resistance and typing results from the reference laboratories at SSI. Isolates are submitted to SSI either as part of the programme on mandatory notifiable diseases or based on voluntary submission of species carrying resistance mechanisms of concern (Table 8.1).

Table 8.1 Summary of species/types, sampling and sources of isolates for national resistance surveillance in humans, Denmark, 2022 DANMAP 2022

Routine diagnostics from all 10 DCMs in Denmark. All data are directly identified and extracted in MiBa	
Species	**Inclusion criteria**
Escherichia coli *Klebsiella pneumoniae*	First isolate per patient per year from blood, cerebrospinal fluid or urine (hospitals and primary health care)
Pseudomonas aeruginosa *Acinetobacter* species *Enterococcus faecalis* *Enterococcus faecium*	First isolate per patient per year from blood or cerebrospinal fluid
Voluntary submissions of isolates to the reference laboratories at SSI	
Species or type	**Inclusion criteria**
Staphylococcus aureus Beta-haemolytic streptococci	One isolate per patient per episode from blood or cerebrospinal fluid
Neisseria gonorrhoeae	One isolate per patient per episode from any sample site
3rd generation cephalosporin-resistant *Escherichia coli*	First isolate per patient within 12 months from blood
Vancomycin-resistant enterococci	First isolate per patient within 12 months from any sample site (excluding screening samples)
Enterococci with exceptional phenotype (e.g. linezolid, daptomycin and tigecycline R)	First isolate per patient within 12 months from any sample site (clinical and screening samples)
All bacterial species with other exceptional phenotypes (e.g. acquired colistin resistance)	One isolate per patient per episode from any sample site
Mandatory submissions of isolates to the reference laboratories at SSI	
Species or type	**Inclusion criteria**
Carbapenemase-producing organisms	First isolate per patient within 12 months from any sample site (clinical and screening samples)
Methicillin-resistant *Staphylococcus aureus*	First isolate from all new cases of MRSA postive patients from any sample site (clinical and screening samples)
Streptococcus pneumoniae	One isolate per patient per episode from blood or cerebrospinal fluid
Haemophilus influenzae serotype b, Hib	All invasive isolates

Regarding submissions of isolates to the reference laboratories often several isolates per patient are received, but for the statistics each patient is counted only once

8.1.1 Surveillance based on data from the Danish Microbiology Database (MiBa)

An important part of the national surveillance programme consists of systematically extracted data from a register (EpiMiBa) linking to the Danish Microbiology Database (MiBa). MiBa was established in 2010 with the aim to build a cross-national database that included and made available all microbiology analyses performed by the individual DCMs. MiBa thus simultaneously delivers real time patient data to the DCMs for clinical use and data to SSI for national surveillance. Until the establishment of MiBa, surveillance was based on voluntary manual reporting from the individual DCMs, beginning with data from just two DCMs in 1995, but quickly expanding to include more than half of the DCMs. Since 2015, all DCMs have contributed to the program resulting in a 100% coverage of hospitalised patients and – since the DCMs perform microbiology analyses for GPs and private specialists as well – also a very high coverage of results from the primary sector.

A description of MiBa and the usage and validation of data from the associated database EpiMiBa can be found on the following homepage [https://miba.ssi.dk/Service/English.aspx] and in DANMAP 2018, Textbox 8.1.

The MiBa-based surveillance includes all invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis,*

Enterococcus faecium, *Pseudomonas aeruginosa* and *Acinetobacter* species and results from urine isolates of *E. coli* and *K. pneumoniae*. For inclusion, the following case definition applies: The first sample of each given bacterial species per individual patient per year, by date of sample collection, an approach identical to the surveillance performed by the European Antimicrobial Resistance Surveillance Network (EARS-Net). All duplicates, patient-related or sample-related within the year of observation, are excluded.

Materials and methods are further described in paragraph 10.10 in Chapter 10.

8.1.2 Surveillance based on data from the reference laboratories

Antimicrobial resistance is also monitored in isolates submitted to the reference laboratories at SSI.

Submission of isolates began in 1957 with the voluntary referral of *S. aureus* from bloodstream infections. Still today, many isolates are sent as part of voluntary programmes; however, the majority of isolates are referred as part of surveillance programmes for notifiable diseases.

The mandatory programme includes invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* serotype b (Hib), methicillin-resistant *S. aureus* (MRSA) and carbapenemase-producing organisms (CPO) from clinical and screening samples, and *Neisseria gonorrhoeae* from all clinical samples (Table 8.1).

The voluntary programme includes day-to-day referral of vancomycin-resistant enterococci and monthly or periodically reporting of ESBL-positive invasive *E. coli,* invasive beta-haemolytic streptococci and invasive *S. aureus*.

In addition, the reference laboratory for antibiotic resistance at SSI receives highly resistant strains or strains carrying atypical antimicrobial resistance mechanisms of specific concern irrespective of bacterial species.

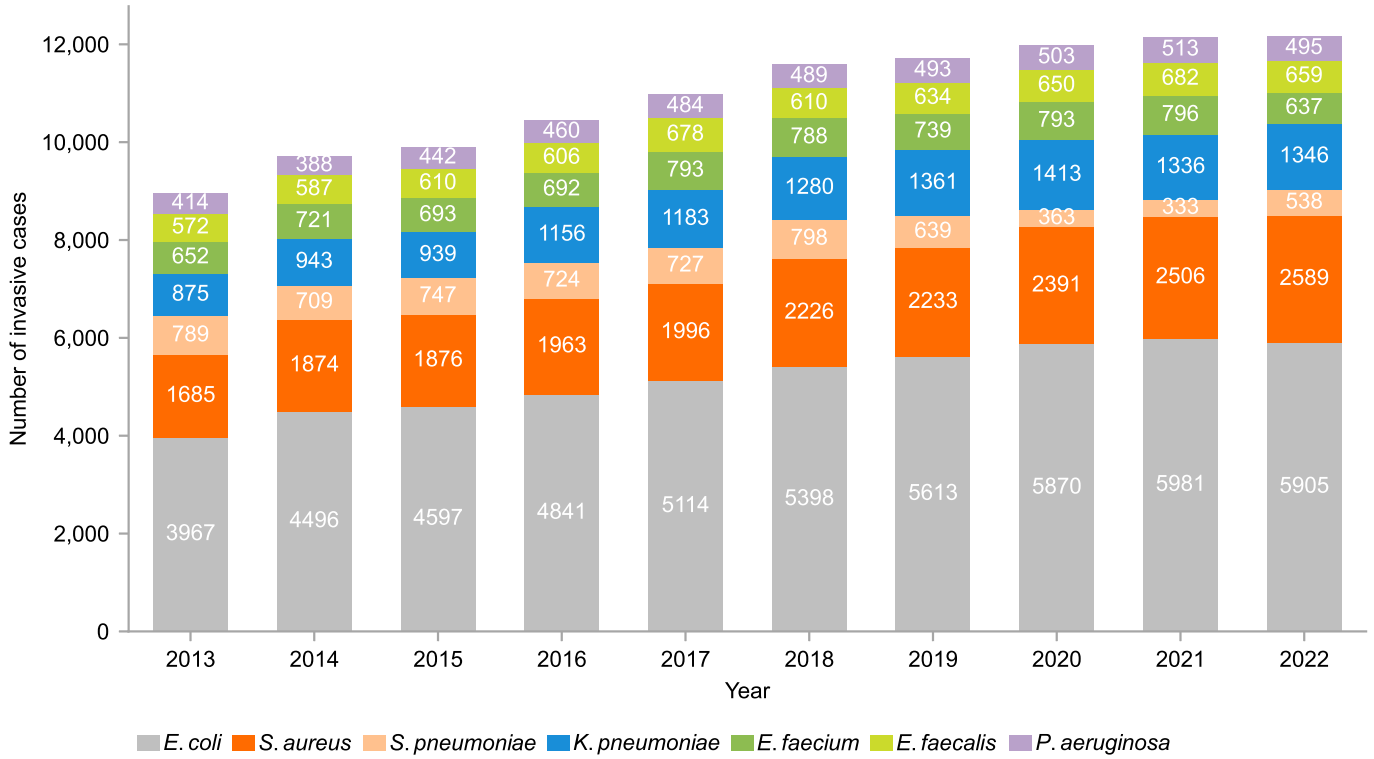
Since 2015/2016, characterisation of antimicrobial resistance mechanisms in bacteria has been performed primarily by whole genome sequencing (WGS).

8.1.3 Number of invasive cases

The total numbers of invasive cases in Denmark bacterial species included in the surveillance programmes for both DANMAP and EARS-Net from 2013 to 2022 are presented in Figure 8.1. Invasive cases caused by *Acinetobacter* species are not shown in the figure due to the small numbers of isolates (ranging from 55 to 97 cases/year between 2013 and 2022).

Between 2013 and 2022, the number of registered individual invasive cases increased by 38% from 8,954 to 12,372 cases in Denmark: *E. coli* 3,967 to 5,905 cases (49% increase), *S. aureus* 1,685 to 2,589 cases (54%) and *K. pneumoniae* 875 to 1,346 cases (54%).

Figure 8.1 Number of invasive cases for bacterial species under surveillance, Denmark, 2013-2022 DANMAP 2022



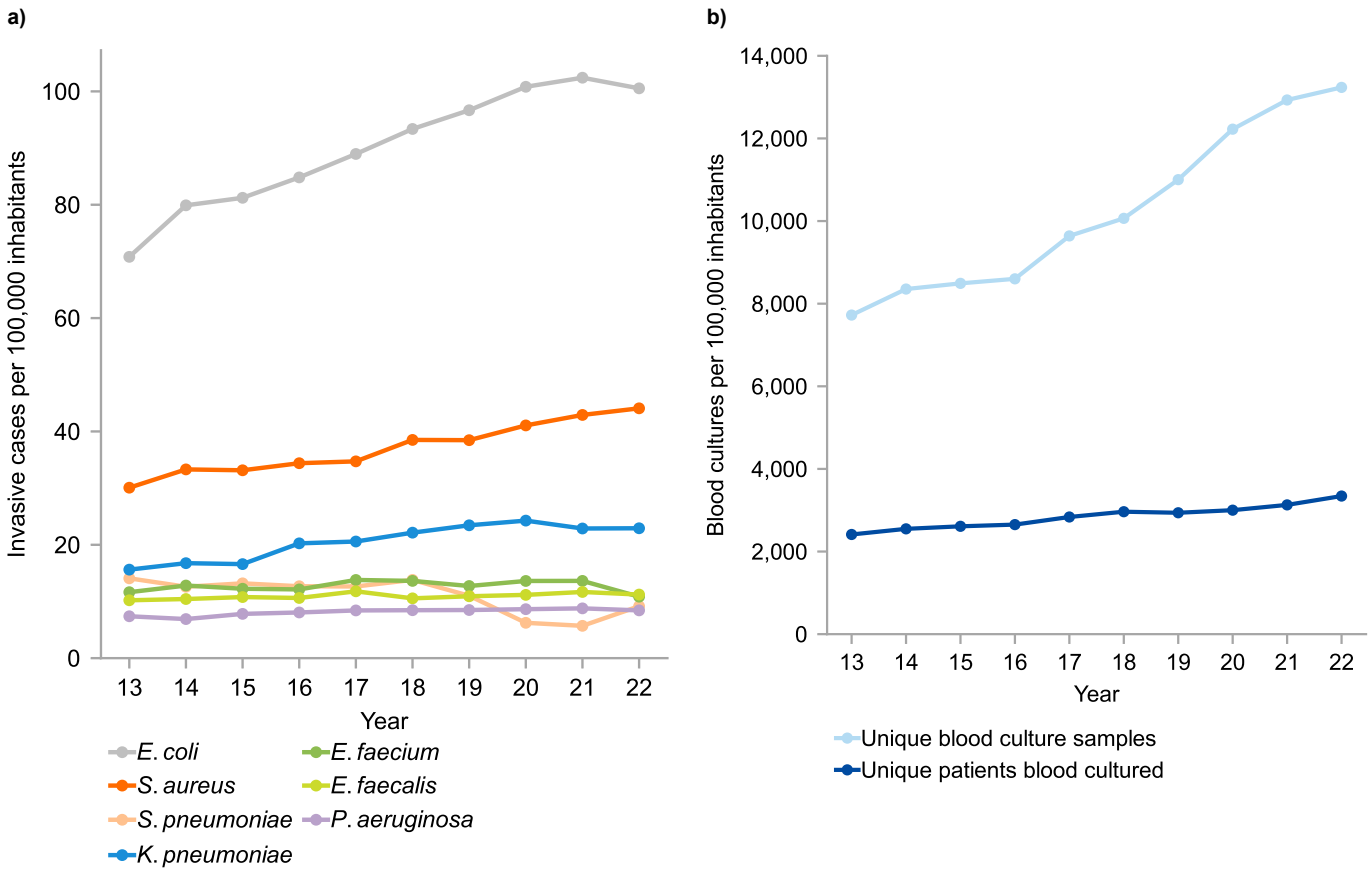
The only species with an overall decreasing number of cases during the past 10 years was *S. pneumoniae*. During the pandemic years 2020 and 2021 an unusual low number of annual invasive cases with *S. pneumoniae* was observed *(363 and 333 cases, respectively)*, but has since increased and the number of cases in 2022 was 538, a 62% increase compared to 2021. This is likely due to a normalization of society post-COVID-19.

Figure 8.2a shows the incidence of invasive cases per 100,000 inhabitants in Denmark per year from 2013 to 2022. During this period, the Danish population increased by 4.8% (from 5,602,628 inhabitants in 2013 to 5,873,420 inhabitants in 2022). For comparison, Figure 8.2b shows the total number of blood cultures taken per 100,000 inhabitants per year for the same period. Additionally, the number of individual patients with minimum one blood culture taken per 100,000 inhabitants per year is shown. In the ten-year period the number of individual patients with at least one blood culture taken per year increased from 2,413 patients per 100,000 inhabitants in 2013 to 3,343 patients per 100,000 inhabitants in 2022 (an increase of 39%). The total number of blood cultures taken per 100,000 inhabitants increased even more (71%). Thus, on average a higher number of patients have an even higher number of blood cultures taken each year.

Changes in hospital workflow (reduced number of bed-days), healthcare-related infections, improved diagnostics and increased numbers of elderly at risk of infections have probably affected the increased number of total infections.

The following sections present results of resistance monitoring in Denmark for the individual bacterial species and/or resistance types under surveillance.

Figure 8.2 a) Invasive cases and b) blood cultures taken per 100,000 inhabitants, Denmark, 2013-2022 DANMAP 2022



8.2 Results from MiBa data surveillance

8.2.1 *Escherichia coli*

Escherichia coli is by far the most frequent cause of community and hospital acquired bacteraemia and urinary tract infections (UTI) in Denmark. The pathogen is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferable resistance mechanisms are frequently seen in these gut bacteria as well as resistance conferred by mutations. Since *E. coli* is the most frequent cause of UTI and bacteraemia, it is also responsible for a large proportion of the antimicrobials used to treat these conditions.

The percentages of resistance in invasive *E. coli* isolates for each key antimicrobial are presented in Table 8.2, as well as in urine samples from hospitals and primary health care (see details in later paragraphs). Figures 8.3a and 8.3b show the total annual number of invasive isolates and proportion of resistant isolates, respectively, between 2013 and 2022. The percentages of isolates resistant to key antimicrobials are presented in Figure 8.3c.

Invasive cases from hospital patients

In 2022, a total of 5,905 individual patients with invasive *E. coli* isolates were identified in MiBa. Antimicrobial susceptibility test results for key antimicrobials were also extracted for these isolates (See Chapter 10 'Material and methods').

As mentioned in the introduction, an increase in the number of invasive *E. coli* cases was observed over the past decade corresponding to 70.8 cases and 100.5 cases per 100,000 inhabitants respectively (a 42% increase, data not shown).

In 2022, 9.9% of invasive *E. coli* isolates were resistant to cefuroxime. This level has been stable (range 8.6-10%) over the last decade. A minor EUCAST breakpoint change in 2017 influenced data from 2017 compared to data from 2016. For 3rd generation cephalosporins, the percentage of resistant isolates was 6.2% with an increase compared to 2021.

The ciprofloxacin resistance for invasive *E. coli* was 10.8% in 2022, and ranged from 10.3% to 13% in the period 2013-2022. The increased ciprofloxacin resistance rate in 2017 compared to 2016 mainly reflected a relatively large EUCAST breakpoints change in 2017. Ciprofloxacin breakpoints were changed (lowered) once again combined with the introduction of the Area of Technical Uncertainty (ATU) in the EUCAST clinical breakpoint table v. 9.0 in 2019. Therefore, trends for ciprofloxacin resistance should be interpreted with caution.

Piperacillin-tazobactam resistance has gradually increased from 3.8% of invasive *E. coli* isolates being reported as resistant in 2018 to 6.3% in 2022. Gentamicin resistance was 4.5% in 2022 and resistance rates have declined over the last decade, however it increased from 2021 to 2022 (Figure 8.3b and 8.3c). The sharp increase for resistance towards amoxicillin-clavulanic acid can be attributed to removal of data from 2021 to 2022: a major DCM did not meet the inclusion criteria of having tested at least 75% of the isolates, which is clearly mirrored in the number of isolates tested (2021: 4226 isolates tested, 2022: 3076 isolates tested).

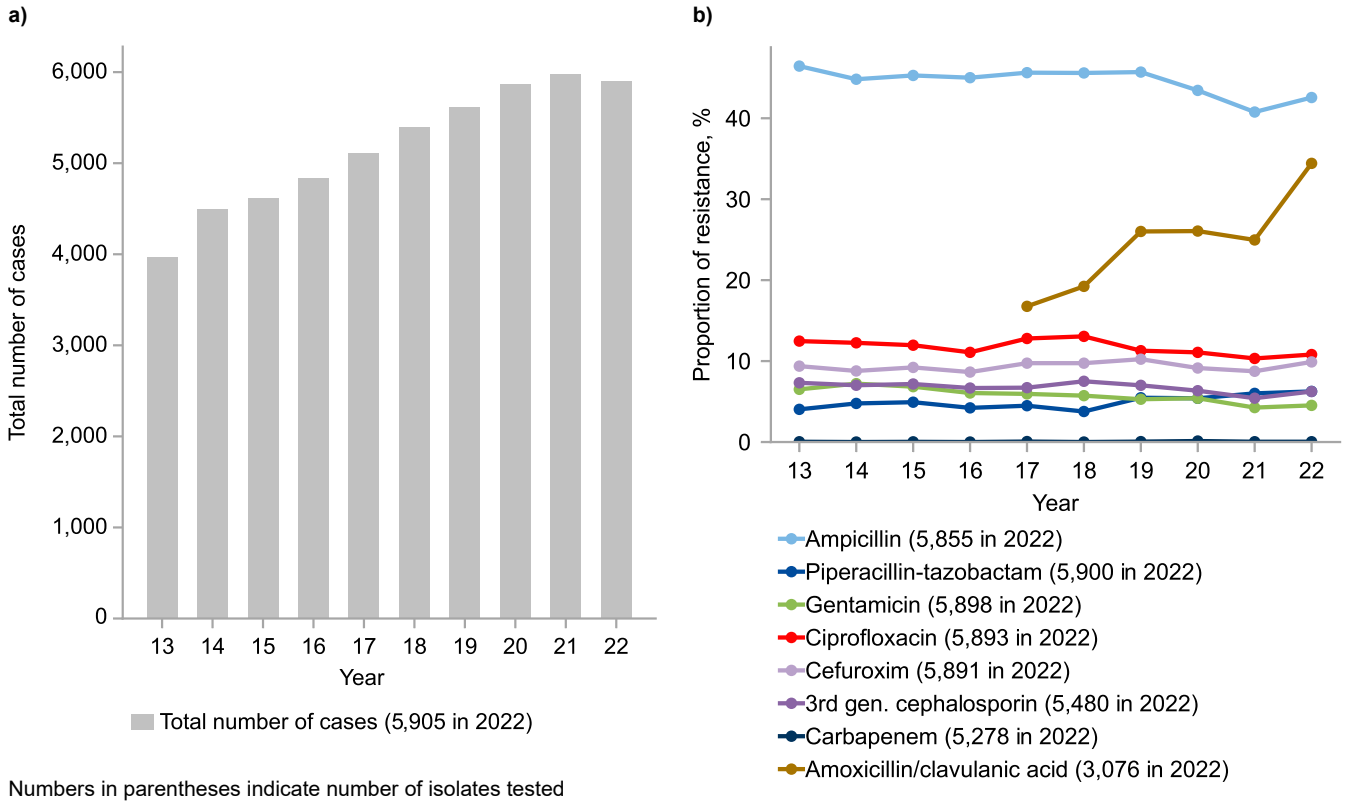
The number of carbapenem-resistant invasive *E. coli* isolates remained low in 2022 with two isolates categorised carbapenem-resistant and six isolates categorised "susceptible, increased exposure" (as per new EUCAST definition of previous intermediate category applicable from January 2019 [www.eucast.org/newsiandr]). The occurrence of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained low at around 1.3% (Table 8.3).

Table 8.2 *Escherichia coli*. Resistance (%) in isolates from humans, Denmark, 2022 DANMAP 2022

	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	43	40	35
Mecillinam	8.8	6.8	4.3
Piperacillin/tazobactam	6.3	5.4	3.7 (2)
Amoxicillin/clavulanic acid	34.4 (6)	12	6.6 (6)
Sulfonamide		28 (5)	25
Trimethoprim		21	20
Nitrofuratoin		1.0 (5)	0.8
Gentamicin	4.5	4.2	3.2 (3)
Ciprofloxacin	11	9.3	6.9
Cefuroxime	9.9	7.5	5.3 (4)
3rd generation cephalosporins	6.2	6.2	4.8
Carbapenem	0.0	0.0	0.0 (2)
Max. number of isolates tested for resistance to the presented antibiotics	5,900	48,469	104,345

Included are results from all DCMs where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parentheses indicate the number of DCMs included if six or less

Figure 8.3 Invasive *Escherichia coli* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

c)

Substance	Percent resistant invasive *E. coli* isolates									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	46.4	44.8	45.3	45	45.6	45.5	45.7	43.4	40.7	42.6
Piperacillin-tazobactam	4	4.8	4.9	4.2	4.5	3.8	5.5	5.4	6.0	6.3
Gentamicin	6.5	7.2	6.8	6.1	6	5.7	5.3	5.4	4.3	4.5
Ciprofloxacin	12.5	12.3	12	11.1	12.8	13	11.3	11.1	10.3	10.8
Cefuroxime	9.4	8.8	9.2	8.6	9.7	9.8	10.2	9.2	8.7	9.9
3rd gen.cephalosporins	7.7	7	7.2	6.7	6.7	7.3	6.9	6.2	5.4	6.2
Carbapenem	0	0	0	0	0.1	0	0.1	0.1	0.0	0.0
Total number of isolates	3,967	4,492	4,618	4,841	5,114	5,398	5,613	5,870	5,981	5,905

Table 8.3 *Escherichia coli*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2022 DANMAP 2022

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)
Resistance	2.3 (93)	1.8 (87)	1.8 (88)	2.0 (100)	1.8 (93)	1.5 (82)	1.1 (60)	1.3 (70)
Percentage (no.) of isolates tested for combined resistance (multidrug-resistance)	88 (4,071)	98 (4,763)	95 (4,883)	93 (4,997)	94 (5,259)	93 (5,470)	93 (5,564)	93 (5,474)
Total number of invasive isolates	4,614	4,841	5,114	5,398	5,613	5,870	5,981	5,905

Urinary cases from hospitals

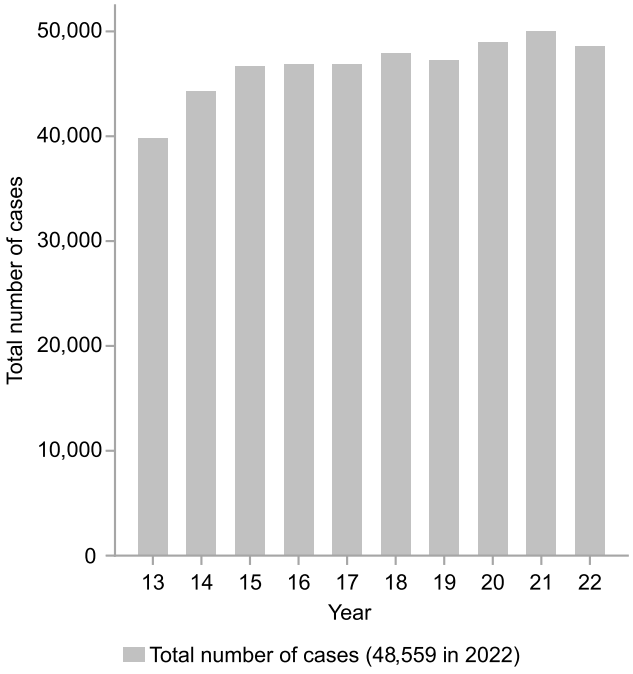
In 2022, 48,559 individual hospital patients had *E. coli* isolated from urine samples, a 22% increase and 2.9% decrease compared to 2013 and 2021, respectively.

Interpretation results from antimicrobial susceptibility test (AST) data on *E. coli* urine isolates were available from all DCMs for ampicillin, mecillinam and ciprofloxacin. In addition, susceptibility test results were reported for: piperacillin-tazobactam (nine DCMs), gentamicin (nine DCMs), cefuroxime (eight DCMs), trimethoprim (eight DCMs), 3rd generation cephalosporins (eight DCMs), amoxicillin/clavulanic acid (eight DCMs), carbapenem (six DCMs), nitrofurantoin (five DCMs) and sulphonamide (four DCMs).

Summarized antimicrobial susceptibility results for *E. coli* isolates (invasive and urine specimen) are shown in Table 8.2. In Figure 8.4, resistance for *E. coli* urine isolates from hospitalised patients are shown for 2013-2022.

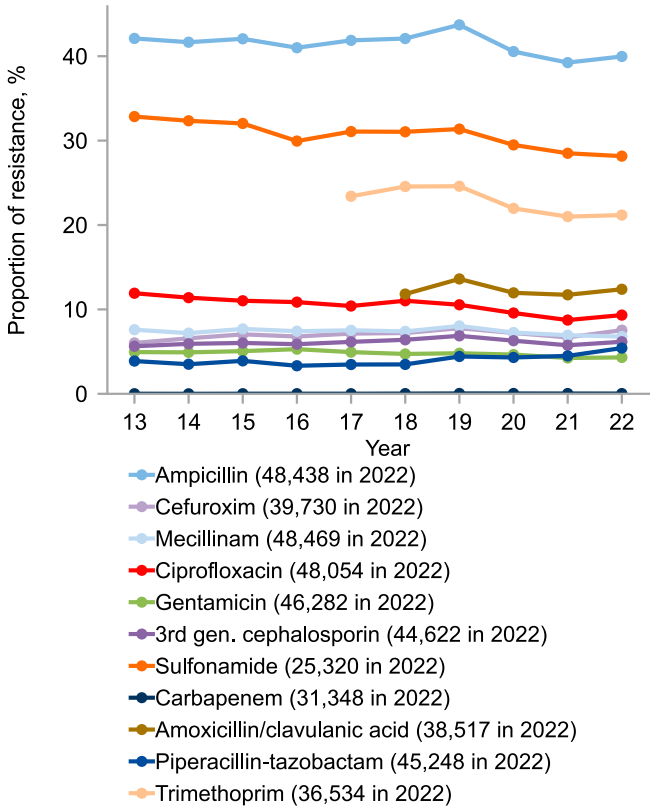
Figure 8.4 Urine *Escherichia coli* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Percent resistant *E. coli* isolates from hospital urine									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	42.1	41.7	42.1	41	41.9	42.1	43.7	40.6	39.2	40.0
Mecillinam	7.6	7.2	7.7	7.4	7.5	7.4	8.1	7.3	6.9	6.8
Piperacillin-tazobactam	3.9	3.5	3.9	3.3	3.7	3.5	4.4	4.3	4.5	5.4
Sulfonamide	32.8*	32.3*	32	34.9	31.1	31*	31.4*	29.4*	28.5*	28.2*
Gentamicin	4.9	4.9	5.1	5.3	4.9	4.7	4.6	4.6	4.2	4.3
Ciprofloxacin	11.9	11.4	11	10.9	10.4	11	10.6	9.6	8.7	9.3
Cefuroxime	6	6.6	7	6.8	7.1	7.2	7.8	7.2	6.7	7.5
3rd gen. cephalosporins	5.7	5.9	5.9	5.9	6.2	6.4	6.9	6.3	5.8	6.2
Carbapenem		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total number of isolates	39,820	44,288	46,723	46,865	46,884	47,914	47,235	48,962	49,986	48,559

* Indicates less than 6 DCMs reported rutine susceptibility testing

As can be seen in Figure 8.4c, a decrease in ciprofloxacin and gentamicin resistance was observed for the past five and ten years. However, for almost all antibiotics in 2022 rising rates of resistance have been observed as compared to 2021.

In 2022, 21 carbapenem-resistant and 21 carbapenem-"susceptible, increased exposure" *E. coli* urine isolates from hospitalised patients were reported, compared to 20 and 10 isolates in 2021, respectively.

Urinary cases from primary health care

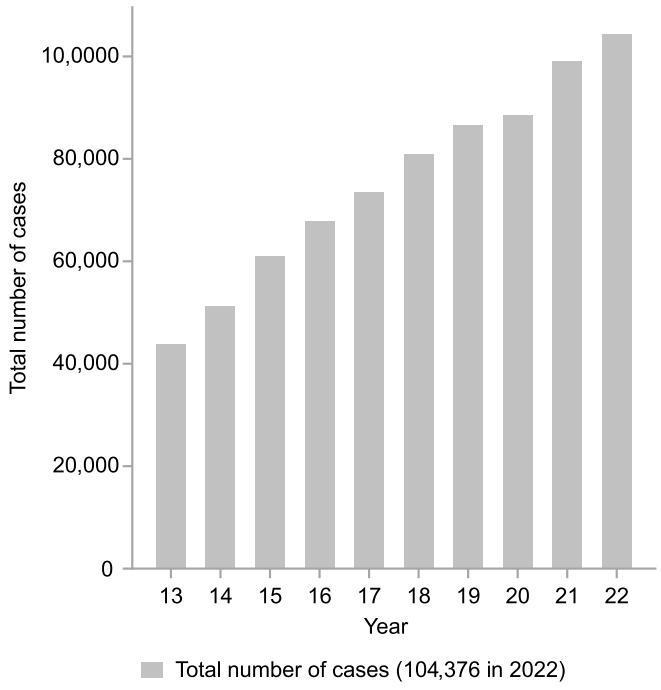
In 2022, 104,376 unique patients in primary health care had *E. coli* isolated from urine samples, a 139% and 5.3% increase compared to 2013 and 2021, respectively.

Susceptibility results for all tested antimicrobials are shown in Table 8.2. In Figure 8.5, resistance for *E. coli* urine isolates from primary health care are shown for the past decade.

As observed with *E. coli* from urinary cases in hospitals, the declining trends in resistance rates observed over the last five years appears to have stagnated or even reversed for most antibiotics and warrants further observation over the coming years.

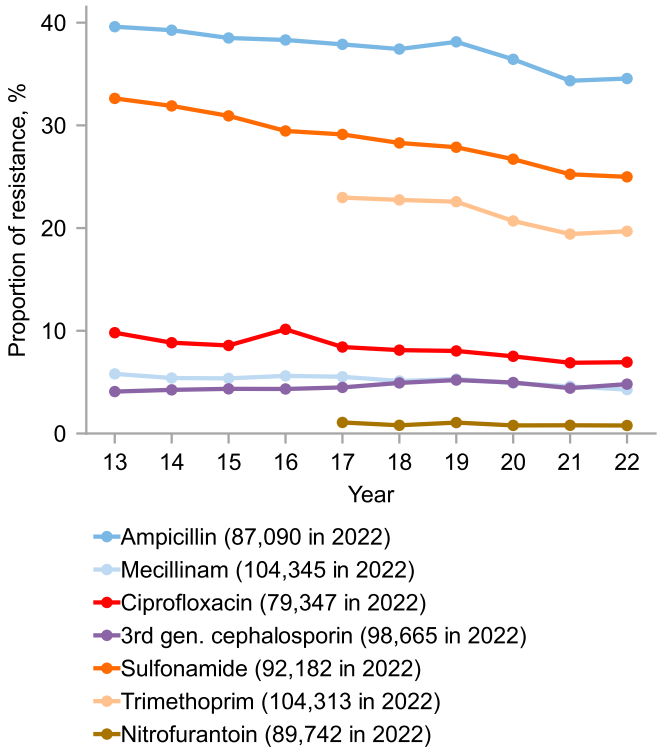
Figure 8.5 Urine *Escherichia coli* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Resistance in *E. coli* urine isolates from primary health care									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Ampicillin	39.6	39.3	38.5	38.3	37.9	37.4	38.1	36.5	34.3	34.6
Mecillinam	5.8	5.2	5.4	5.6	5.5	5.1	5.3	4.9	4.6	4.3
Sulfonamide	32.6	31.9	30.9	29.5	29.1	28.3	27.9	26.7	25.2	25.0
Ciprofloxacin	9.8	8.8	8.6	10.1	8.4	8.1	8	7.5	6.9	6.9
3rd gen. cephalosporins	4.1	4.2	4.3	4.3	4.5	4.9	5.2	5	4.4	4.8
Total number of isolates	43,770	51,272	61,083	67,798	73,497	80,851	86,508	88,462	99,077	104,376

Conclusion

A steady increase in the number of invasive and urinary tract infections (from hospital and primary health care) caused by *E. coli* has been observed since 2013. Around 35-43% of *E. coli* urine isolates are ampicillin-resistant and 7-11% are ciprofloxacin-resistant. Proportion of resistance to cefuroxime and 3rd generation cephalosporins for invasive *E. coli* increased slightly from 8.7% to 9.9% and 5.4% to 6.2% from 2021 to 2022, respectively, reversing the trend from the previous year. Resistance to carbapenems in invasive *E. coli* remains low.

8.2.2 *Klebsiella pneumonia*

Klebsiella pneumoniae is part of the human intestinal tract. The bacteria causes urinary tract infections, intra-abdominal infections, bacteraemia and ventilator-associated pneumonia (VAP). *K. pneumoniae* may cause nosocomial outbreaks. *K. pneumoniae* frequently displays plasmid-mediated resistance mechanisms, which are transferrable to other organisms.

The percentage of resistance in invasive *K. pneumoniae* isolates for each key antimicrobial is presented in Table 8.4. Figures 8.6a and 8.6b show total annual numbers of invasive isolates and percentages of resistance in invasive isolates, respectively, between 2013 and 2022. The proportions of isolates resistant to key antimicrobials are presented in Figure 8.6c.

Invasive cases from hospitals

In 2022, a total of 1,346 unique patients were registered in MiBa with invasive *K. pneumoniae* isolates.

As for invasive *E. coli* cases, a continuous increase in the number of invasive *K. pneumoniae* cases was observed over the last decade, from 875 cases in 2013 to 1346 cases in 2022. This corresponds to 15.6 and 22.9 cases per 100,000 inhabitants, respectively (a 46.6% increase).

Resistance in invasive *K. pneumoniae* isolates has decreased over the past 10 years for gentamicin, cefuroxime and 3rd generation cephalosporins, but have noticeably stabilised. However, resistance to ciprofloxacin and piperacillin-tazobactam has increased. For more details, see Figure 8.6b and 8.6c. The increased proportion of ciprofloxacin-resistant isolates in 2017 when compared to 2016 mainly reflects a change in interpretation of S-I-R due to implementation of new EUCAST breakpoints for ciprofloxacin in most Danish DCMs in January 2017. Ciprofloxacin breakpoints changed (lowered) once again in 2019 combined with the introduction of the "Area of Technical Uncertainty" (ATU) in the EUCAST clinical breakpoint table v. 9.0. Therefore, trends for ciprofloxacin resistance should be interpreted with caution. Resistance for piperacillin-tazobactam should also be interpreted with caution. EUCAST breakpoints did not change since 2012 but an ATU for piperacillin-tazobactam was introduced in 2019.

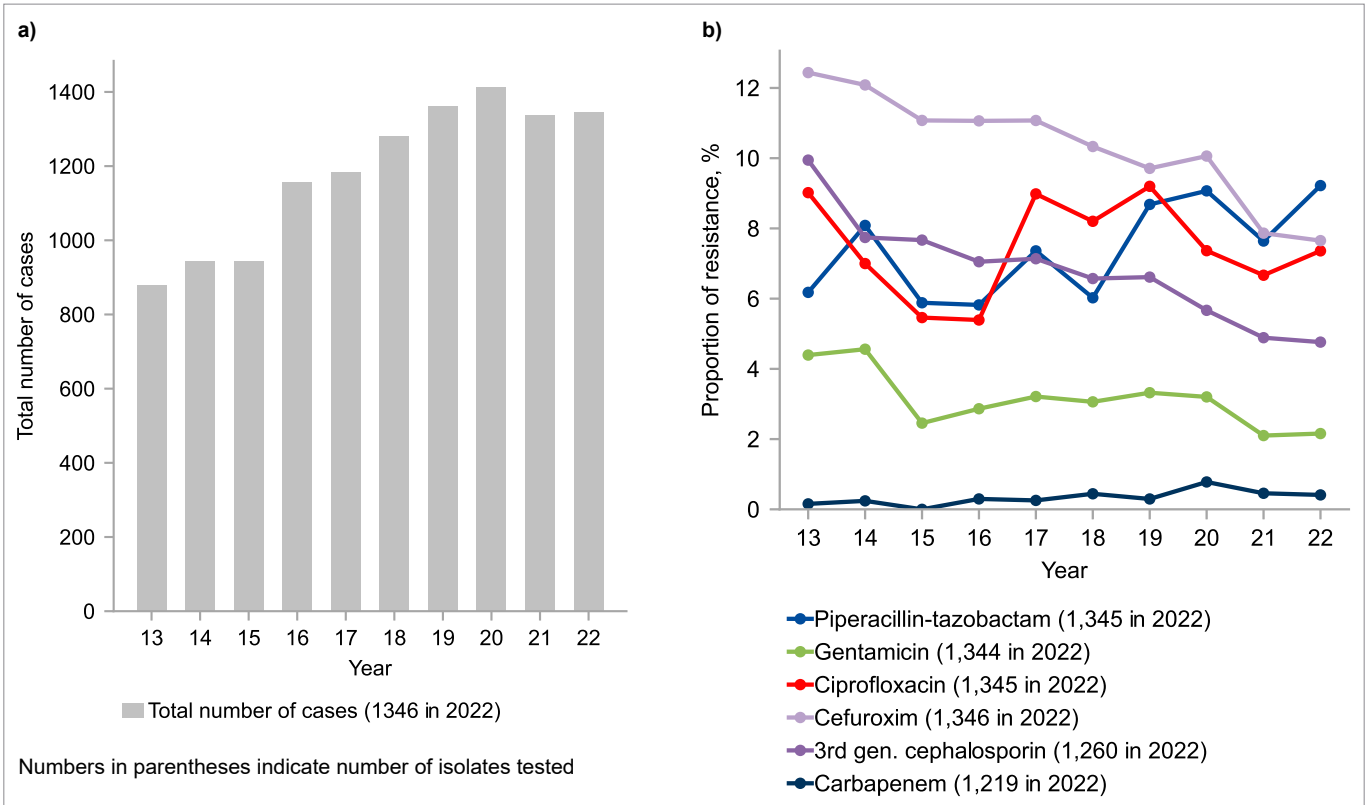
Carbapenem resistance in invasive *K. pneumoniae* is very low (6 resistant isolates out of 1336 tested [0.4%] in 2022). The increase shown in Figure 8.6b and 8.6c in 2020 is mainly due to hospital outbreaks (see Section 8.3.2 Carbapenemase-producing bacteria in Denmark, DANMAP 2020). The level of multidrug-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* was 1.0% in 2022 (Table 8.5). Two invasive *K. pneumoniae* isolates were registered resistant to colistin. Susceptibility to colistin is not routinely tested.

Table 8.4 *Klebsiella pneumoniae*. Resistance (%) in isolates from humans, Denmark, 2022 DANMAP 2022

Substance	Invasive isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Mecillinam	8.7	9.4	7.9
Piperacillin/tazobactam	9.2	9.9	8.1 (1)
Amoxicillin/clavulanic acid	14	8.2	4.4 (5)
Sulfonamide		14.7 (4)	11.9
Trimethoprim		14.5	12.3
Nitrofuratoin		34 (5)	29.7
Gentamicin	2.2	2.3	1.5 (2)
Ciprofloxacin	7.4	7.2	5.1
Cefuroxime	7.7	8.1	5.2 (3)
3rd generation cephalosporins	4.8	4.7	3.4
Carbapenem	0.4	0.4	0.1 (1)
Max. number of isolates tested for resistance to the presented antibiotics	1,346	7,892	11,036

Included are results from all DCMs where >75% of the isolates in each antibiotic/sample group were susceptibility tested
Numbers in parantheses indicate the number of DCMs included if less than six

Figure 8.6 Invasive *Klebsiella pneumoniae* isolates from humans: a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

c)

Substance	Percent resistant invasive *K. pneumoniae* isolates									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Piperacillin/tazobactam	6.2	8.1	5.9	5.8	7.4	6.1	8.7	9.1	7.5	9.2
Gentamicin	4.4	4.6	2.5	2.9	3.2	3.1	3.3	3.2	2.1	2.2
Ciprofloxacin	9.4	7	5.5	5.4	9.0	8.1	9.2	7.4	6.7	7.4
Cefuroxime	12.4	12.1	11.1	11.1	11.1	10.3	9.7	10.1	7.9	7.7
3rd gen. cephalosporins	9.2	7.7	7.7	7.3	7.1	6.1	6.6	5.3	4.9	4.8
Carbapenem	0.2	0.2	0	0.3	0.3	0.5	0.3	0.8	0.5	0.4
Total number of isolates	875	943	939	1,156	1,183	1,280	1,361	1,413	1,336	1,346

Table 8.5 *Klebsiella pneumoniae*. Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multidrug-resistance) in invasive isolates from humans, Denmark, 2015-2022 DANMAP 2022

	2015 % (N)	2016 % (N)	2017 % (N)	2018 % (N)	2019 % (N)	2020 % (N)	2021 % (N)	2022 % (N)
Resistance	1.1 (9)	1.6 (18)	2.4 (27)	1.7 (20)	2.4 (30)	1.5 (19)	1.0 (13)	1.0 (13)
Percentage (no.) of isolates tested for combined resistance (multidrug-resistance)	89 (840)	98 (1,131)	95 (1,122)	93 (1,188)	94 (1,275)	93 (1,308)	93 (1,248)	94 (1,259)
Total number of invasive isolates	943	1,156	1,183	1,280	1,361	1,413	1,336	1,346

Urinary cases from hospitals

In 2022, 7,911 unique hospital patients had *K. pneumoniae* isolated from urine samples in Denmark. This represents a 34% and 2.7% increase compared to 2013 and 2021, respectively.

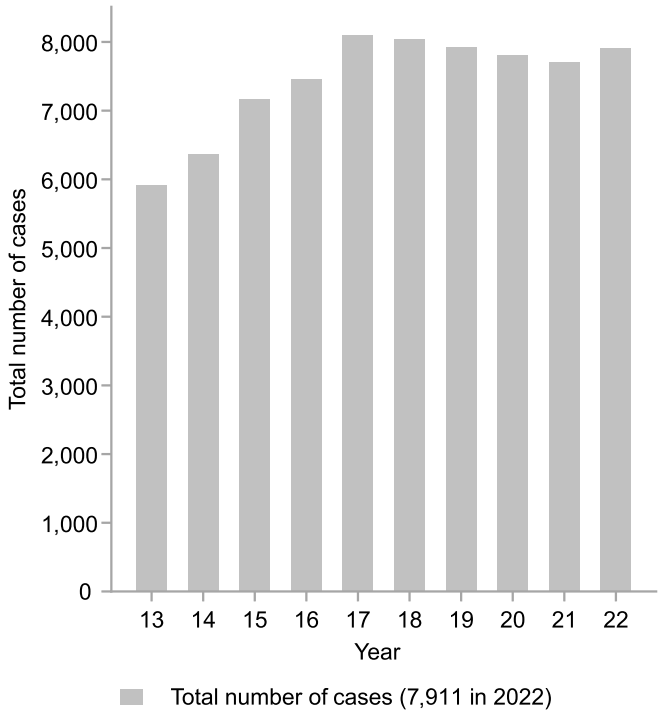
Summarized antibiotic-specific susceptibility results for *K. pneumoniae* isolates (invasive and urine specimen) are shown in Table 8.4. In Figure 8.7, resistance for *K. pneumoniae* urine isolates from hospitals is shown for 2013-2022.

Similar to the changes seen in invasive *K. pneumoniae* isolates, resistance in urine isolates from hospitals has decreased over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins. Meanwhile resistance to piperacillin-tazobactam has been increasing and is now 9.9% The increase in resistance to mecillinam observed in 2017 and 2018 has been followed by a marked decrease to the current 9.4% (Figure 8.7). Amoxicillin/clavulanic acid resistance has only been reported since 2018 in urinary isolates from hospitals, due to less than six DCMs testing isolates previously. The proportion of resistance decreased since then.

In 2022, there were 23 carbapenem-resistant and 13 carbapenem-"susceptible, increased exposure" *K. pneumoniae* urine isolates from hospital patients reported in MiBa, compared to 13 and eight isolates in 2021, respectively.

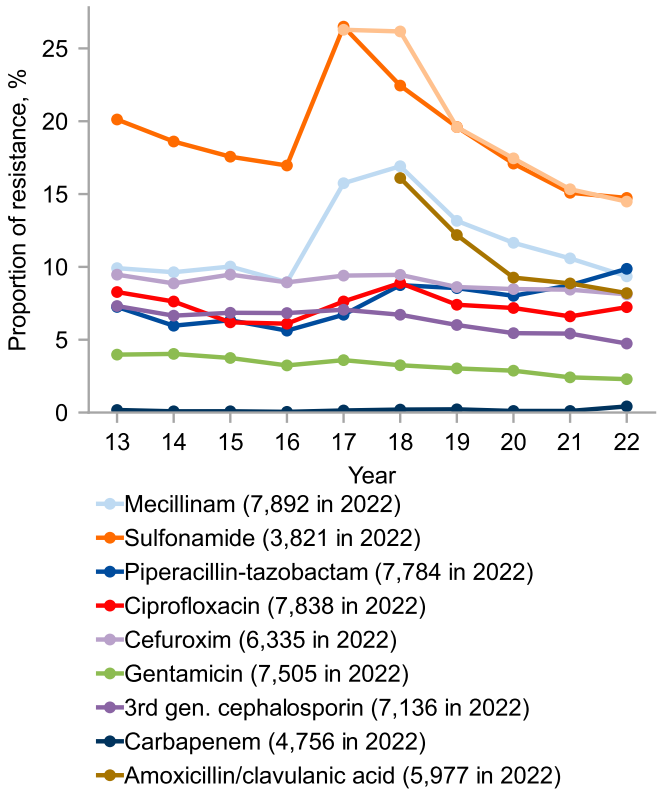
Figure 8.7 Urine *Klebsiella pneumoniae* isolates from humans (hospitals): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Resistance in *K. pneumoniae* urine isolates from hospitals									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Mecillinam	9.9	9.6	10	8.9	15.7	16.9	13.2	11.7	10.6	9.4
Piperacillin/tazobactam	7.2	6	6.3	5.6	6.7	8.7	8.5	8	8.7	9.9
Gentamicin	4	4	3.7	3.2	3.6	3.2	3	2.9	2.4	2.3
Ciprofloxacin	8.3	7.6	6.2	6.1	7.6	8.9	7.4	7.2	7.2	7.2
Cefuroxime	9.5	8.9	9.5	9.1	9.4	9.5	8.6	8.5	8.4	8.1
3rd gen. cephalosporins	7.3	6.6	6.8	6.8	7.1	6.8	6	5.5	5.4	4.7
Total number of isolates	5,919	6,372	7,175	7,467	8,106	8,047	7,926	7,814	7,701	7,911

Urinary cases from primary health care

In 2022, 11,039 unique patients in primary health care had *K. pneumoniae* isolated from urine samples, a 200% and 8.3% increase compared to 2013 and 2021, respectively.

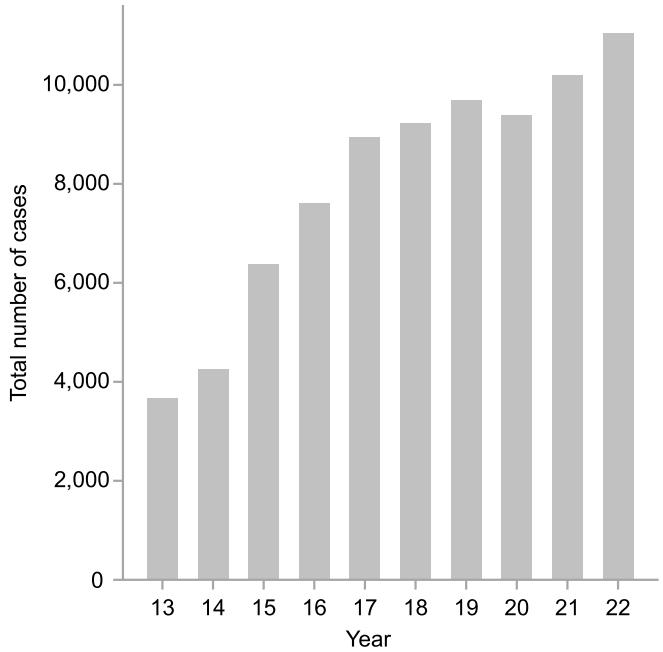
As for the results from invasive isolates and isolates from hospital urine samples, susceptibility results for all tested antimicrobials are shown in Table 8.4. In Figure 8.8, resistance for *K. pneumoniae* urine isolates from primary health care is shown for the past decade.

Following a sharp increase in 2017, resistance to mecillinam and sulfonamides/trimethoprim has since decreased. Additionally, in 2022, the 3rd generation cephalosporins resistance rate has also continued to decline, while resistance towards ciprofloxacin has increased slightly (Figure 8.8).

Five carbapenem-resistant isolates and one carbapenem-"susceptible, increased exposure" isolate were registered in 2022 compared to three and one, respectively, in 2021. However, susceptibility to carbapenems is only routinely reported to MiBa by one DCM.

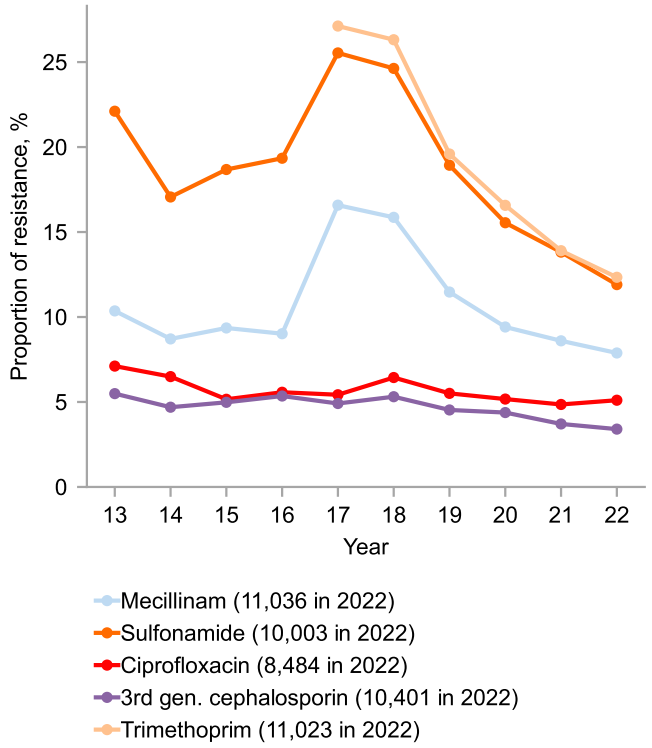
Figure 8.8 Urine *Klebsiella pneumoniae* isolates from humans (primary health care): a) annual number of isolates from unique cases, b) proportion of resistant isolates and c) table of resistance percentages, Denmark, 2013-2022 DANMAP 2022

a)



Numbers in parentheses indicate number of isolates tested

b)



c)

Substance	Percent resistant *K. pneumoniae* isolates from primary health care urine									
	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Mecillinam	10.4	8.8	9.4	9	16.6	15.9	11.5	9.4	8.6	7.9
Sulfonamide	22.1	17.1	18.7	19.3	25.5	24.6	18.9	15.5	13.8	11.9
Ciprofloxacin	7.1	6.5	5.2	5.6	5.4	6.4	5.5	5.2	4.9	5.1
3rd gen. cephalosporins	5.5	4.7	4.8	5.4	4.9	5.3	4.5	4.4	3.7	3.4
Total number of isolates	3,669	4,246	6,372	7,615	8,948	9,227	9,696	9,387	10,196	11,039

Conclusion

The general trend for *K. pneumoniae* in all three specimen types (blood/cerebrospinal fluid, urine [hospital/primary health care]) has been a decrease in resistance to important antimicrobials (cephalosporins, gentamicin and ciprofloxacin) over the last ten years. Following a sharp increase in mecillinam and sulfonamide/trimethoprim resistance in urine samples from hospitals and primary care in 2017 and 2018, the proportion of resistant *K. pneumoniae* isolates has since been decreasing. Resistance to piperacillin-tazobactam in *K. pneumoniae* from hospital urinary samples has been increasing and is now 9.9%. The carbapenem resistance in *K. pneumoniae* continues to be very low (<1%).

8.2.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen *that* can colonise the lung, urinary tract, burn wounds, superficial wounds and can cause invasive infections. The pathogen is capable of biofilm formation and has the ability to colonise medical devices (e.g. indwelling catheters and implants) which in many cases causes complicated infections requiring prolonged and combination treatment. *P. aeruginosa* infections are more prevalent in certain at-risk groups such as immunocompromised, diabetes and cystic fibrosis patients. *P. aeruginosa* exhibits intrinsic resistance to various antimicrobial agents through chromosomal gene mutations and has the ability to acquire β-lactamases (extended-spectrum β-lactamases (ESBLs) and carbapenemases (especially class B carbapenemases or metallo-β-lactamases [MBLs]) by horizontal transmission. The antimicrobial classes which can be used for treatment include: fluoroquinolones, aminoglycosides (tobramycin, gentamicin and amikacin), broad-spectrum beta-lactams (piperacillin-tazobactam, ceftazidime and carbapenems) and colistin. New antibiotic combinations with beta-lactamase inhibitors, such as aztreonam-avibactam and ceftolozane-tazobactam, may be used in serious cases of *Pseudomonas* infections including MBL-producers.

Invasive cases from hospital patients

In 2022, a total of 495 unique patients with invasive *P. aeruginosa* isolates were registered in Denmark. Number of cases over the last decade are presented in Figure 8.9.

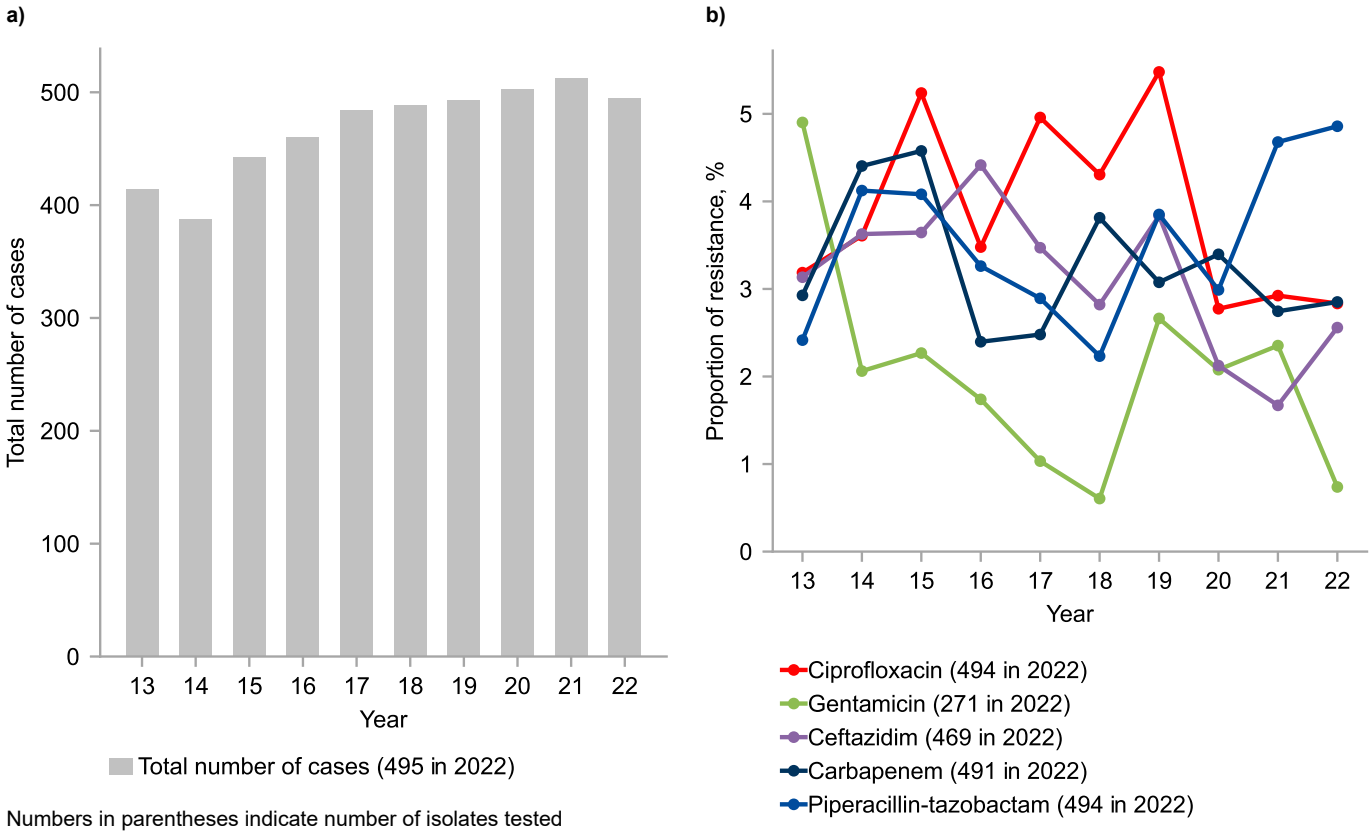
The highest proportion of resistance was reported for ciprofloxacin ranging from 2.8 to 5.4% over the past 10 years. In 2022, meropenem resistance was reported in 2.9% of the cases, but only 0.6% of the cases were resistant to three or more of the five antimicrobials under surveillance.

Conclusion

The occurrence of invasive *P. aeruginosa* in Denmark is stable regarding the number of cases reported and resistance levels are low.

Figure 8.9 Invasive *Pseudomonas aeruginosa* isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2013-2022

DANMAP 2022



Numbers in parentheses indicate number of isolates tested

8.2.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature (soil and water) as well as in animals and humans. In humans, *Acinetobacter* species (spp.) can colonise the skin and wounds but may also cause hospital-acquired infections such as central line-associated bloodstream infections, nosocomial pneumonia, UTIs and wound infections, most often in severely ill patients. Many of the different subspecies are phenotypically alike, and thus identification at species level can be difficult. Species belonging to the *A. baumannii* group are considered as the most clinically important. *Acinetobacter* spp. is intrinsically resistant to a broad range of antimicrobials mediated by a low membrane permeability and constitutive expression of various efflux systems. The antimicrobial classes that are recommended for treatment include fluoroquinolones, aminoglycosides, carbapenems and colistin.

Invasive cases from hospitals

In 2022, a total of 93 unique patients with invasive *Acinetobacter* species were reported. Data are presented in Table 8.6 and in Figure 8.10.

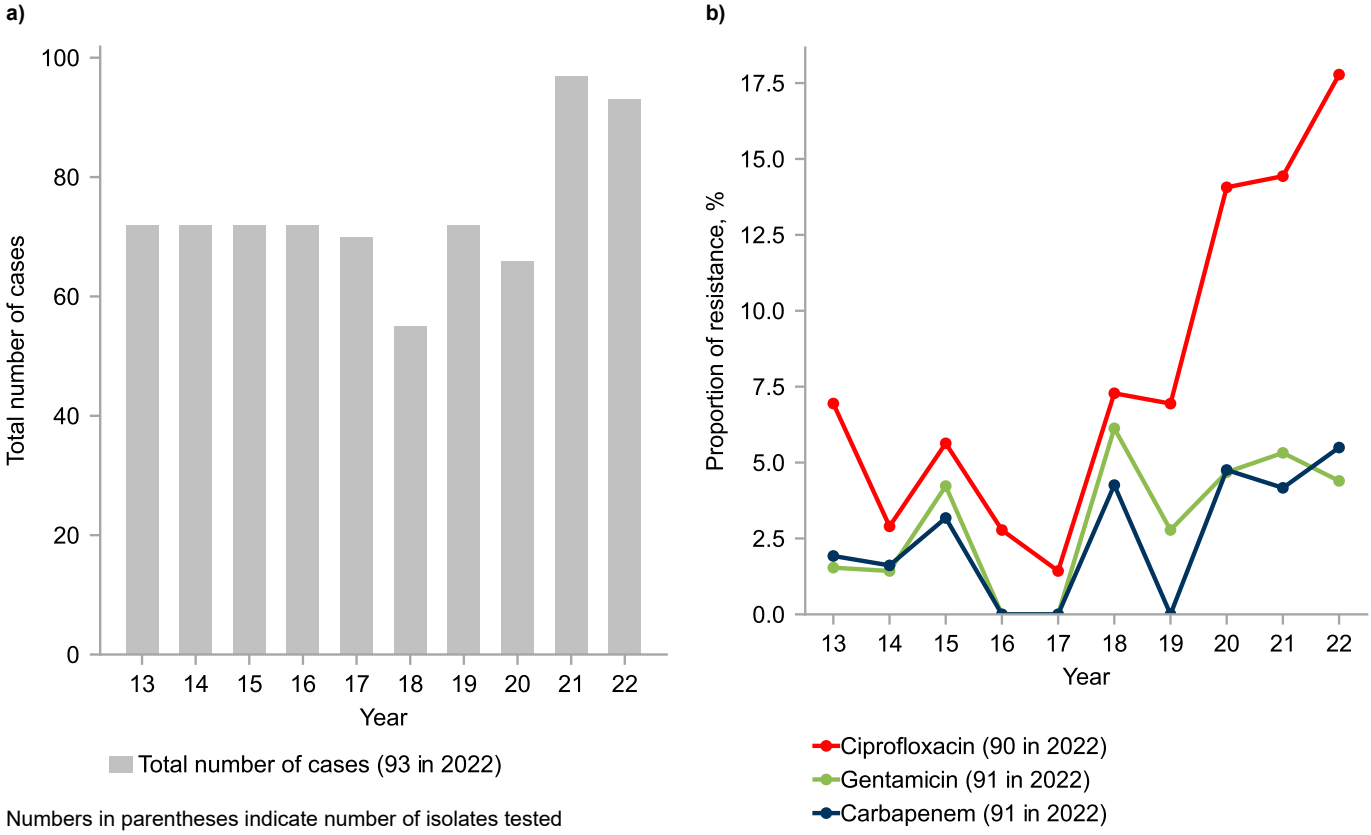
Following the marked increase in the number of invasive *Acinetobacter* spp. cases from 2020 to 2021, the number of cases has stabilized in 2022, but remains high compared to previous years. Five of the 93 isolates identified in 2022 were resistant to meropenem, 16 were resistant to ciprofloxacin and four were resistant to gentamicin. Four isolates had combined resistance to ciprofloxacin and gentamicin and four were reported resistant to colistin, however, susceptibility to colistin, is not routinely tested.

Table 8.6 *Acinetobacter* spp. Tested and resistant invasive isolates, Denmark, 2013-2022 DANMAP 2022

	2013		2014		2015		2016		2017		2018		2019		2020		2021		2022	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	5	72	2	69	4	71	2	72	1	70	4	55	5	72	9	64	14	97	16	92
Gentamicin	1	65	1	70	3	71	0	70	0	70	3	49	2	72	3	64	5	94	4	92
Carbapenem	1	52	1	62	3	68	0	69	0	67	2	47	0	72	3	63	4	96	5	93
Total number of invasive isolates	72		72		71		72		70		55		72		66		97		93	

res. = number of resistant isolates. n = number of tested isolates

Figure 8.10 Invasive *Acinetobacter* spp. isolates from humans: a) annual number of isolates from unique cases and b) proportion of resistant isolates, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

Conclusion

The number of invasive *Acinetobacter* spp. saw a stabilisation in 2022 compared to the marked increase from 2020 to 2021; however, the proportion of invasive isolates resistant to key antimicrobials remained low in Denmark, but with a marked increase in ciprofloxacin resistance..

8.2.5 Enterococci

Enterococci are part of the normal intestinal microbiota in humans and animals. More than 54 species belonging to the genus *Enterococcus* have been described. The majority of human infections are caused by *E. faecalis* and *E. faecium* and in rare cases by *E. casseliflavus*, *E. gallinarum* and *E. raffinosus*. Most common clinical infections include urinary tract infections, intra-abdominal infections, bacteraemia and infective endocarditis. Enterococci are intrinsically resistant to many groups of antimicrobials which gives them a selective advantage in e.g. hospitalised patients under antibiotic treatment, leading to colonization or infection. The source of hospital infection is often associated with invasive medical devices.

Treatment of enterococcal infections may be challenging. Combination therapy based on a synergistic effect of beta-lactam antibiotics (penicillin/ampicillin) and aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is indicated in cases of complicated infection (e.g. endocarditis). In cases of high-level gentamicin resistance, combination of ampicillin and penicillin V may be used for treatment. The vast majority of *E. faecium* are ampicillin-resistant, and therefore most infections are treated with vancomycin. Antimicrobials such as linezolid and daptomycin can be used for treatment of the multidrug-resistant vancomycin-resistant enterococci (VRE).

Invasive cases from hospitals

In 2022, 659 unique patients with invasive *E. faecalis* isolates and 637 unique patients with invasive *E. faecium* isolates were reported in MiBa.

The proportion of resistant invasive *E. faecalis/faecium* isolates in 2022 are presented for each key antimicrobial in Table 8.7. In Figure 8.11, total numbers of invasive isolates of *E. faecalis* and *E. faecium* and the respective percentages of vancomycin resistance are shown for 2013 to 2022.

The total number of invasive cases caused by *E. faecalis* and *E. faecium* increased by 5.8% from 2013 to 2022. However, a marked decrease of 14% was observed from 2021 to 2022.

A continuous high proportion of ampicillin resistance in invasive *E. faecium* has been observed with proportions of resistant isolates ranging between 92% and 95% since 2010. In 2002, the resistance rate was reported to be 65%. The proportion of invasive vancomycin-resistant *E. faecium* isolates continued to increase to 12.0% in 2022 from 10.2% in 2021 after having stabilised at around 9% in the previous past three years.

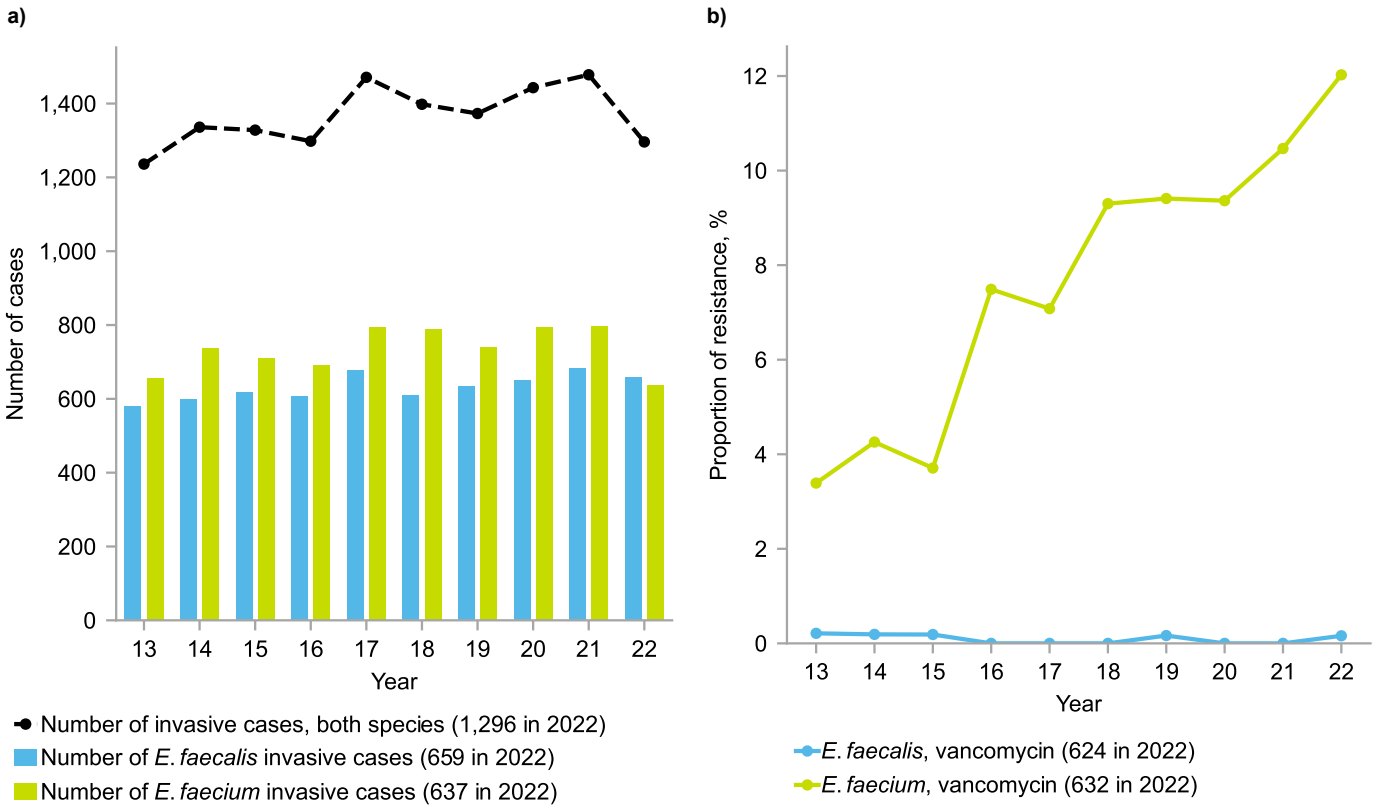
During 2022, five invasive isolates of *E. faecalis* and five invasive isolates of *E. faecium* from ten unique patients were reported linezolid-resistant (Table 8.7). In 2021, the numbers were four *E. faecalis* and two *E. faecium*. Four of the five linezolid-resistant invasive *E. faecium* isolates identified in MiBa in 2022 were also reported resistant towards vancomycin.

Table 8.7 Enterococci. Resistance (%) in invasive isolates from humans, 2022 DANMAP 2022

	E. faecalis	*E. faecium*	Number of included isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0	92.9	658 (10)	607 (9)
Vancomycin	0.2	12.0	624 (9)	632 (10)
Linezolid	1.0	0.8	508 (6)	513 (6)
Teicoplanin	0.4	1.1	233 (2)	177 (2)
Tigecycline	1.0	2.4	104 (1)	82 (1)

All proportions of resistance are presented for each antibiotic as a mean of the resistance rates reported by the DCMs. Included are all DCMs that report routine testing (>75% of the isolates). The number in parentheses tells the number of included DCMs.

Figure 8.11 Invasive *Enterococci faecalis/faecium* isolates from humans: a) annual number of isolates from unique cases and b) proportion of vancomycin-resistant isolates, Denmark, 2013-2022 DANMAP 2022



Numbers in parentheses indicate number of isolates tested

Conclusion

Over the past 20 years, a steady increase in invasive has been observed. However, a noticeable drop occurred from 2021 to 2022. Additionally, a marked increase in invasive *E. faecium* resistant to vancomycin has been observed over the past 10 years.

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8.3 Results from the reference laboratories

8.3.1 Characterization of ESBL- and pAmpC-producing *Escherichia coli* from bloodstream infections

Background

Resistance to third-generation cephalosporins in *Escherichia coli* often occur through production of extended-spectrum beta-lactamases (ESBLs), carbapenemases, plasmid-mediated AmpC (pAmpC) or through mutations within the promoter/attenuator region of chromosomal AmpC (cAmpC) leading to overexpression/hyperproduction.

Since 2014, the Danish Departments of Clinical Microbiology (DCMs) have, on a voluntary basis, submitted third-generation cephalosporin-resistant *E. coli* (3GC-R *Ec*) from bloodstream infections for further characterization at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI) by adhering to the EUCAST criteria for reduced susceptibility towards cefpodoxime, ceftriaxone, cefotaxime or ceftazidime.

At SSI, the 3GC-R Ec's collected in Denmark through 2022, were phenotypically tested for ESBL-production. ESBL- and/or pAmpC-positive isolates from January, March, May, July, September, and November were subjected to whole-genome sequencing and characterized according to Multilocus Sequence Types (MLSTs) and the encoding ESBL-, pAmpC- and carbapenemase genes.

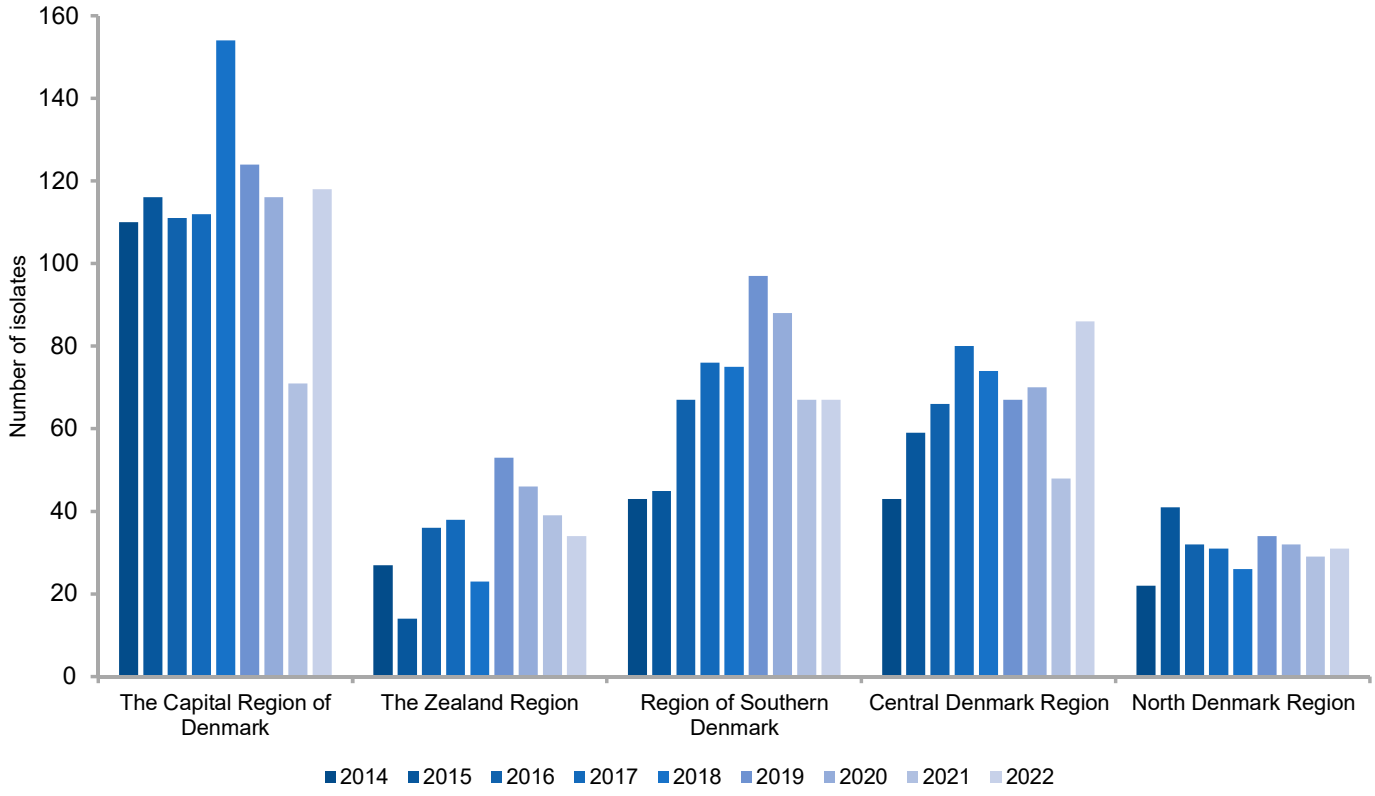
Results

In 2022, a total of 336 *E. coli* isolates from unique patients, were identified with phenotypic test, as ESBL and/or AmpC positive isolates. Demographic data was available for all 336 *E. coli* isolates in 2022; 187 (56%) of the patients were men compared to 147 (58%) in 2021, and 149 (44%) were women compared to 107 (42%) in 2021. The median age at diagnosis was 71 years, ranging from below one year to 96 years. The regional distribution of the 336 isolates with ESBL- and AmpC phenotype was compared to data from previous years (Table 8.8 and Figure 8.12).

Following the overall decreasing trend of reported cases of ESBL/pAmpC *E. coli* in bloodstream infections observed from 2019 to 2021 (32%), the number increased from 2021 to 2022 (32% from 254 to 336 isolates). Increasing numbers were notably observed in The Capital Region and Central Region of Denmark, whereas the numbers in the remaining regions did not change notably.

Whole genome sequencing data were obtained from 181 *E. coli* isolates (as only isolates from every second month and carbapenemase producing *E. coli* were sequenced). Genes encoding ESBL and/or pAmpC were detected in 178 isolates (five having carbapenemase encoding genes detected) and three isolates were AmpC hyperproducers caused by cAmpC mutations. The AmpC hyperproducers will not be analysed further.

Figure 8.12 ESBL/pAmpC producing *E. coli* from bloodstream infections by region, 2014-2022, Denmark DANMAP 2022



In 2022, 20 different genes associated with ESBL-, and pAmpC enzymes were detected among the 178 sequenced isolates encoding ESBL and/or pAmpC genes (Table 8.9). As in previous years, CTX-M-15 was the most prevalent enzyme, remaining relatively stable in occurrence at 52% in 2022, compared to 46% in 2021. In addition, five carbapenemase producers were observed during 2022 among the 181 whole genome sequenced blood infection isolates (3%); two NDM- and three OXA-48-group producing isolates.

Table 8.8 Distribution of ESBL and Carbapenemase producing *E. coli* from bloodstream infections, Denmark, 2015-2022 DANMAP 2022

	DANMAP 2015	DANMAP 2016	DANMAP 2017	DANMAP 2018	DANMAP 2019	DANMAP 2020	DANMAP 2021	DANMAP 2022
Region	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers	Numbers
The Capital Region of Denmark	116	111	112	154	124	116	71	118
The Zealand Region	14	36	38	23	53	46	39	34
Region of Southern Denmark	45	67	76	75	97	88	67	67
Central Denmark Region	59	66	80	74	67	70	48	86
North Denmark Region	41	32	31	26	34	32	29	31
Total Numbers	275	312	337	352	375	352	254	336

Table 8.9 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark 2015-2022 DANMAP 2022

	DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020		DANMAP 2021		DANMAP 2022	
Enzyme	Number	%	Number	%	Number	%	Number	%	Number	%	Number*	%	Number*	%	Number*	%
CTX-M-1	7	3%	8	3%	17	5%	25	7%	8	4%	7	4%	6	4%	1	<1%
CTX-M-14	33	12%	40	13%	48	14%	31	9%	33	17%	15	8%	12	9%	17	9%
CTX-M-14b	5	2%	9	3%	3	1%	10	3%	3	2%	4	2%	0	0%	3	2%
CTX-M-15	139	51%	157	50%	164	49%	200	57%	82	43%	100	52%	63	46%	94	52%
CTX-M-27	33	12%	44	14%	52	15%	53	15%	37	19%	36	19%	29	21%	34	19%
CTX-M-3	4	1%	7	2%	8	2%	5	1%	4	2%	1	1%	3	2%	1	<1%
CTX-M-55	14	5%	6	2%	13	4%	4	1%	8	4%	4	2%	5	4%	3	2%
CMY-2	6	2%	10	3%	7	2%	6	2%	5	3%	5	3%	2	1%	2	1%
DHA-1	3	1%	5	2%	6	2%	10	3%	4	2%	7	4%	3	2%	11	6%
SHV-12	5	2%	5	2%	3	1%	4	1%	2	1%	5	3%	3	2%	3	2%
Other CMY variants	10	4%	3	1%	3	1%	3	1%	5	3%	0	0%	1	1%	1	<1%
Other ESBL enzymes	23	8%	17	5%	10	3%	10	3%	3	2%	8	4%	6	4%	7	4%
Carbapenemase enzymes	3	1%	1	<1%	1	<1%	5	1%	0	0%	7	4%	4	3%	5	3%

In some isolates more than one enzyme was detected
* Numbers based on sequenced data from odd months

Table 8.10 Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark, 2015-2022 DANMAP 2022

	DANMAP 2015		DANMAP 2016		DANMAP 2017		DANMAP 2018		DANMAP 2019		DANMAP 2020		DANMAP 2021		DANMAP 2022	
MLST	Number	%	Number	%	Number	%	Number	%	Number*	%	Number*	%	Number*	%	Number*	%
ST131	135	49%	177	57%	175	52%	189	54%	93	47%	89	46%	64	49%	89	50%
ST38	23	8%	21	7%	23	7%	22	6%	13	7%	8	4%	1	1%	11	6%
ST69	10	4%	16	5%	20	6%	27	8%	14	7%	20	10%	7	5%	9	5%
ST648	10	4%	5	2%	8	2%	6	2%	4	2%	0	0%	1	1%	6	3%
ST1193	5	2%	10	3%	7	2%	8	2%	6	3%	9	5%	9	7%	5	3%
ST73	2	1%	4	1%	2	1%	6	2%	4	2%	8	4%	1	1%	5	3%
ST12	9	3%	14	4%	6	2%	5	1%	5	3%	2	1%	5	4%	4	2%
Other STs[1]	51	19%	48	15%	69	20%	69	20%	38	19%	43	22%	34	26%	46	26%

[1] Found in less than 2% in 2022
* Numbers based on sequenced data from odd months

In 2022, the 172 of the 178 whole genome sequenced *E. coli* isolates belonged to 44 different known MLSTs, with the remaining 6 isolates typed with novel STs. The most common sequence type (ST) was ST131 (50%), followed by ST38 (6%) and ST69 (5%) (Table 8.10).

Among the 89 *E. coli* isolates belonging to ST131, CTX-M-15 (55%) was the most common enzyme, followed by CTX-M-27 (33%), and CTX-M-14 (6%). The distribution of ESBL and/or pAmpC enzymes observed within ST131 remained stable in 2022.

Conclusion

In 2022, the number of ESBL- and/or AmpC positive isolates increased from 254 to 336 isolates (32% increase). CTX-M-15 remained by far the most prevalent ESBL enzyme in Danish *E. coli* from bloodstream infections in 2022. In isolates belonging to ST131, the relative distribution of ESBL/pAmpC enzymes was stable in 2022 compared to 2020 and 2021.

In 2022, five carbapenemase producers were observed among the 178 whole genome sequenced ESBL- and/or pAmpC blood infection isolates. The relative distribution of sequence types for the whole genome isolates were similar to the distributions in the previous years; the worldwide disseminated ST131 clone was still strongly represented in 2022 (50%).

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8.3.2 Carbapenemase-producing organisms (CPO)

Background

Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multidrug-resistant Gram-negative bacteria like *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Treatment options for infections with carbapenem-resistant bacteria are often none or suboptimal. Resistance can be expressed by the presence of various carbapenemases of which the five most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integrin encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM) and Imipenemase (IMP).

Carbapenemase-producing organisms (CPO) comprise two main groups: 1) Intestinal bacteria: Carbapenemase-producing Enterobacterales (CPE), e.g., *E. coli* and *K. pneumoniae*, and 2) environmental bacteria: *P. aeruginosa* and *A. baumannii*. The term CPE is used to designate intestinal bacteria. Thus, CPE are a subset of CPO.

CPO have been notifiable in Denmark since 5th September 2018 [https://www.retsinformation.dk/eli/lta/2018/1091]. Before this date, Danish departments of clinical microbiology (DCMs) submitted carbapenem-resistant isolates for verification and genotyping at the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut on a voluntary basis. This section describes findings for confirmed carbapenemase-producing Enterobacterales (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

During 2022, 392 CPOs were identified from 335 patients compared with 277 CPO isolates from 242 patients in 2021, an increase in isolates of 42%. More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases. Nineteen out of all CPOs were from bloodstream infections compared to 11 out of all CPOs in 2021.

Collection of travel information is part of the CPO surveillance in Denmark. Thus, information of travelling abroad in the period of 6 months prior to testing positive for CPO is requested and reported to SSI, together with the clinical sample information.

Carbapenemase-producing Enterobacterales

In 2022, 350 CPE isolates were reported from 304 patients compared to 251 CPE from 221 patients in 2021 resulting in a 39% increase of CPE isolates compared to 2021. In 2022, 22 of the 350 CPE isolates produced both NDM and OXA-48 group enzymes, 208 produced OXA-48-like enzymes alone and 112 were NDM-producers. Furthermore, four KPC-, one VIM-, one IMP-, one KPC-/VIM- as well as one KPC-/NDM-producing CPE isolate(s) were identified (Figure 8.13).

Carbapenemase-producing *Acinetobacter* spp.

In 2022, 23 carbapenemase-producing *Acinetobacter* spp. isolates were reported from 23 patients, compared to 25 isolates from 25 patients in 2021. Of these, 21 patients had been travelling abroad prior to identification of the carbapenemase-producing *Acinetobacter* spp. In 2022, 22 carbapenemase-producing *Acinetobacter baumannii* with the following enzymes were identified: OXA-23 (16), OXA-72 (4), OXA-58 (1) and NDM-5/OXA-23 (1). Furthermore, one OXA-72-producing *Acinetobacter pittii* was identified. All in all, a steady increase in the number of carbapenemase-producing *Acinetobacter* spp. have been observed since the first Danish isolate was identified in 2008 (Figure 8.14).

Figure 8.13 Numbers of carbapenemase-producing Enterobacterales (CPE), Denmark, 2008-2022 DANMAP 2022

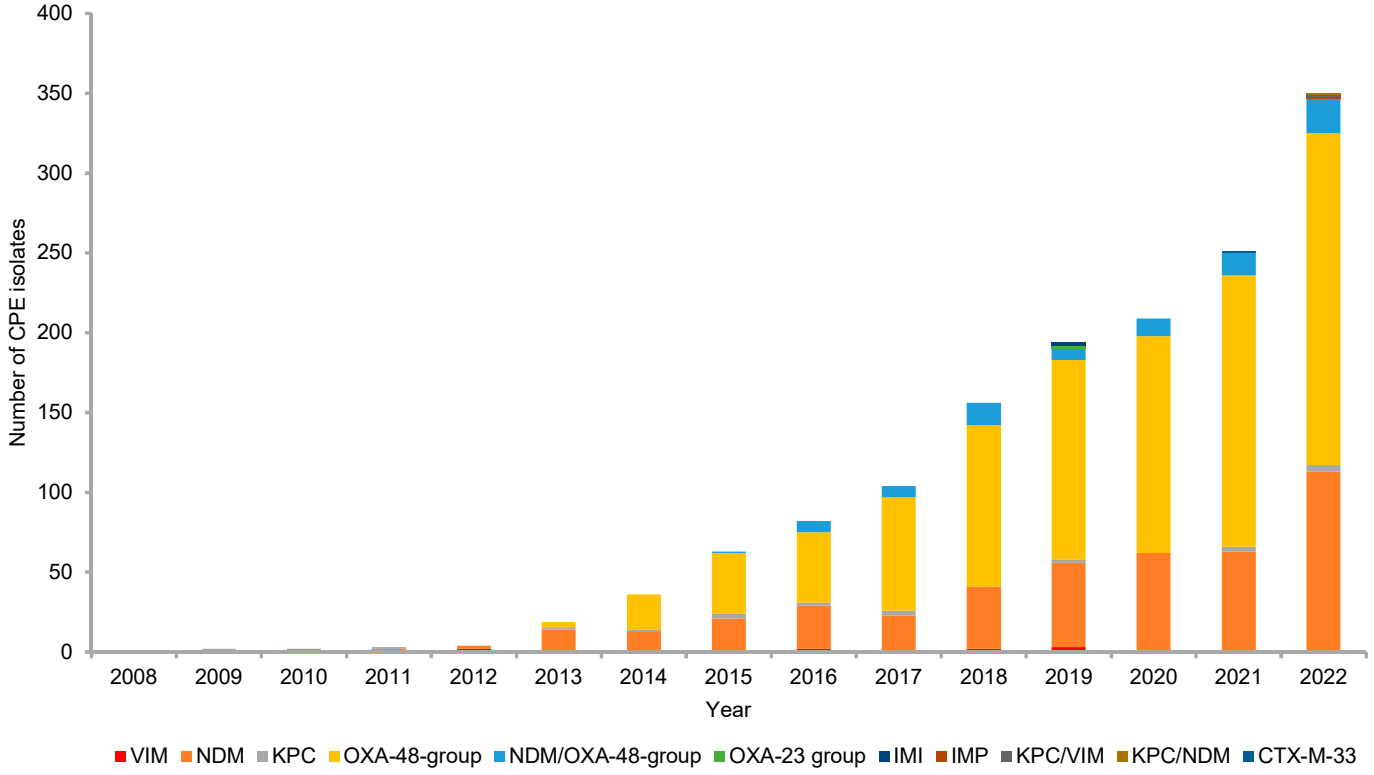
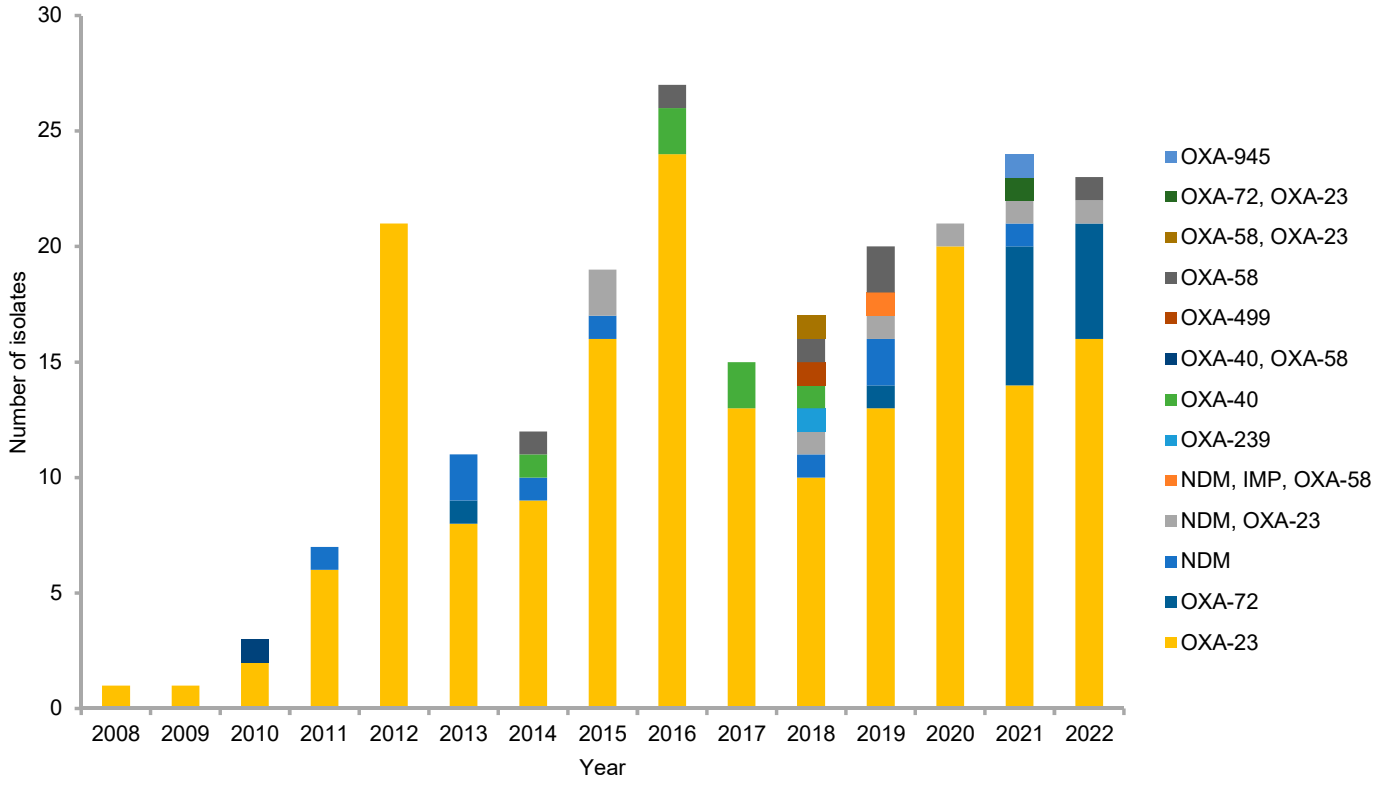


Figure 8.14 Carbapenemase-producing *Acinetobacter* spp. and enzymes identified, Denmark, 2008-2022 DANMAP 2022

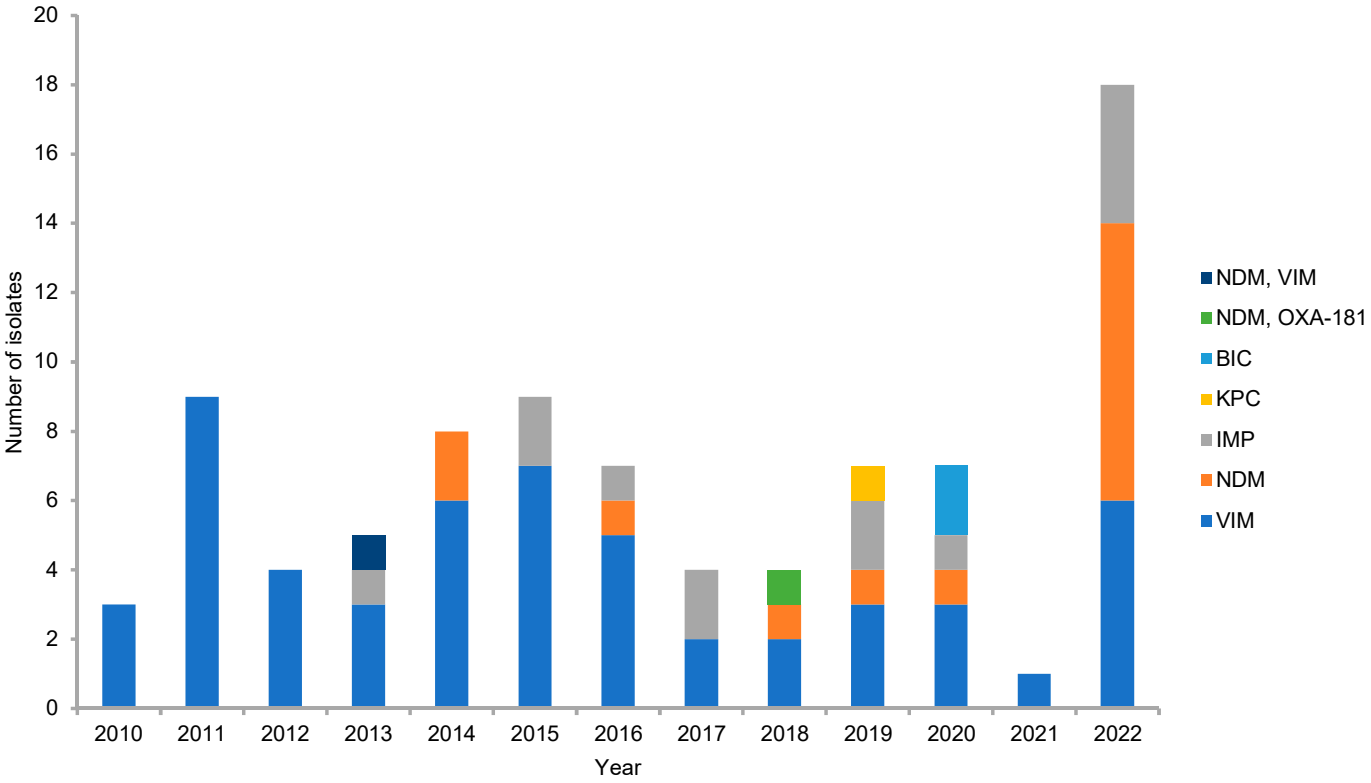


Carbapenemase-producing *Pseudomonas* spp.

In 2022, 19 carbapenemase-producing *Pseudomonas* spp. isolates from 18 patients were reported compared to only one isolate in 2021. In 2022, 18 carbapenemase-producing *Pseudomonas aeruginosa* with the following enzymes were identified: NDM-1 (8), VIM-2 (4), VIM-4 (1), IMP-10 (2), IMP-1 (1), IMP-1/IMP-10 (1) or unknown carbapenemase (1). Furthermore, one VIM-2-producing *Acinetobacter pittii* was identified.

In general, the number of carbapenemase-producing *Pseudomonas* spp. have been relatively stable between 2010 and 2020, with a remarkable decrease in 2021 presumably due to reduced travel activities on account of the COVID-19 pandemic. As opposed to this observation, a large increase in 2022 happened due to the arrival of patients in relation to the current military conflict in Ukraine (Figure 8.15).

Figure 8.15 Carbapenmase-producing *Pseudomonas* spp. and enzymes identified, Denmark, 2010-2022 DANMAP 2022



CPO - Place of origin 2019-2022

It is mandatory for the treating physician to obtain information regarding travel of a CPO-positive patient, if possible, encompassing six months prior to detection. The DCM or a clinical physician can also report a CPO-patient to be colonised in Denmark, implicating that the patient has not been travelling prior to detection of the strain. Depending on this and on the results from WGS performed at the laboratory, the CPO-patient will be classified as belonging to one of five current epidemiological categories: 1) Denmark, sporadic cases 2) Denmark, part of outbreak, 3) travel outside the Nordic countries, 4) patients from Ukraine, and 5) unknown (Figure 8.16).

If a known CPO-patient later is affected by a Danish nosocomial outbreak, the patient will be reclassified as an outbreak-patient. Vice versa, if the index patient (the first patient) in an outbreak was known to be travelling prior to detection of the CPO-strain, the outbreak will be registered according to travel information.

In 2022, the reported travel data showed that 52 (19%) of 279 CPO-positive persons (e.g. patients that were not associated with an outbreak in Denmark) reported travelling outside the Nordic countries, which is is the same proportion as in 2021. In 2019, 43% of the CPO-cases had been travelling outside the Nordic countries. In 2020, supposedly due to the pandemic, the proportion of travel-associated cases had dropped markedly. The most frequent reported travel destinations in 2022 were Asia (11), Africa (10), Middle East (8), and the Mediterranean (7). The most single reported travel destinations were Turkey (10) and Egypt (6).

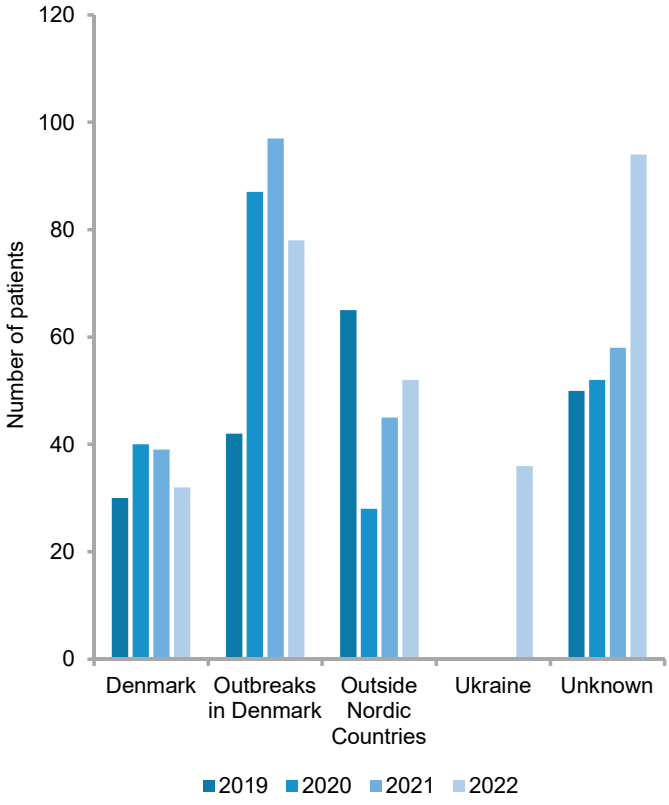
Due to the war in Ukraine, several patients originating from Ukraine have been in contact with the Danish health care system. From February 24th, 2022, through January 23rd, 2023, a total of 371 CPO from 288 patients were obtained as part of the Danish National CPO surveillance. Of the 371 CPO, 77 CPO were collected from 42 patients from Ukraine. The

findings show that the patients originating from Ukraine were colonized and/or infected by many different CPO per patient [Stolberg, et al. 2023, J Glob Antimicrob Resist, 34:15-17].

Outbreaks with CPO during 2022

In Denmark, outbreaks caused by CPO in healthcare settings are registered at SSI in the national database (KURS). At SSI, CPO isolates are routinely characterized by whole genome sequencing (WGS). Cluster analysis is conducted on all isolates using cgMLST to detect possible clustering between the CPO isolates. A genomic cluster is defined as at least two isolates (from different patients) belonging to the same cgMLST cluster (or having less than 15 alleles difference between the isolates). Due to the risk of long-term colonization of the patients, cluster analysis is carried out regardless of the time of isolation. When epidemiological investigations can establish a link (e.g., the patients had been at the same hospital ward at the same time) between at least two patients in a genomic cluster, the patients are classified as belonging to a "verified outbreak". When no epidemiological link can be established, the patients related to the genomic cluster are classified as part of a probable outbreak (Materials and methods, Section 10.12).

Figure 8.16 Place of origin of CPO, 2019-2022 DANMAP 2022



In 2022, a total of 17 CPO-outbreaks were registered compared to 15 CPO-outbreaks in 2021. In seven of the outbreaks, it was possible to establish an epidemiological link between the patients. All epidemiological links were found in healthcare settings, caused by patients sharing the same ward or hospital. Eleven of the seventeen outbreaks had been ongoing for more than two years and two outbreaks more than ten years, meaning that new patients had been identified as belonging to the same cgMLST cluster as found in the previous years.

In 2022, no new outbreak due to *A. baumannii* was detected and no new cases have been detected in the three outbreaks reported in 2021 and were therefore removed from Table 8.11. In total, 78 new patients were affected in 2022 by outbreaks, which is the same level as in 2021. Of the seventeen outbreaks registered in 2022, five new small clusters were identified involving 2-3 patients each, but no epidemiological link could be established in any of the these clusters. Three smaller clusters detected in 2021 had no new patients in 2022 and were removed from the table (Table 8.11).

Outbreaks with CPO of interest

An unusual outbreak with the same unique epitype ST79 *Enterobacter hormaechei* carrying the resistance genes bla_{NDM-5} and bla_{OXA-48} was registered and investigated in Denmark (ID1062) during October 2022 to June 2023. Altogether 15 CPO patients with the epitype and 19 CPO patients with one of the unique resistance genes were detected. Please see Textbox 8.1.

The ST18 NDM-1-producing *Citrobacter freundii* outbreak (ID41), which started in 2012 in the North Denmark Region, continued in 2022 [Hammerum *et al.* 2016, J. Antimicrob. Agents, 71:3117-3124]. Since 2012, the outbreak has spread to four out of five Danish Regions. Until the end of 2022, 93 hospitalized patients have been involved in this outbreak. In all, 20 new patients were identified in 2022 and all had been hospitalized in the North Denmark Region. None of these new outbreak-cases had a prior history of travel.

Since 2015, another large outbreak (ID21) has been ongoing mainly in the Zealand Region and in the Capital Region with spread of ST410 NDM-5/OXA-181 *E. coli*. [Roer *et al.* 2018, mSphere;3(4)]. By the end of 2022, 84 patients in total have been involved in this outbreak. During 2022, twelve new patients were affected by this outbreak. Apart from the first reported patient in 2015, who had been traveling to Egypt [Overballe-Petersen *et al.* 2018, Genome Announc.6(5): e01542-17] none of the patients have a prior history of travel.

Table 8.11 Outbreaks of carbapenemase-producing Enterobacterales (CPE) during 2022, n=17, Denmark DANMAP 2022

Outbreak ID	Year	Patients total	Patients 2022	Carbapenemase	Type of Outbreak	Species (clonal spread)	Regions[1]	Status
41	2012-2022	93	20	NDM-1	Clonal/ plasmid	ST18 *C. freundii*	1 / 2 / 3 / 4	Verified
48	2013-2022	34	4	OXA-436/OXA-48	Clonal/ plasmid	ST90 *E. cloacae*/ ST22 *C. freundii*	1 / 4 / 5	Verified
21	2015-2022	84	12	NDM-5/OXA-181	Clonal	ST410 *E. coli*	1 / 2 / 5	Verified
22	2015-2022	13	3	OXA-181	Clonal	ST440 *E. coli*	1 / 2	Possible
42	2015-2022	13	1	OXA-48	Clonal	ST65 *C. freundii*	1 / 3 / 5	Verified
47	2015-2022	11	3	VIM-2	Clonal	ST111 *P. aeruginosa*	2 / 3	Possible
43	2019-2022	5	2	OXA-48	Clonal	ST323 *C. freundii*	5	Possible
1061	2020-2022	10	4	OXA-181	Clonal	ST22 *C. freundii*	2	Possible
1062	2020-2022	14	11	NDM-5/OXA-48	Clonal/ plasmid	ST79 *E. hormaechei*	1 / 2 / 3 / 4 / 5	Verified
1068	2020-2022	10	1	OXA-48	Clonal	ST18 *C. freundii*	1	Verified
1052	2020-2021	5	1	NDM-1	Clonal	ST18 *C. freundii*	2	Possible
1089	2021-2022	6	4	OXA-244	Clonal	ST131 *E. coli*	2	Verified
10972	2022	3	3	OXA-48/OXA-181	Clonal	ST698 *C. freundii*	1	Possible
10992	2022	2	2	OXA-244	Clonal	ST131 *E. coli*	2 / 4	Possible
11032	2022	3	3	NDM-5	Clonal	ST17 *K. pneumoniae*	1	Possible
11072	2022	2	2	OXA-181	Clonal	ST636 *C. freundii*	5	Possible
11132	2022	2	2	OXA-48	Clonal	ST22 *C. freundii*	5	Possible

[1] Regions will be listed as: 1) Capital Region, 2) Central Denmark Region, 3) North Denmark Region, 4) Southern Denmark Region and 5) Zealand Region

Conclusion

The occurrence of carbapenemase-producing bacteria in Denmark continued to increase throughout 2022. The number of patients received from Ukraine contributed to this increase. The level of new nosocomial outbreaks in 2022 was the same as in 2021. The number of patients belonging to the two largest outbreaks in hospital settings continued to increase, highlighting the importance to start early interventions with infection prevention control (IPC) in order to prevent further spread of an outbreak. The spread of CPO among patients in Denmark is of concern, since Enterobacterales can be carried in the intestine for a long time (years) without any symptoms of infection, which makes outbreak detection and control difficult. For the first time in Denmark, an outbreak due to a pharmaceutical product was revealed, emphasizing the importance of national surveillance and the collaboration between national and regional level.

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8.3.3 Vancomycin-resistant/vancomycin-variable enterococci

Background

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents, including cephalosporins, which increases the risk of enterococci being selected for in hospital environments and emerging in patients that receive cephalosporins for other conditions. Most hospital-acquired *Enterococcus faecium* are resistant to ampicillin, further limiting the treatment options. Vancomycin is an important drug for the treatment of severe *E. faecium* infections, however an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed within the last decade, both internationally as well as in Denmark. Newer antibiotics such as linezolid and daptomycin can be used for treatment of VRE, but both antimicrobial agents may lead to adverse events, and the development of resistance, particularly against linezolid, is relatively common. In recent years, isolates of phenotypically vancomycin-susceptible *E. faecium* have been described to harbor a *vanA*-gene complex, in various countries. These isolates are referred to as vancomycin-variable enterococci (VVE). It has been demonstrated that VVE retain the ability to become vancomycin-resistant upon exposure to vancomycin [Kohler, et al, 2018, PLoS One. 2018 Mar 22;13(3)], and are often associated with nosocomial outbreaks. However, VVE cannot be selectively cultured on vancomycin-containing media and can only be detected by molecular methods. In 2015 and 2016, sporadic VVE isolates with different genetic background in relation to concurrent VRE-outbreaks were detected in the Capital Region of Denmark [Hammerum *et al.* Euro Surveill. 2020;25(18)]. In 2016, a new VVE clone belonging to ST1421- CT1134, displaying variable vancomycin susceptibility due to a deletion in the *vanX* gene was detected. [Hansen *et al.*, J. Antimicrob. Agents, 2018, 73: 2936–2940]. Conclusively, VVE may be of clinical relevance and timely detection as well as continuous monitoring are critical to avoid treatment failure and further transmission.

Surveillance of VRE/VVE

Since 2005, Danish departments of clinical microbiology (DCMs) have voluntarily submitted clinical VRE (one VRE/VVE isolate per patient, isolated within 12 months) for species identification, genotyping and surveillance purposes to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI).

Since 2015, the received clinical VRE/VVE isolates have been analysed using whole-genome sequencing (WGS). The WGS data are used for *in silico* genotyping of isolates characteristics such as species identification, multilocus sequence typing (MLST), detection of *van*-genes and core genome sequence typing (cgMLST) used for clonal detection see (Section 10.13.3).

To determine any underreporting in the submissions of VRE isolates, the number of VRE/VVE submitted to SSI since 2016 were compared to data from clinical VRE reported by the DCMs to MiBa. This comparison showed that the number of submitted VRE/VVE isolates was not complete (Figure 8.17). In 2022, 667 VRE/VVE isolates were submitted to SSI, and were genotyped by WGS. Furthermore, 178 VRE/VVE isolates were identified in MiBa, which had not been submitted to SSI and hence have not been genotyped. This was an increase compared to 2021, were 578 VRE/VVE isolates were sent to SSI and 164 VRE/VVE isolates were identified in MiBa. (Figure 8.17).

Of the 667 clinical VRE/VVE isolates sequenced in 2022, 60 were *vanA E. faecium*, 604 *vanB E. faecium*, 1 *vanA*/*vanB E. faecium*, and 2 *vanB E. faecalis* (Figure 8.17). Until 2020, *vanA E. faecium* were most common, but during the last years this has changed. In 2022, 91% of the *E. faecium* isolates had the *vanB* gene.

WGS-based cgMLST analysis was performed on the 665 E. faecium isolates using SeqSphere+ (Ridom), where a total of 137 unique clonal types (CTs) were observed. When investigating the composition of CTs for *E. faecium*, we observed a clustering tendency between isolates, where CTs were diverging while the allelic differences were minimal within each cluster. To investigate further, Local Single Linkage clustering (SLC) was set up in SeqSphere+, setting the maximal allelic distances to 20. A total of 110 SLCs were detected, of which 70 clusters consisted of 5 or fewer isolates. Each SLC was named according to the ST and CT of the first observed isolate within each cluster. Of these clusters, one SLC (covering several different CTs, but presumably originating from the same clone) was predominant in the Danish surveillance: The ST80-CT2406 *vanB E. faecium* group containing 468 isolates (Table 8.12).

Figure 8.17 Overview and distribution of vancomycin resistance genes in vancomycin-resistant isolates, Denmark, 2015-2022

DANMAP 2022

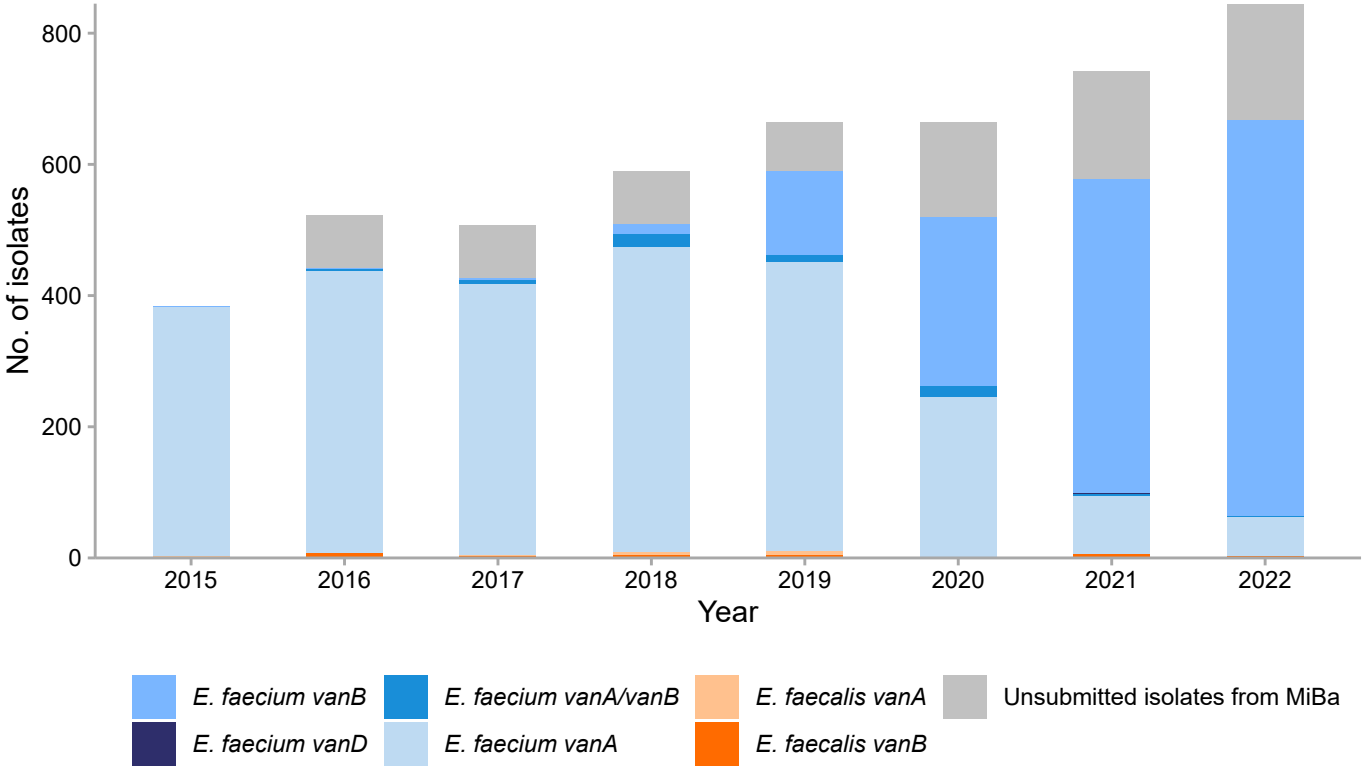


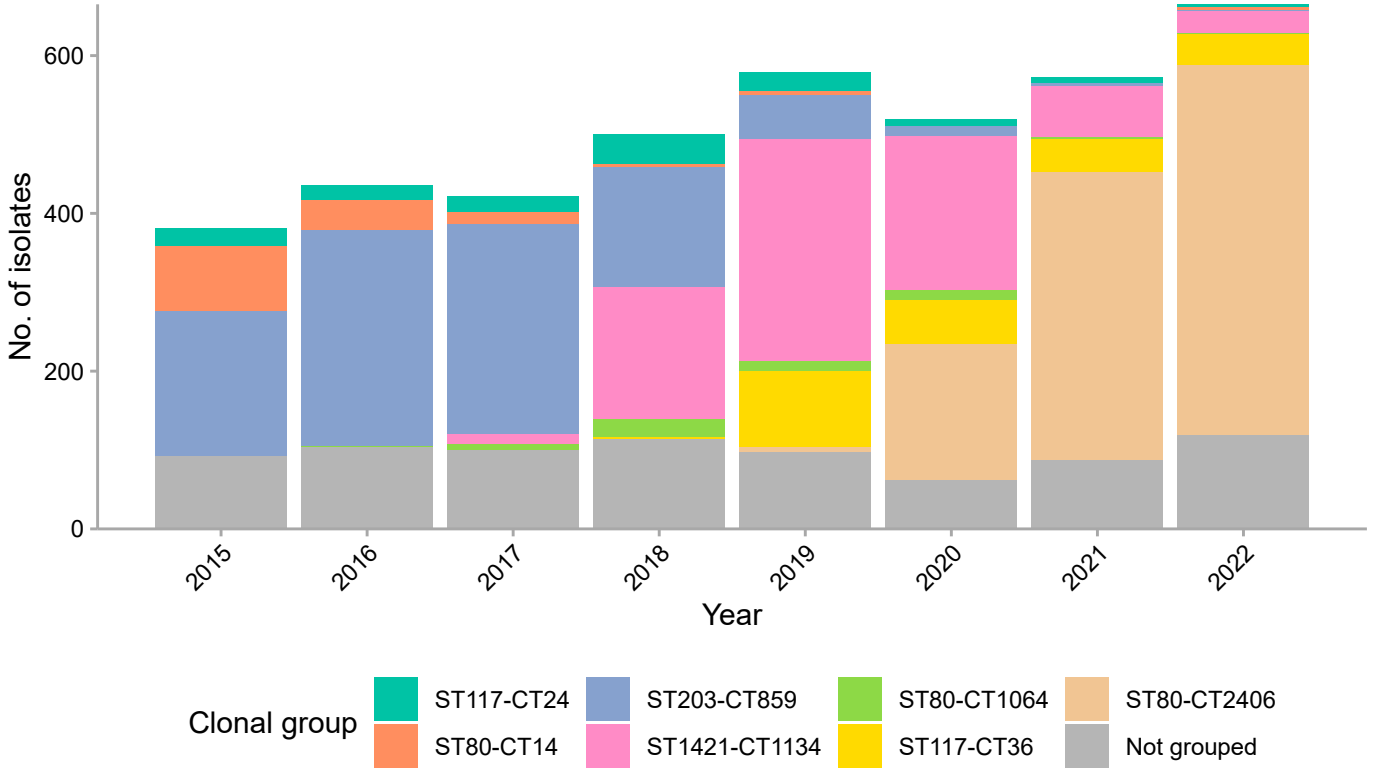
Table 8.12 Description of the most common types of *vanA* and/or *vanB Enterococcus faecium* according to MLST and cgMLST, Denmark, 2016-2022

DANMAP 2022

Types [a]	2016		2017		2018		2019		2020		2021		2022		All years
	(n = 435)		(n = 426)		(n = 518)		(n = 584)		(n = 519)		(n = 565)		(n = 609)		Total
ST117-CT24 group [b]	19	4%	20	5%	38	7%	26	4%	8	2%	7	1%	4	1%	147
ST80-CT14 group [c]	39	9%	16	4%	3	1%	3	1%	1	0%	0	0%	3	0%	147
ST203-CT859 group [d]	273	63%	265	62%	156	30%	57	10%	12	2%	3	1%	2	0%	952
ST1421-CT1134 group [e]	1	0%	12	3%	167	32%	285	49%	197	38%	63	11%	27	4%	752
ST80-CT1064 group [f]	2	0%	7	2%	23	4%	12	2%	13	3%	3	1%	2	0%	62
ST117-CT36 groupG	0	0%	0	0%	3	1%	95	16%	56	11%	43	8%	40	6%	237
ST80-CT2406 group [h]	0	0%	0	0%	0	0%	7	1%	174	34%	356	63%	468	68%	1,005
ST117-CT1686	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	15	2%	15
ST80-CT6438	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	10	1%	10
Other types	101	23%	106	25%	128	25%	99	17%	58	11%	90	16%	120	17%	927

a) ST, sequence type (MLST); CT, cluster type (cgMLST)
b) **CT24**,CT875,CT1180,CT1487,CT1834,CT2456,CT6018
c) **CT14**,CT869,CT1530,CT1797,CT2019
d) **CT859**,CT1051,CT1507,CT1688,CT2257,CT2758,CT5973
e) **CT1134**,CT1749,CT1854,CT2545,CT2911,CT3379,CT5936,CT6048
f) **CT1064**,CT2496,CT6123,CT6520
g) **CT36**,CT991,CT1526,CT2531,CT2659,CT2979
h) **CT2406**,CT2946,CT2949,CT3024,CT3234,CT4189,CT4835,CT5120,CT5143,CT5166,CT5211,CT5215,CT5928,CT5972,CT5974, CT5999,CT6117,CT6132,CT6253,CT6254,CT6417,CT6435,CT6435,CT6436,CT6494,CT6507,CT6531,CT6547,CT6598,CT6610

Figure 8.18 Timeline of the clonal group prevalence in all sequenced VRE isolates. Clonal groups are named according to sequence type and clonal type of the earliest observed member, Denmark, 2015-2022 DANMAP 2022



Retrospectively, three clonal groups were conspicuous in the sense of being predominant for a limited time period (see Table 8.12 and Figure 8.18). During 2015-2017, the ST203-CT859 *vanA E. faecium* clonal group was the most prevalent clone. It has since decreased in prevalence and in 2022 less than 1% of the VRE/VVE *E. faecium* isolates belonged to ST203-CT859. In 2017, testing for the presence of *vanA*/*vanB* genes by use of PCR in phenotypically vancomycin-susceptible *E. faecium* isolates from blood cultures was introduced in the DCMs in the Capital Region as a mean of detecting possible VVE [Hammerum *et al.* Euro Surveill. 2020;25(18)]. At that time, the ST1421-CT1134 *vanA E. faecium* clonal group only constituted 3% of the total clinical VRE/VVE *E. faecium* isolates. This clone was initially only detected from clinical samples from the Capital Region, yet in 2018 and 2019 its prevalence increased to 32% and 49%, respectively, and it was now observed in the Capital Region and Zealand Region [Hammerum *et al.* Euro Surveill. 2020;25(18)]. It has since been found in all five regions of Denmark. While the ST1421-CT1134 *vanA E. faecium* VVE clone became the predominant clone throughout 2019 and 2020, it was overtaken by the ST80-CT2406 *vanB E. faecium* clone in 2021, where it decreased to 11%. The earliest detection of the ST80-CT2406 *vanB E. faecium* clone in Denmark was in 2019, where it was present in 1% of the isolates. From 2020 to 2022, the ST80-CT2406 *vanB E. faecium* clone increased from 34% to 68%. Since its introduction, the clone has been detected in all of the five regions, where 74% of cases has been observed on Zealand and in the Capital Region. It is the most frequently occurring clone of the ST80-CT2406 clonal group, and the group itself is the most diversified clonal group, spanning 20 different CT clones. Detailed in Figure 8.18.

Conclusion

The number of VRE/VVE cases increased again in 2022 compared to 2021. Thus it remains clear that more prevention strategies are required to break the annual trend of increasing occurrence of VRE in the Danish health care system.

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8.3.4 Detection of linezolid-resistant enterococci and linezolid-vancomycin-resistant enterococci

Background

Linezolid is an antimicrobial belonging to the oxazolidinones. Its indication of use are nosocomial pneumonia and complicated skin and soft tissue infections caused by Gram-positive bacteria. It belongs to the defined last line choices and should be used with caution, based on microbiological testing, and only if other antibiotics are not available. In Denmark it is primarily used in combination treatments for patients with very complicated Gram-positive infections, and as treatment against vancomycin-resistant enterococci. Resistance to linezolid in enterococci is often due to mutations in the V domain of the 23S rRNA genes. The G2576T mutation (*Escherichia coli* numbering) is the most common cause of the linezolid-resistant enterococci (LRE) phenotype. Another mutation in the 23S rRNA, G2505A (*E. coli* numbering) has also been reported for LRE, but seems to be less frequent than the G2576T mutation. Furthermore, transferable resistance genes (*cfr*, *cfr(B)*, *optrA* and *poxtA*) encoding linezolid resistance have been described in enterococci, whereas amino acid substitutions in the ribosomal proteins L3, L4 and/or L22, suspected to cause decreased susceptibility to linezolid in staphylococci, are rare in enterococci.

Particular interest has been paid to the transferable resistance genes. These are monitored in the Danish surveillance system due to a potential risk of a shared pool of resistance genes with enterococci stemming from animals that have been treated with pleuromutilines, a group of antimicrobials related to the oxazolidinones.

In 2018, a web tool for detection of the 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and *poxtA* genes from whole genome sequences from enterococci was developed [Hasman *et al.*, J. Antimicrob. Chemother., 2019, 74:1473-1476].

Surveillance of linezolid-resistant enterococci (LRE)

Danish departments of clinical microbiology (DCMs) have, on voluntarily basis, submitted LRE for surveillance to the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (SSI).

In 2022, four linezolid-resistant *E. faecalis* isolates and one linezolid-resistant *E. faecium* were sent to SSI. WGS data from the LRE isolates were investigated for 23S rRNA mutations (G2576T and G2505A), and the *optrA*, *cfr*, *cfr(B)* and poxtA genes using the LRE-Finder (https://cge.cbs.dtu.dk/services/LRE-Finder/).

The four *E. faecalis* isolates and the *E. faecium* were all positive for *optrA* (Table 8.13). During the period 2015-2021, 15 linezolid-resistant *E. faecalis* were detected, whereas eight linezolid-resistant *E. faecium* were identified.

Surveillance of linezolid-vancomycin-resistant Enterococci (LVRE)

In order to detect linezolid-resistant VRE isolates (LVRE), the LRE-Finder was also implemented in the automated surveillance at SSI for detection of LRE mutations and LRE genes in genomes from VRE isolates submitted by the DCMs directly for the national VRE Surveillance. During the period 2015-2021, no linezolid-vancomycin-resistant *E. faecalis* were detected, whereas 36 linezolid-vancomycin-resistant *E. faecium* were identified.

In 2022, 15 linezolid-vancomycin-resistant *E. faecium* were identified. All 15 linezolid-resistant *E. faecium* isolates had the G2576T mutation, five of these were positive for the *vanA* gene and 10 were positive *vanB* (Table 8.13).

Conclusion

As in previous years the numbers of LRE and LVRE have been low, however the findings are of concern as linezolid is important for the treatment of VRE. Often, only one antimicrobial agent is available for treatment of infections with LVRE.

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Table 8.13 Characterization of the 5 linezolid-resistant enterococci (LRE) and the 15 linezolid-vancomycin-resistant enterococci (LVRE), 2022, Denmark DANMAP 2022

	No. of isolates	Species	Linezolid resistance mechanism	Vancomycin resistance gene
LRE	4	*E. faecalis*	*optrA*	none
	1	*E. faecium*	*optrA*	none
LVRE	5	*E. faecium*	G2576T	*vanA*
	10	*E. faecium*	G2576T	*vanB*

8.3.5 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa. Approximately 50% of humans will be colonized with *S. aureus* in their nose or throat at any given time, some of these carrying the strain only intermittently, others for longer periods of time. *S. aureus* causes infections ranging from superficial skin infections, e.g. impetigo and boils, to deeper and more complicated infections. These may be health care associated such as post-operative wound infections and infections related to intravenous catheters and prosthetic devices but they can also be caused by endogenous spread of bacteria causing septic arthritis, osteomyelitis, endocarditis and bacteraemia. Most of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteraemia cases was established in 1957. By comparison with the numbers of bacteraemia cases registered in the MiBa since 2010, the number of reported cases and bacterial isolates submitted to SSI has been almost complete (94-97%). Laboratory and clinical notification of all cases of methicillin-resistant *S. aureus* (MRSA) has existed since November 2006.

Surveillance of *S. aureus* bacteraemia

The number of *S. aureus* bacteraemia cases were 2,578 in 2022 corresponding to 44 cases per 100,000 inhabitants. This is almost the same number as in 2021 (2,511). Fifty (1.9%) of the bacteraemia cases were caused by MRSA. During the last decade this proportion has been between 1.3% (2012) and 2.9% (2014) and remains below most other European countries participating in EARS-Net [EARS-Net 2021]. Livestock-associated (LA) - MRSA CC398 caused seven of the 50 MRSA bacteraemia cases. Within 30 days from the bacteraemia onset, 627 (24%) patients died (all-cause mortality). The mortality for the MRSA bacteraemia cases was 18%.

Table 8.14 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases 2013-2022, Denmark DANMAP 2022

	2013	2014	2015	2016	2017	2018	2019	2020*	2021	2022
Antimicrobial agent	%	%	%	%	%	%	%	%	%	%
Methicillin	1.7	2.9	1.5	2.1	2.2	1.6	2.1	1.6	1.6	1.9
Penicillin	76	77	71	71	72	72	72	72	69	68
Erythromycin	7	8	7	7	6	5	9	7	7	9
Clindamycin	6	8	7	6	5	4	8	7	7	8
Tetracycline	3	5	4	3	3	3	2	3	2	3
Fusidic acid	15	15	16	12	14	17	14	14	13	13
Rifampicin	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Moxifloxacin#	5	6	6	4	4	4	5	6	4	4
Kanamycin	2	2	3	1	1	2	<1	nt	nt	nt
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	0	<1	0	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	1	1	<1	<1	<1	0	<1	<1	<1	<1

nt = not tested. * From 2020 and onwards, resistance data was extracted from the Danish Microbiology database, MiBa. # Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Table 8.15 The ten most prevalent *spa* types demonstrated in SAB and in non-LA-CC398 MRSA cases, Denmark 2022 DANMAP 2022

SAB			MRSA			
spa type	CC group	No. of cases	*spa* type	CC group	No. of cases	No. causing infections (%)
t127	CC1	120	t304	CC8	229	114 (50)
t084	CC15	115	t127	CC1	168	79 (47)
t091	CC7	101	t223	CC22	122	55 (45)
t002	CC5	96	t002	CC5	121	71 (59)
t230	CC45	78	t008	CC8	110	74 (67)
t008	CC8	73	t4549	CC8	85	69 (81)
t012	CC30	69	t005	CC22	56	33 (59)
t701	CC8	55	t021	CC30	49	31 (63)
t015	CC45	51	t688	CC5	42	29 (69)
t1451	CC398	48	t1476	CC8	37	23 (62)

CC = Clonal complex, SAB = *S. aureus* bacteraemia

The antimicrobial susceptibility remained at the same level as the previous years for most agents (Table 8.16). Resistance to penicillin in 2022 was 68%, which confirms the decreasing trend since the beginning of the 1990s, where resistance to penicillin was around 86%. The highest frequency of resistance to antimicrobials other than penicillin was observed for fusidic acid (13%), erythromycin (9%) and clindamycin (8%).

Typing revealed a high diversity with 727 different *spa* types distributed in 27 different clonal complexes (CCs). The ten most prevalent *spa* types, representing 31% of the total number, are presented in Table 8.17 together with *spa* types from the most prominent types of MRSA. The distribution of the most prevalent *spa* types has been very stable over the last decade with only minor changes in the relative order. The Panton-Valentine Leukocidin (PVL) toxin was present in 21 (1%) cases of which six were MRSA. The 21 isolates with the PVL gene were distributed among 15 different *spa* types.

Surveillance of methicillin-resistant *S. aureus*

In 2022, 2,996 MRSA cases were detected (51 per 100,000 inhabitants), a 10% increase compared to 2021 (2,712; Figure 8.21a). The number of new cases was still below the pre-covid levels (3,657 cases registered in 2019). A case was defined when a person for the first time tested positive for a specific MRSA strain regardless of clinical situation (infection or colonisation). Infections constituted 51% of the cases. The proportion of infections in the years 2013 to 2022 has varied between 38% to 51% (Figure 8.21b).

CC398 cases constituted 28% (n = 853) of new MRSA cases, of which 828 belonged to the livestock-associated clone (LA-MRSA CC398) and the remaining 25 to a human adapted variant harbouring the PVL encoding genes. More LA-MRSA CC398 isolates (72%) were found in healthy carriers compared to MRSA of other types (41%), which likely reflects the active screening of patients with contact to livestock at admission to healthcare.

MRSA isolates carrying *mecC* were detected in 55 cases (1.8%). Thirty-nine of the cases (71%) had infections at the time of diagnosis. Three patients reported contact to hedgehogs, which recently has been shown to be a major environmental reservoir for *mecC* MRSA (see Textbox 3.3, Chapter 3, DANMAP 2021). One patient worked with livestock, one patient had a family member working with livestock, while the remaining 50 patients did not report any contact to livestock or other animals.

spa typing revealed 357 different strain types, not including isolates belonging to LA-CC398. Among the infections, 299 *spa* types were identified. The 10 dominating non-LA-CC398 *spa* types isolated in 2022 are listed in Table 8.17. They constituted 28% of the total number of non-LA-CC398 MRSA isolates. Table 8.17 does not list *spa* types in CC398, which for many years has been the dominating CC. *spa* types t304 and t223 have been among the five most prevalent *spa* types since 2016, when their increase was linked to the refugee crisis following the civil war in Syria. No change in *spa* type distribution has so far been registered following the war in Ukraine.

Figure 8.19a Number of new MRSA cases 1994-2022, Denmark, with a three years moving average DANMAP 2022

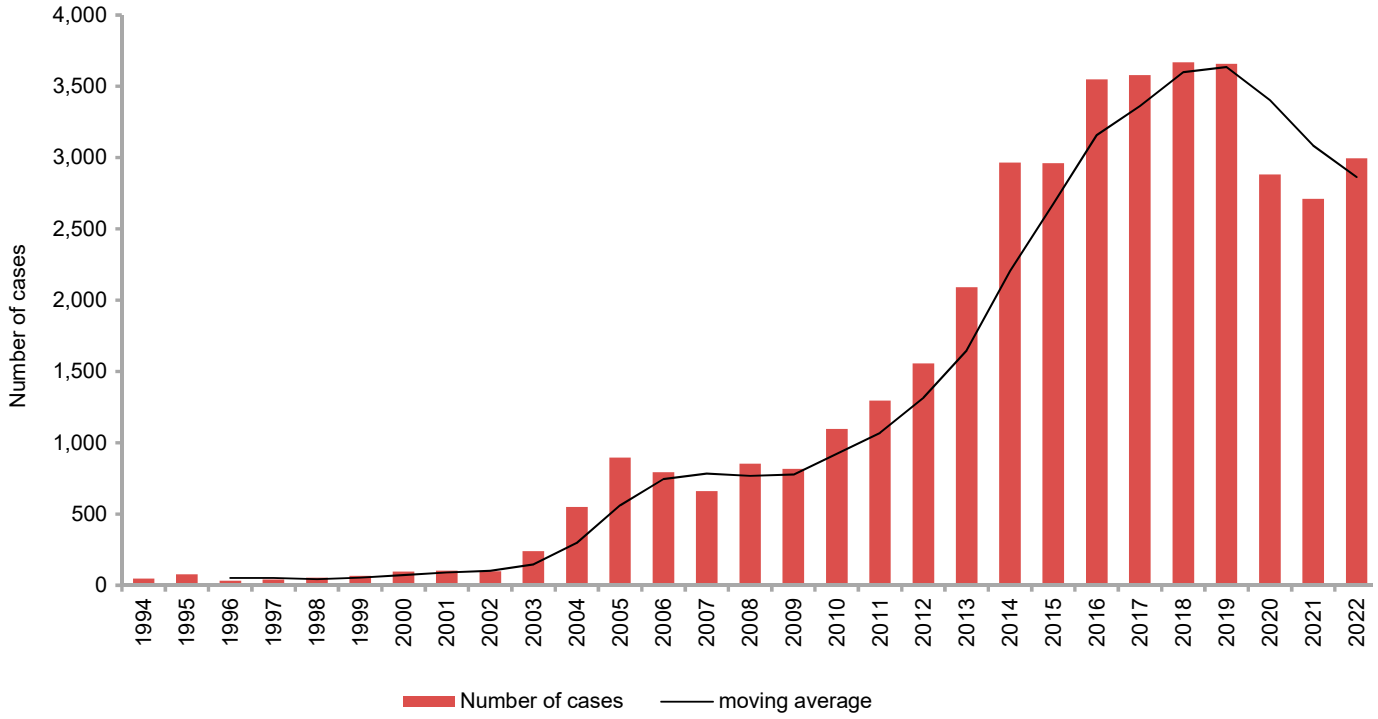

Figure 8.19b Number of new MRSA cases 2013-2022, Denmark, divided in infection and screening samples DANMAP 2022

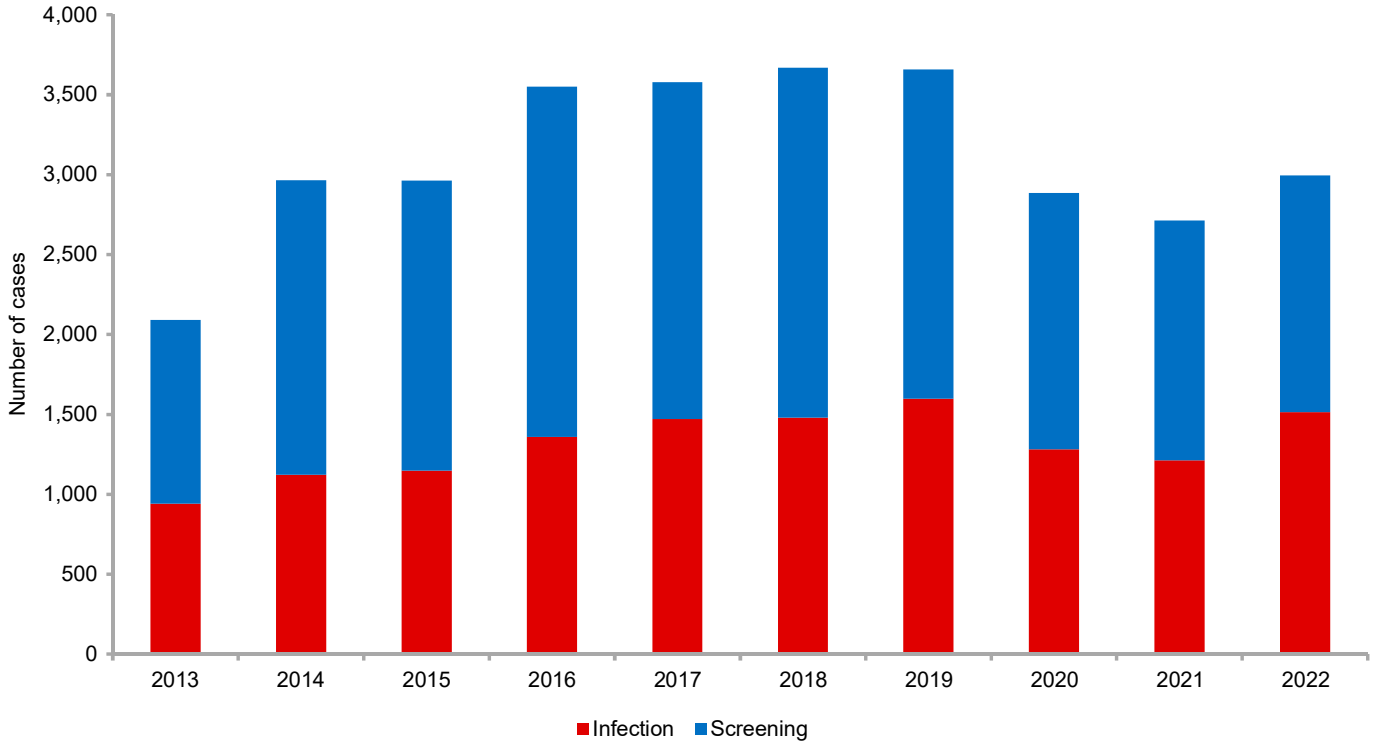

Table 8.16 Epidemiological classification of new MRSA cases, Denmark, 2022 DANMAP 2022

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections (% of cases in same epi. class)
Imported (IMP)		**423 (14)**	279 (66)
Hospital-acquired (HA)		**55**	30 (55)
"Health-care associated, community onset (HACO)"		**232 (8)**	
	with known exposure	14	6 (43)
	without known	218	184 (84)
Health care worker		**28 (1)**	20 (71)
Community-acquired (CA)		**1,430 (48)**	
	with known exposure	653	99 (15)
	without known	777	666 (86)
LA-MRSA CC398		**828 (28)**	
	with known exposure	697	138 (20)
	without known	131	92 (70)
Total		**2,996**	**1,514 (51)**

Numbers shown in bold are totals

The PVL encoding gene was detected in 24% of the infections and in 13% of the asymptomatic carriers and most often in relation to isolates with *spa* types t008 (n = 67), t005 (n = 39), t021 (n = 37), t002 (n = 35) and t127 (n = 23).

Thirty-nine MRSA outbreaks were registered at hospitals, nursing homes and other institutions. These outbreaks comprised a total of 143 cases of which 70 had an infection. Seven of the outbreaks occurred in neonatal departments, comprising a total of 46 cases. Additionally, eight outbreaks were registered in other hospital departments, comprising 17 patients and thirteen outbreaks were observed in nursing homes (counting a total of 30 residents).

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.16. Most of the cases (86%) were acquired in Denmark.

Figure 8.20 Number of MRSA infections according to epidemiological classification, 2013-2022 Denmark DANMAP 2022

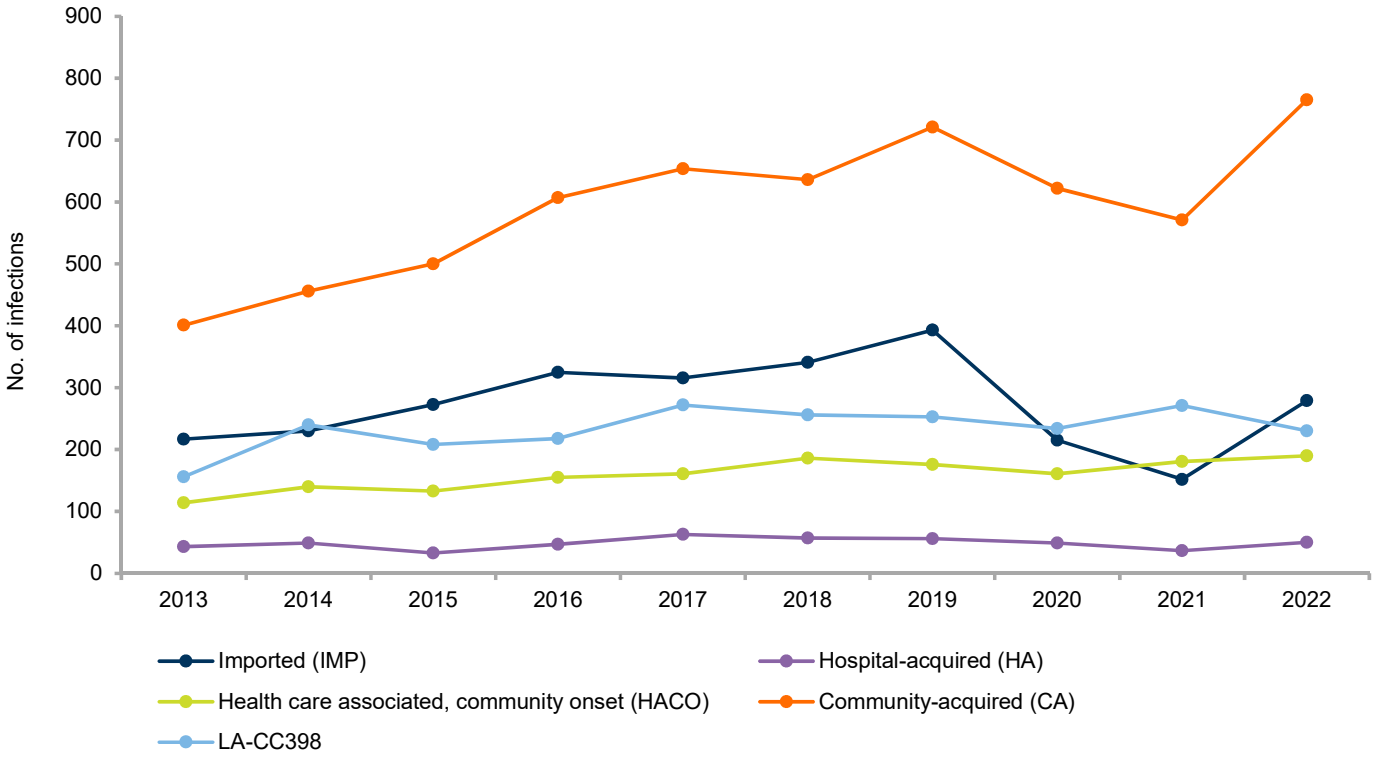

Table 8.17 Resistance (%) in non LA-CC398 MRSA isolates, 2013-2022, Denmark DANMAP 2022

	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Erythromycin	32	33	37	34	34	33	33	30	29	34
Clindamycin	24	23	29	25	27	28	23	22	19	21
Tetracycline	20	21	24	26	24	26	22	22	21	23
Fusidic acid	17	17	19	18	16	18	23	22	21	22
Rifampicin	1	<1	<1	1	1	1	<1	<1	<1	<1
Moxifloxacin#	23	27	21	19	20	21	21	17	19	23
Linezolid	<1	<1	0	<1	0	<1	0	<1	<1	<1
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim-sulfamethoxazole	3	3	4	2	3	3	4	2	<1	1
Number of tested isolates	1,451	1,616	1,242	1,184	1,193	1,233	1,025	1,920*	1,520*	2,043*

* Not all isolates were tested for all listed antimicrobials
\# Prior to 2021 testing for fluoroquinolone resistance was reported for norfloxacin

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates are presented in Table 8.17. Resistance prevalences were similar to previous years, with relatively high resistance to erythromycin (34%), fusidic acid (22%), clindamycin (21%), tetracycline (23%) and moxifloxacin (23%), and low resistance (<1%-1) to trimethroprim-sulfamethoxazole, linezolid, mupirocin and rifampicin.

Conclusion

The number of *S. aureus* bacteraemia cases (N=2,578) was almost the same in 2022 as in 2021, with a still low proportion of MRSA <2%. The number of MRSA increased in 2022 after two years of lower numbers due to COVID-19 related restrictions. This was also reflected in an increased number of imported and community-acquired cases, while number of LA-MRSA CC398 decreased.

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The Danish Infection prevention and control guidelines for MRSA, CPO, VRE and other MDRO

The growing numbers of multidrug-resistant bacteria globally demand increased and continued focus on the use of infection prevention and control measures at all levels in the health care system. Although Denmark is regarded a low-prevalent country, it takes efforts to keep the number of outbreaks low and, despite the efforts some of these outbreaks continue to develop. Historically, the emergence of MRSA at Danish hospitals in the 1980´ies paved the way for both surveillance of *S. aureus* infections and infection prevention and control programmes at hospitals, but only within the recent ten to fifteen years, IPC programmes have become more formalised and nationwide. Currently, the following specific programmes exist:

The Guidance on Preventing the Spread of MRSA by the Danish Health Authority was issued in 2006, the year MRSA became notifiable. The applicable 3rd edition of the guideline is from 2016 (Guidance on Preventing the Spread of MRSA - The Danish Health Authority (sst.dk)). The guideline represents the national recommendations and strategic framework for preventing the spread of MRSA at hospitals and in other healthcare settings and nursing homes. Upon referral to a hospital the following high-risk situations are screened for in a patient: being previously diagnosed with MRSA, household-like contact with persons tested positive for MRSA, travelling outside the Nordic countries with admission to a hospital or clinic, and frequent contact with live pigs (for the particular risk of being a carrier of livestock-associated MRSA). Individual risk factors for acquiring colonization or infection with MRSA are wounds, chronic skin diseases and invasive devices etc. As strategy the guideline applies a `search and destroy´ approach: MRSA-carriers can be treated and be declared MRSA-free (in contrast to persons carrying resistant bacteria in the gut, e.g. CPE). Being MRSA-free demands testing negative thrice within a minimum period of six months after completion of treatment. In the case of an outbreak at hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-mrsa]. All patients with MRSA are isolated in single rooms or cohort isolation in case of outbreak at hospitals (with contact isolation regime). This is not the case at nursing homes.

The first **national guideline on preventing the spread of CPO by** the Danish Health Authority was issued in 2018 (www.sst.dk/da/udgivelser/2018). The guideline provides a national strategic framework for detection and management of CPO at hospitals. The main purpose of the guideline is to maintain a low prevalence of disease caused by CPO associated with certain high-risk situations. Upon admission to a hospital a patient is screened for: previous diagnosis of CPO, household-like contact with a CPO positive person, travelling outside the Nordic countries with admission to a hospital or clinic and/or receiving antibiotic treatment during the stay.

Individual risk factors for acquiring colonization or infection with CPO are older age (above 80 years), immunosuppression caused by medication or certain chronic and cancer diseases, antibiotic treatment and invasive devices. It is noteworthy that patients are known to be carriers of CPE for several years (in some of the Danish outbreaks more than five years) with no treatment for CPE carrier state available. As a result, compliance with IPC guidelines is extremely important in order to prevent further spread of CPE, and correct hand hygiene and use of personal protective equipment (PPE) are among the most important control measures.

In hospitals, nursing homes and home care, it is necessary to supplement the general precautions with transmission based (isolation) precautions in the case of an outbreak [https://hygiejne.ssi.dk/retningslinjer/infektionshygiejniske-retningslinjer-for-cpo]. All patients with CPO are isolated in single rooms or cohort isolation in case of outbreak at hospitals (with contact isolation regime). This is not the case at nursing homes.

In relation to hospital outbreaks, environmental spread of CPO has occurred through bath drains and toilets. After intensive cleaning and disinfection of these parts it has in some cases been possible to stop an outbreak. This emphasizes the importance of more frequent cleaning and disinfection in an outbreak situation and the importance of well-functioning drains in general.

Infection prevention and control guidelines for VRE and other multidrug-resistant microorganisms (MDRO)
All healthcare professionals are expected to be familiar with and act in compliance with the national guidelines for infection prevention and control (published by the National Centre for Infection Control at SSI); [https://hygiejne. ssi.dk/NIRgenerelle]. The supplemental national guideline includes specific guidance on VRE/VVE and other multidrug-resistant microorganisms and should be followed when being in contact with a patient, for which either clinical infection or carriage of MDRO is suspected or known [https://hygiejne.ssi.dk/NIRsupplerende]. Precautions include the use of PPE such as a disposable gown and gloves and proper cleaning of the environment [https://hygiejne.ssi.dk/NIRdesinfektion]. As for MRSA and CPO screening of a patient for either VRE or other MDRO is recommended on admission to hospital for defined risk situations, such as travel and hospitalisation outside the Nordic countries or known exposure to a patient with VRE or other MDRO at a Danish ward. Isolation is recommended in the national supplemental IPC-guideline upon verification of VRE in the patient.

All IPC guidelines emphasise the importance of all health care staff, irrespective of profession, to contribute to the management and prevention of infections and all prescribers to prescribe antibiotics with care.

8.3.6 *Streptococcus pneumoniae*

Streptococcus pneumoniae is known to cause various diseases that can be classified into two main groups: non-invasive and invasive. Among these, invasive pneumococcal diseases (IPD) are considered the most severe, while non-invasive pneumococcal infections are more commonly observed. Pneumonia, which can be categorized as non-invasive or invasive, is estimated to be the leading pneumococcal disease worldwide, affecting both children and adults. This is followed by the severe cases of invasive bacteraemia and meningitis. Pneumococci often cause the non-invasive acute otitis media (AOM) in children, with more than half of Danish children estimated to experience this condition. AOM is frequently treated with antibiotics, although their effectiveness is uncertain. In addition to these well-known pneumococcal-related diseases, pneumococci are also associated with other common infections such as non-invasive sinusitis and bronchitis, as well as invasive diseases like endocarditis, peritonitis, and septic arthritis.

The surveillance of pneumococci (*S. pneumoniae*) causing invasive disease in Denmark is conducted through mandatory submission of clinical isolates from invasive cases to Statens Serum Institut (SSI). At SSI, the isolates are serotyped and tested for antimicrobial susceptibility. Any remaining cases without a submitted isolate are identified through MiBa.

Table 8.18 Number of invasive pneumococcal isolates (IPD) observed in Denmark, 2022 DANMAP 2022

Serotype	Included in pneumococcal vaccines	N 2022	PEN-S	PEN-NON-S	Unknown	% Suscep
Unknown		46	0	0	46	0
4	PCV13, PCV15, PCV20, and PPV23	4	4	0	0	100%
6B	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
9V	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
14	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
18C	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
19F	PCV13, PCV15, PCV20, and PPV23	10	10	0	0	100%
23F	PCV13, PCV15, PCV20, and PPV23	1	0	1	0	0%
1	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
3	PCV13, PCV15, PCV20, and PPV23	107	106	1	0	99%
5	PCV13, PCV15, PCV20, and PPV23	0	0	0	0	0%
6A	PCV13, PCV15, and PCV20	2	1	1	0	50%
7F	PCV13, PCV15, PCV20, and PPV23	1	1	0	0	100%
19A	PCV13, PCV15, PCV20, and PPV23	14	13	1	0	93%
2	PPV23	0	0	0	0	0%
8	PCV20 and PPV23	81	81	0	0	100%
9N	PPV23	16	14	2	0	88%
10A	PCV20 and PPV23	14	14	0	0	100%
11A	PCV20 and PPV23	24	23	1	0	96%
12F	PCV20 and PPV23	4	4	0	0	100%
15B	PCV20 and PPV23	7	7	0	0	100%
17F	PPV23	9	6	3	0	67%
20	PPV23	4	4	0	0	100%
22F	PCV15, PCV20, and PPV23	39	39	0	0	100%
33F	PCV15, PCV20, and PPV23	14	14	0	0	100%
15A		18	16	2	0	89%
7C		18	18	0	0	100%
23B		17	7	10	0	41%
24F		15	15	0	0	100%
35F		15	15	0	0	100%
16F		15	15	0	0	100%
Other		64	58	6	0	91%
Sum		560	486	28	46	95%*

N = number of isolates, PEN-S = Penicillin-susceptible, PEN-NON-S = Penicillin non-susceptible, % Suscep = percentage of IPD isolates susceptible to penicillin. For serotypes not covered by vaccines, isolates were grouped in other if there were fewer than 15
* Calculation = 486 / (560-46).

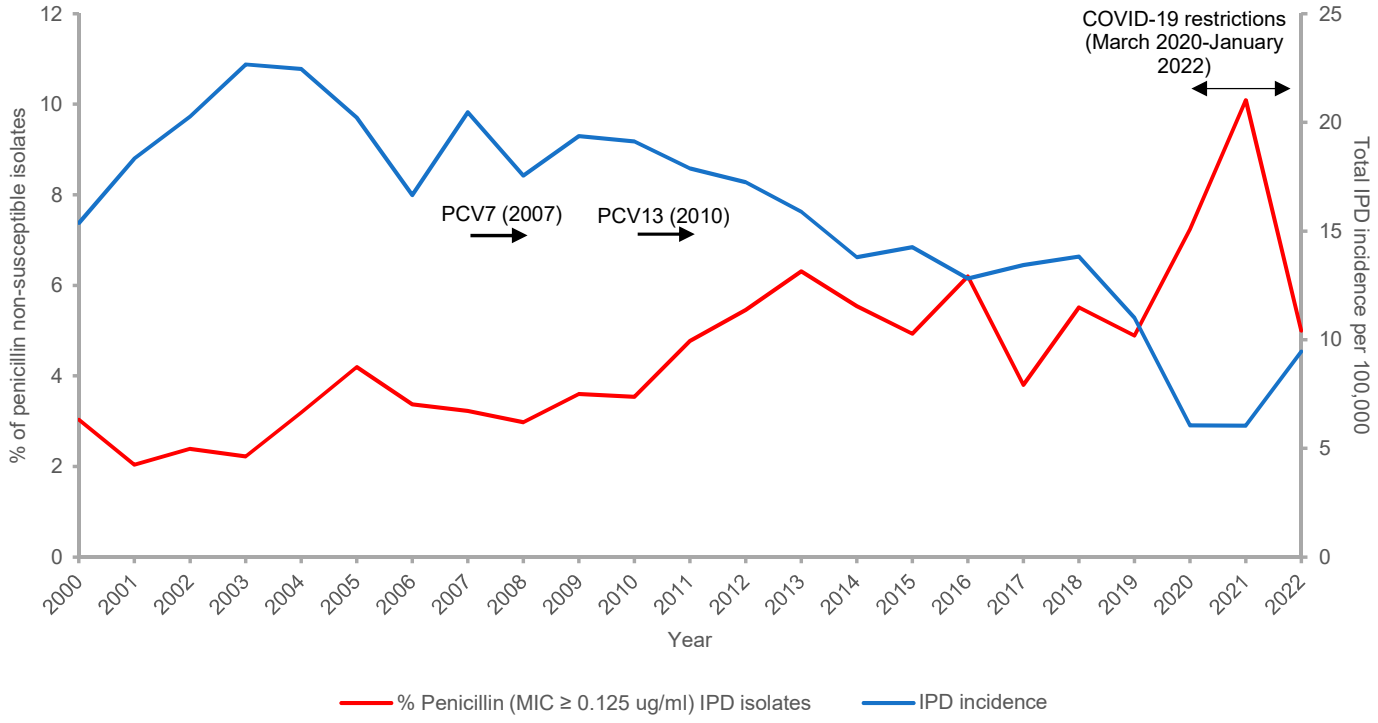
In Denmark, 560 cases of invasive pneumococcal disease (IPD) were registered in 2022 (Table 8.18). The cases were mainly from pneumococci found in either blood (479) or cerebrospinal fluid (70). For 11 cases, pneumococci had been found in other, normally sterile sites. Traditionally, the IPD cases found in other sterile sites than blood and cerebrospinal fluid, have not been included in the DANMAP report, however in 2022 these cases are included in the report. Of the 560 IPD cases identified in MiBa, 514 isolates were received at the reference laboratory. Data for the 46 remaining cases, where isolates were not provided, were retrieved from MiBa and serotypes for these cases are unknown. Failure of submitting an isolate for serotyping was mainly due to non-viable isolates or diagnosis through PCR. In total, data for serotypes and antimicrobial susceptibility data for penicillin were available for 514 cases.

The IPD isolates belonged to 49 different serotypes. For the 514 cases with available penicillin susceptibility data, 486 isolates were susceptible to penicillin (94.6%), and 28 isolates (5.4%) were classified as non-susceptible to penicillin.

The pneumococcal vaccine introduced in the childhood vaccination programme in 2007 has had a profound effect on the serotypes that are causing invasive pneumococcal disease in Denmark, as it is clear that IPD caused by the seven serotypes included in the first vaccine (PCV7 vaccine from 2007) and later 13 serotypes included in the second vaccine (PCV13 vaccine from 2010) has decreased. The predominant serotype in 2022 was serotype 3 (20.8%) (Table 8.18). One serotype 3 isolate was classified as non-susceptible, while the remaining 106 isolates were susceptible to penicillin. The second and third most frequently isolated serotypes are serotype 8 (81 isolates in 2022) and serotype 22F (39 isolates in 2022), and these isolates were all fully sensitive to penicillin.

Figure 8.21 The incidence of invasive pneumococcal infection (IPD) and the percentage of penicillin non-susceptible IPD isolates in Denmark, 2000–2022. PCV-7 was introduced into the child vaccination program in 2007, and replaced with PCV13 in 2010

DANMAP 2022



Conclusion

The level of penicillin non-susceptible IPD isolates returned to pre covid-19 level in 2022 (5%) compared to the two previous years (10.1% (2021) and 7.2% (2020)). The incidence of invasive pneumococcal disease has also increased to pre-pandemic level, compared to the years 2021 and 2020. Both the great fluctuation in the prevalence of penicillin non-susceptible IPD isolates and the general IPD incidence in the years 2020 and 2021 is assumed to be an effect of the COVID-19 restrictions on the general prevalence of infectious diseases associated with respiratory droplet transmission. Such fluctuations have been seen in other countries as well [Shaw, et al., Lancet Digit Health. 2023 Sep;5(9):e582-e593].

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8.3.7 Beta-haemolytic streptococci

Beta-haemolytic streptococci (BHS) are classified into serological groups according to antigenic properties of the polysaccharide capsule. In human infections, groups A, B, C and G are the most frequent species. Approximately 10% of humans are asymptomatic throat carriers of BHS of any group.

Streptococcus pyogenes (group A streptococci; GAS) cause pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections, e.g., bacteraemia, necrotizing soft tissue infections, and rarely meningitis. In Denmark, the rate of asymptomatic throat carriage of GAS is approximately 2%.

Streptococcus agalactiae (group B streptococci; GBS) may be present in the vaginal flora of 20-25% of women in the childbearing age. If these bacteria are transferred to the child during labour, meningitis and septicaemia may develop in the newborn. Such infections also occur in elderly or immunocompromised patients.

Streptococcus dysgalactiae subsp. *equisimilis* (group C streptococci; GCS, and group G streptococci; GGS) predominantly cause soft tissue infections and sometimes bacteraemia.

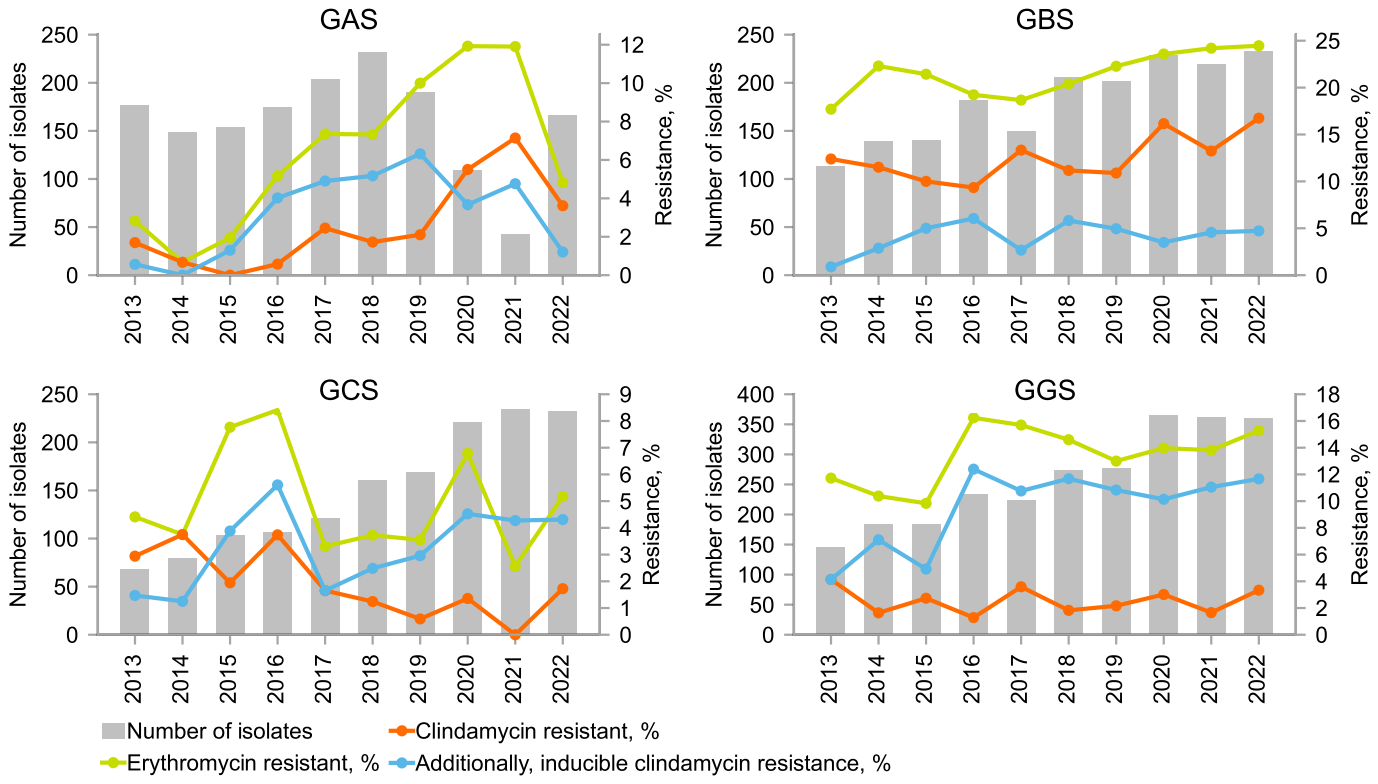
This report presents data on antimicrobial resistance in invasive isolates (i.e. from blood, cerebrospinal fluid or other normally sterile anatomical sites) submitted from the departments of clinical microbiology (DCMs) in 2022 to the Neisseria and Streptococcus Reference laboratory (NSR). This report includes only non-duplicate isolates, i.e. isolates obtained with an interval of more than 30 days. Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of BHS.

Infections with BHS are usually treated with penicillins or macrolides. All submitted isolates of BHS were therefore tested for susceptibility to penicillin, erythromycin, and clindamycin as well as inducible clindamycin resistance. Susceptibility testing was performed with disk diffusion methods. If resistance was observed, MIC was determined with Etest. All results were interpreted according to MIC breakpoints issued by EUCAST (https://www.eucast.org/) (version 12.0). For all isolates of GAS the *emm* type was determined by whole genome sequencing of the portion of the *emm* gene that dictates the M protein serotype.

In 2022, a total of 1,046 isolates were received. The number of isolates from unique cases was 991, an increase of 16% compared to 2021 (857). Corresponding changes for individual serogroups were: GAS, + 295%; GBS, + 6%; GCS, - 0,9%; and GGS, -0,6%.

Figure 8.22 shows the resistance findings for the years 2013 through 2022. All isolates were fully susceptible to penicillin. Comparing 2022 to 2021, erythromycin resistance as well as clindamycin resistance declined somewhat for GAS and increased for GCS. The percentage of strains with inducible clindamycin resistance was virtually unchanged for all serogroups: GAS, 1.2%; GBS, 4.7%, GCS 4.3%, and GGS, 12%. The percentage of fully susceptible isolates was unchanged compared to 2021 for all four serogroups.

Figure 8.22 Beta-haemolytic streptococci: Antimicrobial resistance testing results, Denmark, 2013-2022 DANMAP 2022



Results of genotyping of *Streptococcus pyogenes*

The GAS isolates belonged to 55 different multi-locus sequence types (MLSTs) and 40 different *emm* types. As shown in Table 8.19 nearly two-thirds (110) of the isolates belonged to eight different combinations of MLSTs and *emm* types. None of these were resistant to erythromycin or clindamycin. The variants 28 / *emm* 1.0 and 36 / *emm* 12.0 accounted for 41% of all isolates. The remaining 56 isolates belonged to 41 different combinations of MLSTs and *emm* types

Table 8.19 *Streptococcus pyogenes*: Combinations of MLSTs and *emm* types DANMAP 2022

MLST / *emm* type	Number
36 / 12.0	36
28 / 1.0	32
101 / 89.0	10
52 / 28.0	10
62 / 87.0	8
44 / 66.0	6
1146 / 1.0	4
28 / 1.25	4

Nine isolates contained genes encoding erythromycin resistance. Seven of these isolates were phenotypically resistant as well as one without detected resistance genes.

Comments and conclusions

The substantial increase from 2021 to 2022 for GAS was part of the national as well as international surge of invasive and non-invasive GAS infections, including a high proportion of cases with a serious course. The increase in the number of submitted GAS isolates is probably a consequence of discontinued COVID-19 related restrictions on upper respiratory tract colonization and droplet transmission of this species.

The increase cannot be explained by changes in the prevalence of antimicrobial resistance. All isolates were fully susceptible to penicillin. Comparing 2022 to 2021, erythromycin resistance as well as clindamycin resistance declined somewhat for GAS and increased for GCS.

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8.3.8 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhoea, which is usually located in the urethra in males and cervix in females. *N. gonorrhoeae* (gonococci) may also sometimes be demonstrated in specimens from the pharynx or rectum in either gender. In females, the finding of gonococci in rectal specimens is usually due to contamination with vaginal secretion, while in men who have sex with men (MSM) it is due to unprotected anal sex leading to infection. In both genders, the condition is generally asymptomatic. Pharyngeal gonorrhoea is virtually always asymptomatic.

Complications of gonorrhoea include e.g. salpingitis, epididymitis, orchitis, and prostatitis. Furthermore, conjunctivitis may occur in newborns after transmission from an infected mother during labour and rarely in adults following direct or indirect inoculation.

Gonococcal surveillance

Since 1962, the departments of clinical microbiology in Denmark have voluntarily submitted isolates of gonococci to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance.

Both resistant and intermediary susceptible isolates were categorized as resistant in this report. However, isolates with intermediary susceptibility are non-existing according to EUCAST breakpoints for ceftriaxone and azithromycin and they are exceedingly rare regarding ciprofloxacin.

As part of NSR's participation in ECDC's surveillance (Euro-GASP) of sexually transmitted infections since 2009, 110-120 gonococcus isolates are collected consecutively each year in September-October and investigated for susceptibility to an expanded panel of antimicrobial agents. This panel includes cefixime and in selected years also spectinomycin and sometimes gentamicin.

Most of the isolates received in 2022 were from urethra or cervix, while clinicians only rarely obtained specimens from rectum and pharynx. Occasionally, the NSR laboratory receives isolates from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

Repetitive detections of gonococci in a patient are considered to represent unique cases of gonorrhoea if separated by more than 21 days. The NSR laboratory received 2,145 isolates from 1,841 unique cases of gonorrhoea diagnosed in 2022 (1,131 males, 708 females, and 2 of unknown gender). Only one isolate from each unique case is counted in this report.

Results and discussion

The annual number of received isolates increased considerably from 2011 through 2017 (Figure 8.23). This is most likely due to the widespread use of combined nucleic acid amplifications tests (NAATs) for *Chlamydia trachomatis* and *N. gonorrhoeae* which have identified unexpected cases of gonorrhoea (sometimes followed by culture), and also to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion until 2016 were women. The proportion of female cases has decreased slightly since 2017, but it is still substantially higher than in the beginning of the observation period (2003-2022). A decrease in the annual number of isolates from unique cases was seen in 2018 followed by an increase in 2019-2021 and in particular from 2021 to 2022. It should be recalled, however, that many cases diagnosed by NAATs are not followed-up by culture.

The ciprofloxacin resistance rate was 40% in 2022 (48% in 2021 and 43% in 2020), thus still considerably lower than the peak of 75% in 2009 (Figure 8.23). Only 0.7% of the isolates were classified as ciprofloxacin intermediate (MIC 0.047-0.064 mg/L).

The percentage of strains producing penicillinase was 14% (23% in 2021 and 17% in 2020). Previously, it has fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance (MIC above the present ECOFF >1 mg/L) was found in 2.9% of the tested isolates (2.8% in 2021 and 2.0% in 2020). In 2018, azithromycin resistance was observed in 6% of the isolates and intermediary susceptibility in 4%. However, EUCAST breakpoints were changed as of January 1, 2019 leading to a greater proportion of strains being classified as sensitive, compared to previously, albeit only if used in conjunction with another effective agent.

Ceftriaxone-resistant gonococci (MIC >0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea have occurred in several countries during recent years. As shown in Figure 8.24, the ceftriaxone MIC distribution changed from 2003 through 2009 with the highly susceptible isolates becoming gradually less frequent. However, since then, this change has completely reversed. In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L (i.e. resistant) and azithromycin MIC of 0.25 mg/L. Secondary cases were not reported and no strains with ceftriaxone resistance have been encountered among the submitted isolates since 2017.

The Danish National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of ceftriaxone (500 mg i.m.) and azithromycin (2 g p.o.) was recommended for the empiric treatment of gonorrhoea. This therapeutic regimen has gradually been abandoned during 2019 and onwards by many clinicians and has been replaced by ceftriaxone 1 g i.m.

Ciprofloxacin (500 mg p.o. as a single dose) may still be used for treatment if the strain is fully susceptible.

In a subset of 134 isolates tested as part of the Danish participation in Euro-GASP, resistance against cefixime (MIC >0.125 mg/L) was 0% in 2022 (Table 8.20), like in 2021. Cefixime is an oral cephalosporin that has never been used in Denmark. Resistance to spectinomycin was also 0%, like in all previous years where data for Euro-GASP were produced. The gentamicin MIC values were in the range 0.5 to 4 mg/L, but clinical breakpoints for this agent have not been established.

Figure 8.23 Number of submitted gonococci isolates from males and females, and the percentage of isolates with ciprofloxacin resistance, azithromycin resistance, and penicillinase production, Denmark, 2003-2022 DANMAP 2022

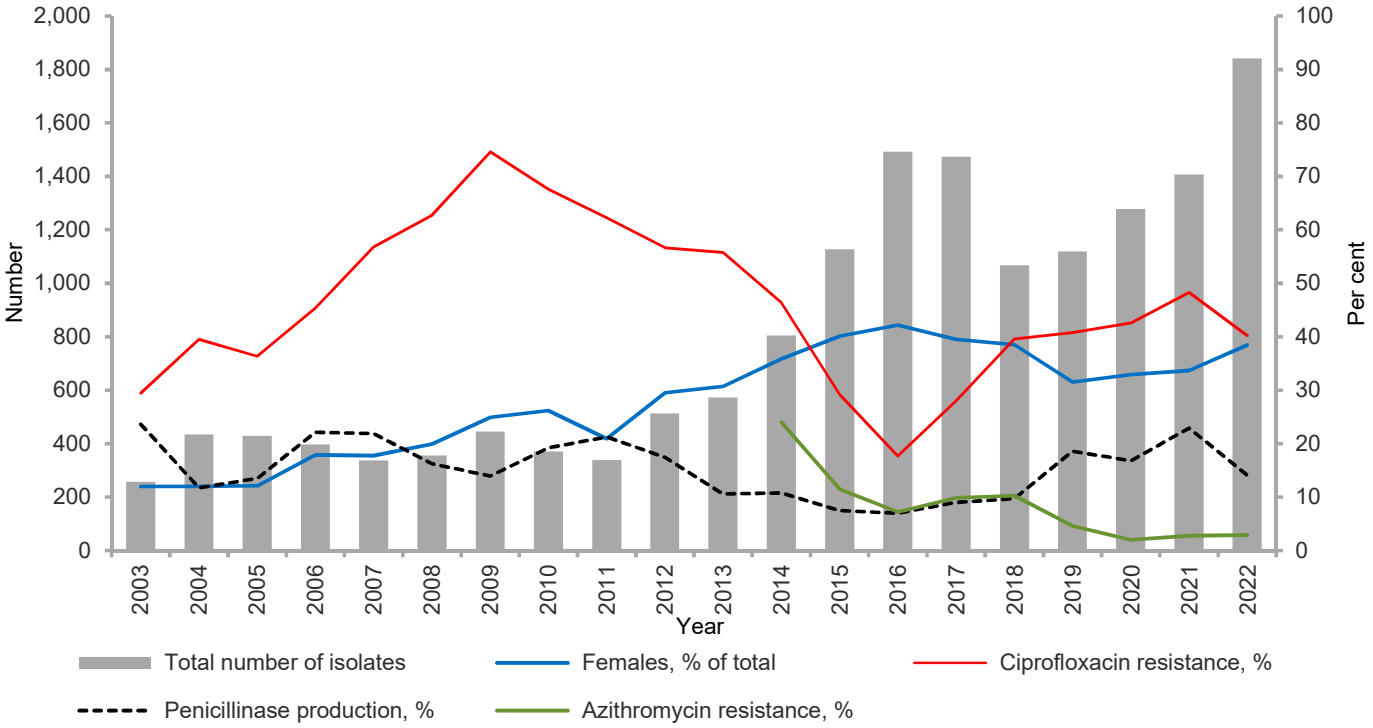

Figure 8.24 Distribution of ceftriaxone MIC (mg/L) values in gonococci, Denmark, 2003–2022 DANMAP 2022

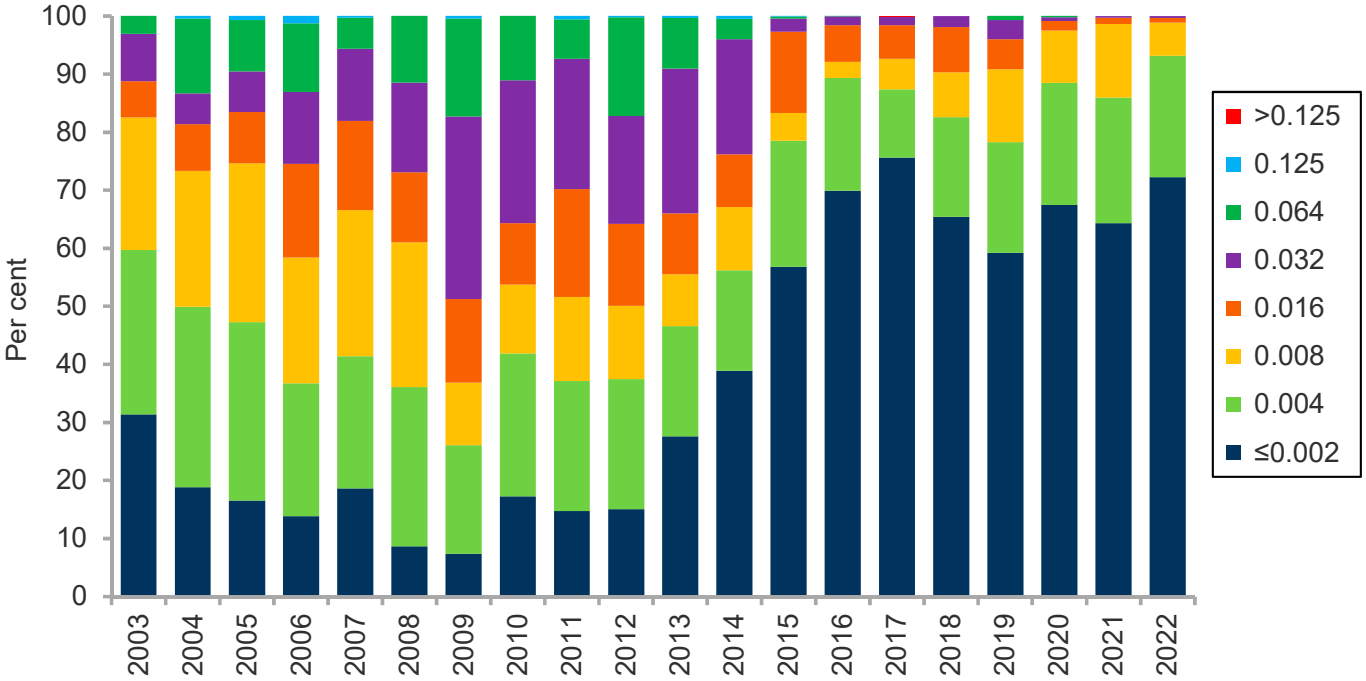

Table 8.20 Distribution of 134 gonococcal isolates according to MIC values for cefixime, gentamicin, and spectinomycin; number of isolates DANMAP 2022

	MIC values (mg/L)							
	≤0.016	0.032	0.064	0.5	1	2	4	8
Cefixime	131	1	2					
Gentamicin				1	35	95	3	
Spectinomycin						2	65	67

In both genders, the ciprofloxacin resistance rate was higher in anorectal isolates than in urogenital and pharyngeal isolates (Table 8.21).

In both genders, the azithromycin resistance rates was higher in pharyngeal isolates than in anorectal and urogenital isolates (Table 8.22).

Penicillinase production was demonstrated at a higher rate among anorectal isolates than among urogenital and pharyngeal isolates (Table 8.23).

Table 8.21 Ciprofloxacin resistance rates by gender and anatomical origin of the isolates DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	622 / 867	72	391 / 616	64	1,013 / 1,483	68
Anorectal	111 / 135	82	11 / 13	85	122 / 148	82
Pharynx	51 / 69	74	34 / 53	64	86 / 122	70
Blood	1 / 1	100	0 / 0	-	1 / 1	100
Eye	4 / 4	100	0 / 0	-	4 / 4	100
Unknown	41 / 55	75	16 / 26	62	57 / 81	70
Total	830 / 1,131	73	452 / 708	64	1,282 / 1,839	70

Table 8.22 Azithromycin resistance rates by gender and anatomical origin of the isolates DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	25 / 867	2.9	14 / 616	2.3	39 / 1,483	2.6
Anorectal	6 / 135	4.4	0 / 13	0	6 / 148	4.1
Pharynx	6 / 69	8.7	4 / 53	7.5	10 / 122	8.2
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	0 / 4	0	0 / 0	-	0 / 4	0
Unknown	0 / 55	0	0 / 26	0	0 / 81	0
Total	37 / 1,131	3.3	18 / 708	2.5	55 / 1,839	3.0

Table 8.23 Penicillinase production by gender and anatomical origin of the isolate DANMAP 2022

	Males		Females		Total	
	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant	No. resistant / total no.	% resistant
Urogenital	119 / 867	14	69 / 616	11	188 / 1,483	13
Anorectal	34 / 135	25	4 / 13	31	38 / 148	26
Pharynx	14 / 69	20	8 / 53	15	22 / 122	18
Blood	0 / 1	0	0 / 0	-	0 / 1	0
Eye	0 / 4	0	0 / 0	-	0 / 4	0
Unknown	7 / 55	13	4 / 26	15	11 / 81	14
Total	1,74 / 1,131	15	85 / 708	12	55 / 1,839	3.0

Conclusions

The ciprofloxacin resistance rate was somewhat lower in 2022 than in 2021 and the ceftriaxone MIC distribution was virtually unchanged. Although resistance problems among gonococci are still not present in Denmark, the frequent emergence globally of gonococcal resistance to antibiotics underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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8.3.9 *Haemophilus influenzae*

Haemophilus influenzae is part of the normal upper respiratory tract flora, with colonization varying by age. *H. influenzae* frequently causes upper and lower respiratory infections, while invasive infections are relatively rare and primarily occur in young children and elderly individuals, particularly those with underlying conditions like chronic obstructive pulmonary disease or cancer. Bacteremia/sepsis is the predominant form (80-90%), while meningitis is observed in approximately 10% of cases. *H. influenzae* is categorized into six capsular serotypes (a, b, c, d, e, and f), also known as Hia, Hib, Hic, Hid, Hie, and Hif, and there are noncapsular (non-typeable, NTHi) strains as well. The introduction of the polysaccharide type b vaccine in 1993 significantly reduced the incidence of systemic infection caused by *H. influenzae* type b (Hib) isolates, as the vaccine specifically protects against Hib.

Invasive *Haemophilus influenzae*

The surveillance of *H. influenzae* causing invasive disease in Denmark involves submitting clinical isolates to Statens Serum Institut (SSI). Cases without a submitted isolate are identified through MiBa. At SSI, the isolates are now (2022) subjected to whole-genome sequencing to extract the serotype, sequence type, and beta-lactam resistance genes (beta-lactamase genes and mutations in the *ftsI* gene encoding penicillin-binding protein 3). Phenotypic beta-lactam susceptibility is retrieved through MiBa, if available.

In 2022, a total of 118 cases of invasive *H. influenzae* were registered. The majority of cases were in blood (103) or cerebrospinal fluid (12), with some found in other normally sterile sites (3). Out of the 118 *H. influenzae* cases identified in MiBa, 98 isolates were received at the reference laboratory. The age and serotype distribution of the submitted isolates can be seen in Figure 8.25. Invasive *H. influenzae* infections are most commonly observed in the elderly.

Non-capsular *H. influenzae* is still the most commonly tested type (55%), with Hif being the most common serotype (18%), followed by Hib (8%), Hie (2%), and Hic (1%).

Sequence type (ST) and clonal complex (CC) were linked to the serotype of *H. influenzae* (Table 8.24). Thus, the MLST type can indicate the correct identification of the genotype because the capsular isolates appear to be part of only a few clonal complexes. This was not the case for the non-capsular *H. influenzae* isolates which had 39 different STs, including three novel STs.

Figure 8.25 The distribution of age and serotype of *H. influenzae* in 2022 DANMAP 2022

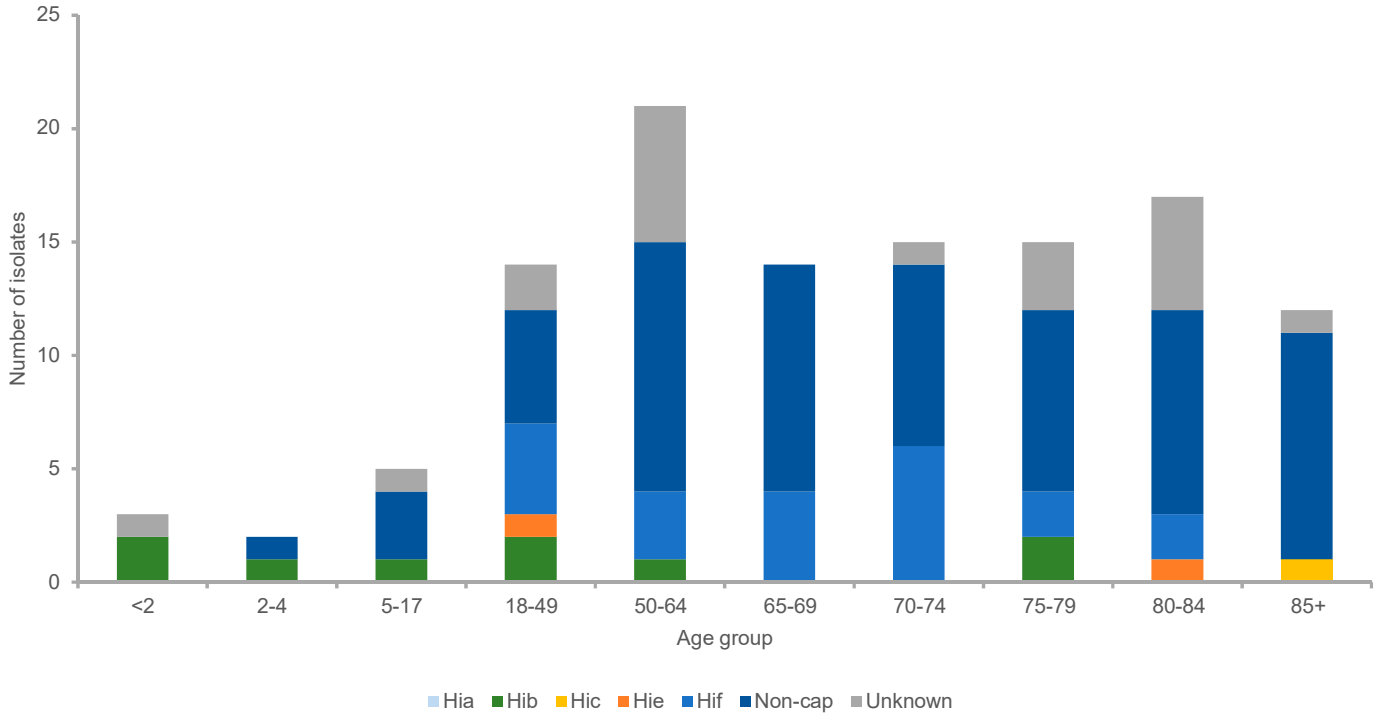

Table 8.24 Sequence type and clonal complex found among the *H. influenzae* isolates with a capsule DANMAP 2022

ST	Clonal complex	A	B	C	D	E	F	Noncap
6	ST-6 complex	0	1	0	0	0	0	0
92	ST-6 complex	0	1	0	0	0	0	0
95	ST-6 complex	0	1	0	0	0	0	0
190	ST-6 complex	0	6	0	0	0	0	0
103	ST-11 complex	0	0	1	0	0	0	0
Novel	ST-18 complex	0	0	0	0	1	0	0
18	ST-18 complex	0	0	0	0	1	0	0
124	ST-124 complex	0	0	0	0	0	19	0
598	ST-124 complex	0	0	0	0	0	1	0
1739	ST-124 complex	0	0	0	0	0	1	0

Table 8.25 Phenotypic resistance against penicillin/ampicillin DANMAP 2022

Sensitivity	Number (%)
Resistant	29 (30)
Susceptible	67 (70)
Total	96

Table 8.26 Phenotypic resistance against penicillin/ampicillin vs. beta-lactam resistance mechanism DANMAP 2022

Beta-lactam resistance mechanism	Resistant	Susceptible	Total (%)
BLNAS	0	67	67 (70)
BLPAR	9	0	9 (9)
BLNAR	18	0	18 (19)
BLPACR	2	0	2 (2)
Total	29	67	96

Data on both molecular and phenotypic antimicrobial susceptibility were available for 96 *H. influenzae* isolates. Of *these*, 30% were penicillin/ampicillin-resistant (Table 8.25). Eleven isolates tested positive for TEM beta-lactamase genes (ten were TEM-1 and one was TEM-234). BLNAR-defining mutations in the *ftsI* gene were found in 20 isolates (19 had the N526K mutation and one had the R517H mutation). The most common *ftsI* types were *IIb* (11), followed by *IIa* (3), *IId* (3), I (2), and *IIc* (1).

A comparison between phenotypic beta-lactam susceptibility and beta-lactam resistance mechanisms (BLNAS=beta-lactamase-negative ampicillin-susceptible; BLPAR=beta-lactamase-positive ampicillin-resistant; BLNAR=beta-lactamase negative ampicillin-resistant; BLPACR=beta-lactamase-positive amoxicillin-clavulanic acid-resistant) can be found in Table 8.26. A 100% concordance was observed between phenotypic susceptibility testing and molecular resistance gene detection.

Conclusions

The number of invasive *H. influenzae* cases in 2022 were 118 comparable to 101 cases in 2021 and an increase from 59 cases in 2020. The majority of isolates are still of the non-capsular type (55%) while Hif was the most common serotype (18%). Resistance towards penicillin and ampicillin was 30% with 19% BLNAR, 9% BLPAR and 2% BLPACR.

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8.3.10 Meningococci

Neisseria meningitidis (meningococci) are capable of causing invasive meningococcal disease (IMD), usually meningitis or septicaemia or both, which may be life threatening and may cause permanent neurological sequelae. Asymptomatic throat carriage is frequent. IMD is usually treated with intravenous penicillin or ceftriaxone. Single-dose ciprofloxacin or rarely rifampicin may be used for prophylaxis to close contacts to patients with IMD.

Meningococci are classified into serological groups (A, B, C, W, X, Y, Z, and 29E) according to antigenic properties of the polysaccharide capsule. In Denmark, serogroups B and C are the most frequent ones in most years, although serogroup W was quite frequent in 2016 through 2019. Vaccination against meningococci has never been part of the Danish childhood immunization programme but is used for prophylaxis to close contacts to patients with IMD. Specific vaccines exist for group B and groups A, C, W and Y.

This report presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. usually from blood, cerebrospinal fluid) submitted from the departments of clinical microbiology (DCMs) during 2012-2022 to the Neisseria and Streptococcus Reference laboratory (NSR). Isolates are received from all DCMs in Denmark. It is voluntary to submit isolates of meningococci, but the coverage rate is estimated to be 100% when compared to the clinical notification system. The two surveillance systems continuously supplement each other.

Surveillance of meningococci and resistance

During the years 2012 through 2019, 18 to 52 isolates were received annually. In 2020, where restrictions to counteract the spread of COVID-19 were implemented, the number was 14, decreasing to 8 and 11, respectively, in 2021 and 2022. Figure 8.26 shows the number of isolates of groups B, C, W, and Y received during 2012-2022. Because of low numbers the following has been omitted: One isolate of group 29E (2017), two isolates of group X (2016 and 2019), and one isolate which was non-groupable (2019). The susceptibility pattern of these four isolates did not show any specific deviations from the remaining findings and are therefore not further included in the present report.

All isolates were susceptible to ceftriaxone (MIC ≤0.125 mg/L) (Figure 8.27).

Figure 8.26 Number and serogroup of meningococcal isolates received during 2012-2022 DANMAP 2022

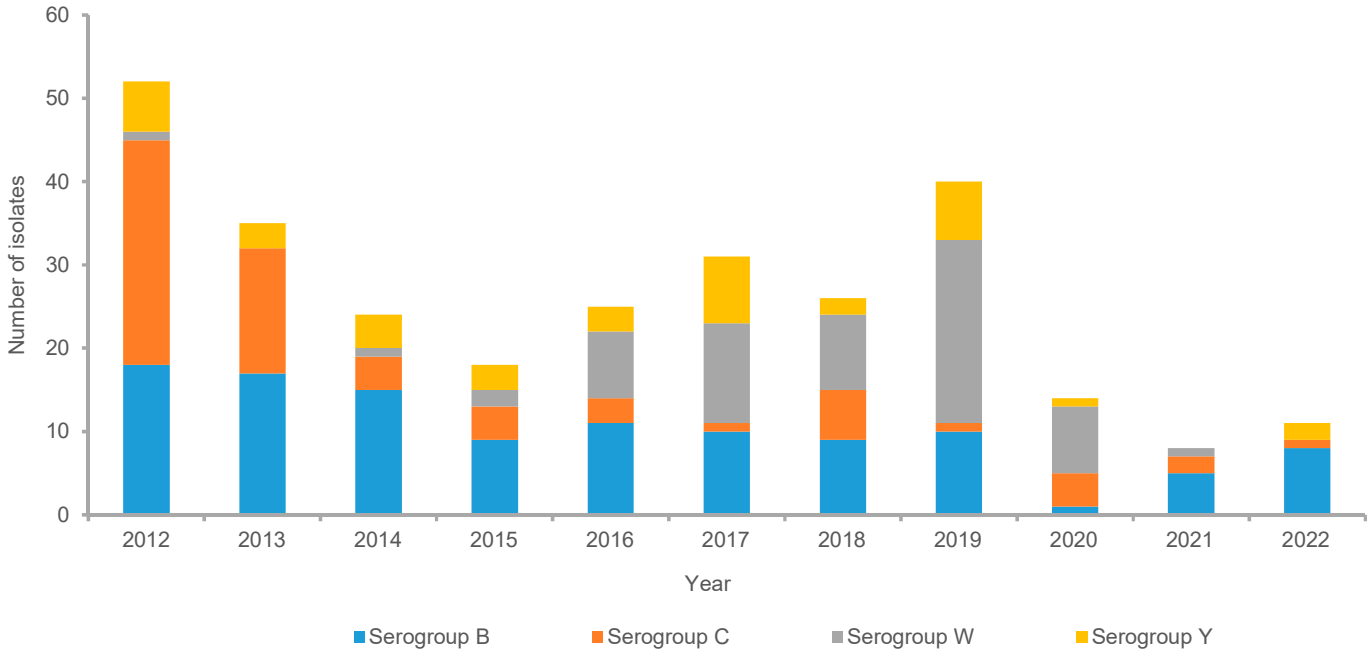


Figure 8.27 Distribution of ceftriaxone MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022

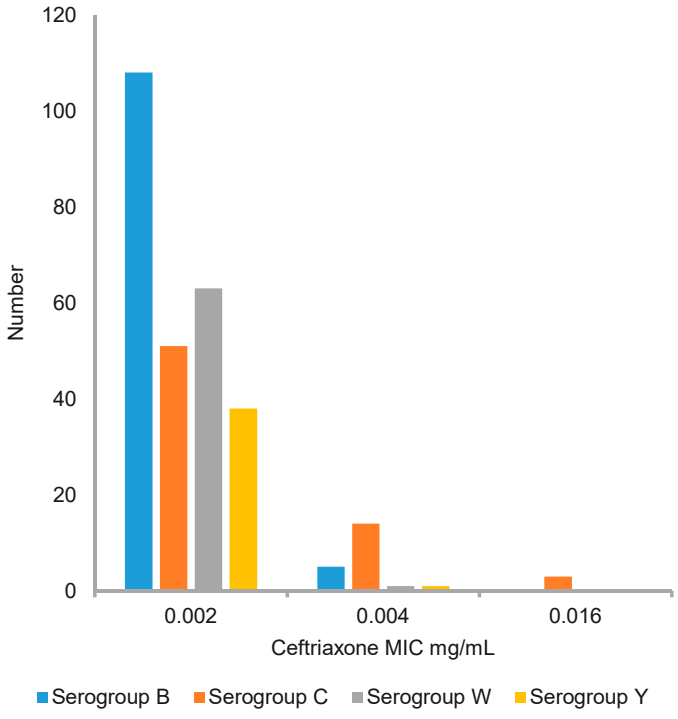
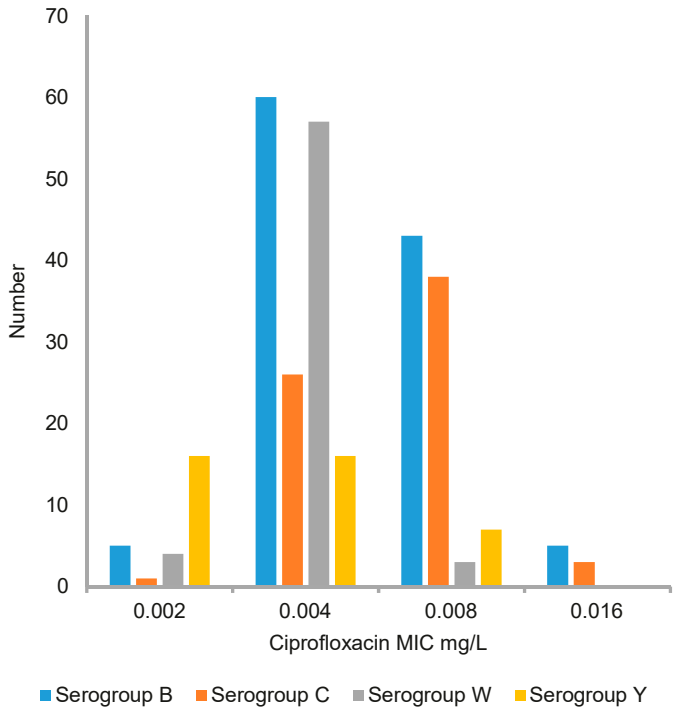


Figure 8.28 Distribution of ciprofloxacin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022



All isolates were susceptible to ciprofloxacin (MIC ≤0.016 mg/L) (Figure 8.28). Isolates of serogroup W and Y tended to have slightly lower levels of ciprofloxacin MIC values than isolates of serogroup B and C.

In total, 95% of the isolates during the study period were susceptible to penicillin (MIC ≤0.25 mg/L) (Figure 8.29). Seven (6%) isolates of serogroup B and six (9%) isolates of serogroup C were penicillin-resistant (MIC >0,25 mg/L) (Table 8.27).

Nearly all isolates during the study period were susceptible to rifampicin (MIC ≤ 0.25 mg/L) (Figure 8.30). Only one (from 2017) was resistant (MIC = 32 mg/L).

Figure 8.29 Distribution of penicillin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022

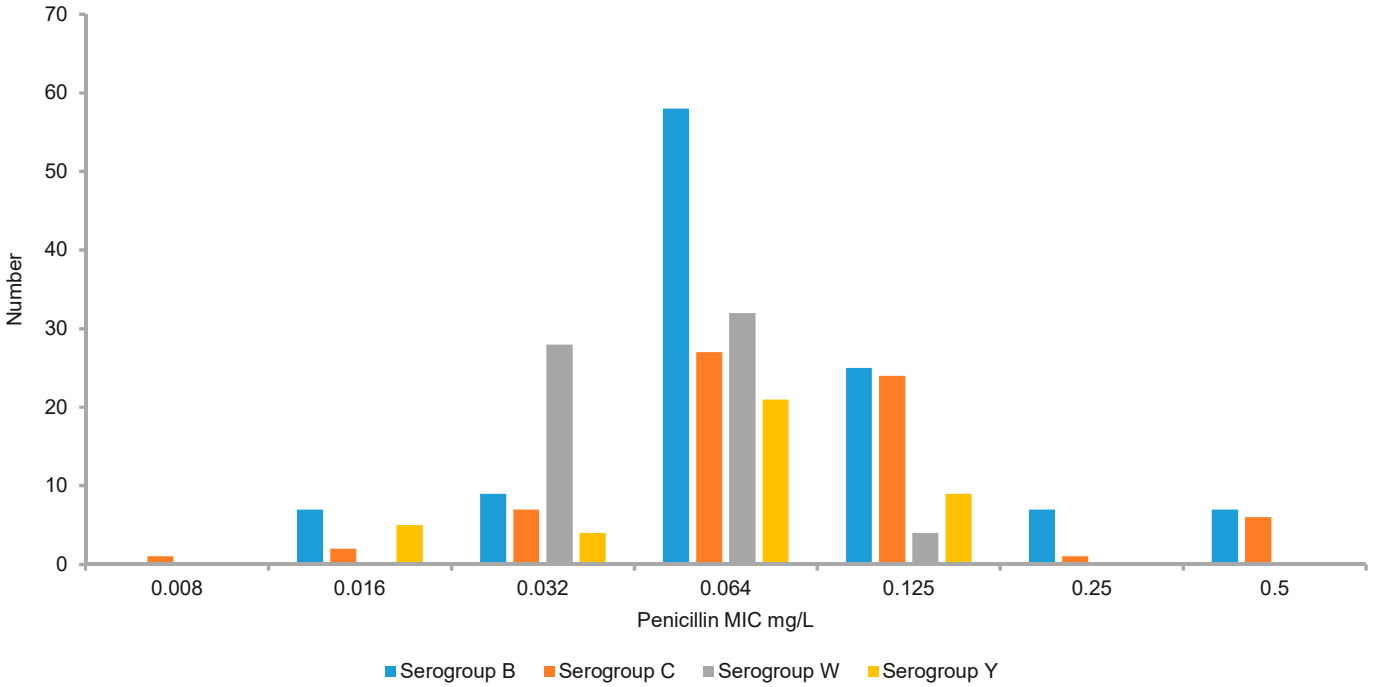
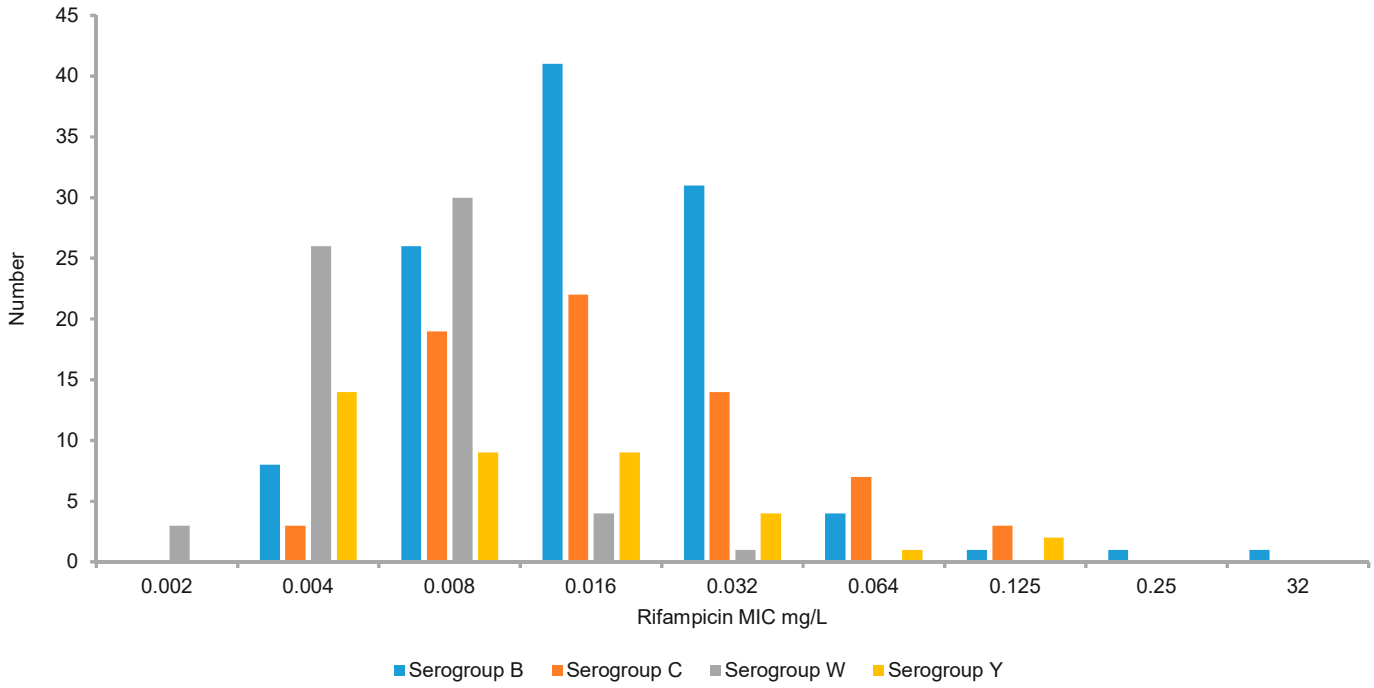


Table 8.27 Number of penicillin-resistant meningococci (MIC = 0.5 mg/L), serogroups B and C, 2013-2022 DANMAP 2022

	2013	2014	2016	2017	2018	2020	2022
Serogroup B	1	2	1	2			1
Serogroup C		1			2	3	

Figure 8.30 Distribution of rifampicin MIC values (mg/L) by serogroup, 2012-2022 DANMAP 2022



Comments and conclusions

The substantial decrease of received meningococcal isolates during and following 2020 are most likely due to the social restrictions implemented in April 2020 because of COVID-19. The antibiotic susceptibility patterns of meningococci do not reveal any imposing threats of resistance.

During the first six months of 2023 nearly 20 cases of IMD have been diagnosed (not described in this report).

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Textbox 8.1

Dicloxacillin capsules contaminated with CPE in Denmark and Iceland

The prevalence of carbapenemase producing Enterobacterales (CPE) has been increasing in Denmark (Figure 8.13), as well as in many other countries, for the last decade. The source for CPE in Danish patients was initially mostly attributed to travel and hospitalization in countries with high(er) prevalence of CPE than Denmark, but in recent years nosocomial spread within Denmark account for more and more cases as part of local or regional outbreaks (Table 8.11). Community onset of CPE may also occur, but here investigations are often difficult to conduct due to sparse epidemiological data as well as lack of national and international surveillance programs in the community.

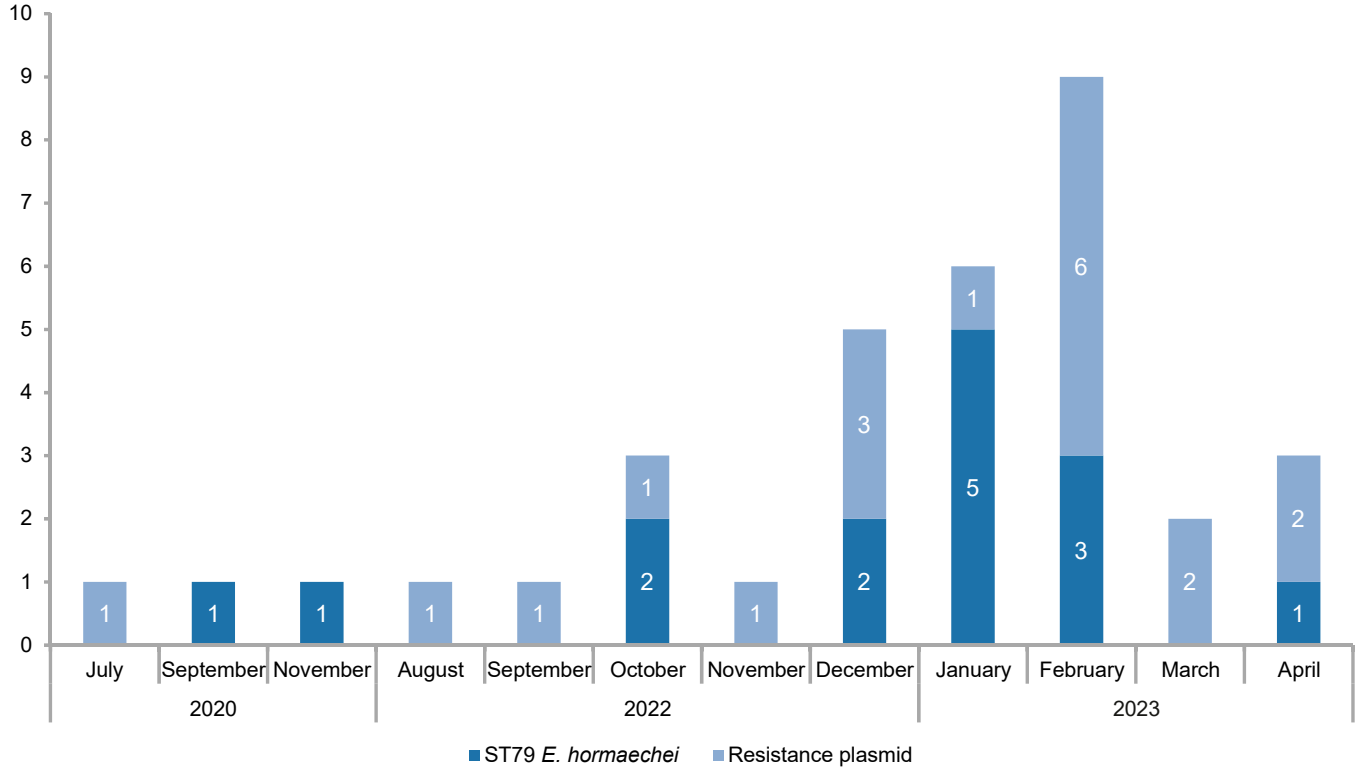
During the winter of 2022/2023, a sudden increase in NDM-5 + OXA-48 producing *Enterobacter hormaechei* ST79 was observed in the national surveillance data. Whole genome sequencing (WGS) analysis showed a strong clonal relationship between the 13 isolates as well as to two highly similar isolates from the autumn 2020 thus suggesting these 15 patients were part of the same outbreak. The isolates were submitted from four of five Danish regions, they originated in most cases from uncomplicated urinary tract infections and the patients had not been hospitalized neither in Denmark nor abroad, indicative of a community source for the outbreak. In the Region of Southern Denmark, three patients submitted samples a few days apart and therefore these three patients were interviewed by the staff of the Department of Clinical Microbiology at Odense University Hospital (OUH). Here, investigation of the interview data identified consumption of the same brand of dicloxacillin product (Dicillin®) prior to the urinary tract infection as a common denominator. This finding was immediately reported to the national outbreak team at Statens Serum Institut (SSI), who contacted the remaining three regions who confirmed that the remaining patients had also been administered Dicillin®. [1]

In parallel with the epidemiological investigations, OUH retracted unused Dicillin® capsules from two of the three patients in the Region of Southern Denmark and was able to culture a CPE *E. hormaechei* ST79 from the surface of one of the capsules. Subsequent WGS analysis confirmed that this capsule isolate clustered with the outbreak isolates, thereby confirming a direct link between the Dicillin capsules and infected patients. Dicillin® was only marketed in Denmark and Iceland (under a different brand name, though) and contact with the Icelandic authorities led to the identification of one identical CPE *E. hormaechei* ST79 isolated from a patient there. This led to retraction of all Dicillin products from the Danish and Icelandic market by February 6th 2023, while the possible source of contamination was being investigated by testing available batches at the company producing Dicillin®. Based on this investigation, at least 12 contaminated batches could be identified between February 2022 and February 2023. The most likely explanation for the contamination was a set of brushes on the packing line even though no viable bacteria were extracted from these. In total, approximately 79 thousand Danish citizens may have ingested the capsules between February 2022 and February 2023.

The two carbapenemase genes ($bla_{\text{NDM-5}}$ and $bla_{\text{OXA-48}}$) were located on a 45,048 bp IncX3 plasmid and a 79,966 bp IncL plasmid, respectively. Both of these two plasmid types were conjugative, and transfer of either one or both plasmids to other Enterobacterales species such as *E. coli*, *Klebsiella* spp. and *R. ornithinolytica* were identified for at least three of the patients carrying the outbreak strain. As Denmark has had an extensive monitoring program of CPE since 2014, where all CPE isolates are submitted to SSI for WGS analysis, a retrospective search within the existing data identified an additional 19 patients between 2020 and 2023, who were carrying other bacterial species than *E. hormaechei*, but with identical plasmids (one or both) as the outbreak strain. As these two plasmids seem to be extremely rare or even unique compared to the data in the international databases such as NCBI and PATRIC, it was most likely that these 19 patients also had received Dicillin®, which was later confirmed through interviews with the patients or their general practitioner (Figure 1).

A decrease in CPE-positive patients with the outbreak strain as well as other bacterial species with the outbreak plasmids has been observed after the contaminated Dicillin® capsules were removed from the market. Therefore, there is a good agreement between the availability of contaminated capsules and the number of new cases. Patients who have ingested the contaminated capsules may therefore to some extent have eliminated the CPE from their digestive system, but it is known from similar cases that patients may be healthy carriers for a longer time [2]. It is therefore important that the national surveillance system is designed to detect both the presence of the outbreak strain and its plasmids in the future.

Figure 1 Epicurve of patients carrying either the outbreak *E. hormaechei* ST79 strain (dark blue) or other bacterial species with either one or both outbreak plasmids (light blue)
DANMAP 2022



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Textbox 8.2

Fungaemia epidemiology, resistance rates and human antifungal consumption: a 2021-2022 update

Candidemia is the most common form of fungemia. It mainly occurs in hospitalized patients with risk factors such as abdominal surgery, presence of intravenous lines and immunosuppression [1] [2]. The 30-day mortality rate is approximately 40% in Denmark and higher in ICU [2].

A Danish nationwide surveillance has existed since 2004. All fungal blood culture isolates from Danish Departments of Microbiology are referred to Statens Serum Institut for confirmatory species identification, susceptibility testing and sequencing (when relevant).

Denmark is a high incidence country compared to our Nordic neighbours. After a peak at 10.1/100.000 inhabitants in 2011, the incidence stabilised at around 8/100.000 but appears to have risen again to 9.1/100.000 (2020-2021) and 9.6 (2022) [3] [4]. The age specific peak incidence in men has risen from 70 to 80-90 years age (Figure 1). The overall incidence changes could be influenced by an ageing population, the COVID-19 epidemic, changed treatment regimens or other factors including change in blood culture system use [5].

The first line treatment for candidaemia is an echinocandin (iv administration only). Acquired echinocandin resistance emerged after the introduction of the echinocandins [6]. However, despite a continued increase in echinocandin usage (+21% since 2013) the acquired echinocandin resistance rate 2021-22 in the five most common species remained low (1.0%). For infection with susceptible isolates, de-escalation to fluconazole is possible and allows outpatient oral treatment. A marked shift from the susceptible species *C. albicans* to the less azole-susceptible species *C. glabrata* has been evident and coupled to a high azole (mainly fluconazole) use [4] [6]. Despite a 34% decline in use 2013-2022, *C. albicans* accounted for only 38% in 2021-2022 (Figure 2). An increase in *C. glabrata* to 34% of the isolates is the main cause for only ~57% of all isolates being fully susceptible to fluconazole.

Figure 1 Incidence by age and gender on 3 or 4-years intervals 2004-2022. M: Males; F: Females; MF: Both genders DANMAP 2022

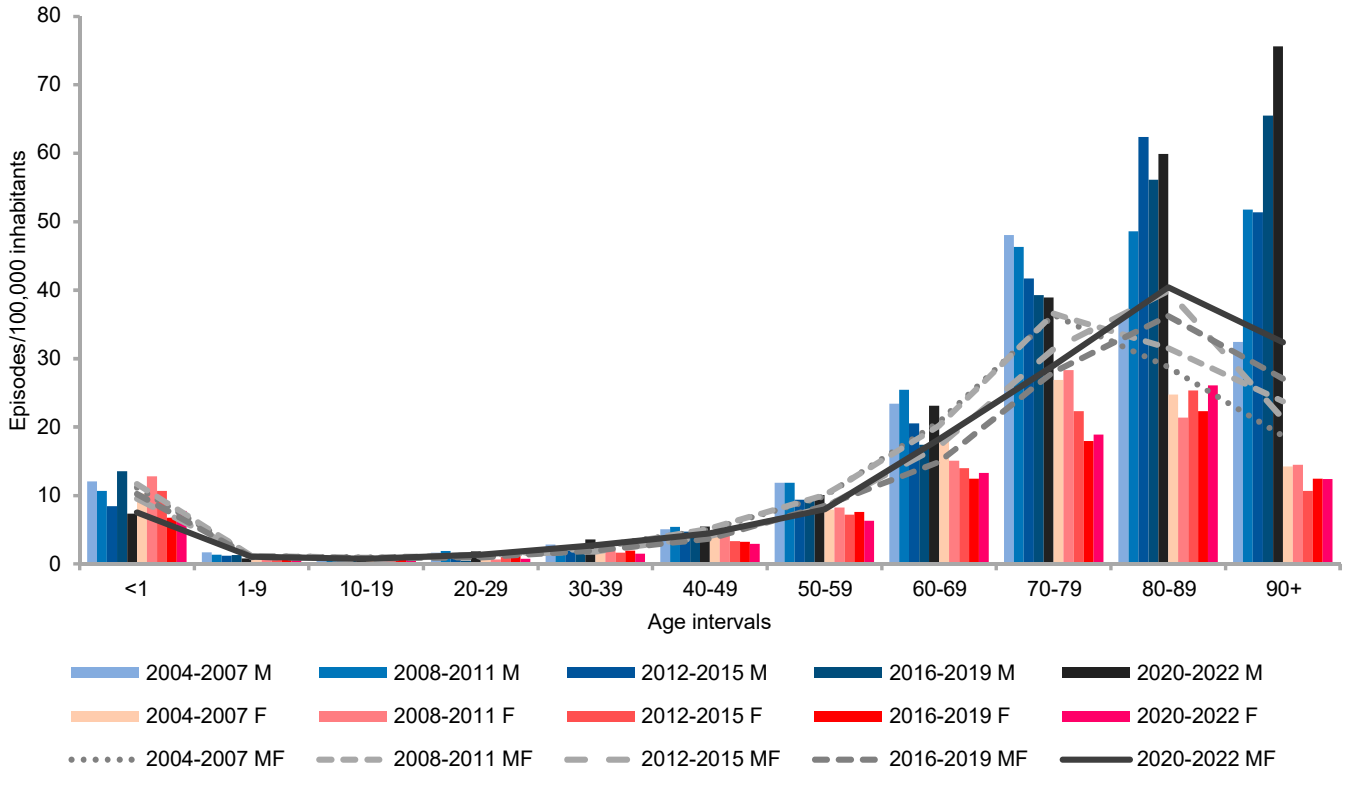
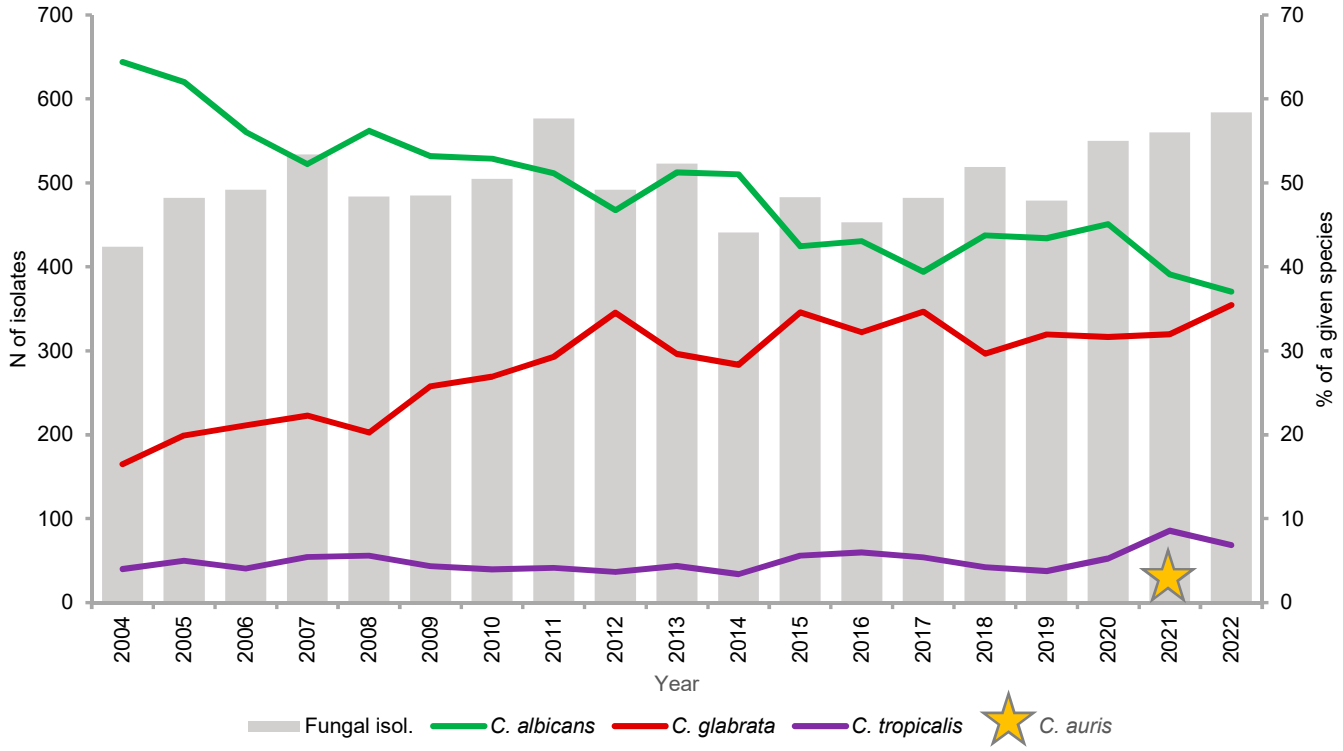


Figure 2 Number of annual isolates and selected trends in species distribution DANMAP 2022



In an international context, emergence of less susceptible species or clones causes concern and poses a threat to standard treatment regimens. *C. auris* is renowned for its near-universal fluconazole resistance and ability to rapidly acquire echinocandin resistance. It has caused hospital outbreaks in multiple countries in Asia, Africa, South and North America and also within Europe [7]. The first and so far only Danish *C. auris* candidemia case occurred in 2021 and involved acquisition of echinocandin resistance [8]. Screening for colonization has been recommended for patients previously admitted in foreign hospitals/countries with known transmission [9] [10]. Moreover, fluconazole-resistant *C. parapsilosis* infections involving clonal outbreaks (and occasionally also echinocandin resistance) have emerged in Southern Europe, Asia, Brazil, South Africa and North America [11].

In conclusion, in Denmark the overall incidence and high proportion of *C. glabrata* remained stable despite a reduction in azole use. Resistance levels remain overall stable but *C. auris* has for the first time been detected in the fungemia surveillance and the global emergence and spreading of resistance in *Candida* is concerning. Consequently, continued surveillance of the shifting epidemiology is important.

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continued ... Textbox 8.2

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Textbox 8.3

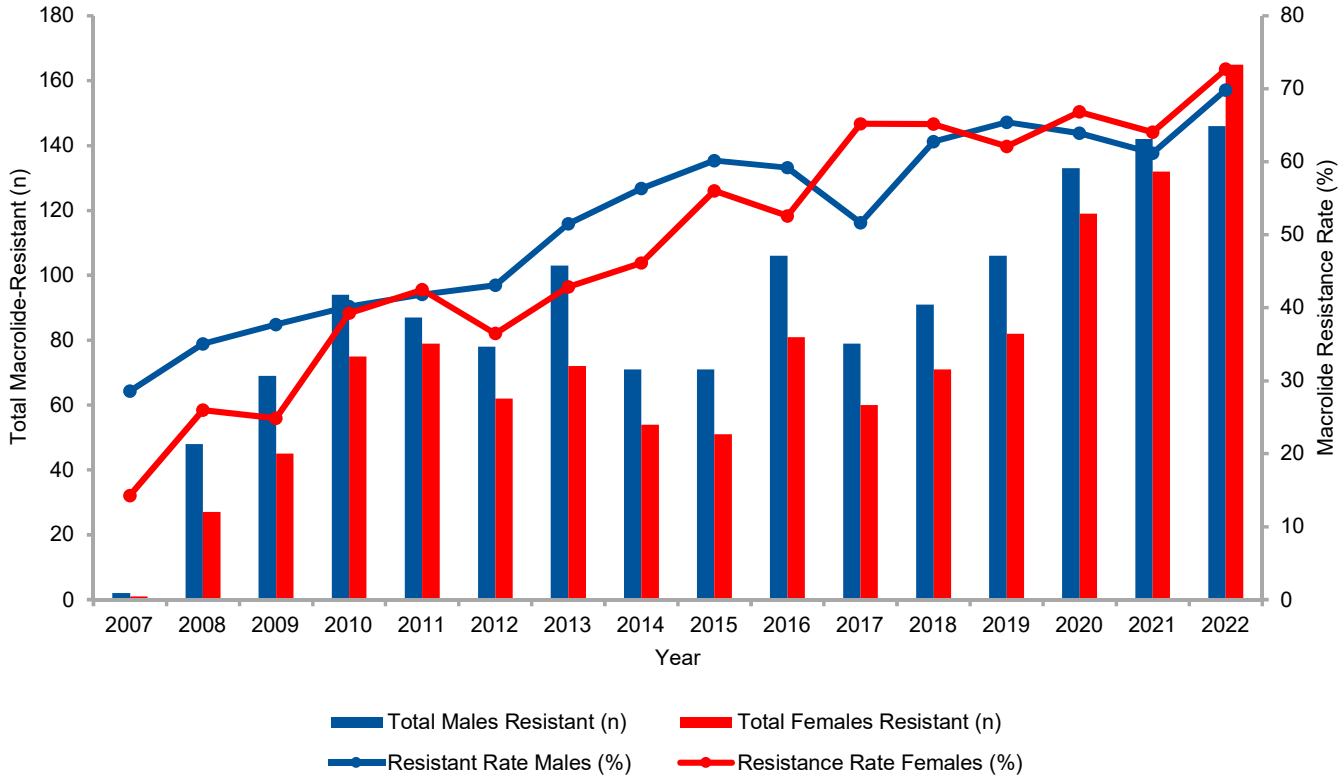
Mycoplasma genitalium

Mycoplasma genitalium (MG) is a sexually transmitted bacterium that was first discovered in 1980. The bacterium causes urethritis, cervicitis, pelvic inflammatory disease and is suspected to be a cause of infertility. The symptoms of an active infection include genital discharge and pain on micturition, however, a large number of infected individuals are asymptomatic. Data show that MG is quite common in the general population, second only to *Chlamydia trachomatis*, with a positive rate of 11.5% of individuals tested in 2022. The prevalence of MG in men with non-gonococcal, non-chlamydial urethritis approaches 25-35%.

Treatment and antimicrobial resistance in *Mycoplasma genitalium*

Treatment for MG in Denmark is based on the European guidelines described by the International Union against Sexually Transmitted Infections (IUSTI), backed by the WHO and ECDC. Broadly, the guidelines recommend that testing for MG is accompanied with molecular detection of resistance to macrolides prior to commencing treatment. For susceptible infections, the first-line treatment is 500 mg azithromycin on Day 1 followed by 250 mg for 2-5 days. Where macrolide resistance mutations (MRM) are detected, the recommended second-line treatment is 400 mg moxifloxacin once daily (fluoroquinolone) for seven days. In cases where moxifloxacin treatment fails, detection of quinolone resistance associated mutations (QRAM) is recommended, in order to discriminate between reinfection and resistance. Third-line treatment requires antimicrobials not registered in Denmark such as pristinamycin or minocycline. Unfortunately, both options are expensive and have an efficacy of only 60-75%. Denmark, like several other countries across the world, has seen an increase in the rate of macrolide resistance. In 2022, 71.3% of all MG positive samples tested at Statens Serum Insitut (SSI) had MRM detected, compared to 21.4% in 2007 when MRM testing was first implemented in Denmark (see Figure 1). The increase has been attributed to selective pressure from azithromycin prescribed for chlamydia.

Figure 1 Number (n) of patients with macrolide resistance mutations (bars) and rate of resistance (%) to macrolides for *Mycoplasma genitalium* infections in Denmark for samples from males and females tested at Statens Serum Institut from 2007-2022 DANMAP 2022



continued ... Textbox 8.3

Prior to 2018, the recommended treatment for uncomplicated chlamydia in Denmark was 1 g single dose azithromycin. Due to an increased awareness of the correlation between the treatment of *Chlamydia trachomatis* with azithromycin and resistance to macrolides in MG infections as well as a lower treatment efficacy in rectal infection, IUSTI and SSI guidelines changed from azithromycin single dose to 100 mg doxycycline twice a day for seven days.

Compared to 2019, 2020 saw a significant decline in the number of azithromycin prescriptions for *C. trachomatis* infections in Denmark. 1.47 prescriptions (per 1000 inhabitants) were issued in 2020 compared to 5 prescriptions (per 1000 inhabitants). At the same time, the number of doxycycline prescriptions issued per 1000 inhabitants saw an increase from 0.26 to 3.43. However, 2021 and 2022 have seen a gradual increase in the number of azithromycin prescriptions issued for *C. trachomatis* infections to 2.88 and 3.14 scripts per 1000 inhabitants, respectively. Consequently, it may be too soon to conclude that medical practitioners in Denmark have changed the treatment regimen for uncomplicated chlamydial infections. Of note, the dramatic decline in the number of azithromycin prescriptions was observed over the COVID-19 period when prescription of macrolides was prohibited.

Testing for resistance to quinolones is not standard practice and a test for detection of QRAM using DNA sequencing methods is currently only offered at the SSI. A retrospective evaluation of MG positive samples submitted to the SSI since 2003 is currently underway. Preliminary results indicate a QRAM rate of 3.7% in 2022 (108 samples tested for both, MRM and QRAM). Of these, two (1.9%) carried mutations for both macrolides and quinolones (dual class resistance). Given that global trends for dual resistance rates exceed 70% in some reports from China, it is of considerable interest to monitor resistance rates for quinolones in Denmark.

Surveillance and treatment strategy for MG

Unlike chlamydia and gonorrhoea, MG is currently not a notifiable disease in Denmark. SSI undertakes testing for MG, MRM and QRAM, however, since 2014 Region Hovedstaden (Capital Region of Denmark) and since 2017 Region Midtjylland (Central Denmark Region) have performed MG and MRM detection. Data from the SSI are, consequently, incomplete since 2014 but suggest that there has been a noteworthy increase in the macrolide resistance rate since 2007. Surveillance of QRAM rates and, preferably, a reduction in the prescription of moxifloxacin would be of significant public health importance considering the absence of an effective third-line treatment. To some extent, this can be obtained by diagnostic stewardship limiting testing for MG to symptomatic patients only, but new, safe, effective, and affordable antimicrobials are also urgently needed.

9

RESISTANCE IN ANIMAL PATHOGENS

9. Resistance in animal pathogens

Highlights

Surveillance of antimicrobial resistance in 2022 focused on pathogenic bacteria from pigs and included results obtained through antimicrobial susceptibility (AST) testing and/or whole genome sequencing (WGS) of isolates belonging to *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS).

AST showed that most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years.

A notable exception was the increased frequency of neomycin resistance in haemolytic *E. coli*, from 6.9% in 2016 to 43.2% in 2022. This is concerning because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. The rapid increase in neomycin resistance might, at least in part, be due to increased use of neomycin in weaners.

WGS demonstrated that resistance towards antimicrobial agents considered critically important for human medicine remained at a low level.

The observed concordance between AST results and WGS-based detection of resistance genes and point mutations was 99.7% for *A. pleuropneumoniae*, 64.5% for *B. bronchiseptica*, 92.8% for haemolytic *E. coli*, 93.9% for non-haemolytic *E. coli*, 61.7% for *K. pneumoniae*, 95.7% for *S. enterica*, 92.6% for *S. hyicus* and 94.0% for *S. suis*.

9.1 Introduction

Antimicrobial susceptibility testing (AST) and surveillance of antimicrobial resistance (AMR) in pathogenic bacteria from pigs, including *Actinobacillus pleuropneumoniae*, haemolytic *Escherichia coli* and *Streptococcus suis*, have been part of the DANMAP programme since 2015. In 2020, the Danish Veterinary and Food Administration (DVFA) asked the Danish Veterinary Consortium (DK-VET) to investigate whether it would be possible to implement whole genome sequencing (WGS) in the surveillance of AMR in pathogenic bacteria from food-producing animals as a basis to detect resistance genes and point mutations. WGS-based AMR surveillance in pathogenic bacteria from pigs commenced in January 2021 and included AST and/or WGS of isolates belonging to *A. pleuropneumoniae* (AST and WGS) *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *E. coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *S. suis* (AST and WGS), which were identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

9.2 Temporal trends of AMR in pathogenic bacteria from pigs

The Veterinary Laboratory performed AST of isolates belonging to *A. pleuropneumoniae*, *B. bronchiseptica*, haemolytic and non-haemolytic *E. coli*, *K. pneumoniae*, *S. enterica*, *S. hyicus* and *S. suis*. Table 9.1 shows the frequencies of resistant isolates in 2022, while results from 2016-2021 can be found on DK-VET's homepage (https://www.vetssi.dk/). Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/).

Table 9.1 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2022 DANMAP 2022

Antimicrobial agent	Ap	Bb	H-Ec	NH-Ec	Kp	Se	Sh	Ss
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
Amoxicillin	0.0%	ND	70.8%	85.6%	ND	68.6%	75.0%	ND
Amoxicillin/clavulanic acid	ND	ND	13.5%	18.0%	5.6%*	7.1%	ND	ND
Ampicillin	0.0%	100.0%*	60.9%	71.4%	100.0%*	85.7%	ND	ND
Cefotaxime	ND	ND	4.3%	2.9%	0.0%*	0.0%	ND	ND
Cefpodoxime	ND	ND	0.0%	0.0%	ND	ND	ND	ND
Cefquinome	ND	ND	ND	ND	0.0%*	ND	ND	ND
Ceftiofur	1.1%	ND	0.0%	0.0%	0.0%*	0.0%	ND	ND
Chloramphenicol	ND	ND	21.7%	22.9%	50.0%*	42.9%	ND	1.1%
Ciprofloxacin	ND	ND	13.0%	0.0%	0.0%*	0.0%	ND	ND
Colistin	ND	ND	1.0%	0.0%	0.0%*	ND	ND	ND
Doxycycline	0.0%	ND	51.6%	51.0%	28.6%*	74.3%	ND	39.0%
Enrofloxacin	3.4%	ND	9.2%	3.8%	ND	ND	0.0%*	0.0%
Erythromycin	0.0%	ND	ND	ND	ND	ND	ND	68.9%
Florfenicol	0.0%	0.0%	16.9%	16.5%	11.1%*	26.2%	0.0%*	1.2%
Gentamicin	ND	ND	19.3%	13.0%	0.0%*	19.0%	ND	ND
Lincomycin	ND	ND	ND	ND	ND	ND	100.0%*	ND
Nalidixic acid	ND	ND	4.3%	0.0%	ND	0.0%	ND	ND
Neomycin	ND	ND	43.2%	26.1%	ND	28.6%	ND	ND
Penicillin	1.1%	ND	ND	ND	ND	ND	75.0%*	0.6%
Spectinomycin	ND	ND	57.4%	44.9%	ND	ND	ND	ND
Streptomycin	ND	ND	78.0%	82.0%	ND	81.0%	ND	ND
Tetracycline	3.4%	ND	73.3%	70.3%	27.8%*	76.2%	ND	35.6%
Tiamulin	0.0%	ND	ND	ND	ND	ND	100.0%*	ND
Tildipirosin	0.0%	0.0%*	ND	ND	ND	ND	ND	ND
Tilmicosin	0.0%	ND	ND	ND	ND	ND	0.0%*	ND
Trimethoprim	ND	ND	58.7%	65.7%	50.0%*	71.4%	ND	ND
Trimethoprim/sulfamethoxazole	0.0%	ND	54.8%	72.1%	42.9%*	45.7%	50.0%*	16.3%
Tulathromycin	0.0%	3.3%	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	0.0%*	ND

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; Bb, *Bordetella bronchiseptica*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; Ss, *Streptococcus suis,* R, resistant; ND, not determined

Most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years. Table 9.2 and Figure 9.1 show all significant changes in phenotypic resistance over a 1-year period (2022 vs. 2021) or a 5-year period (2022 vs. 2017).

Haemolytic *E. coli* displayed significantly increased resistance to florfenicol, gentamicin, neomycin and tetracycline (Figure 9.1). The high frequency of neomycin resistance in haemolytic *E. coli* (43.2%) is particularly worrisome because neomycin is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. Furthermore, haemolytic *E. coli* also displayed medium to high frequencies of resistance to the other first-choice drugs, including amoxicillin/clavulanic acid (13.5%), ampicillin (60.9%), spectinomycin (57.4%), trimethoprim/sulfamethoxazole (54.8%) and streptomycin (78.0%). The rapid increase in neomycin resistance might, at least in part, be due to increased use of neomycin in weaners (Figure 9.2) following two recent decisions to restrict the use of alternative drugs in pigs: 1) the Danish Yellow Card initiative to reduce the use of colistin in 2016 and 2) the European Union-wide ban of medicinal zinc in 2022. It should be noted that we also observed a significant increase in neomycin resistance in non-haemolytic *E. coli* (Figure 9.1). *S. enterica* displayed significantly increased resistance to florfenicol and trimethoprim, while *S. suis* displayed significantly increased resistance to erythromycin (Figure 9.1).

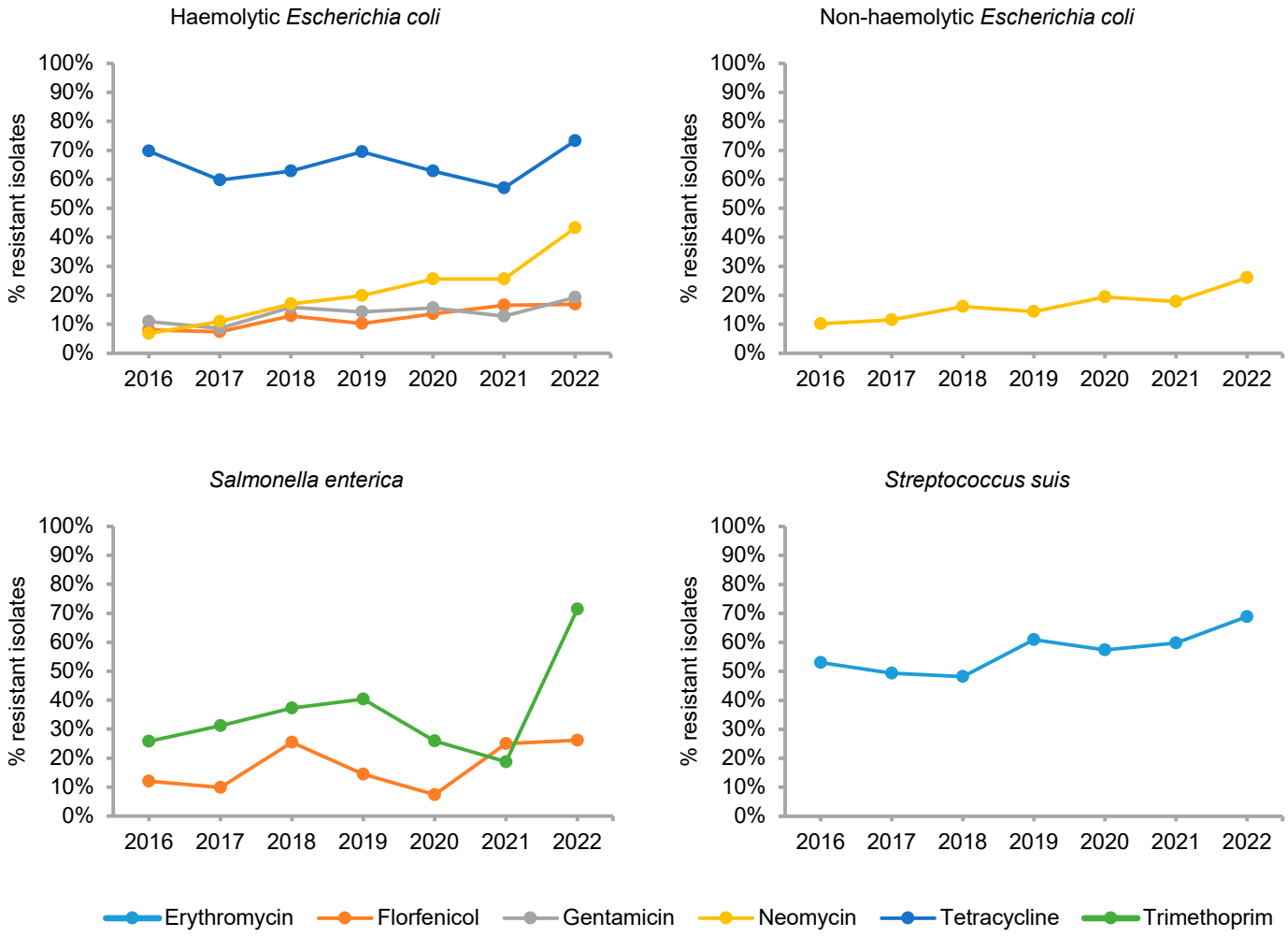
Table 9.2 Temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2021-2022 and 2017-2022

DANMAP 2022

Pathogen	Antimicrobial agent	2016	2017	2018	2019	2020	2021	2022	2022 vs. 2021	2022 vs. 2017
		R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	P value	P value
H-Ec	Florfenicol	8.1%	7.4%	12.9%	10.3%	13.6%	16.5%	16.9%	1.0000	0.0008
H-Ec	Gentamicin	10.9%	8.6%	15.9%	14.3%	15.6%	12.8%	19.3%	0.0470	0.0004
H-Ec	Neomycin	6.9%	10.9%	17.0%	19.8%	25.6%	25.6%	43.2%	0.0000	0.0000
H-Ec	Tetracycline	69.8%	59.8%	62.9%	69.4%	62.8%	57.0%	73.3%	0.0001	0.0008
NH-Ec	Neomycin	10.2%	11.5%	16.1%	14.4%	19.4%	17.8%	26.1%	0.0638	0.0008
Se	Florfenicol	12.1%	9.8%	25.5%	14.5%	7.4%	25.0%	26.2%	1.0000	0.0338
Se	Trimethoprim	25.9%	31.1%	37.3%	40.3%	25.9%	18.8%	71.4%*	0.0122	0.0875
Ss	Erythromycin	53.0%	49.3%	48.2%	61.0%	57.3%	59.7%	68.9%	0.1684	0.0032

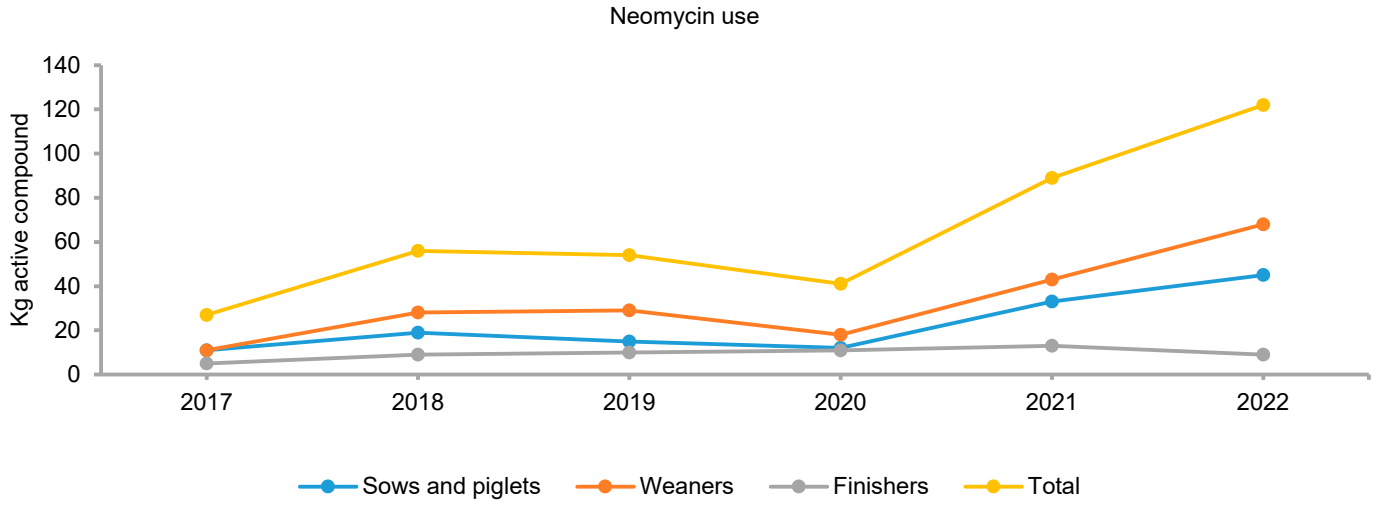
Antimicrobial resistance phenotypes that remained at the same level during 2021-2022 and 2017-2022 were excluded (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Se, *Salmonella enterica*; Ss, *Streptococcus suis;* R, resistant

Figure 9.1 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2016-2022 DANMAP 2022



Antimicrobial resistance phenotypes that remained at the same level during 2021-2022 and 2017-2022 were excluded (https://www.vetssi.dk/)
The percentages of trimethoprim-resistant *Salmonella enterica* isolates are based on small sample sizes (n<20) and should therefore be interpreted with caution

Figure 9.2 Neomycin use in the total pig population and in each age group, Denmark 2017-2022 DANMAP 2022



9.3 WGS-based detection of resistance genes and point mutations

WGS of a randomly selected subset of *A. pleuropneumoniae* (n=218), *B. bronchiseptica* (n=54), *C. perfringens* (n=140), *E. rhusiopathiae* (n=2), haemolytic *E. coli* (n=214), non-haemolytic *E. coli* (n=144), *G. parasuis* (n=90), *K. pneumoniae* (n=28), S. enterica (n=58), *S. hyicus* (n=11) and *S. suis* (n=250) isolates from 2021 and 2022 was subjected to WGS. Table 9.3 provides a list of the detected resistance genes and point mutations in *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis* from both 2021 and 2022. A full list of resistance genes and point mutations detected in isolates from both 2021 and 2022 can be found on DK-VET's homepage (https://www.vetssi.dk/).

Of note, *aph(3')-Ia* encoding resistance to neomycin was present in 33.9% and 31.4% of the haemolytic *E. coli* isolates and in 21.2% and 17.4% of the non-haemolytic *E. coli* isolates from 2022 and 2021, respectively. No other neomycin resistance genes were detected in *E. coli*. Interestingly, *aph(3')-Ia* was also present in 12.5% of the *K. pneumoniae* isolates from 2022 and in 28.2% and 21.1% of the *S. enterica* isolates from 2022 and 2021, respectively, while it was absent in *K. pneumoniae* isolates from 2021. In addition, *aph(3')-Ia* was present in 2.0% of the *G. parasuis* isolates from 2021 but absent in *G. parasuis* isolates from 2022. These observations suggest that *aph(3')-Ia* is present on a mobile genetic element that can be horizontally transferred within and between different species. Most of the susceptibility tested *aph(3')-Ia*-positive *E. coli* and *S. enterica* isolates were phenotypically resistant to neomycin, except from two out of 68 haemolytic *E. coli* isolates, one out of 26 non-haemolytic *E. coli* isolates and one out of 14 *S. enterica* isolates. *K. pneumoniae* and *G. parasuis* isolates were not tested for susceptibility to neomycin, and their phenotype is therefore unknown.

Some isolates harboured genes/point mutations associated with resistance towards antimicrobial agents considered critically important for human medicine by the World Health Organization. Here we focus on genes and mutations that confer resistance to carbapenems, 3rd, 4th and 5th generation cephalosporins (e.g., the 3rd generation cephalosporin cefotaxime), oxazolidinones (e.g., linezolid) and polymyxins (e.g., colistin).

For *C. perfringens*, *cfr*(B) and *cfr*(E) associated with resistance to linezolid were present in 1.8% and 3.6% of the isolates from 2022 but absent in isolates from 2021, while *cfr*(C) was present in 8.3% of the isolates from 2021 but absent in isolates from 2022. *cfr* genes are usually plasmid-borne and confer transferable resistance not only to linezolid and other oxazolidinone but also to lincosamides, phenicols, pleuromutilins and streptogramins, and it is therefore possible that use of other antimicrobial agents can co-select for linezolid resistance.

optrA, another gene associated with transferable resistance to linezolid and other oxazolidinone as well as to phenicols, was present in 1.4% and 1.9% of the *S. suis* isolates from 2022 and 2021, respectively. *C. perfringens* and *S. suis* isolates were not tested for susceptibility to linezolid, and their phenotype is therefore unknown.

The extended-spectrum β-lactamase (ESBL)-encoding $bla_{TEM-169}$ gene was present in 1.9% of the non-haemolytic *E. coli* isolates from 2022, while it was absent in non-haemolytic *E. coli* isolates from 2021. The ESBL-encoding $bla_{CTX-M-1}$ gene was present in 1.0% of the haemolytic *E. coli* isolates from 2021 but absent in haemolytic *E. coli* isolates from 2022. In addition, we detected two distinct extended-spectrum β-lactam resistance-associated mutations in the *ampC* promoter, a -32T→A transversion and a -42C→T transition, in 2.8% and 0.9% of the haemolytic *E. coli* isolates from 2022, compared with 1.9% and 3.8% of the haemolytic *E. coli* isolates from 2021. Seven out of eight susceptibility tested haemolytic *E. coli* isolates harbouring $bla_{CTX-M-1}$ and/or point mutations in the *ampC* promoter were phenotypically resistant to cefotaxime, with the only exception being a single haemolytic *E. coli* isolate harbouring a -32T→A transversion in the *ampC* promoter. The single non-haemolytic *E. coli* isolate harbouring $bla_{TEM-169}$ was not susceptibility tested, and its phenotype is therefore unknown. bla_{SHV-27}, $bla_{SHV-110}$ and $bla_{SHV-185}$ were detected in 6.3%, 87.5% and 6.3% of the *K. pneumoniae* isolates from 2022, compared with 0.0%, 100.0% and 0.0% of the *K. pneumoniae* isolates from 2021. These genes are considered to be naturally occurring in *K. pneumoniae*, where they encode an ESBL, a broad-spectrum β-lactamase and a hitherto uncharacterised β-lactamase, respectively (http://bldb.eu/). In addition, all *K. pneumoniae* isolates from both 2022 and 2021 harboured point mutations in *ompK36* and/or *ompK37* associated with resistance to cephalosporins. However, none of the 15 susceptibility tested *K. pneumoniae* isolates harbouring bla_{SHV} genes and/or point mutations in *ompK36* and *ompK37* were phenotypically resistant to cefotaxime.

We did not detect any genes encoding resistance to carbapenems or colistin, although it should be noted that we identified a point mutation in *pmrB* associated with resistance to colistin in 14.7% and 14.3% of the haemolytic *E. coli* isolates and 1.9% and 3.3% of the non-haemolytic *E. coli* isolates from 2022 and 2021, respectively. However, only two out of 35 susceptibility tested *E. coli* isolates harbouring the point mutation in *pmrB* were phenotypically resistant to colistin. All *K. pneumoniae* isolates harboured point mutations in *ompK36* and/or *ompK37* associated with resistance to carbapenems, but their phenotype is unknown as they were not tested for susceptibility to this antimicrobial subclass.

Table 9.3 Antimicrobial resistance genes and mutations identified through whole genome sequencing of pathogenic bacteria from pigs, Denmark, 2021-2022 DANMAP 2022

Pathogen	Resistance gene/mutation	Class	Phenotype	2021 %	2022 %	2022 vs. 2021 P value
Ap	*aph(3")-Ib*	Aminoglycoside	Streptomycin	0.0%	1.1%	0.4128
	bla$_{ROB-1}$	β-lactam	Penicillin, amoxicillin, ampicillin	0.8%	1.1%	1.0000
	sul2	Folate pathway antagonist	Sulfamethoxazole	0.0%	1.1%	0.4128
	tet(B)	Tetracycline	Doxycycline, tetracycline, minocycline	3.1%	3.3%	1.0000
H-Ec	*aac(3)-IId*	Aminoglycoside	Apramycin, gentamicin, tobramycin, dibekacin, netilmicin, sisomicin	1.9%	0.9%	0.6163
	aac(3)-IV	Aminoglycoside	Gentamicin, tobramycin	9.5%	22.0%	0.0148
	aadA1	Aminoglycoside	Spectinomycin, streptomycin	41.0%	44.0%	0.6798
	aadA2	Aminoglycoside	Spectinomycin, streptomycin	17.1%	11.0%	0.2387
	aadA3	Aminoglycoside	Spectinomycin, streptomycin	2.9%	4.6%	0.7217
	aadA4	Aminoglycoside	Spectinomycin, streptomycin	0.0%	1.8%	0.4978
	aadA5	Aminoglycoside	Spectinomycin, streptomycin	1.0%	3.7%	0.3695
	aadA7	Aminoglycoside	Spectinomycin, streptomycin	0.0%	0.9%	1.0000
	aadA11	Aminoglycoside	Spectinomycin, streptomycin	1.0%	1.8%	1.0000
	aadA12	Aminoglycoside	Spectinomycin, streptomycin	17.1%	11.9%	0.3330
	aadA13	Aminoglycoside	Spectinomycin, streptomycin	1.9%	3.7%	0.6834
	aadA17	Aminoglycoside	Spectinomycin, streptomycin	0.0%	3.7%	0.1217
	aadA22	Aminoglycoside	Spectinomycin, streptomycin	1.0%	0.9%	1.0000
	ant(2")-Ia	Aminoglycoside	Gentamicin, tobramycin	1.0%	1.8%	1.0000
	ant(3")-Ia	Aminoglycoside	Streptomycin	26.7%	30.3%	0.6499
	aph(3")-Ib	Aminoglycoside	Streptomycin	46.7%	57.8%	0.1320
	aph(3')-Ia	Aminoglycoside	Neomycin, kanamycin, lividomycin, paromomycin, ribostamycin	31.4%	33.9%	0.7711
	aph(4)-Ia	Aminoglycoside	Hygromycin	9.5%	20.2%	0.0349
	aph(6)-Id	Aminoglycoside	Streptomycin	44.8%	51.4%	0.3420
	bla$_{CTX-M-1}$	β-lactam	Amoxicillin, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin, ticarcillin	1.0%	0.0%	0.4907
	bla$_{TEM-1A}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	1.0%	4.6%	0.2126
	bla$_{TEM-1B}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	61.9%	63.3%	0.8880
	bla$_{TEM-1C}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	1.0%	1.8%	1.0000
	bla$_{TEM-30}$	β-lactam	Amoxicillin, amoxicillin/clavulanic acid, ampicillin, ampicillin/clavulanic acid, piperacillin,piperacillin/ tazobactam, ticarcillin, ticarcillin/clavulanic acid	1.9%	2.8%	1.0000
	bla$_{TEM-127}$	β-lactam	Amoxicillin, ampicillin, cephalothin, piperacillin, ticarcillin	0.0%	0.9%	1.0000
	bla$_{TEM-176}$	β-lactam	Unknown β-lactam	0.0%	1.8%	0.4978
	bleO	Glycopeptide	Bleomycin	1.0%	0.9%	1.0000
	catA1	Amphenicol	Chloramphenicol	1.9%	3.7%	0.6834
	cmlA1	Amphenicol	Chloramphenicol	15.2%	6.4%	0.0468
	dfrA1	Folate pathway antagonist	Trimethoprim	31.4%	43.1%	0.0904
	dfrA5	Folate pathway antagonist	Trimethoprim	5.7%	2.8%	0.3255
	dfrA8	Folate pathway antagonist	Trimethoprim	0.0%	1.8%	0.4978
	dfrA10	Folate pathway antagonist	Trimethoprim	0.0%	0.9%	1.0000
	dfrA12	Folate pathway antagonist	Trimethoprim	8.6%	4.6%	0.2780
	dfrA14	Folate pathway antagonist	Trimethoprim	5.7%	2.8%	0.3255
	dfrA16	Folate pathway antagonist	Trimethoprim	0.0%	0.9%	1.0000
	dfrA17	Folate pathway antagonist	Trimethoprim	1.0%	2.8%	0.6217
	dfrA32	Folate pathway antagonist	Trimethoprim	1.0%	0.0%	0.4907
	dfrA36	Folate pathway antagonist	Trimethoprim	1.0%	0.9%	1.0000
	ere(A)	Macrolide	Erythromycin	1.0%	0.0%	0.4907
	erm(42)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	0.0%	1.8%	0.4978

Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis*

continued ... Table 9.3 Antimicrobial resistance genes and mutations identified through whole genome sequencing of pathogenic bacteria from pigs, Denmark, 2021-2022 DANMAP 2022

Pathogen	Resistance gene/mutation	Class	Phenotype	2021 %	2022 %	2022 vs. 2021 P value
H-Ec	*erm*(B)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	13.3%	14.7%	0.8452
	floR	Amphenicol	Chloramphenicol, florfenicol	16.2%	17.4%	0.8563
	lnu(F)	Lincosamide	Lincomycin	1.0%	5.5%	0.1193
	lnu(G)	Lincosamide	Lincomycin	2.9%	4.6%	0.7217
	mef(B)	Macrolide	Erythromycin, azithromycin	1.9%	0.9%	0.6163
	mph(A)	Macrolide	Erythromycin, azithromycin, spiramycin, telithromycin	15.2%	19.3%	0.4735
	mph(B)	Macrolide	Erythromycin, spiramycin, telithromycin	2.9%	6.4%	0.3329
	qnrS1	Quinolone	Ciprofloxacin	5.7%	8.3%	0.5948
	sul1	Folate pathway antagonist	Sulfamethoxazole	44.8%	50.5%	0.4151
	sul2	Folate pathway antagonist	Sulfamethoxazole	38.1%	50.5%	0.0750
	sul3	Folate pathway antagonist	Sulfamethoxazole	15.2%	12.8%	0.6952
	tet(A)	Tetracycline	Doxycycline, tetracycline	40.0%	43.1%	0.6787
	tet(B)	Tetracycline	Doxycycline, tetracycline, minocycline	21.0%	35.8%	0.0227
	tet(C)	Tetracycline	Doxycycline, tetracycline	1.0%	0.0%	0.4907
	tet(G)	Tetracycline	Doxycycline, tetracycline	1.0%	0.0%	0.4907
	tet(M)	Tetracycline	Doxycycline, tetracycline, minocycline	1.0%	1.8%	1.0000
	ampC promoter T-32A	β-lactam	Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin	1.9%	2.8%	1.0000
	ampC promoter C-42T	β-lactam	Ampicillin, ampicillin/clavulanic acid, amoxicillin, amoxicillin/clavulanic acid, cefixime, cefotaxime, cefoxitin, ceftazidime, piperacillin	3.8%	0.9%	0.2058
	gyrA S83L	Quinolone	Nalidixic acid, ciprofloxacin	2.9%	4.6%	0.7217
	gyrA D87Y	Quinolone	Nalidixic acid	0.0%	0.9%	1.0000
	parC F60I	Quinolone	Nalidixic acid, ciprofloxacin	0.0%	0.9%	1.0000
	parC S80I	Quinolone	Nalidixic acid, ciprofloxacin	1.0%	0.0%	0.4907
	parC S80R	Quinolone	Nalidixic acid, ciprofloxacin	0.0%	1.8%	0.4978
	parC E84K	Quinolone	Nalidixic acid, ciprofloxacin	1.9%	1.8%	1.0000
	parE I355T	Quinolone	Nalidixic acid, ciprofloxacin	0.0%	0.9%	1.0000
	parE I529L	Quinolone	Nalidixic acid, ciprofloxacin	3.8%	0.9%	0.2058
	pmrB V161G	Polymyxin	Colistin	14.3%	14.7%	1.0000
Ss	*ant(6)-Ia*	Aminoglycoside	Streptomycin	19.4%	19.0%	1.0000
	ant(6)-Ib	Aminoglycoside	Streptomycin	0.9%	0.7%	1.0000
	aph(3')-III	Aminoglycoside	Kanamycin, amikacin, neomycin, butirosin, isepamicin, lividomycin, paromomycin, ribostamycin	7.4%	7.0%	1.0000
	erm(47)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	1.9%	2.1%	1.0000
	erm(B)	Macrolide, lincosamide, streptogramin B	Erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin IA, virginiamycin S	57.4%	64.8%	0.2409
	lnu(B)	Lincosamide	Lincomycin, clindamycin	25.9%	16.9%	0.0862
	lnu(C)	Lincosamide	Lincomycin	0.0%	0.7%	1.0000
	lsa(E)	Lincosamide, streptogramin A, pleuromutilin	Lincomycin, clindamycin, dalfopristin, pristinamycin IIA, virginiamycin M, tiamulin	25.9%	16.9%	0.0862
	mef(A)	Macrolide	Erythromycin, azithromycin	4.6%	2.1%	0.2970
	msr(D)	Macrolide, streptogramin B	Erythromycin, azithromycin, telithromycin, quinupristin, pristinamycin IA, virginiamycin S	3.7%	0.7%	0.1689
	optrA	Oxazolidinone, amphenicol	Linezolid, chloramphenicol, florfenicol	1.9%	1.4%	1.0000
	tet(40)	Tetracycline	Doxycycline, tetracycline	0.0%	0.7%	1.0000
	tet(M)	Tetracycline	Doxycycline, tetracycline, minocycline	7.4%	3.5%	0.2497
	tet(O)	Tetracycline	Doxycycline, tetracycline, minocycline	30.6%	16.9%	0.0146

Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis*

9.4 WGS-based prediction of AMR

WGS-based prediction of AMR was assessed by determining the concordance, sensitivity, specificity, positive predictive value, negative predictive value, major error rate and very major error rate between the results obtained through AST and WGS using the genotype-to-phenotype translations in the ResFinder 4.1 database. Table 9.4 shows the results for *A. pleuropneumoniae*, haemolytic *E. coli* and *S. suis* isolates from both 2021 and 2022, while results for all pathogen-drug combinations can be found on DK-VET's homepage (https://www.vetssi.dk/). The observed concordance was 99.7% for *A. pleuropneumoniae*, 64.5% for *B. bronchiseptica*, 92.8% for haemolytic *E. coli*, 93.9% for non-haemolytic *E. coli*, 61.7% for *K. pneumoniae*, 95.7% for *S. enterica*, 92.6% for *S. hyicus* and 94.0% for *S. suis*.

Table 9.4 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark, 2021-2022 DANMAP 2022

Pathogen	Antimicrobial agent	P+/G+	P-/G-	G+/P-	G-/P+	Concordance	Sensitivity	Specificity	PPV	NPV	ME rate	VME rate
Ap	Amoxicillin	0	28	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Ampicillin	1	183	0	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Ceftiofur	0	210	0	2	99.1	0.0	100.0	NA	99.1	0.0	100.0
	Doxycycline	0	27	1	0	96.4	NA	96.4	0.0	100.0	3.6	NA
	Enrofloxacin	0	27	0	1	96.4	0.0	100.0	NA	96.4	0.0	100.0
	Erythromycin	0	184	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Florfenicol	0	212	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Penicillin	1	209	0	2	99.1	33.3	100.0	100.0	99.1	0.0	66.7
	Tetracycline	6	178	0	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Tiamulin	0	212	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Tildipirosin	0	28	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Tilmicosin	0	212	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Trimethoprim/ sulfamethoxazol	0	212	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Tulathromycin	0	199	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Total	8	2121	1	5	99.7	61.5	100.0	88.9	99.8	0.0	38.5
H-Ec	Amoxicillin	63	22	0	1	98.8	98.4	100.0	100.0	95.7	0.0	1.6
	Amoxicillin/clavulanic acid	14	179	0	18	91.5	43.8	100.0	100.0	90.9	0.0	56.3
	Ampicillin	85	39	0	1	99.2	98.8	100.0	100.0	97.5	0.0	1.2
	Cefotaxime	7	117	1	0	99.2	100.0	99.2	87.5	100.0	0.8	0.0
	Cefpodoxime	0	86	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Ceftiofur	0	124	0	1	99.2	0.0	100.0	NA	99.2	0.0	100.0
	Chloramphenicol	30	91	4	0	96.8	100.0	95.8	88.2	100.0	4.2	0.0
	Ciprofloxacin	14	105	6	0	95.2	100.0	94.6	70.0	100.0	5.4	0.0
	Colistin	2	180	29	0	86.3	100.0	86.1	6.5	100.0	13.9	0.0
	Doxycycline	46	24	15	1	81.4	97.9	61.5	75.4	96.0	38.5	2.1
	Enrofloxacin	0	78	0	8	90.7	0.0	100.0	NA	90.7	0.0	100.0
	Florfenicol	35	173	1	2	98.6	94.6	99.4	97.2	98.9	0.6	5.4
	Gentamicin	38	173	0	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Nalidixic acid	4	115	6	0	95.2	100.0	95.0	40.0	100.0	5.0	0.0
	Neomycin	66	143	2	0	99.1	100.0	98.6	97.1	100.0	1.4	0.0
	Spectinomycin	117	61	24	9	84.4	92.9	71.8	83.0	87.1	28.2	7.1
	Streptomycin	161	27	20	3	89.1	98.2	57.4	89.0	90.0	42.6	1.8
	Tetracycline	135	71	4	1	97.6	99.3	94.7	97.1	98.6	5.3	0.7
	Trimethoprim	63	53	1	8	92.8	88.7	98.1	98.4	86.9	1.9	11.3
	Trimethoprim/ sulfamethoxazol	0	38	0	48	44.2	0.0	100.0	NA	44.2	0.0	100.0
	Total	880	1899	113	101	92.8	89.7	94.4	88.6	95.0	5.6	10.3

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis;* P+, resistant phenotype; P-, susceptible phenotype; G+, resistant gentotype; G-, susceptible genotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error; NA, not applicable

continued ... Table 9.4 Diagnostic performance of ResFinder 4.1 as an antimicrobial resistance prediction tool for pathogenic bacteria from pigs, Denmark, 2021-2022 DANMAP 2022

Pathogen	Antimicrobial agent	P+/G+	P-/G-	G+/P-	G-/P+	Concordance	Sensitivity	Specificity	PPV	NPV	ME rate	VME rate
Ss	Chloramphenicol	0	183	2	1	98.4	0.0	98.9	0.0	99.5	1.1	100.0
	Doxycycline	12	33	0	10	81.8	54.5	100.0	100.0	76.7	0.0	45.5
	Enrofloxacin	0	55	0	0	100.0	NA	100.0	NA	100.0	0.0	NA
	Erythromycin	115	68	1	2	98.4	98.3	98.6	99.1	97.1	1.4	1.7
	Florfenicol	4	237	0	0	100.0	100.0	100.0	100.0	100.0	0.0	0.0
	Penicillin	0	237	0	4	98.3	0.0	100.0	NA	98.3	0.0	100.0
	Tetracycline	53	103	0	20	88.6	72.6	100.0	100.0	83.7	0.0	27.4
	Trimethoprim/ sulfamethoxazol	0	198	0	43	82.2	0.0	100.0	NA	82.2	0.0	100.0
	Total	184	1114	3	80	94.0	69.7	99.7	98.4	93.3	0.3	30.3

Data are based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/)
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; H-Ec, haemolytic *Escherichia coli*; Ss, *Streptococcus suis;* P+, resistant phenotype; P-, susceptible phenotype; G+, resistant gentotype; G-, susceptible genotype; PPV, positive predictive value; NPV, negative predictive value; ME, major error; VME, very major error; NA, not applicable

9.5 Conclusions and perspectives

AST showed that most pathogenic bacteria from pigs displayed similar frequencies of phenotypic resistance as in previous years.

The high frequency of resistance in haemolytic *E. coli* to first-choice drugs for treatment of *E. coli*-associated post-weaning diarrhoea is worrisome and should be monitored closely in the coming years. Of note, our interpretation was based on ECOFFs as animal-specific clinical breakpoints for these drugs are currently lacking. ECOFFs are based on microbiological studies and do not necessarily indicate whether a drug will be clinically active. Future studies should therefore seek to establish animal-specific clinical breakpoints to antimicrobial agents of veterinary importance by considering what happens to the drug within a specific animal and body site (pharmacokinetics).

WGS demonstrated that resistance towards antimicrobial agents considered critically important for human medicine remained at a low level.

WGS seems to be a promising tool for prediction and surveillance of AMR in pathogenic bacteria from pigs. However, it was not always possible to compare AST and WGS results due to the lack of ECOFFs and clinical breakpoints for many antimicrobial agents of veterinary importance, and due to limited knowledge on genes and mutations conferring resistance to these drugs. In addition, the ResFinder 4.1 genotype-to-phenotype translation scheme for point mutations in *K. pneumoniae* is under development and the phenotypes are currently based on antimicrobial classes rather than agents, which might explain some of its poor performance in this species. Closing these gaps could substantially improve the usefulness of WGS for AMR prediction and surveillance in pathogenic bacteria from animals. WGS is also a useful tool for monitoring resistance mechanisms in pathogenic bacteria, for which AST is unavailable, and for tracing the spread of specific resistance genes and pathogenic bacteria within and between animal and human populations.

It has been agreed not to mention additional material in this year's report.

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Textbox 9.1

Antimicrobial resistance in dogs and cats: focus on extended-spectrum cephalosporinase-producing *Escherichia coli* and their resemblance to human clinical isolates

Background

In Denmark, reports on antimicrobial resistance in canine and feline pathogens have been published sporadically over the years, last time in DANMAP 2019. Traditionally, susceptibility results have been reported for *Escherichia coli* and *Staphylococcus pseudintermedius*, which are the most frequently isolated bacterial pathogens of companion animals and the primary causes of urinary tract and skin infections, respectively. These species can have important resistant and multidrug-resistant phenotypes. Of particular concern are extended-spectrum cephalosporinase- (ESC) producing *E. coli* and methicillin-resistant *S. pseudintermedius* (MRSP), which have emerged worldwide in the last two decades and constitute a threat to animal health, as they can be resistant to all veterinary licensed antibiotics [1]. *E. coli* and to a lesser extent MRSP are also pathogens of public health relevance due to the risk of zoonotic transmission.

Materials and Methods

Antimicrobial susceptibility data were retrieved for 726 *S. pseudintermedius* and 559 *E. coli* isolates obtained in 2020-2022 from various infections in dogs and cats (Table 1). Diagnostic specimens had been shipped from primary care and referral veterinary hospitals from across Denmark to the diagnostic laboratory Sund Vet Diagnostik at the University of Copenhagen. Susceptibility testing was done using broth microdilution with commercial Sensititre plates (ThermoFisher Scientific). Interpretation of MIC data was according to clinical breakpoints published by the Clinical and Laboratory Standards Institute [2]. Sixteen cefpodoxime-resistant *E. coli* isolates stored in the period 2012-2022 were subjected to Illumina MiSeq sequencing, followed by genome assembly using SPAdes v.3.13.1 [3]. Multi-locus sequence typing and screening for genes encoding ESBL or plasmid-borne AmpC genes was performed using mlst v2.19.0 (https://github.com/tseemann/mlst), and Abricate v1.0.1. (https://github.com/tseemann/abricate), respectively. Sequences were then imported into the software SeqSphere+ (Ridom) for construction of core-genome (CG) MLST phylogeny based on analysis of 2,513 genes. Here, cgMLST profiles were compared to corresponding profiles of all 1,243 Danish human ESBL/AmpC-producing *E. coli* isolates obtained from blood infections in 2014-2023 and representing the sequence types (STs) found in dogs and cats.

Table 1 Origin of *E. coli* and *S. pseudintermedius* isolates obtained from clinical specimens in Sund Vet Diagnostik, 2020-2022

DANMAP 2022

	Staphylococcus pseudintermedius		*Escherichia coli*	
	Dogs	Cats	Dogs	Cats
Skin, wounds and ears	594	13	114	16
Urinary tract	48	1	288	73
Other	68	2	56	12
Total	710	16	458	101

Results and Discussion

Except for minor fluctuations, levels of antimicrobial resistance were very similar to those encountered in the latest surveillance periods, namely 2016-17 and 2018-19 (Table 2). One example to highlight for *S. pseudintermedius* is oxacillin (6%), which is used as diagnostic indicator for MRSP. In *E. coli*, no isolates displayed carbapenem resistance, whereas 4% and 3% of isolates were resistant to fluoroquinolone and 3rd generation cephalosporin (3GC), respectively. The latter drug class (3GC) is used as diagnostic indicator for ESC production (i.e. ESBLs and AmpCs), hence the level of these resistant bacteria remains stable or may even be decreasing slightly. Sequencing of 3GC-resistant *E. coli* revealed that CMY-2 and CTX-M-15 are the most common ESCs, being present in 7 (44%) and 4 (25%) isolates, respectively. This is in line with previous studies reporting these to be among the predominant ESCs in companion animals [4;5]. Five of seven CMY-2-producing isolates belonged to ST372,

continued ... Textbox 9.1

which has recently been described as a major dog-adapted *E. coli* lineage [5]. The second most common sequence type was ST131 (n=3), which in humans predominates as a multi-resistant and hyper-virulent CTX-M-15-producing lineage, typically of the O25:4 *fimH*30 variant. Only one of the three canine ST131 isolates was positive for CTX-M-15. Although this strain was multi-resistant and carried 36 predicted virulence genes, the strain was classified as O16:J5 *fimH*41, hence different from the worldwide dominating ST131 lineage, and different from human clinical isolates in Denmark.

Using an arbitrary cut-off of 30 alleles, we found by cgMLST i) 2 allele differences between the canine ST155/DHA-1 isolate and a human isolate, ii) 21-25 allele differences between the canine ST162/CTX-M-15 isolate and three human isolates, and iii) 8 allele differences between the canine ST131/CTX-M-27 isolate and one human isolate. Remaining dog and cat isolates had between 32 and several hundred allele differences to human isolates. It is unlikely that any of the ESBL/AmpC-producing isolates originate from pets and humans living together. Nevertheless, these findings support previous research indicating that pets may be reservoirs of human-infectious *E. coli* lineages, and that zoonotic transmission within households is possible [6;7]. In extension to that, a recent Danish study indicated that approximately one out of ten dog owners with community-associated UTI share the infectious *E. coli* strain with their dog [6].

Table 2 Percentages of antimicrobial-resistant clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats in Denmark

DANMAP 2022

	Escherichia coli			*Staphylococcus pseudintermedius*		
Antimicrobial agent	2016-2017 (N=394)	2018-2019 (N=441)	2020-2022 (n=559)	2016-2017 (N=486)	2018-2019 (N=602)	2020-2022 (n=726)
	%	%	%	%	%	%
Amikacin	2	2	1	1	1	1
Ampicillin [1]	14	25	22	59	70	65
Amoxicillin/clavulanic acid [1]	4	5	6	8	7	6
Cefazolin	-	-	-	8	7	6
Cefpodoxime	4	5	3	-	-	-
Chloramphenicol	4	4	2	16	21	20
Clindamycin	-	-	-	25	27	26
Doxycycline	7	8	4	33	29	28
Enrofloxacin	3	4	4	3	2	4
Erythromycin	-	-	-	26	28	26
Gentamicin	4	4	3	3	2	5
Imipenem	0	0	0	-	-	-
Marbofloxacin	3	3	4	3	3	4
Oxacillin	-	-	-	8	6	6
Sulfamethoxazole/trimethoprim	7	9	8	5	6	8

1) Susceptibility data for ampicillin and amoxicillin/clavulanic acid in *E. coli* have been determined only for isolates from urinary tract infections, as isolates from other infections are unequivocally classified as resistant to these drugs according to CLSI breakpoints

Table 3. Multilocus sequence types and extended-spectrum cephalosporinases detected in the 16 stored *E. coli* isolates resistant to cefpodoxime
DANMAP 2022

Isolate	Extended-spectrum cephalosporinase	Sequence type	Origin	Year
1	CMY-2	ST372	Dog	2012
2	CTX-M-14	ST448	Dog	2015
3	CTX-M-27	ST131	Dog	2015
4	CMY-2	ST963	Dog	2016
5	CTX-M-15	ST648	Cat	2017
6	CMY-2	ST372	Dog	2018
7	CMY-2	ST372	Dog	2019
8	CTX-M-15	ST131	Dog	2019
9	CMY-2	ST372	Dog	2019
10	CMY-2	ST14967	Dog	2019
11	CMY-2	ST372	Dog	2021
12	DHA-1	ST155	Dog	2021
13	CTX-M-15	ST998	Dog	2022
14	CTX-M-1	ST88	Dog	2022
15	CTX-M-3	ST131	Dog	2022
16	CTX-M-15	ST162	Dog	2022

Conclusion

Antimicrobial resistance in clinical *E. coli* and *S. pseudintermedius* from dogs and cats in Denmark has been stable over the last six years. The close genetic similarities between canine and human clinical ESC-producing *E. coli* isolates indicates a potential risk of transmission between the two hosts. Further research is needed to understand if the close genetic similarities detected are limited to ESC-producing strains like ST131, and if this and other human pathogenic lineages also circulate in the canine population as non-ESC producers.

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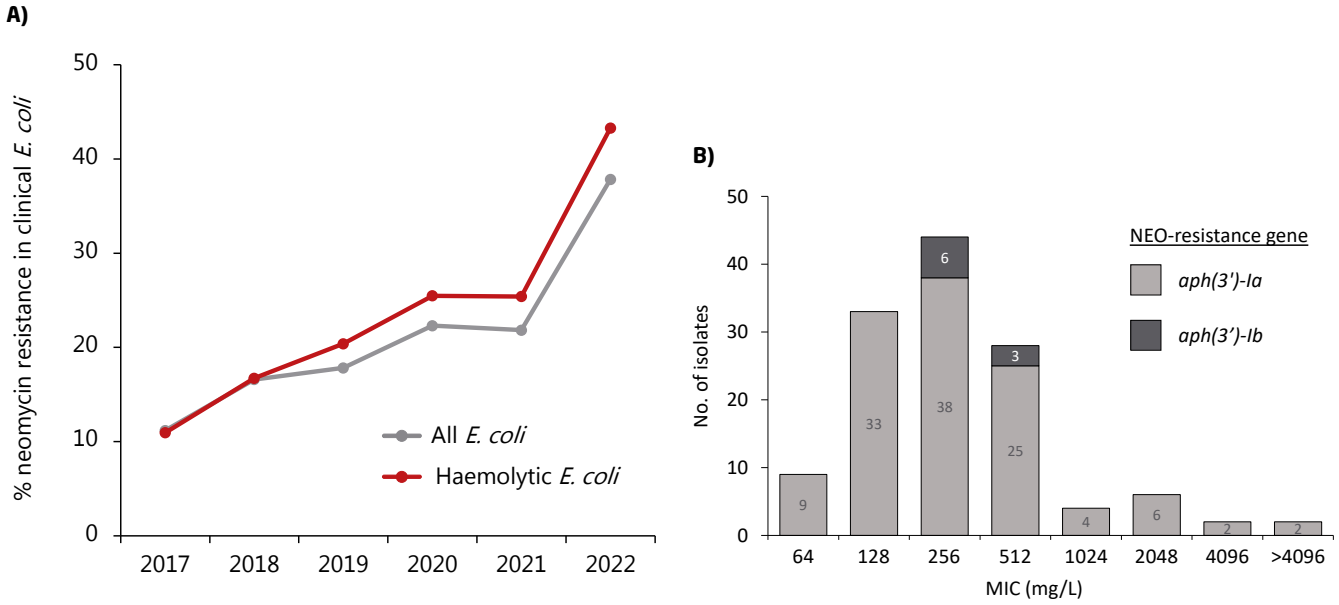
Textbox 9.2

Insights into the genetic basis of neomycin resistance in clinical *Escherichia coli* isolated from pigs

Background

Neomycin is commonly used as a first choice antibiotic for treating porcine enteritis caused by enterotoxigenic *Escherichia coli* (ETEC). After the ban on zinc oxide, a rise in neomycin resistance has been observed in Denmark (Figure 1A), likely due to increased neomycin use [1]. In this study, we elucidated the mechanisms of neomycin resistance by characterizing a collection of 128 neomycin-resistant clinical *E. coli* isolated from Danish pig farms between 2015 and 2020 [2].

Figure 1 Prevalence of neomycin-resistance among porcine clinical *E. coli* isolates after the reintroduction of neomycin in 2017 (A) and MIC distribution in the 128 neomycin-resistant strains analyzed in this study (B) DANMAP 2022



Source: Danish Agriculture and Food Council, Veterinary Laboratory, Kjellerup

Methods

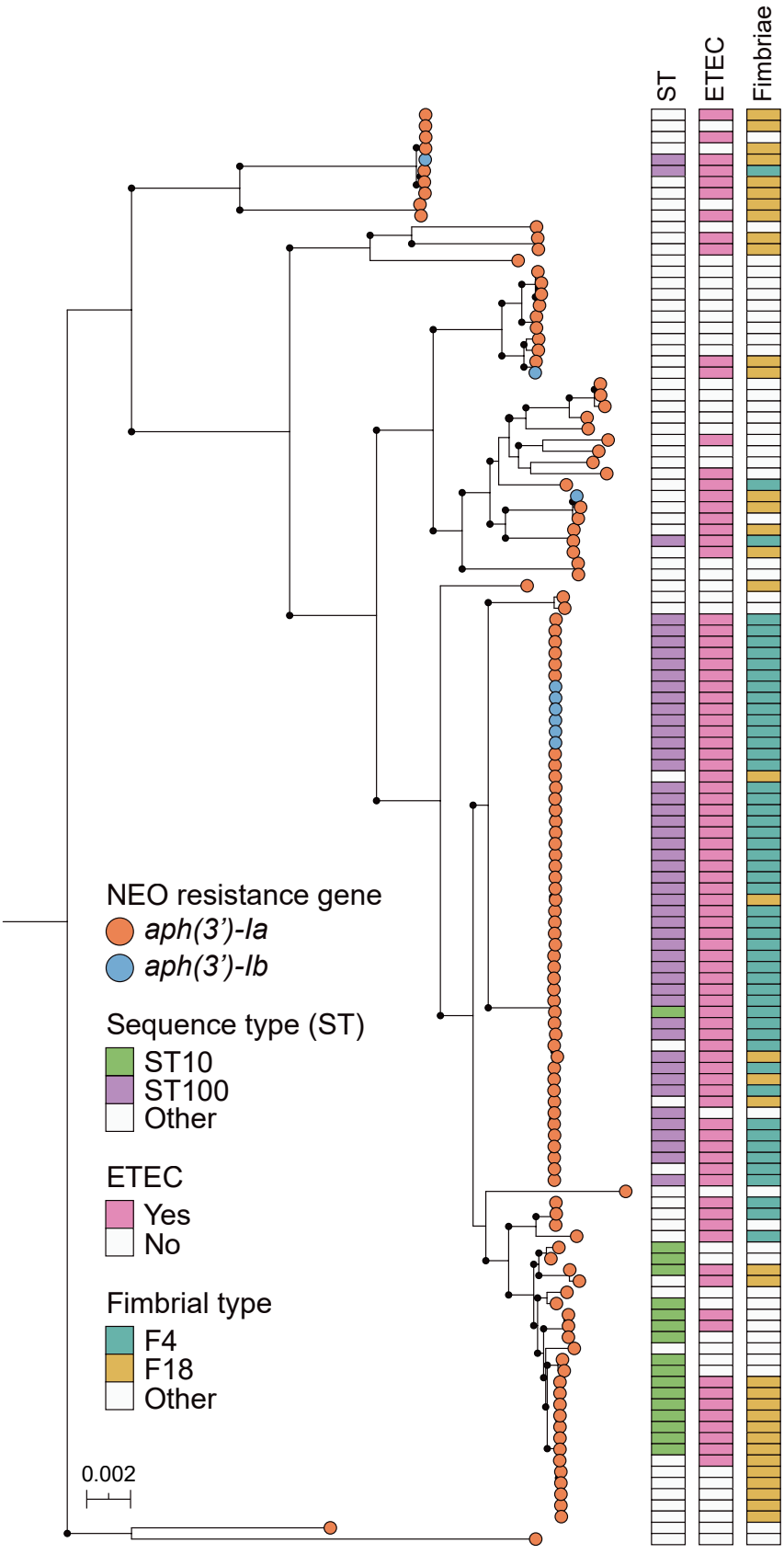
All isolates were analysed by Illumina sequencing and antimicrobial susceptibility testing. Conjugation experiments were performed on 32 strains selected based on phylogenetic analysis to assess plasmid transferability. For further understanding of the structures and associated mobile genetic elements in the plasmids encoding neomycin resistance, eight selected representative strains were subjected to long-read sequencing using Nanopore.

Results

We identified 35 different *E. coli* lineages with sequence types ST100 (38.3%) and ST10 (22.7%) accounting for approximately 61% of the isolates. While ST100 was strongly associated with ETEC displaying fimbria type F4, ST10 occurred in both ETEC with fimbria type F18 and non-ETEC strains (Figure 2). Most isolates (95.3%) were resistant to three or more antimicrobial classes in addition to neomycin. The MICs of neomycin were extremely variable (64 to ≥4096 mg/L) with most isolates (82%) displaying MICs of 128-512 mg/L (Figure 1B). Neomycin resistance was transferable under laboratory conditions from 25 out of the 32 selected strains. The genes encoding neomycin resistance were *aph(3')-Ia* (93%) and *aph(3')-Ib* (7%). While the former gene was associated with two types of transposons, Tn903 or Tn4352, which were distributed on a variety of conjugative plasmid backbones (mainly IncI1α but also IncHI1, IncHI2, IncN and ColRNAI), the second gene was not flanked by any transposable element and was consistently found on a small (19 kb) non-conjugative but mobilizable plasmid that was traced back to distantly related Gram-negative bacteria like *Achromobacter* and *Pseudomonas putida*.

Figure 2 Phylogenetic tree of the 128 sequenced neomycin-resistant *E. coli* isolates using core-genome alignment. The tree displays neomycin resistance gene, sequence type (ST), ETEC status and fimbrial type for each strain (modified from reference [2])

DANMAP 2022



continued ... Textbox 9.2

Discussion

The results show that the spread of neomycin resistance recently observed in clinical *E. coli* from Danish pig farms is driven by two resistance determinants that are located on different plasmid scaffolds capable of spreading across many different *E. coli* lineages. The two most common lineages, ST100 and ST10, have previously been reported as prevalent among clinical porcine ETEC strains in various regions of the world, including Denmark [3]. The latter lineage has zoonotic potential, since it is one of the five most common epidemic lineages responsible for human extraintestinal infections globally [4].

Neomycin-resistant strains displayed high rates of resistance to alternative antibiotics that can be used to manage porcine ETEC enteritis, such as spectinomycin (89.8%), sulfamethoxazole (85.9%), and tetracycline (78.9%). This result highlights the lack of effective alternatives to neomycin for treatment of this common disease in pig production. The situation will unlikely improve in the future, since our study shows that *aph(3')-Ia* is usually located on plasmids carrying genes conferring resistance to other antimicrobials such as tetracyclines, providing evidence that neomycin resistance may be co-selected by the use of other antimicrobials and vice versa. The lack of effective alternatives to neomycin underscores the importance of implementing strategies to preserve the efficacy of neomycin and explore novel approaches to managing this common infection in pig production, including alternatives to antimicrobials (see Chapter 4, Textbox 4.3).

This study provides valuable insights into the genetic basis of neomycin resistance in porcine clinical *E. coli* strains. In the absence of a validated clinical breakpoint, it is still unclear if all strains tested *in vitro* as neomycin-resistant are *in vivo* resistant, especially due to the low oral bioavailability of this aminoglycoside and the high concentrations achieved in the intestinal tract following oral administration [5]. The high variability of MICs observed in this study highlights the importance of assessing the clinical efficacy of neomycin in the field while monitoring the evolution of the neomycin resistance phenotype in the years to come.

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Textbox 9.3

Assessing the burden of Antimicrobial Resistance and Usage in the Global Burden of Animal Disease programme: the start of the Danish case study

Background

The Global Burden of Animal Diseases programme (GBADs) [1] is an international collaboration of partners that aims to assess the burden of animal disease from an economic perspective in terms of net loss of production, expenditure, and impacts on the economy and trade within the context of food systems. It measures the burden of disease in livestock in terms of Animal Health Loss Envelope (AHLE), an approach to calculate the absolute cost of disease against a zero-cost ideal [2].

This overall loss envelope can then be disaggregated into specific causes, including infectious diseases and within those, antimicrobial resistance (AMR). A better understanding of the socio-economic impact of AMR and of antimicrobial usage (AMU) in livestock is key to efficiently tackling the threat of AMR. Burden data provides baseline information and underpins cost-effectiveness assessments for changes in practices. Yet gaps remain in our understanding, including how AMR leads to livestock production and animal health losses, veterinary expenditure, and externalities in public health and the environment. The GBADs' work on AMU and AMR aims to address these gaps, by providing a methodology nested within the AHLE framework, identifying data requirements, and generating burden estimates in selected case studies.

In the Danish case study, the component of the AHLE attributable to AMU and AMR in the pig sector will be estimated. As the first GBADs case study on AMU/AMR, the results will be particularly relevant for further applications of the methodology. Lessons learned will be used to refine the analytical framework developed and to better understand data challenges. This textbox expands on the analytical approach that is being taken for this assessment and describes the next stages of the work.

Analytical approach

AMR and AMU can contribute to the AHLE component attributable to infectious diseases through different pathways. On one hand, the usage of antimicrobials in farmed animals constitutes a component of the health expenditure accrued when mitigating or preventing infectious diseases' impact. On the other hand, AMR's potential negative effects on the severity and/or duration of illness in animals will be a contributor to mortality and productivity losses associated with infectious diseases. If treated, those resistant infections will contribute further to the AHLE as additional healthcare expenditure due to treatment failures, including repeated treatments and treatment with potentially costlier therapeutic alternatives. Figure 1 summarizes these pathways for losses.

Methods and next steps in the Danish case study

The Danish case study will follow the analytical framework described above and will focus specifically on the pig sector.

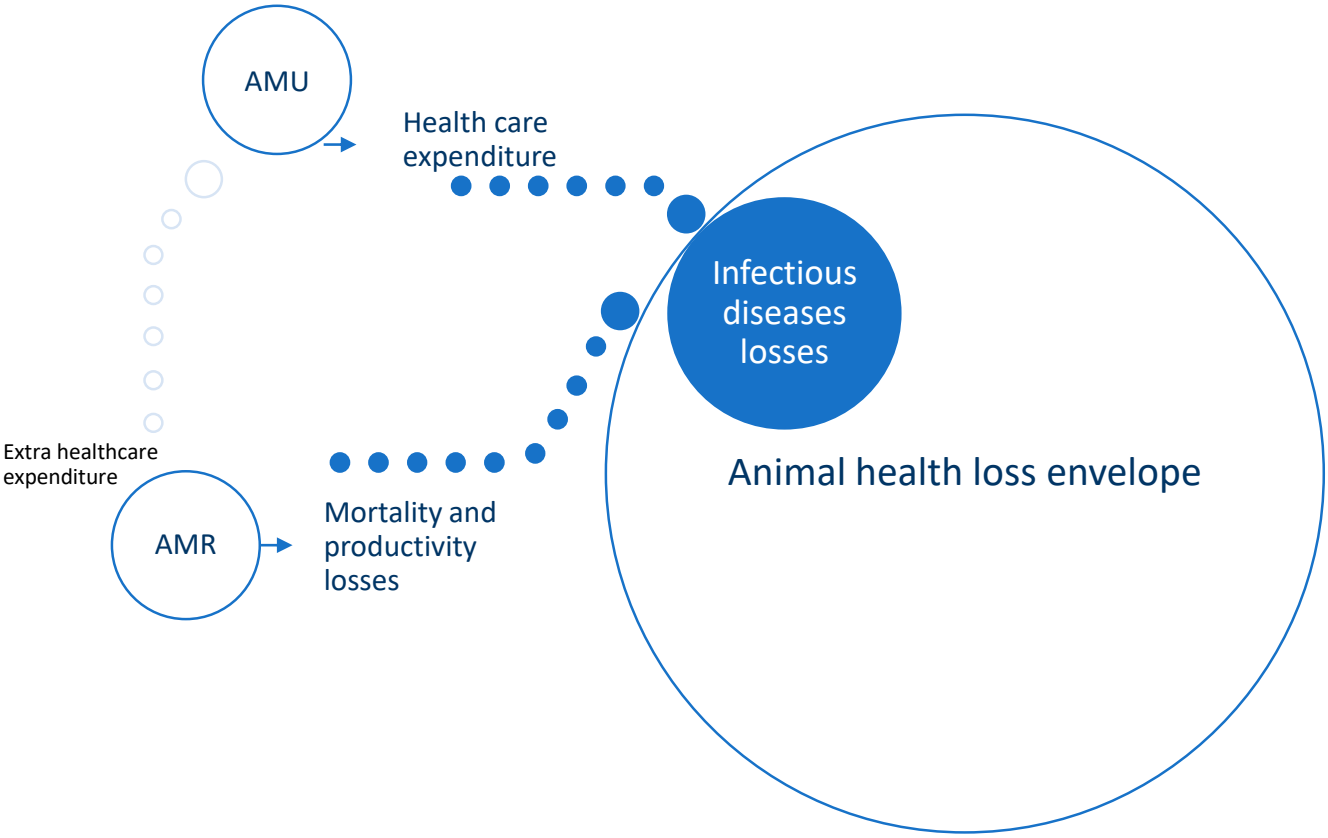
The first stage of the assessment is focusing on the assessment of the AMU burden. Data on antimicrobial consumption and sales for 2021 in pigs has been sourced from the VetStat database. Expenditure is currently being estimated by combining the VetStat data on consumption and sales and pricing data published by Medicin Til Dyr [3]. The pricing data has been extracted using Web Scraping scripts developed to automate the process. Web Scraping scripts were developed in Python using the Selenium package [4] and are available in the GBADs GitHub [5].

The work will move to assess the AMR burden, in terms of contribution to mortality and productivity losses, and extra health care expenditure within the AHLE, with data inputs from DANMAP. Currently available AHLE estimates for swine production in Denmark [6] will also be refined in the next stages.

continued ... Textbox 9.3

Figure 1 Analytical approach used to estimate the burden of AMR and AMU in the Global Burden of Animal Disease programme

DANMAP 2022



The animal health loss envelope includes mortality and morbidity-associated losses and healthcare expenditure attributable to infectious diseases, non-infectious diseases and external hazards. AMU: antimicrobial usage; AMR: antimicrobial resistance

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10

MATERIALS AND METHODS

10. Materials and methods

10.1 General information

For the DANMAP 2022 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on the number of general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined at the individual farm level, meaning that only one isolate per bacterial species per farm was included in the report. The individual flock of broilers was defined as the epidemiological unit, and for food, the epidemiological unit was defined as the individual meat sample.

For humans, the epidemiological unit was defined as the individual patient and the first isolate per species per patient per year was included for analyses of AMR trends. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 2.4.

10.2 Data on antimicrobial consumption in animals

10.2.1 Data

In Denmark, all antimicrobial agents used for treatment are available on prescription only. Until 2007, antimicrobial agents were exclusively sold by pharmacies or as medicated feed from the feed mills. This monopoly was suspended in April 2007, and since then private companies have been able to obtain license to sell prescribed veterinary medicinal products for animals, if they adhere to the same guidelines that apply to pharmacies. A pharmacy or licensed company either sells the medicine to veterinarians for use in their practice or for resale to farmers or sells the medicine directly to the animal owner upon presentation of a prescription.

In 2022, 97.35% of all antimicrobial agents were purchased through pharmacies and the drug trading companies, while 2.65% were purchased from feed mills. For cattle in 2022, 14.3% of antimicrobial agents were used by veterinarians to treat cattle in farms, compared to only 7% in 2004. In aquaculture, approximately 93.7% were purchased through feed mills.

Data on all sales of veterinary prescription medicine from pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database, VetStat, which is hosted by the Danish Veterinary and Food Administration. Veterinarians are required by law to report all use of antibiotics and prescriptions for production animals to VetStat monthly.

For most veterinarians, the registration of data is linked to their invoice system. Electronic registration of the sales at pharmacies is linked to the billing process and stock accounts at the pharmacy. This ensures a very detailed data of high quality. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at The Danish Health Authority and to VetStat.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not used since 2000) are also collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark since 2000. In very rare instances, medicine is prescribed on special license, i.e. medicines not approved for marketing in Denmark. These are not included in VetStat data.

VetStat contains detailed information about source (veterinarian/pharmacy/feed mill) and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age group, disease category and code for farm-identity (CHR Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" (DADD) a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human sector [www.whocc.no]. The data presented in DANMAP 2022 were extracted from VetStat on 22 May 2023.

10.2.2 Methods

In DANMAP, we report use of antimicrobials in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound. This enables an overall crude comparison of consumption among different animal species and between the veterinary and human sectors.

Furthermore, a more detailed comparison of antimicrobial use is performed, taking into account potency, formulation, route of administration and the age of the animals (where relevant), by generating defined animal daily doses (DADDs). For these calculations, we select data with relevant animal and age group codes and relevant codes for dispensation. For example, when calculating the antimicrobial use for systemic treatment in pigs, we select consumption data where the age groups are defined as finishers, weaners, and sows (including piglets and boars) and exclude antimicrobials dispensed as tablets, products for topical use, intramammaries and gynaecologicals.

Numerator - DADD

Defined animal daily dose (DADD) is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but as mg active compound per kg live animal for each antimicrobial agent, administration route and animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The following principles are applied when setting the DADDs:

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products is given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group is maintained as the common DADD even if a new product is approved with a higher dosage;
4. If the dosage for a group shows large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] are applied;
5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age groups, indications or formulations.

When principles 3 and 4 are conflicting, principle 5 is applied.

Denominator - live biomass

The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species. For DANMAP 2022, only the live biomass for pigs and cattle were updated. Pig production: The estimation was based on the number of pigs produced, including exports at different ages, productivity data for each year [Statistics Denmark; Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) was estimated from the annual productivity numbers [Danish Agriculture and Food]. The size of the breeding animals (sows and boars) has probably increased over the last decade, but this was not accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age-gender categories.

Treatment proportion - DAPD

The treatment proportion is a statistical measure for AMU in animal populations, calculated as the annual number of DADDs administered in the population, divided by the estimated total population live biomass. For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight. The treatment proportions, therefore, also represents the proportion of animals treated daily with an average maintenance- dose of a particular antimicrobial agent. These are reported as Defined animal daily dose per 1,000 animals per day (DAPD).

For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. In principle, the metric DAPD is parallel to the metric DID, defined daily dose per 1,000 inhabitants per day (DID), used in pharmaco-epidemiology for the human sector, see Section 10.8.2.

In 2022, DAPD calculations were carried out for pigs and cattle.

For example, the antimicrobial use per pig produced is calculated as:

$$DAPD = \frac{DADD_{sows} + DADD_{weaners} + DADD_{finishers}}{\Sigma biomassdays}$$

Where DADDs, DADDw, and DADDf are amounts of antimicrobial agents used in finishers, weaners, and sows (including piglets and boars).

10.3 Collection of bacterial isolates from animals and meat

In DANMAP, samples originate both from the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria, and the national *Salmonella* surveillance programs. Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring, according to Decision 2013/652/EU. This Decision was repealed by Decision 2020/1729/EU, applied from 1 January 2021. With the aim to ensure continuity in assessing future trends in antimicrobial resistance, the new Implementing Decision includes adaptations of food categories to be sampled, sampling design to be followed, bacterial species to be tested and the analytical methods to be used.

EU harmonized monitoring from 2021 to 2027 shall cover *Salmonella* spp., *Campylobacter coli*, *Campylobacter jejuni*, indicator commensal *Escherichia coli*, ESBL-, AmpC- or carbapenemase- producing *Salmonella* spp. and *E. coli*, and may cover *Enterococcus faecalis* and *Enterococcus faecium*. Previously, monitoring of *Campylobacter coli* was voluntary. For the monitoring of *Salmonella* in poultry, it is now possible to report only samples collected within the national control programme in poultry farms, while the monitoring of *Salmonella* in fattening pigs at slaughter is still required for most countries, including Denmark, due to the inexistence of an implemented national surveillance programme which has been approved at EU level.

Additionally to monitoring of fresh meat at retail, the present EU legislation requires monitoring of indicator *E. coli* and ESBL-, AmpC- or CP-producing *E. coli* on fresh imported meat sampled at border control posts, and the fresh meat categories to be monitored include turkey, both at retail and at the border. In 2022, due to miscommunication, no meat samples were collected at the border control posts.

Decision 2020/1729/EU further allows the use of whole genome sequencing as an alternative method for the specific monitoring of ESBL-, AmpC- or CP-producing *E. coli* or for further testing of indicator *E. coli* and *Salmonella* showing resistance to cefotaxime, ceftazidime or meropenem.

The legislation continues to require mandatory sampling of broilers and fattening turkeys and meat thereof in even years (2022, 2024, 2026), and sampling of fattening pigs and cattle <1 year, and meat thereof in odd years (2021, 2023, 2025, 2027). In Denmark, fattening turkeys are not sampled at slaughter as part of the EU harmonised monitoring, because the national production of turkey meat is below 10.000 tonnes per year.

10.3.1 Animals

In 2022, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from broilers at slaughter, and additional sampling of caeca from pigs and cattle (<1 year) was also carried out.

Caecal samples from healthy broilers, cattle (<1 year) and pigs were collected by meat inspection staff at the slaughterhouses. Samples were collected throughout the year, in major Danish slaughterhouses slaughtering conventionally produced chicken (three slaughterhouses), pigs (eight slaughterhouses) and cattle (six slaughterhouses).

These slaughterhouses handled at least 90% of the total number of broilers, cattle and pigs slaughtered in Denmark in 2022.

Sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals per slaughterhouse, proportionally to the annual throughput of the slaughterhouse. For broiler flocks, ten intact caeca were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal.

All samples were processed by the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted or by a DVFA-approved private laboratory. Samples from all three animal species were examined for indicator *E. coli*.

Broiler and cattle samples were also examined for *Campylobacter coli* and *Campylobacter jejuni*. Furthermore, broiler samples were also examined for ESBL/AmpC/carbapenemase-producing *E. coli*, and *Enterococcus faecium* and *Enterococcus faecalis* (Table 10.1).

Pathogenic bacteria comprised *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, *Clostridium perfringens*, *Erysipelothrix rhusiopathiae*, haemolytic *Escherichia coli*, non-haemolytic *E. coli*, *Haemophilus parasuis*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Staphylococcus hyicus* and *Streptococcus suis* isolates identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

10.3.2 Meat

In 2022, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled broiler and turkey meat collected in Danish wholesale and retail outlets. These samples were collected throughout the year by DVFA officers (Table 10.1). Products with added saltwater or other types of marinade as well as minced meat were not included. Packages of meat were selected at retail without pre-selecting by country of origin, as requested for the harmonised EU monitoring.

The number of establishments and samples selected by each regional DVFA control unit was proportional to the number of establishments in the region in relation to the total number of establishments in the country. One unit of meat (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory.

Salmonella isolates from domestically produced pork originated from the national control programme at the slaughterhouses (Table 10.1). Pig carcases were swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10 cm). All samples were processed at DVFA-approved Industry laboratories and isolates were sent to the DVFA laboratory. *Salmonella* isolates from imported pork originated from samples collected at wholesale and retail outlets and processed at the DVFA laboratory.

10.4 Microbiological methods – isolates from animals and meat

10.4.1 *Salmonella*

Salmonella from pork not originating from the national *Salmonella* surveillance program were isolated at DVFA in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] and in accordance with Annex D, ISO 6579-1 [ISO6579-1:2017]. Serotyping of those isolates was performed at DVFA by whole genome sequencing using the Illumina MiSeq platform, paired-end sequencing 2x250 cycles. For bioinformatics, a CGE service (Centre for Genomic Epidemiology, DTU) for *Salmonella* serotyping was applied based on the genetic background for antigenic formulas given by the White-Kauffmann-Le Minor scheme.

Salmonella from carcasses originating from the national *Salmonella* surveillance program were isolated and serotyped according to the White-Kauffmann-Le Minor scheme at DVFA-approved Industry laboratories.

10.4.2 *Campylobacter*

Campylobacter from broiler and cattle caeca was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] with modifications, with pre-enrichment in Bolton broth, and followed by species-determination by BAX® rtPCR assay (Hygiena, BAX® System PCR Assays for Campylobacter). Only one *Campylobacter* isolate per broiler flock or cattle herd was selected for antimicrobial susceptibility testing.

Table 10.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contributed with isolates to DANMAP 2022 DANMAP 2022

Bacteria	Origin of isolates	Legislative reporting frequency (2020/1729/EU)	Number of tested and positive samples in 2022
Campylobacter spp.	Caecal samples from broilers [a] Caecal samples from cattle <1 yr [a]	Even years Odd years	669 flocks (259 positive) 159 animals (135 positive)
Enterococcus spp.	Caecal samples from broilers [b]		336 flocks (331 positive)
Indicator *E. coli*	Caecal samples from broilers Caecal samples from fattening pigs Caecal samples from cattle <1 yr	Even years Odd years Odd years	204 flocks (195 positive) 188 animals (176 positive) 117 animals (112 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from broilers Fresh broiler meat at retail (Imported) Fresh broiler meat at retail (Danish) Fresh turkey meat at retail (Imported) WGS data for collected ESBL/AmpC isolates	Even years Even years Even years Even years Even years	697 flocks (9 positive) [c] 45 units (6 positive) [c] 301 units (6 positive) [c] 113 units (59 positive) [c] 76 isolates [d]
Salmonella spp.	Fresh pork at retail (Imported) Carcase swabs from fattening pigs [e]	Odd years	214 units (10 positive) 18,934 animals (103 positive)

a) Broilers: *C. jejuni* (n=170), *C. coli* (n=56), 30 unspecified isolates ; Cattle: *C. jejuni* (n=104), *C. coli* (n=6), *C. lari* (n=2), 23 unspecified isolates
b) Broilers: *E. faecalis* (n=28), *E. faecium* (n=305)
c) Positive for ESBL/AmpC-producing *E. coli* and negative for carbapenemase-producing *E. coli*
d) 76 isolates from the positive samples were sequenced, (12 from fresh broiler meat at retail, 57 from fresh turkey meat at retail, 7 from broilers)
e) Carcass swab samples are part of the national *Salmonella* surveillance program and are classified in DANMAP as meat of domestic origin. Samples collected at slaughterhouses slaughtering more than 30,000 pigs are analysed in pools of 5 individual samples.

10.4.3 *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread onto violet red bile agar incubated for 24h at 44 °C. Presumptive *E. coli* was identified on TBX agar incubated at 44 °C o/n. Only one indicator *E. coli* isolate per flock or herd was selected. The specific isolation of ESBL/AmpC or carbapenemase-producing *E. coli* from poultry meat and caecal samples of broilers occurred within 96 h after sample collection, applying the current EURL-AR laboratory protocol [https://www.eurl-ar.eu/protocols.aspx]. Carbapenemase-producing *E. coli* screening was done with ChromID CARBA and ChromID OXA-48 plates. ESBL/AmpC-producing *E. coli* screening was done with MCA cefotaxime plates. All presumptive ESBL/AmpC or carbapenemase producing *E. coli* isolates were sequenced by WGS using the Illumina MiSeq platform (paired-end sequencing 2x250 cycles), followed by bioinformatics analysis using the WGS Portal v1.24.0. for EU harmonised monitoring. Only one ESBL/AmpC-producing *E. coli* isolate per broiler flock and meat sample was selected for antimicrobial susceptibility testing.

10.4.4 Enterococci

Indicator enterococci were isolated from broiler caeca by adding 10 ml buffered peptone water to the content of a pool of ten caeca and placing it in the stomacher for 30 seconds, after which 10 µl were inoculated onto Slanetz agar and incubated for 48 h at 41,5 °C. Presumptive *E. faecium/E. faecalis* were identified by real-time PCR assay. When present, only one *E. faecalis* or *E. faecium* isolate per flock was selected for antimicrobial susceptibility testing.

10.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella, Campylobacter* and *E. coli* was carried out by Minimum Inhibitory Concentration (MIC) determination, using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were performed in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard [ISO 20776-1:2020]. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2020/1729/EU about the EU harmonised monitoring of antimicrobial resistance.

The quality control strains used were: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *C. jejuni* ATCC 33560 and *P. aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted, which is accredited by DANAK (the national body for accreditation).

Antimicrobial susceptibility testing of pathogenic bacteria from pigs was performed at the Veterinary Laboratory, The Danish Agriculture and Food Council. In brief, MICs were determined by broth microdilution using customised Sensititre panels according to CLSI standards. The analysis is accredited by DANAK.

10.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing, whole genome sequencing (WGS) and in silico bioinformatics tools were also used to detect the genetic background of the ESBL/AmpC and carbapenemase-producing *E. coli*. At the DVFA laboratory in Ringsted, strains were sequenced using the Illumina MiSeq platform and the bioinfor- matics analysis was conducted at DTU National Food Institute using the WGS portal for EU harmonized AMR monitoring [https://wgsportal.efsa.europa.eu]. This pipeline follows the protocol provided by EURL-AR [https://www.eurl-ar.eu/protocols.aspx], to extract AMR genes in a standardized format from raw sequencing reads using ResFinder 4.1, which includes chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari *et al.* 2012. J Antimicrob Chemother. 67(11):2640; Zankari *et al.* 2017. J Antimicrob Chemother. 72(10):2764]. The service also detects ST types based on MLST.

WGS of pathogenic bacteria from pigs was performed on Illumina platforms at Statens Serum Institut. Acquired resistance genes and point mutations were detected by mapping sequence reads against the ResFinder 4.1 database [Bortolaia *et al.* 2020. J. Antimicrob. Chemother 75(12):3491-3500) using the k-mer alignment (KMA) tool 1.3 (Clausen *et al.* 2018. BMC Bioinformatics 19(1):397], setting both length match and similarity match to 0.9 and excluding hits with less than 10X coverage.

10.7 Data handling - isolates from animals and meat

For the samples processed at the DVFA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were sent to DTU National Food Institute (Excel sheets). At the DTU National Food Institute, data were harmonised and one isolate per epidemiological unit was selected for reporting.

For the samples processed at the Veterinary Laboratory, The Danish Agriculture and Food Council, sampling details and laboratory results were stored in the information management system used at the Veterinary Laboratory. Following internal validation and anonymisation, data were sent to DK-VET (Excel sheets). At DK-VET, data were harmonised and one isolate per epidemiological unit was selected for reporting.

Table 10.2 Interpretation criteriae for MIC-testing by EUCAST- and EFSA-provided epidemiological cut-off values (ECOFFs)

DANMAP 2022

Antimicrobial agent	*Salmonella*	*E. coli*	*E. faecalis*	*C. jejuni*	*C. coli*
	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml
Amikacin	>4	>8	Not tested	Not tested	Not tested
Ampicillin	>8	>8	>4	Not tested	Not tested
Azithromycin	>16	>16 [a]	Not tested	Not tested	Not tested
Cefepime	>0.125 [a]	>0.125 [a]	Not tested	Not tested	Not tested
Cefotaxime	>0.5	>0.25	Not tested	Not tested	Not tested
Cefotaxime-clavulanic acid	>0.5 [a]	>0.25	Not tested	Not tested	Not tested
Cefoxitin	>8	>8	Not tested	Not tested	Not tested
Ceftazidime	>2	>0.5	Not tested	Not tested	Not tested
Ceftazidime-clavulanic acid	>2 [a]	>0.5	Not tested	Not tested	Not tested
Chloramphenicol	>16	>16	>32	>16 [d]	>16 [d]
Ciprofloxacin	>0.064	>0.064	>4	>0.5	>0.5
Colistin	>2 [a] [b]	>2	Not tested	Not tested	Not tested
Daptomycin	Not tested	Not tested	>4	Not tested	Not tested
Ertapenem	>0.064 [a]	>0.064 [a]	Not tested	>0.5 [a] [d]	>0.5 [a] [d]
Erythromycin	Not tested	Not tested	>4	>4	>8
Gentamicin	>2	>2	>64	>2 [a]	>2 [a]
Imipenem	>1	>0.5	Not tested	Not tested	Not tested
Linezolid	Not tested	Not tested	>4	Not tested	Not tested
Meropenem	>0.125 [a]	>0.125	Not tested	Not tested	Not tested
Nalidixic acid	>8	>8	Not tested	Not tested	Not tested
Quinopristin-dalfopristin	Not tested	Not tested	>1 [a] [c]	Not tested	Not tested
Sulfamethoxazole	>256 [a]	>64 [a]	Not tested	Not tested	Not tested
Teicoplanin	Not tested	Not tested	>2	Not tested	Not tested
Temocillin	>16 [a]	>16	Not tested	Not tested	Not tested
Tetracycline	>8	>8	>4	>1	>2
Tigecycline	>0.5 [a]	>0.5	>0.25	Not tested	Not tested
Trimethoprim	>2	>2	Not tested	Not tested	Not tested
Vancomycin	Not tested	Not tested	>4	Not tested	Not tested

EUCAST epidemiological cut-off values (ECOFFs) and ECOFFs provided by EFSA for EU harmonized reporting

a) ECOFF as provided by EFSA [EFSA Supporting publication 2023:EN-7826]

b) For colistin, a tentative ECOFF of 16 µg/ml for *Salmonella* Dublin is established by EUCAST. The same ECOFF is used in DANMAP to interpret results of *Salmonella* Enteritidis. Both serotypes belong to the O-group (O:1, 9,12), which has been associated with increased MIC for colistin [https://www.doi.org/10.1089/fpd.2011.1015]

c) For quinopristin-dalfopristin, ECOFF only applies for *E. faecium*. ECOFF >1 for *E. faecalis* (intrinsically resistant to quinopristin-dalfopristin) is used only for the purpose of EU harmonized reporting

d) In 2021, chloramphenicol and ertapenem were introduced in the test panel for *Campylobacter* spp.

Table 10.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*
DANMAP 2022

Antimicrobial classes	*Salmonella* and *E. coli*
Beta-lactam penicillins	Ampicillin
Macrolides	Azithromycin
Cephalosporins	Cefotaxime and/or ceftazidime
Phenicols	Chloramphenicol
Quinolones	Ciprofloxacin and/or nalidixic acid
Polymyxins	Colistin
Aminoglycosides	Gentamicin and/or amikacin
Carbapenems	Meropenem
Sulfonamides	Sulfamethoxazole
Tetracyclines	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

An isolate is considered multidrug-resistant if resistant to three or more of the defined antimicrobial classes and fully sensitive if susceptible to all antimicrobial agents included in the test panel; The aminoglycoside antimicrobial amikacin has been introduced in the test panel in 2021

10.7.1 Interpretation of MIC values

MIC values were retained as continuous variables, from which binary variables (resistant/sensitive) were created using the relevant cut-off. Since 2007, MIC results have been interpreted using EUCAST epidemiological cut-off (ECOFF) values, with a few exceptions, as described in Table 10.2. An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 10.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

For pathogenic bacteria from pigs, MIC values were interpreted with ECOFFs (1st choice) or tentative ECOFFs (2nd choice) established by EUCAST. When ECOFFs were unavailable, interpretation was based on CLSI-approved animal-specific or human clinical breakpoints (3rd and 4th choice, respectively) (available at https://www.vetssi.dk/).

10.7.2 ESBL/AmpC phenotypes

Classification of CP-, ESBL- or AmpC-producing phenotypes was done according to the scheme provided by EFSA. [EFSA 2023. EFSA Journal 21(3):7867].

1. ESBL phenotype if cefotaxime and/or ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC <=8 µg/ml and synergy (clavulanic acid and cefotaxime/ ceftazidime);
2. AmpC phenotype if cefotaxime and/or ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ ml and no synergy (clavulanic acid and cefotaxime/ ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime and/or ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ ceftazidime);
4. CP phenotype if meropenem MIC >0.12 µg/ml;
5. Other phenotype if not in 1-4.

Synergy is defined as ≥3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

10.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate depending on sample size. Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test, using the DescTools R package version 0.99.45. One-sided tests were chosen because of preliminary expected trend directions. A significance level of 0.05 was considered in all significance tests.

Analyses were done using R statistical software version 3.6.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org].

10.8 Data on antimicrobial consumption in humans

10.8.1 Data registration

Annual data on antimicrobial consumption in Denmark has been provided to DANMAP by the Register of Medicinal Product Statistics at the Danish Health Data Authority every year since 1997. Since 2020, DANMAP also reports monthly antimicrobial consumption data to allow analysis of the impact of the Covid-19 pandemic on antimicrobial consumption in humans since 2020.

Until 2012, data from hospitals on certain infusion substances such as cephalosporins, carbapenems and trimethoprim were obtained by DANMAP directly from hospital pharmacies. Since 2013, all data from hospitals are reported to and provided to DANMAP by The Register of Medicinal Product Statistics at the Danish Health Data Authority.

Reports of human antimicrobial consumption in Denmark existed already before 1997. These were prepared by the Association of Medicine Importers (Medicinimportørforeningen, MEDIF) and the Association of Danish Medicinal Factories (Foreningen af danske Medicinfabrikker, MEFA) based on whole sales data to pharmacies. These reports became less reliable over time since there was an increasing amount of parallel imported drugs from the late 1980s, which were not covered by MEDIF/MEFA.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported by pharmacies using a code relating to the defined package. The code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The report also includes age, gender and regional residence of the patient. Since 2004, the sales registration has included a code for indication of the prescription as well. However, clinical indications provided for the treatment of infectious diseases were often quite unspecific ("against infection"). Since 2016, the use of more specific indication codes has increased following the implementation of electronic prescribing via the "common medicine card" (fælles medicinkortet, FMK), a digital pharmacy platform which is mandatory to be used by all medical doctors. In 2022, indication codes were available for 94% of prescriptions, but specific indication codes still only accounted for 75%.

For hospitals, reporting is based on deliveries from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between different medical suppliers and Amgros, a private company under agreement with the five Danish Regions. Amgros is responsible for harmonisation of prices and for ensuring deliveries to all hospitals and works closely together with the Regions' Joint Procurement. Detailed information is given on the different drugs delivered on ATC5 level. For surveillance purposes, it has to be assumed that the amount of delivered antimicrobials is similar to the consumption at the different departments. In reality, antimicrobials may be exchanged between different specialties and departments belonging to the same hospital making precise calculations of the consumption on specialty level difficult. In DANMAP, reporting of data on hospital consumption is therefore kept at national and regional level. In case of production failures and shortages in delivery of specific products, the hospitals have to apply for special delivery through the Danish Medicines Agency (Figure A5.2 in web annex). These special deliveries are reported separately to DANMAP through the hospital pharmacies. An example is the shortages in delivery of piperacillin with tazobactam and pivmecillinam as well as mecillinam in 2017. The shortage in piperacillin with tazobactam had significant impact on the amount used, while the shortage in (piv)mecillinam could not be clearly tracked in changes in consumption. In 2022, 126.490 DDD (3%) of the total antimicrobial consumption were special deliveries. Data on consumption at patient level are available at some hospitals and have so far been used in local quality assurance only but have not been available to DANMAP.

10.8.2 Method

Primarily somatic hospitals were included in the DANMAP reporting. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP in most calculations since their activity and functions are not comparable to public, somatic hospitals and therefor may skew the data. Their consumption accounts for approximately 3% of the antimicrobial consumption at hospitals in Denmark.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2022 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary healthcare and in hospitals as well as consumption of oral and rectal preparations of metronidazole (P01AB01) and for hospitals oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary healthcare is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day.

The consumption in hospital healthcare is expressed as the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days or DBD). Since reporting in DBD does not necessarily reflect changes in hospital activity and production, consumption at hospitals is also presented as DAD (the number of DDDs per 100 admissions) and crude DDD. Finally, the consumption of antibacterial agents at hospitals has also been calculated in DIDs, primarily for comparison with primary healthcare.

10.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for it's main indication in adults. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [https://www.whocc.no/atc_ddd_index/].

Per January 2019 the WHO updated the DDDs for seven main antimicrobial agents, based on recommendations from an expert working group in collaboration with the European Center for Disease Control (ECDC) (Table 10.5). From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

10.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the exact duration of a hospital stay in hours divided by 24 hours. Number of bed-days was extracted from the National Patient Registry at the Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019 and data are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific hospital for ≥12 hours. If a patient is transferred between wards within 4 hours, it will not count as a new admission. The admissions were extracted from the National Patient Registry at the Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019 and data are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.6 Antimicrobial consumption for elderly living in long care facilities

Data from the Care Home Register were combined with data from the Danish Civil Registration System (CPR) and with data from the Register of Medicinal Product Statistics in order to determine the antimicrobial consumption for elderly people living in care homes and for elderly people living in their own homes.

10.9 *Salmonella* and *Campylobacter* isolates from humans

10.9.1 Data source

Antimicrobial susceptibility was performed on human clinical isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all clinical microbiology laboratories in Denmark and *Campylobacter* isolates were submitted from clinical microbiology laboratories representing the island of Zealand excluding the Capital Region, Funen, and Northern Jutland. As in previous years, SSI collected information on travel history from the patients. Cases were categorised as "domestically acquired" if the patients had not travelled abroad within the week prior to the onset of disease.

10.9.2 Microbiological methods

Salmonella isolates were analysed by whole genome sequencing and the serotypes were derived from the DNA sequences. In a few cases, the DNA information was supplemented with slide agglutination according to the Kauffman-White Scheme. *Campylobacter* species identification was performed by the use of MALDI-TOFF.

10.9.3 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* was performed as Minimum Inhibitory Concentration (MIC) determination using the Sensititre broth microdilution system (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

Table 10.5 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2022

ATC5 code	ATC level name	Previous DDD			New DDD		
		Weight	Unit	Route of administration	Weight	Unit	Route of administration
J01CA01	Ampicillin	2.0	g	Parenteral	6.0	g	Parenteral
J01CA04	Amoxicillin	1.0	g	Oral	1.5	g	Oral
J01CA04	Amoxicillin	1.0	g	Parenteral	3.0	g	Parenteral
J01CA17	Temocillin	2.0	g	Parenteral	4.0	g	Parenteral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.0	g	Oral	1.5	g	Oral
J01DE01	Cefepime	2.0	g	Parenteral	4.0	g	Parenteral
J01DH02	Meropenem	2.0	g	Parenteral	3.0	g	Parenteral
J01MA02	Ciprofloxacin	0.5	g	Parenteral	0.8	g	Parenteral
J01XB01	Colistin	3.0	MU	Parenteral	9.0	MU	Parenteral

10.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) that is maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

10.10 *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans

10.10.1 Data source

The surveillance of invasive isolates of *E. coli, K. pneumoniae, E. faecalis* and *faecium, P. aeruginosa* and A. spp. and urine isolates of *E. coli* and *K. pneumoniae* are all based on data from routine diagnostics at the ten Departments of Clinical Microbiology (DCMs) in Denmark. All data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk]. Before 2018, data were reported by the individual DCM to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1 in DANMAP 2018 [www.danmap.org].

10.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCM. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST clinical breakpoints and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs where local rules were applied to the susceptibility interpretations in specific cases - e.g. susceptibility to mecillinam in invasive cases. In 2019 EUCAST introduced the Area of Technical Uncertainty (ATU) for some combinations of species and agents reflecting problematic areas regarding variations and uncertainty of susceptibility categorisation. Piperacillin-tazobactam, ciprofloxacin and amoxicillin-clavulanic acid (in systemic breakpoints) and Enterobacterales are examples where ATUs were applied. ATUs can be handled differently by individual DCMs and may influence interpretation results. This was commented on when necessary in the affected sections.

To be included in resistance surveillance more than 75% of respective isolates need to be antimicrobial susceptibility tested for a given antibiotic, if not stated otherwise. Data of antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the respective DCM, based on the S-I-R system. In addition, zone diameters have also been registered since 2015 and will be commented upon in specific cases.

Urine specimen taken in primary health care are also being tested at DCMs except for some samples taken by GPs in the Capital Region of Denmark that are being tested at a private laboratory.

All enterococci isolates reported as VRE in MiBa (based on PCR results for *vanA/B* genes) were reported as vancomycin-resistant independent of the actual zone/MIC result. It was not distinguished between VRE and VVE (vancomycin-variable enterococci). High-level resistance to gentamicin was defined using EUCAST break points (MIC >128 mg/L and/or zone diameters <8 mm) for MIC and/or zone diameters reported in MiBa. Gentamicin MIC and/or zone diameters were routinely reported by three DCMs in 2020.

10.10.3 Data handling

Cases and susceptibility results were extracted from MiBa and analysed in Python 3.8.10.

The case definition has been harmonised with the definition used by the European Antimicrobial Resistance Surveillance Network (EARS-Net): The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates from the same patient, within the year of observation, were removed. Thereby only resistance data on the first isolate per patient per specimen per year were included. Resistance data from DCMs were excluded if not tested or registered in MiBa routinely (minimum 75% of the specific species/antimicrobial agent combination). Samples were either invasive (including blood cultures or cerebrospinal fluid) or urinary samples from patients at hospitals or primary healthcare settings.

10.11 ESBL-producing bacterial isolates from humans

10.11.1 Data source

Since 2014, the Danish DCMs have on a voluntary basis submitted 3rd generation cephalosporin-resistant *Escherichia coli* isolates from bloodstream infections for verification and genotyping at the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

10.11.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and in silico bioinformatics analysis have been applied for isolates predicted to carry ESBL and/or AmpC genes based on initial phenotypic tests, to characterize the genetic background of the ESBL and/or AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

10.11.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder/src/master/] was used for the in silico detection of acquired ESBL genes, pAmpCs, carbapenemase genes and MLST from assembled WGS data. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate chromosomal AmpC by the use of myDb-Finder version 1.2 [https://cge.cbs.dtu.dk]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

10.12 CPO isolates from humans

10.12.1 Data source

Historically, Danish DCMs have submitted carbapenem-resistant isolates for verification and genotyping on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at the Statens Serum Institut. Since 5 September 2018, notification of CPO has been mandatory in Denmark. For outbreak investi-gation Data from The National Patient Register (LPR), informa-tion gathered at the hospitals and information of residence from the Danish Civil Registration System (CPR) has been included in the analysis for this report.

10.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were analysed using WGS. More than one isolate from the same patient was only included in the dataset if the isolates belonged to different bacterial species and/or if isolates within the same species harboured different carbapenemases.

10.12.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the in silico detection of acquired CPO genes and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types where such schemes are available (*E. coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*). For outbreak investigations, identified clonal clusters were linked with patient data like time and place of hospitalization and place of residence. Identification of isolates from two or more persons (cases) sharing the same unique genotype was defined as an outbreak. An outbreak was defined as a verified outbreak if an epidemiological link could be established between two or more cases in the cluster, e.g. the patients had been at the same hospital ward at the same time or lived at the same geographical location such as a nursing home. When no epidemiological link could be establish between cases with the same unique genotype, the outbreak was classified as a possible outbreak. A possible outbreak can be reclassified as a verified outbreak if new cases or information providing an epidemiological link between two or more of the cases becomes available. Both, possible and verified outbreaks, are registered in the CPO-outbreak database KURS (coordinated outbreak registration).

Outbreak investigations of a cluster of cases are closed when no new cases have been reported within 6 months after the last reported case, but can be reopened, if new cases are being detected.

10.13 VRE isolates from humans

10.13.1 Data source

Danish DCMs are submitting VRE for species identification, genotyping and surveillance on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

10.13.2 Microbiological methods

All clinical VRE isolates have been whole-genome sequenced. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

10.13.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], was used for the in silico detection of genes related to vancomycin resistance in enterococci and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types.

10.14 Invasive *Streptococcus pneumoniae* isolates from humans

10.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark and it is mandatory to submit all invasive isolates of *S. pneumoniae* for serotyping and susceptibility testing to the Neisseria and Streptococci Reference Laboratory at Statens Serum Institut. For cases of invasive pneumococcal disease, where isolates from blood/spinal fluid could not be submitted, identification and registration of cases is conducted by extracting the required information from the Danish Microbiology Database (MiBa).

10.14.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* was based on: visual evaluation of colonies, positive optochin test and test with either latex omni test (ImmuLex™ *S. pneumoniae* Omni, SSI Diagnostica, Denmark) or Neufeld based Omni serum (SSI Diagnostica, Denmark). If challenging results occurred, MALDI-TOF, bile solubility test, or whole genome sequencing were performed to further confirm the correct species identification. For non-viable isolates, species identification was based on the detection of the lytA and Ply gene by the use of PCR.

Serotype identification of invasive *S. pneumoniae* was performed by the use of latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Denmark) and serotype specific antisera by the Neufeld test (SSI Diagnostica, Denmark). For non-viable isolates, serotyping was often possible by the use of PCR.

10.14.3 Susceptibility testing

Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, SSI Diagnostica, Denmark). Breakpoints were according to EUCAST Clinical Breakpoint Tables v. 11.0. Isolates, that were found non-susceptible by screening were further analysed for penicillin and erythromycin MICs by broth microdilution using the STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST (Eucast Clinical Breakpoint Tables v.11.0). For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

10.14.4 Data handling

Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

10.15 Isolates of beta-haemolytic streptococci of groups A, B, C, and G from invasive infections in humans

10.15.1 Data source

All invasive isolates of beta-haemolytic streptococci (BHS) (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) were submitted to SSI from the departments of clinical microbiology on a volun-tary basis.

10.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark). Genomic DNA was extracted using an enzymatic pre-lysis step before automated purification on MagNA Pure 96 DNA Small Volume Kit (Roche Diagnostics). Fragment libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA), followed by 150-bp paired-end sequencing on a NextSeq (Il- lumina) according to manufacturer's instructions. The sequencing reads were assembled using SKESA [https://github.com/ncbi/SKESA]. The isolates were species typed using Kraken [https://ccb.jhu.edu], and MLST typed using https://github.com/tseemann/mlst.

For Group A Streptococcus (GAS), isolates were *emm* typed by performing a BLAST search to all published *emm* types by CDC [https://www.cdc.gov/streplab/protocol-emm-type.html]. For Group B Streptococcus (GBS), all isolates were serotyped by latex agglutination test and, if needed, confirmed using Lancefield tests. In addition, blasting of capsular sequencing was used for identification of genotypes. No additional identification tests were performed for isolates from Group C or G.

10.15.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin re-sistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Den- mark) on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with ETEST (Biomérieux), with erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 11.0).

Isolates that were either resistant or susceptible to increased exposure were categorised together as resistant.

10.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery). A new case was defined as an invasive isolate with a different Lancefield group within 30 days from the first one or an invasive isolate of any Lancefield group more than 30 days after the first episode, or the isolation of a new type (*emm*-type or GBS serotype) if the group was identical on both occasions.

Only one isolate from each unique case of BHS infection was included in the DANMAP report.

10.16 Invasive *Haemophilus influenzae* isolates from humans

10.16.1 Data source

Invasive infections with *Haemophilus influenzae* type b (Hib) is a notifiable disease in Denmark and all invasive isolates nationwide are sent to the reference laboratory at SSI. By tradition, invasive isolates of other serotypes have also been submitted on a voluntary basis, and thus a broader picture of invasive *H. influenzae* in Denmark can be obtained. All cases were identified through MiBa and registered in the surveillance database at SSI. Cases, where isolates were not submitted to the reference laboratory were registered as "unknown serotype".

10.16.2 Microbiological methods

At SSI, the received isolates were analysed by whole-genome sequencing, from which serotype and biotype were extracted.

10.16.3 Susceptibility testing

Susceptibility data for the 2022 isolates were retrieved from MiBa. In cases where a series of isolates from the same episode developed non-susceptibility over time, the most non-susceptible profile was used for the analysis in DANMAP. In addition, for isolates received at SSI, whole-genome sequencing data was analysed for the presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 as well as for the presence of mutations in the *ftsI* gene that encodes for penicillin-binding protein 3 (PBP3).

10.16.4 Data handling

A case was defined as isolation of *H. influenzae* from normally sterile sites (e.g. blood, spinal fluid, pleura, joint). Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

10.17 *Staphylococcus aureus* including MRSA isolates from humans

10.17.1 Data source

Blood isolates were referred on a voluntary basis by all DCMs to the National reference laboratory for antimicrobial resistance at SSI. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types were sent to the reference laboratory.

10.17.2 Microbiological methods

At SSI, all isolates were initially tested using a multiplex PCR detecting the *spa, mecA, hsd, scn* and *pvl* (LukF-PV) genes [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611–614; Stegger *et al.* 2012. Clin Microbiol Infect. 18: 395–400]. *spa* was used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing [Harmsen *et al.* 2003. J Clin Microbiol. 41: 5442–5448], mecA to determine MRSA status, and scn and *hsd* as markers for human adaptation and relation to CC398, respectively. All bacteremia cases and *mecA* negative presumed MRSA were tested for presence of the *mecC* gene. *spa*-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the *spa* type and known association with MLST typing, each isolate was assigned to a clonal complex (CC).

10.17.3 Susceptibility testing

Data on antimicrobial susceptibility was extracted from MiBa.

10.17.4 Data handling

For blood isolates, a case was defined as a patient with a positive blood culture. Subsequent isolates from the same patient was only included if the positive blood cultures were obtained at least one month apart (new episode).

For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset). MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

10.18 Gonococci isolates

10.18.1 Data source

Isolates of gonococci (*Neisseria gonorrhoeae*) were submitted from the departments of clinical microbiology to SSI on a voluntary basis. The isolates were obtained by culture of specimens from a variety of anatomical locations, most often urethra, cervix, rectum, throat, and rarely from other sites, e.g. eyes, joint fluid, and blood.

10.18.2 Microbiological methods

The bacteriological identification of the received isolates was performed by MALDI-TOF.

10.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar incubated at 35 °C in 5% CO_2. The breakpoints used were those defined by EUCAST (EUCAST version 11.0).

The breakpoints for azithromycin were changed as of January 1, 2019 (EUCAST version 9.0) (S: MIC ≤1 mg/L; R: MIC >1 mg/L) and it was advised that azithromycin should only be used in conjunction with another efficient agent. Until January 1, 2019, S was defined by MIC ≤0.25 mg/L and R by MIC >0.5 mg/L.

In addition to the above, the MIC of cefixime was determined for 117 consecutive isolates as part of an ECDC project on gonococcal antimicrobial resistance (Euro-GASP). The breakpoints used were those defined by EUCAST (EUCAST Clinical Breakpoint Tables v. 11.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

10.18.4 Data handling

Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens was considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.

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TERMINOLOGY

List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
ATC	Anatomical Therapeutic Chemical Classification System
ATCvet	Anatomical Therapeutic Chemical Classification System for veterinary medicines
ATU	Area of Technical Uncertainty
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobacterales
CPO	Carbapenemase producing organisms
CPR	Danish Civil Registry, register for social security numbers
DAD	Defined Daily Doses per 100 admissions
DADD	Defined Animal Daily Dose
DaDDD	Danish adjusted Defined Daily Doses
DAPD	Defined Animal Daily Dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of clinical microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1,000 inhabitants/day)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
ESC	Extended Spectrum cephalosporinase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GP	General Practitioner
HAI	Hospital-acquired infections
HCAI	Health care associated infections
HACO	Health care associated community onset
HAIBA	Hospital Acquired Infections Database
MiBa	The Danish Microbiology Database
MIC	Minimum inhibitory concentration
MDR	Multidrug-resistant
MRSA	Methicillin-resistant *Staphylococcus aureus*
NAAT	Nucleic acid amplification test
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PHC	Primary health care
RFCA	Regional Veterinary and Food Control Authorities
SEGES	Knowledge Centre for Agriculture
SSI	Statens Serum Institut
ST	Serotype/Sequence type
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin-resistant enterococci
VVE	Vancomycin-variable enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization

Glossary

Anatomical Therapeutic Chemical (ATC) classification: An international classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) [www.whocc.no/atcddd/indexdatabase/]. The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology [www.whocc.no/atcvet/database/].

Antibacterial agents: Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. In the section on human consumption, 'antibacterial agents' are referred to as 'antimicrobial agents' (see below).

Antimicrobial agents: The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is generally used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only). In the chapter on human consumption, the term 'antimicrobial agents' refers to all antibacterial agents for systemic use (J01 in the ATC system) including metronidazole and vancomycin, which are used for systemic treatment but registered under the ATC code P01AB01 and A07AA09, respectively.

Broiler: A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.51 kg.

CentralHusbandryRegister (CHR): This is a register of all Danish farms defined as geographical sites housing production animals. It contains information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined Daily Dose (DDD): This is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology [https://www.whocc.no/atc_ddd_index/].

Defined Daily Dose per 100 admissions (DAD): DAD measures the amount of daily doses consumed per 100 admitted patients at hospitals during a given timeframe (one year). It is used for benchmarking consumption related to the hospital activity and will typically be compared to the consumption measured in DBD (see below). DAD and DBD will generally be applied to comparing individual hospitals and consumption of individual drug classes over time. In DANMAP DAD covers all patients attended at somatic hospitals only. Admission-days are extracted from the National Patient Registry (Landspatientregistret, LPR).

Defined animal daily dose (DADD): DADD is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC). The DADD is defined as mg active compound per kg live biomass for each antimicrobial agent, administration route and animal species. In DANMAP 2012, the DADD replaced the ADD (as defined in VetStat and assigned at product level). For more details, see section 9.2, Materials and Methods and the applied DADD's are listed in the web annex.

DADD per 1,000 animals per day (DAPD): Trends in veterinary consumption, both within and across species, are presented in DAPD, which allows for comparison between sectors and adjustment for changes in live biomass. The estimated live biomass is expressed as the number of standard animals with an estimated average weight and lifetime. This may also be referred to as the 'standard-animals-at-risk' and takes into account species differences in body-mass and lifespan. The DAPD is a statistical measure, providing an estimate of the proportion of animals (in thousands) treated daily with a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day (see section 9.2, Materials and Methods).

Defined Daily Doses per 100 occupied bed-days (DBD): DBD is the consumption calculated in defined daily doses at hospitals, divided through the number of bed-days. This allows comparison of hospitals related to the length of patient stays. The number of bed-days is extracted from The National Patient Registry (Landspatientregistret, LPR). Time of reporting

differs between hospitals and closing time for reporting is later than extraction time for the DANMAP report. Every patient admitted to a hospital accounts for the exact length of the hospital stay. This corresponds to the actual hours at hospital divided by 24 hours.

DDD per 1,000 inhabitants per day (DID): Consumption in both primary health care, hospital care and the overall total consumption is presented in DID, allowing for comparison between sectors and for illustration of the consumption in hospital care without taking hospital activity (discharges and length of stays) into account. Data presented in DID provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DIDs indicate that 1% of the population on average gets a certain treatment daily. In figures presented as DDD/1,000 inhabitants/day.

ESBL: In the DANMAP report, 'ESBL' describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC and OXA-ESBLs [Giske et al. 2009. J. Antimicrob. Chemother. 63: 1-4].

Finishers: Pigs from 30-100 kg live weight from after the weaner stage to time of slaughter.

Fully sensitive: An isolate will be referred to as fully sensitive if susceptible to all antimicrobial agents included in the test panel for the specific bacteria.

Human clinical samples/isolates: In the DANMAP report, human clinical samples and/or isolates refers to the sample being taken in a clinical situation, meaning in the course of diagnosing and treating a possible infection in a patient.

Human screening samples/isolates: In the DANMAP report, human screening samples and/or isolates refers to sampling being performed for monitoring purposes in a defined group of asymptomatic individuals. Examples are rectal swaps to determine carriage of multi-resistant bacteria in the intestine or swaps from the throat, nostrils or perineum to determine carriage of methicillin-resistant *Staphylococcus areus* (MRSA).

Intramammaries: Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Layer: A hen raised to produce eggs for consumption.

Minimum inhibitory concentration (MIC): This is the lowest concentration of antimicrobial agent in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

Multi-resistant: A *Salmonella*, *Campylobacter*, *Enterococcus* or *E. coli* isolate is assumed multi-resistant if it is resistant to three or more of the main antimicrobial classes. The number of antimicrobial classes and antimicrobial agents included in this definition depends on the test panel for each bacterium.

Pets or pet animals: Dogs, cats, birds, mice, Guinea pigs and more exotic species kept at home for pleasure, rather than kept for work or food. Horses are not included as pet animals. The live biomasses of Danish pets used for estimating veterinary consumption only include dogs and cat.

Piglet: The new-born pig is called a piglet from birth till they are permanently separated from the sow at 3-4 weeks of age. The weight of the piglet at weaning is approximately 7 kg.

Poultry: The major production species are fowl *Gallus gallus* (broilers, layers, including breeding and rearing) and turkey. Regarding antimicrobial consumption, 'poultry' also includes domesticated ducks, geese, game birds and pigeons.

Sow: Any breeding female pig on the farm.

Weaner: Any pig of 7–30 kg live weight after it has been weaned (dry diet and water only).

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