DANMAP 2017 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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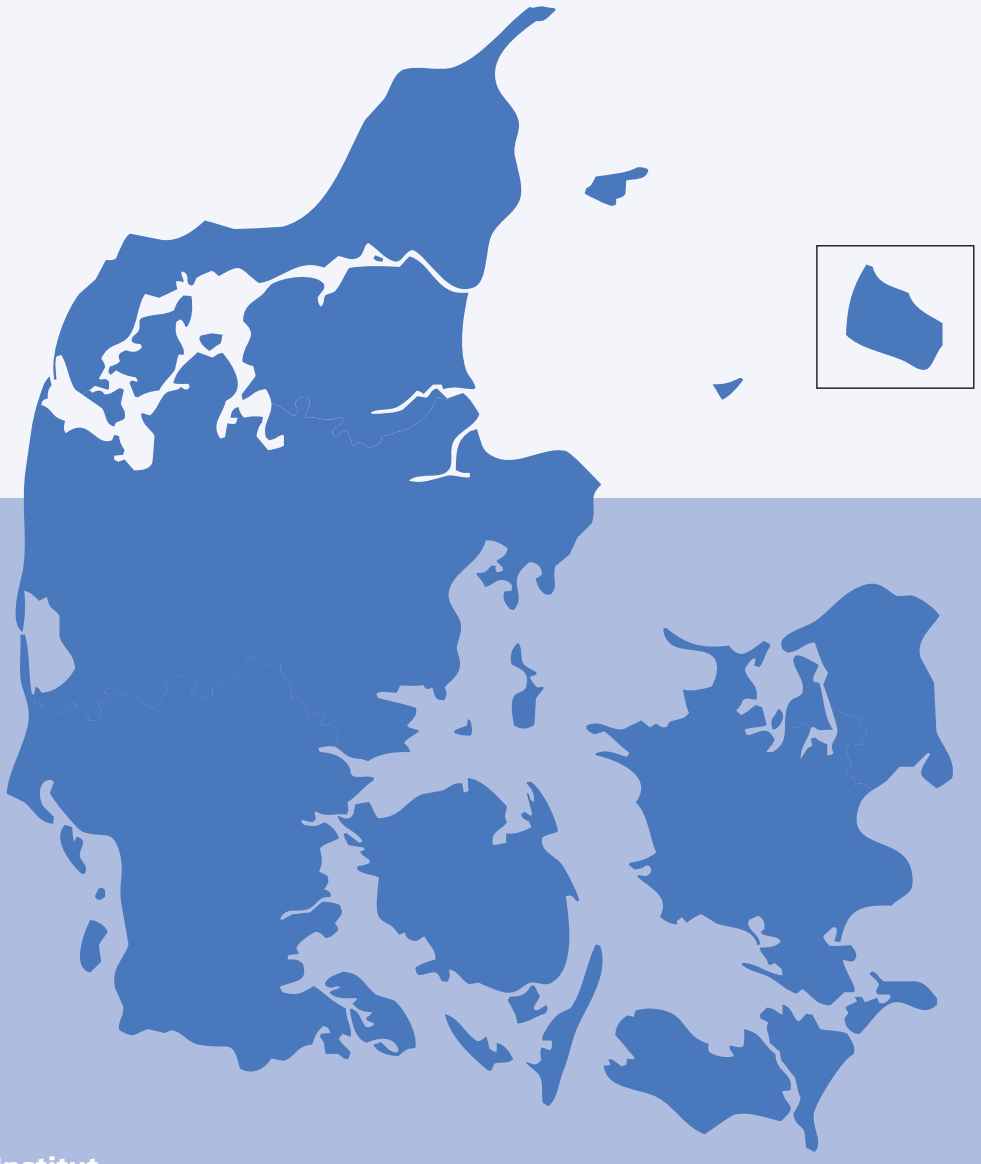

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DANMAP 2017

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STATENS SERUM INSTITUT

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1. Editorial

It has been 22 years, since DANMAP was established. Since then, the need for an integrated approach to control antimicrobial resistance has only become more apparent.

Published estimations for the global burden of antimicrobial resistance (AMR) show that by 2050, 10 million people will die each year due to infection with bacteria that are resistant to antimicrobials. That is 2 million more than estimated to die from cancer. Furthermore, routine surgical interventions will be associated with an increased risk of serious complications. Although there is the need for data to improve the parameters and estimates in such reports, there is no doubt that increasing levels of AMR are a considerable health threat worldwide and must be addressed. Yet, in 2013, it was also estimated that about 5.7 million people, particularly in low and middle income countries, die each year, because they don't have access to antibiotics. Providing such access, without exacerbating the AMR situation, represents a significant challenge.

In the agricultural domain, very large quantities of antimicrobials are used in food animals for treatment, to prevent disease or to promote growth, as well as in plant production against pests. Sick animals need treatment with antimicrobials, which is justified by both an animal welfare perspective and the need to ensure worldwide food security. However, using antimicrobials as a cheap alternative to good animal husbandry is difficult to support these days. The AMR threat also includes environmental issues, as polluted water for drinking and for irrigation of ready-to-eat crops represents a source of AMR. However, pointing fingers and passing blame will be counterproductive in the face of the current crisis; instead all sectors need to unite to address the threat of AMR.

Apart from restricting and improving the use of antimicrobials, we also need a change of the worldwide perception of antimicrobials. Instead of perceiving antimicrobials as common goods, safety net or replacement for hygiene, they need to be regarded as precious agents that should be used sparingly in quantities that are just adequate to safeguard health.

Surveillance is paramount to understanding the situation. To enable intervention, there is a strong need for good quality data on consumption of antimicrobials and knowledge of trends in AMR in as much detail as possible. The historical DANMAP has demonstrated that curbing the use of one specific antimicrobial can result in the reduction of the resistance phenotype to very low levels. However, a more typical outcome will often be a mere stabilising of resistance levels, because of co-selection by other antimicrobials. Thus, the effort may primarily result in preventing further rise in resistance rather than actually lowering the number. This illustrates that comprehensive and detailed surveillance of AMR does not always give the answer as to whether an intervention has been efficient. However, information on resistance levels in relevant reservoirs is still desirable and so is the ability to spot new or emerging resistances in new reservoirs, if the aim is to control.

In Denmark, DANMAP 2017 shows – again – that the level of acquired carbapenemase producing microorganisms in humans continues to increase, despite a highly restricted use of carbapenems in Denmark. It is clear that AMR represents a global public health challenge that can be tackled only by all sectors and countries working together. The threat from antimicrobial resistance has been acknowledged internationally and the united work of WHO, FAO and OIE are good examples of a way forward. DANMAP has been mentioned in these fora as one of the initiatives demonstrating that One Health surveillance is possible and can make a change.

While the integrated approach to monitoring remains valid, the details deserve scrutiny. Better use of available data and future access to more comprehensive data are areas, where DANMAP works to expand. Whole genome sequencing and increased data analyses are also opening new doors to further understanding and lead the way towards development, expansion and increased relevance of the DANMAP surveillance.

DANMAP Steering Committee

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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 Department of Medication Statistics and Research Support at the Danish Health Data Authority (formerly the Danish Medicines Agency and SSI) for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies
- the Danish Veterinary and Food Administration for collecting and transmitting data on veterinary consumption of antimi crobial agents from VetStat, including statistics on consumption measured in tonnage
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SUMMARY

2. Summary

The Danish integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) was founded in 1995, providing a unique One Health platform for the continuous surveillance and research of antimicrobial consumption and resistance. A key objective of the DANMAP programme is to provide an evidence base for decision-making and to further understand the associations between antimicrobial usage (AMU) and the occurrence of antimicrobial resistance (AMR).

In Denmark, antibiotic treatment - of both humans and animals - is available by prescription only, and all prescriptions are recorded in national databases (MEDSTAT and VetStat). Through many years, Denmark has maintained a strong focus on transmission of AMR from production animals to humans. Prescription and treatment guidelines for human and animal use have ensured effective treatments even with very restrictive use of critically important antimicrobials. In hospital settings, effective control measures have been initiated, whereas ongoing research is trying to find the most effective ways to minimise the public health risks in the community.

The report DANMAP 2017 summarises the results of susceptibility testing of isolates obtained by hospitals, general practice, veterinary practice and the National Food and Veterinary Authority; as well as records of the types and amount of antimicrobials prescribed by physicians and veterinarians in Denmark during 2017.

Human isolates cover all bacteremias caused by the most important pathogenic bacteria, based on reporting of the first isolate per species per patient per year representing complete data from all Denmark. Also included are a representative number of isolates from urinary tract infections at hospitals and in the community. Statens Serum Institut collate and interpret data from the human sectors.

Samples from production animals and meat are collected and analysed in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU]. AMR data from additional surveys conducted by the Danish Veterinary and Food Administration and zoonotic bacteria from national control programmes are also included. DTU Technical University of Denmark collate and interpret data from the food and animals sectors.

Antimicrobial consumption in animals

The overall use of antimicrobials for animals decreased for the fourth consecutive year and has since 2013 been reduced by more than 16 tonnes. From 2016 to 2017, the antimicrobial consumption decreased by approximately 3%.

The decrease was driven by the use of antimicrobials for pigs, which was approximately 4% (3.4 tonnes active compound) less than the year before, with a total of 74.8 tonnes. In 2017, the export of weaner pigs continued to increase, while the total number of pigs produced remained at approximately the same level. The use of tetracyclines for pigs has been reduced significantly and almost consistently since 2009. From 2016 to 2017 the use of tetracyclines was further reduced by approximately one third (7.2 tonnes), following the revision of the differentiated "Yellow Card" initiative, where the use of tetracyclines is multiplied with a factor 1.5. The differentiated "Yellow Card" initiative has also resulted in a close to zero use of colistin after the first quarter of 2017. However, the decrease in the use of tetracylines and colistin, was mirrored by less marked but clear increases in the use of macrolides, pleuromutilines and aminoglycosides.

To take into account changes in prescription patterns, changes in production, and differences between age groups, the antimicrobial use in pigs was also calculated as treatment intensity (DAPD). Thus, on any given day in 2017, approximately 2% of sows and piglets, 1-2% of finisher pigs and 10% of weaner pigs were treated with antimicrobials. Also measured in DAPD, the antimicrobial use in pigs was 4% lower compared with 2016 and 29% lower than in 2009, when adjusted for export.

Both the poultry and the aquaculture industry saw further decreases in the use of antimicrobials from 2016 to 2017. In poultry, there was a decrease from 1,560 kg in 2016 to 1,488 kg in 2017. In the aquaculture industry, the antimicrobial consumption was the lowest ever recorded in VetStat at 1,697 kg (2,303 kg in 2016). The decreasing trend in aquaculture is likely to be positive effects of favourable weather conditions i.e. low summer temperatures combined with the implemented vaccination strategies

The fur animal industry continued to increase their antimicrobial use with a total use of 6,134 kg, corresponding to an increase of 15% compared with 2016. However, in 2017 there was no apparent increase in diagnostic submissions to provide an explanation for the increased use.

The estimated use of antimicrobials for companion animals was 1,296 kg in 2017. The use of critically important antimicrobials is still relatively high compared with other species. Almost all fluoroquinolones (87%, equivalent to 13 kg) and more than half of the cephalosporins (62%, equivalent to 111 kg) used for animals are prescribed for dogs and cats. Despite a small increase in use from 2015 to 2016, there has been an overall decreasing trend in the use of antimicrobials for dogs and cats since 2011. The shift in the use of antimicrobials continued in 2017 with a marked reduction in the relative use of cephalosporins and an increase in the use of aminopenicillins.

Antimicrobial consumption in humans

In 2017, the total consumption of antimicrobials in humans was 17.55 defined daily doses per 1000 inhabitants per day

(DID), lower than the consumption in 2016 (18.44 DID) and a decade ago in 2008 (18.09 DID). Overall, the consumption of antimicrobials increased from its first registrations in the DANMAP report 1996 (13.60 DID) until 2011 (19.80 DID) and has since levelled off.

Decreases in the past ten years were observed for all age groups (excluding the eldest > 80 years) and for both genders, regardless of the indicators used. The biggest decrease was observed in the youngest (0 to 4 year olds), where the number of treated patients per 1000 inhabitants decreased with 30%. In 2017, there were 298 per 1000 treated 0 to 4 year olds corresponding to 507 prescriptions redeemed per 1000 inhabitants.

In women, the number of treated patients per 1000 inhabitants decreased with 16% and in men with 20%. In 2017, the average number of patients treated (regardless of age and gender) was 255 per 1000 inhabitants corresponding to 489 prescriptions redeemed per 1000 inhabitants.

The total antimicrobial consumption at hospitals was measured at 110.28 defined daily doses per bed-days (DBD) and 323.53 defined daily doses per 100 admissions (DAD), respectively, a rise from 104.21 DBD and 310.68 DAD the previous year. From 2008 to 2017, the total consumption at hospitals increased with 43% and 2.8%, when measured in DBD and DAD, respectively.

Penicillins remained the most frequently used antimicrobial agents in both primary health care (67%) and in hospital care (53%), but the changes in consumption observed within this drug group in the last decade continued. Thus in 2008, beta-lactamase sensitive penicillins constituted 53% of all penicillins consumed in primary health care (5.31 DID of 9.99 DID), while in 2017 this had decreased to 38% (3.88 DID of 10.29 DID), a decrease of close to 27%. Simultaneously, consumption of combination penicillins increased markedly in both sectors for that decade. Thus in 2017, combination penicillins constituted 7.7% of the antimicrobial consumption in primary health care and 16% of the consumption in hospital care. Together with the penicillins with extended spectrum, combination penicillins were in 2017 the largest antimicrobial drug group consumed at hospitals.

In Denmark, fluoroquinolones, cephalosporins and carbapenems are defined as antimicrobials of critical interest and cephalosporins and carbapenems are only used at hospitals. In 2017, the consumption of the three drug classes constituted altogether 23% of the consumption at hospitals, a slight increase from 22% observed the year before, but a decrease from 31% in 2008. The increase in 2017 was observed for all five regions of Denmark and was mainly driven by an increase in the use of cephalosporins from 10.58 DBD to 12.49 DBD (18 %) and a slighter increase in the use of carbapenems from 4.06 DBD to 4.26 DBD (6.0%). These increases are probably linked to a long-term shortage in supply of piperacillin with tazobactam, where the consumption fell from 9.32 DBD to 7.75 DBD (- 17%).

Fluoroquinolones continued the decreasing trend observed since 2010, but with more pronounced reductions for the last two years. In 2016, fluoroquinolones accounted for a consumption of 8.37 DBD, corresponding to 8.2% of the total consumption at hospital. In 2017, this had decreased to 8.06 DBD, corresponding to 7.3% of the consumption at hospitals. In primary care, fluoroquinolones accounted for 0.44 DID equal to 2.9% of the total consumption.

Resistance in zoonotic- and indicator bacteria

Salmonella

Salmonella isolates from Danish pigs and domestically-produced pork were included in the DANMAP 2017, and *Salmonella* Typhimurium remained the most prevalent serotype. The resistance profiles were dominated by the large proportion of monophasic *Salmonella* Typhimurium isolates, exhibiting resistance to tetracycline, ampicillin and sulfonamides. The trend in resistance to these three antibiotics has been increasing steadily with approximately 30% increase since 2010. This steady increase was mirrored in the domestically acquired human isolates. Resistance to ciprofloxacin and 3rd generation cephalosporins was present in low levels in human isolates, but not found pigs or domestically-produced pork.

Campylobacter

Campylobacter isolates from humans (domestically acquired), broilers and cattle exhibited similar resistance patterns. Ciprofloxacin resistance was the most prevalent resistance, despite very limited veterinary use of quinolones. Tetracycline resistance also occurred in similar levels in all three populations. Isolates from travel-associated human cases were much more likely to be resistant than the domestically acquired isolates.

Enterococcus

In 2017, resistance to erythromycin and tetracycline was observed in 55% and 78% of the *Enterococcus faecalis* isolates from Danish pigs, respectively. A few gentamicin-resistant isolates (non-HLGR) were found, but no resistance to other antimicrobial agents critical to human medicine was detected.

Indicator *E. coli*

The proportion of fully susceptible indicator *E. coli* increased slightly in poultry and pigs, and decreased slightly in cattle in 2017 compared to 2016. Resistance patterns and levels in indicator *E. coli* from poultry, pigs and cattle were overall similar to previous years and no resistance to colistin, meropenem and tigecycline was detected.

ESBL/AmpC-producing *E. coli*

ESBL/AmpC-producing *E. coli* were recovered from 25% and 7% of the samples from Danish pigs and cattle, from 1% and 4% of the samples from domestically produced pork and beef, and

from 14% and 3% of the samples from imported pork and beef. The ESBL/AmpC occurrence in *E. coli* from the sources monitored in 2017 was comparable to the level observed in 2015. ESBL/AmpC genotypes were determined for isolates from meat and CTX-M-1 was the most common ESBL in *E. coli* from domestically produced pork and imported pork and beef. CTX-M-14, CTX-M-15, and CTX-M-1 were equally prevalent in domestically produced beef isolates. No plasmid-mediated AmpCs such as CMY was detected in the samples of pork and beef.

Resistance in human clinical bacteria

Since the beginning of DANMAP and especially during the past decade, the number of human invasive infections has increased remarkably.

Escherichia coli

For *Escherichia coli*, which causes approximately 50% of the monitored infections, the number of invasive isolates from hospital patients has increased continuously and almost doubled throughout the past decade, reaching a total of 5114 individual cases in 2017. Until 2016, the resistance trends were slightly decreasing, despite the increasing number of submitted isolates. However, the resistance trends for invasive *E. coli* reversed from 2016 to 2017 and showed slight increases for almost all drugs tested. In 2017, the following resistance percentages were observed: ampicillin 46%, mecillinam 14%, piperacillin/tazobactam 4.5%, gentamicin 6.0%, cefuroxime 9.7%, third-generation cephalosporins 6.7% and carbapenems < 1%.

In addition, the number of reported isolates from urinary tract infections increased markedly during the past decade and trends in resistance rates in these followed the trends observed in invasive isolates.

Klebsiella pneumoniae

In 2017, the total number of invasive *Klebsiella pneumoniae* isolates continued the worrying increase, reaching 1183 individual cases. As for *E.coli*, increases were also observed in the number of reported isolates from urinary tract infection. Eye-catching increases in resistance to mecillinam and sulphonamide were observed in urinary samples from 2016 to 2017, from 10% to 16% and from 18% to 26%, respectively. Despite these trends, a general tendency of decrease regarding critically important antimicrobials was observed for *K. pneumoniae* for the last decade. In 2017, the following resistances were noted in invasive isolates: mecillinam 19%, piperacillin/tazobactam 7.4%, gentamicin 3.2%, cefuroxime 11%, third-generation cephalosporins 7% and carbapenems < 1%.

Staphylococcus aureus

As for other invasive infections, the number of bloodstream infections with *Staphylococcus aureus* increased, reaching a total of 1996 individual cases in 2017, while the percentage of MRSA among all invasive *S. aureus* isolates has kept stable, between 0-3% throughout the years.

Still, attention has been drawn towards methicillin-resistant *Staphylococcus aureus* (MRSA), which, in number of incident cases, has grown by 420% (from 853 to 3,579 cases) from 2008 to 2017. This may partly be a result of changes in sampling methods due to extended guidelines, but also an increase in community-acquired infections was observed.

Carbapenemase-producing organisms (CPO)

During 2017, all carbapenemase-producing organisms (CPO) found in either clinical or screenings samples were sent to SSI for characterization based on whole genome sequencing. Altogether 123 CPO were detected from 115 patients compared with 115 CPO from 99 patients in 2016, leading to a 6% overall increase of submitted CPO isolates compared to 2016. More than one isolate from the same patient were included in the reporting, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases.

The 123 CPO consisted of 104 CPE (mainly *E.coli*, *K.pneumoniae* and *Citrobacter freundii*), 15 *Acinetobacter* spp and 4 *Pseudomonas* spp. For 40 of the cases with CPE a travel history could be obtained – 27 of the patients reported of travelling outside the Nordic countries, while 13 had not travelled. Nine of the persons with *Acinetobacter* spp reported of travelling and one of the patients with *Pseudomonas* spp. For several of the CPE and two of the *Acinetobacter* spp spread within hospital setting was reported.

Vancomycin-resistant *Enterococcus faecium* (VRE)

Since 2005, Danish Departments of Clinical Microbiology (DCM) have voluntarily submitted VRE for species identification, genotyping and surveillance to SSI. Since 2013, an increase in clinical VRE isolates has been observed, mainly driven by increases observed in the Capital Region. For the report 2017, the number of submitted isolates was supplemented with the number of VRE registered in the Danish microbiological database (MiBa), resulting in a total of 508 VRE, a slight decrease compared to 515 VRE in 2016. For 66 of the patients, bloodstream infection was reported. While this is the first time in the observation period without an increase in the number, the geographical dispersing to other regions continued, which is worrisome.

Other key findings in 2017

Besides the mentioned bacteria, DANMAP 2017 presents AMR findings in: *Enterococcus faecalis* and *faecium, Streptococcus pneumoniae,* beta-haemolytic streptococci, *Pseudomonas aeruginosa, Acinetobacter spp.,* and for the first time, *Haemophilus influenza* from invasive infections in humans (textbox 8.4). It also includes resistance findings in sexually acquired *Neisseria gonorrhoeae* and *Mycoplasma genitalium* (textbox 8.6).

Also for the first time the report introduces reporting on consumption of antifungals and development of resistance in human clinical isolates of *Candida* and *Aspergillus* (textbox 5.2).

Textbox 6.1 describes resistance in clinical isolates from animals for *E.coli, Streptococcus suis* and *Actinobacillus pleuropneumonia.*

Finally, the report provides updates on control initiatives and presents some research study outcomes.

Future improvements and developments

DANMAP demonstrates that a well-established surveillance program is important to understand the development of AMR and where prudent use of antimicrobials is necessary.

The general use of antibiotics in humans and food animals is relatively low in Denmark compared to EU and the rest of the world. Even so, we observe an increasing number of multi-resistant organisms in farm animals and humans. International travel and trade are considered an important factor in introducing new bacteria and resistance traits that may be maintained and spread in the Danish populations. Once introduced, high levels of consumed antimicrobials in subpopulations of humans or animals may maintain these resistance traits and even contribute to their dispersion. Monitoring of multi-resistance pathogenic bacteria as MRSA, ESBL, CPE and VRE in all relevant reservoirs provides essential information on when and where control measures are needed.

DANMAP 2017 provides a solid foundation for the Danish surveillance and methods to monitor the antimicrobial resistance situation over time. Moreover, the report reveals some gaps and areas that need further improvements to improve our understanding.

3

INTRODUCTION TO DANMAP

3. Introduction to DANMAP

3.1 Background

DANMAP was established in 1995 at initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries. Participants of the programme count: the National Food Institute and the National Veterinary Institute (both situated at Technical University of Denmark (DTU)) as well as Statens Serum Institut (SSI). The DANMAP programme is funded jointly by the Ministry of Health, the Ministry of Higher Education and Science, and the Ministry of Environment and Food.

A main purpose of DANMAP, has been to implement a One Health approach, comprising the entire chain from farm to fork to sickbed, since 1995. The organisation and collection of DANMAP data is presented in Figure 3.1. The diagram shows the interdisciplinarity and close cooperation between the participants.

The key objectives of DANMAP are:

- To monitor the consumption of antimicrobial agents in food animals and humans
- To monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin (e.g. meat) and humans
- To study associations between antimicrobial consumption and antimicrobial resistance
- To identify routes of transmission and areas for further research studies

A major part of the report concerns antimicrobial resistance in different contexts. The monitoring of resistance is based on three categories of bacteria:

- Human and animal pathogens that cause infections and are thought to reflect resistance caused by use of antimicrobial agents in their respective reservoirs
- Zoonotic bacteria capable of developing resistance in the animal reservoir, which may subsequently compromise treatment outcome when causing infection in humans
- Indicator bacteria (enterococci and *E. coli*) due to their ubiquitous nature in animals, food and humans, and their ability to readily develop or transfer antimicrobial resistance in response to selective pressure in both reservoirs

All pathogens may be considered reservoirs of resistance determinants – genes – that may be disseminated independently of the bacterial hosts.

A web annex presenting minimum inhibitory concentration (MIC) distributions, detailed tables of antimicrobial consumption and other additional data are available for download at www.danmap.org. Current and previous DANMAP reports are also available at the website (PDF versions).

Public health risks

Bacteria can be inherent resistant or become resistant either by spontaneous mutation or by transfer of resistance genes from other bacteria. Resistant strains are favoured when use of antimicrobial agents provide a selective pressure. This occurs in humans as well as in animals undergoing antimicrobial treatment. Resistant bacteria can spread between humans in the community, at healthcare centres and at hospitals. Furthermore, resistant bacteria from animals can be transmitted to humans either through direct contact with animals and their environment or through ingestion of contaminated food or other contaminated vehicles.

Antimicrobial treatment failure may occur if the ingested resistant bacteria are a direct cause of disease, or if resistance determinants are transferred to pathogenic bacteria causing the disease. Bacteria may be resistant to several – sometimes all – antimicrobial agents available for treatment, increasing the risk of treatment failure.

Currently there is only a limited number of antimicrobial agents, with novel modes of actions, under development by the pharmaceutical industry. Therefore, it is vital for public health organisations to ensure the continued effectiveness of compounds considered critically important to human treatment by ensuring prudent use for both humans and animals.

Prudent use should include considerations on possible restrictions of critical antimicrobial agents, so these can be reserved for use in humans primarily, to consider the introduction of new compounds for use in one sector only, as well as to eliminate all overuse. Only humans and animals suffering from an infection responsive to antimicrobial treatment should be exposed to antimicrobial agents.

3.2 Antimicrobial surveillance in Denmark

The following sections present some general information about the human population in Denmark in 2017, the production of food animals and the amount of meat available for human consumption in Denmark through the past decade. It also provides an overview of the antimicrobial agents for systemic and intramammary therapeutic use in humans and animals in 2017.

3.2.1 Populations and productions

During the past two decades, the human population in Denmark has increased from approximately 5.2 million inhabitants in 1995 to almost 5.8 million in 2017 (www.dst.dk).The population, which could potentially have received antimicrobial treatment in 2017, is shown as regional distribution in Figure 3.2. The five healthcare regions and the 10 Departments of Clinical Microbiology (DCM) in Denmark are pictured as well.

The production of food animals and the production of meat and milk are presented in Table 3.1. In 2017, the number of pigs produced was approximately the same as in 2016, but the number of exported fattening pigs (15-50 kg) continued to increase by approximately 7%. Since 2004, the total exports of fattening pigs have increased more than seven-fold.

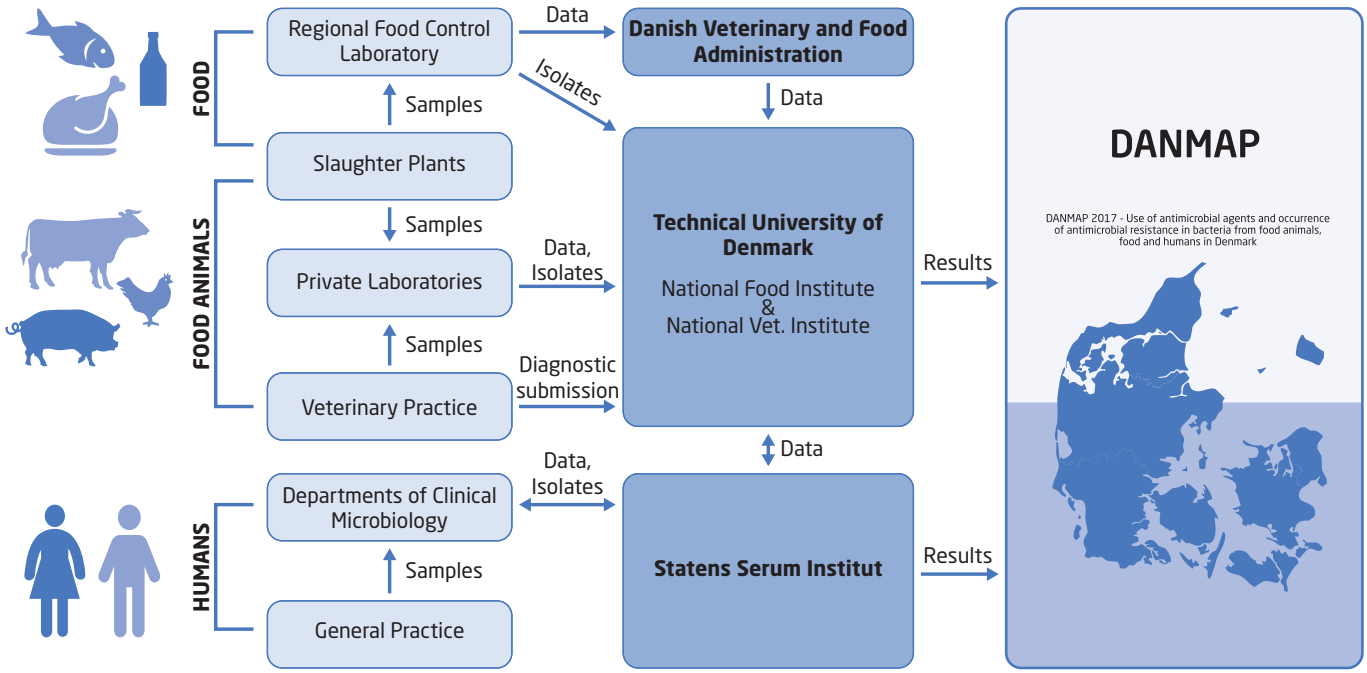
From 2016 to 2017, the number of cattle slaughtered decreased, while the number of dairy cows remained at the same level as in 2016, and the amount of milk produced increased by approximately 2%.

The number of broilers produced decreased and approximately 16% of the broilers produced in Denmark in 2017 were exported for slaughter. The production of turkeys has fluctuated considerably over the past decade. Since 2006, more than 99% of the turkeys produced have been exported for slaughter, thus the majority of turkey meat available for sale in Denmark is listed as imported.

3.2.2 Registered antimicrobial agents

Table 3.2 shows the antimicrobial agents registered to treat bacterial infections in humans and animals respectively. 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, 5.revision, WHO 2017]. In order to be considered critically important, an antimicrobial must be the only- or one of a limited number of compounds available to treat serious human disease. Critically important antimicrobial agents are also used to treat diseases in food animals and pets, so the reservoir of resistance-potential bacteria is not restricted to humans only. Since bacteria may be transmitted from animals to humans, and bacteria that cause human disease are capable of acquiring resistance genes from bacteria of animal origin, resistance against the critically important antimicrobials can be spread widely.

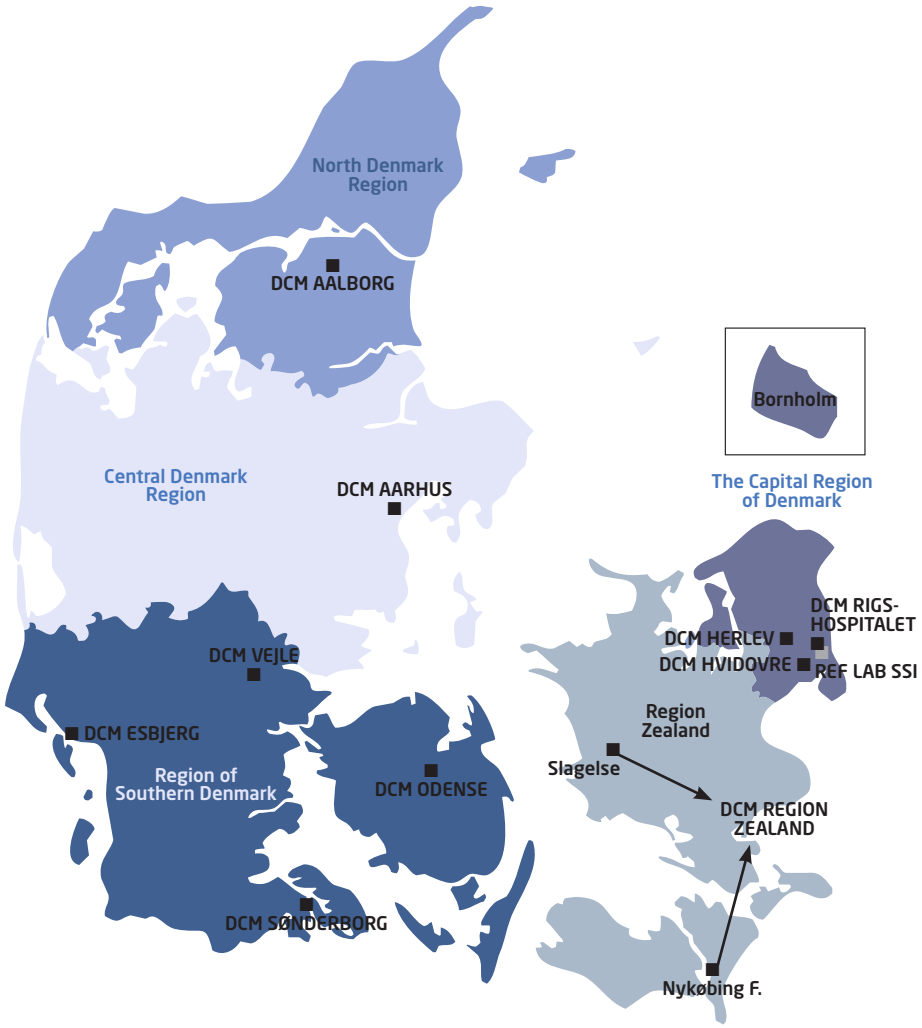
Figure 3.1 Organisation of DANMAP DANMAP 2017



In the newest revision from 2017, five drug classes were considered to be critically important: fluoroquinolones, 3rd, 4th and 5th generation cephalosporins, macrolides, glycopeptides and polymyxins. In Denmark, in food animals the use of these drug classes has in general been low or been reduced through either voluntary or legislative restrictions, see chapter 4 for more information. For trends and traditions in the antimicrobial treatment of humans and informations on recent national action plans see chapter 5.

Growth promoters (no longer used for animals in Denmark) are shown in parentheses in Table 3.2. Most of these influenced Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance towards growth promoters.

Figure 3.2 The five Danish healthcare regions and their respective population distributions. In addition, the 10 DCM are marked by black squares. The grey square indicates the reference laboratory situated at SSI. DANMAP 2017



North Denmark Region

No. of inhabitants	587,335
No. of inhabitants/km²	75
No. of inhabitants/GP	1,938

Central Denmark Region

No. of inhabitants	1,304,253
No. of inhabitants/km²	100
No. of inhabitants/GP	1,608

Capital Region of Denmark

No. of inhabitants	1,807,404
No. of inhabitants/km²	706
No. of inhabitants/GP	1,713

Region Zealand

No. of inhabitants	832,553
No. of inhabitants/km²	115
No. of inhabitants/GP	1,727

Region of Southern Denmark

No. of inhabitants	1,217,224
No. of inhabitants/km²	99
No. of inhabitants/GP	1,550

GP = general practitioner.

Source: Statistics Denmark (www.dst.dk) and the Danish Medical Association (www.laeger.dk)

Table 3.1 Production of food animals and the production of meat and milk, Denmark DANMAP 2017

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish[a]	
												Fresh water	Marine
	1,000 heads	mill. kg[b]	1,000 heads	mill. kg	1,000 heads	mill. kg	1,000 heads	mill. kg milk	1,000 heads	Export 1,000 heads[c]	mill. kg	mill. kg	mill. kg
1990	94560	116	571	2,5	789	219	753	4542	16425	-	1260	-	-
1992	107188	137	761	5,4	862	236	712	4405	18442	-	1442	35	7
1994	116036	152	1091	8,6	813	210	700	4442	20651	-	1604	35	7
1996	107895	149	961	9,3	789	198	701	4494	20424	-	1592	32	8
1998	126063	168	1124	11,6	732	179	669	4468	22738	-	1770	32	7
2000	133987	181	1042	10,3	691	171	636	4520	22414	-	1748	32	7
2001	136603	192	1086	13,2	653	169	623	4418	23199	-	1836	31	8
2002	136350	190	1073	12,8	668	169	611	4455	24203	-	1892	32	8
2003	129861	197	777	11,2	625	161	596	4540	24434	-	1898	34	8
2004	130674	198	1086	19,6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	183	1237	17,4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	161	785	11,3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	163	1009	14,4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	163	1068	12,3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	165	1175	11,1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	178	1184	14,0	519	142	574	4830	28505	7074	1974	28	10
2011	115454	175	960	9,4	551	145	575	4801	29399	7632	2008	28	11
2012	111080	168	1103	12,4	539	138	580	4928	29047	8794	1902	28	13
2013	117315	177	692	8,3	551	140	574	5025	28996	9318	1896	28	12
2014	115497	174	595	8,8	556	143	563	5113	29926	10517	1924	30	11
2015	114238	172	598	8,8	513	135	561	5270	30874	12133	1954	32	12
2016	120685	182	834	9,9	540	142	571	5276	31660	13280	1943	33	12
2017	117602	178	601	7,3	509	135	570	5478	31662	14173	1896	32	12

Source: Statistics Denmark (www.dst.dk) and The Danish AgriFish Agency. Production data for farmed fish was not available for 2017. Live animals exported prior to slaughter are included in number of animals and amount of meat produced. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg live pigs from the Danish Agriculture and Food Council

a) The numbers for 2017 are not final. The production of farmed fish includes fish transferred from one production facility to another

b) Assume a final slaughtered weight of 1.51 kg per broiler produced (Danish Agriculture and Food, 2013)

c) Export of 15-50 kg live pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside of Denmark

Table 3.2 Antimicrobial agents registered for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark

DANMAP 2017

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
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, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins and beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanic acid, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	1st generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin, cefazolin
J01DC	2nd generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DD	3rd generation cephalosporins incl. comb. with beta-lactamase inhibitors	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone, ceftazidime/avibactam
J01DE / QJ51DE	4th generation cephalosporins	Cefquinome	Cefepime
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem
J01DI	5th 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, telithromycine
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
QJ01XX [b]	Streptogramins	(Virginiamycin)	
J01GB / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin
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, oritavancin
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, tedizolide, 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

4

ANTIMICROBIAL CONSUMPTION IN ANIMALS

4. Antimicrobial consumption in animals

Highlights: The overall use of antimicrobials for animals decreased for the fourth consecutive year and has since 2013 been reduced by more than 16 tonnes. From 2016 to 2017, the antimicrobial consumption decreased by approximately 3%.

The decrease was driven by the use of antimicrobials for pigs, which was approximately 4% (3.4 tonnes) less than the year before. In 2017 the export of weaner pigs continued to increase, while the total number of pigs produced remained at approximately at the same level. The use of tetracyclines for pigs has been reduced significantly and almost consistently since 2009, and in particular from 2016 to 2017, following the implementation of the differentiated "Yellow Card". The differentiated "Yellow Card" initiative has also resulted in a close to zero use of colistin after the first quarter of 2017. However, the decrease in the use of tetracylines and colistin, was mirrored by less marked but clear increases in the use of macrolides, pleuromutilines and aminoglycosides.

Both the poultry and aquaculture industry further decreased their use of antimicrobials from 2016 to 2017. Use of antimicrobials in the aquaculture industry was the lowest ever recorded in VetStat. The decreasing trend is likely to be positive effects of favourable weather conditions i.e low summer temperatures combined with the implemented vaccination strategies.

The fur animal industry continued to increase their antimicrobial use, which increased by 15% in 2017, equivalent to approximately 800 kg. There was no apparent increase in diagnostic submissions that could provide an explanation for the increased use.

In companion animals, the use of critically important antimicrobials is still relatively high compared with other species. Almost all fluoroquinolones and more than half of the cephalosporins used for animals are used for dogs and cats. Despite a small increase in use from 2015 to 2016, there has been an overall decreasing trend in the use of antimicrobials for dogs and cats since 2011. The shift in the use of antimicrobials continued in 2017 with a marked reduction in the relative use of cephalosporins and an increase in the use of aminopenicillins.

4.1 Introduction

The use of antimicrobial agents in humans and animals has been monitored by the DANMAP programme since 1995. Since the early 1990s, there has been both political and public focus on the use of antimicrobial agents in the Danish animal production. This resulted in discontinued use of antimicrobial agents for growth promotion from 1994-1999, and more recently, in a voluntary ban of use of cephalosporins in the pig and dairy cattle production, as well as in regulatory legislation regarding therapeutic use [DANMAP 2010].

Figure 4.1 shows the total use of antimicrobials for animals and humans since 1994 and 1997, respectively. Changes in the antimicrobial consumption patterns for animals can be explained in part by an increase in pig production over the years, but risk management measures to reduce consumption have also played a role. In addition, the downward trend in consumption in animals has been affected by increasing export of live pigs at 30-40 kg live weight in recent years.

The prescription patterns for animals have clearly been influenced by risk management decisions during the period. For example, the decrease in antimicrobial consumption after 1994 was likely the result of 1) limitation of veterinary practitioners' profit from sales of medicine, 2) implementation of preventive veterinary strategies with Veterinary Advisory Service contracts (VASCs) and regular visits from the veterinarian in order to promote preventive veterinary strategies and optimize antimicrobial use, and 3) enforcement of the so called "cascade rule" [Order (DK) 142/1993], which limits the use of (cheaper)

extemporaneously produced medicines. The latter particularly affected the use of tetracyclines from 1994. Another important intervention was the restriction on the use of fluoroquinolones in production animals through legislation implemented in 2002 and 2003. Furthermore, in July 2010, the pig industry imposed a voluntary ban on the use of cephalosporins. This was followed by a similar initiative by the cattle industry in July 2014.

From 2010 to 2011, consumption decreased following the introduction of threshold values for antimicrobial consumption adopted within the "Yellow Card Initiative". This enforces legal actions on pig farmers with high antimicrobial use per pig [DANMAP 2010]. The "Yellow Card" was revised in 2016 and further adjusted in 2017 and the latest adjustments are described in Textbox 4.2 Effects from other parts of the legislation may be less obvious, but are also likely to have affected prescription patterns. For example, the rules for group medication in pig herds were tightened in 2014, requiring intensive laboratory diagnoses and frequent veterinary visits when antimicrobials are prescribed for groups of pigs.

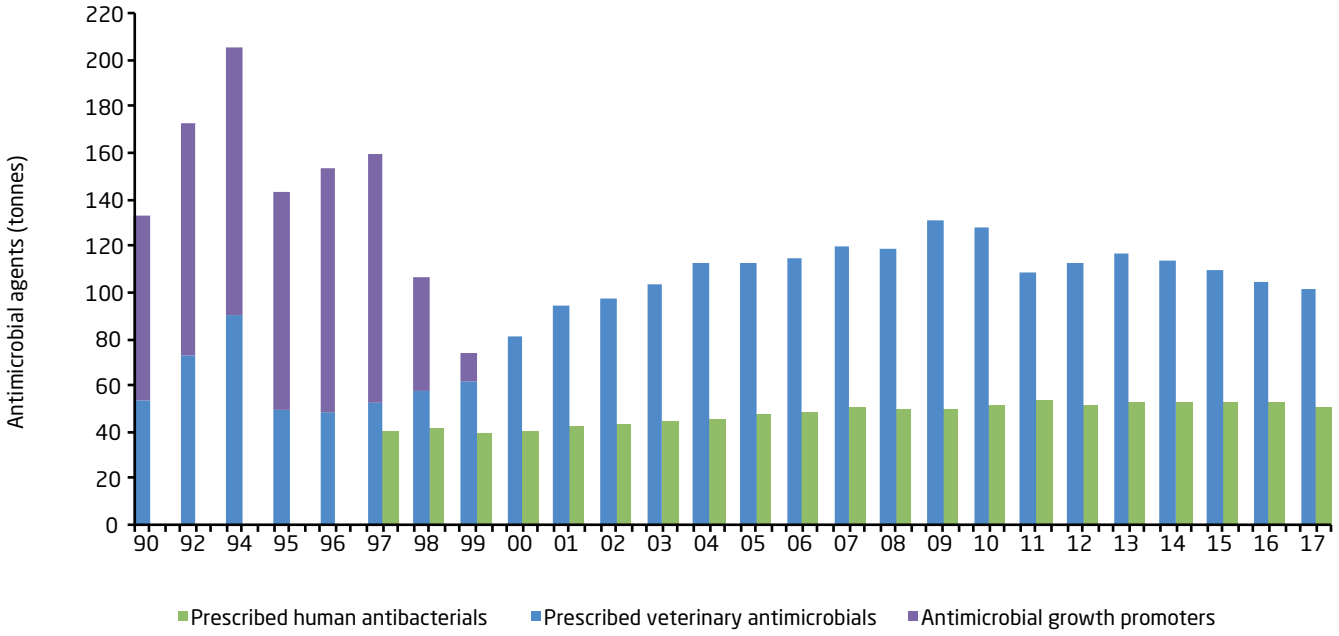
Official guidelines regarding the selection of antimicrobial agents for pigs and cattle have been available since 1996. The guidelines provide specific recommendations for selection of the appropriate antimicrobial treatment of all common indications in the major production animal species. Initially, guidelines were developed by the National Veterinary Laboratory (presently, DTU National Veterinary Institute). Since 2005, the guidelines have been updated by the Danish Veterinary and Food Administration (DVFA) 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.fvst.dk], and were further revised in 2017 and the new version published in April 2018. In 2012, the Danish Veterinary Association published treatment guidelines to promote prudent use of antimicrobials in dogs and cats. The guidelines were prepared by clinical specialists and expert scientists from the Faculty of Health and Medical Sciences at the University of Copenhagen and DTU National Food Institute. The treatment guidelines for dogs and cats was under revision in 2017. Similarly, the Danish Veterinary Association have also published treatment guidelines for use of antimicrobials in horses.

4.1.1 Data sources

Data on antimicrobial use at the product level have been collected in Denmark since 1996, including historical data back to 1990. In Denmark, antimicrobials are available by prescription only.

Since 2001, data on all medicine prescribed for use in animals, including vaccines, antimicrobial growth promoters (no longer permitted) and coccidiostatic agents (non-prescription) have

Figure 4.1 Prescribed antimicrobial agents for humans, and for all animal species, Denmark DANMAP 2017



Sources: Human therapeutics: The Danish Medicines Agency. Antimicrobials for animals: Until 2001, data are based on reports from the pharmaceutical industry of total annual sales from the Federation of Danish pig producers and slaughterhouses (1994-1995) and Danish Medicines Agency and Danish Plant Directorate (1996-2000). Data from 2004-2017 are based on data extracted from VetStat. Data for DANMAP 2017 was extracted from VetStat 6th August 2018. This figure includes all antimicrobial agents registered for use in animals.

been recorded in the national database VetStat. The VetStat database is hosted and maintained by Danish Veterinary and Food Administration (DVFA). Data in VetStat are validated by the DVFA.

The data presented in this report were extracted from VetStat on 6th of August 2018. Data have been summarized for DANMAP by DTU National Food Institute.

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 overall amount of antimicrobial agents is measured in kg active compound and is used in Section 4.2 for the purpose of

Table 4.1 Antimicrobial agents sold (kg active compound) by animal species and age group, Denmark DANMAP 2017

Therapeutic group	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others(a)	Pleuromutilins	Sulfonamides and trimethoprim(b)	Tetracyclines	Total 2016	Total 2017
Pigs	6240	381	<1	<1	2100	11584	315	0	15910	8733	7669	6615	15212	78150	74758
Sows and piglets	1709	279	<1	<1	433	427	34	0	8447	3580	694	4739	1272	22300	21616
Weaners	4328	95	<1	0	937	7928	276	0	1869	4373	2986	1661	9337	34450	33791
Finishers	203	7	<1	0	729	3228	4	0	5594	780	3989	214	4603	21401	19352
Cattle	723	737	63	<1	5	235	4	0	7421	742	0	922	1514	12948	12366
Intramammaries	40	0	53	0	4	0	<1	0	301	102	0	2	0	498	503
Cows and bulls excl. intramammaries	213	15	9	<1	<1	98	<1	0	6520	491	0	791	922	9656	9060
Calves <12 months	444	706	<1	<1	<1	131	4	0	448	139	0	118	556	2562	2547
Heifers and steers	26	16	<1	0	<1	5	<1	0	151	10	0	10	36	233	256
Poultry	65	5	0	<1	32	205	0	0	321	292	<1	85	483	1560	1488
All poultry excl. turkeys	47	1	0	<1	23	96	0	0	234	164	<1	85	198	942	848
Turkeys	18	4	0	0	9	109	0	0	87	128	0	0	285	619	640
Other production animal species	429	350	0	<1	134	932	<1	637	<1	2799	0	1587	962	7648	7831
Aquaculture	0	350	0	<1	<1	0	<1	637	0	31	0	679	<1	2303	1697
Fur animals	429	<1	0	<1	134	932	<1	0	<1	2768	0	908	962	5345	6134
Companion animals[b]	19	1	111	13	67	2	29	0	28	718	<1	1452	40	2458	2481
Pets(estimated)[c]	18	1	111	13	67	2	29	0	19	718	<1	280	38	1326	1296
Horses (estimated)	<1	<1	<1	<1	0	<1	<1	0	9	<1	0	1172	3	1131	1184
Other[d]	234	7	4	2	10	240	3	4	865	147	6	293	153	1622	1967
Total	7709	1482	178	15	2347	13198	351	640	24544	13431	7676	10953	18364	104387	100890

Note: Data for 2016 and 2017 were extracted from VetStat 6 August 2018. Only the ATCvet group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds

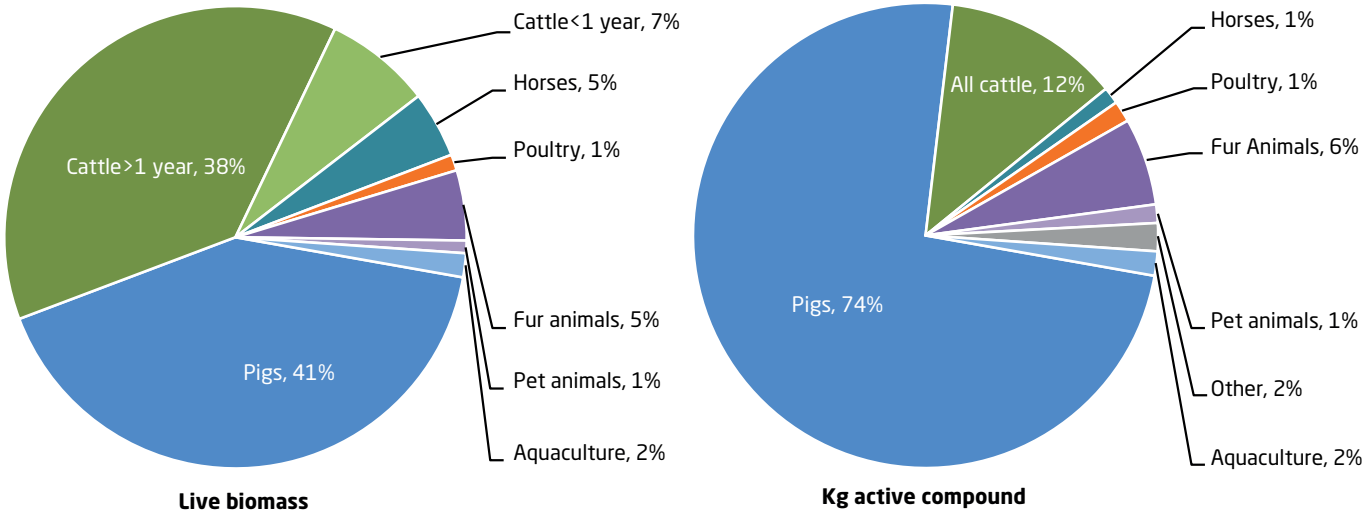
a) Penicillins with extended spectrum and combination penicilins, incl. b-lactamase inhibitors

b) Since DANMAP 2016, new principles have been used to estimate the antimicrobial use for companion animals, see section 4.3.4

c) Approximately 242 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

d) This includes data on sheep and goats (13 kg), data where the animal species has not been defined or where the age group does apply to the designated animal species

Figure 4.2 Live biomass (mill. kg) and antimicrobial consumption (kg) in main animal species, Denmark DANMAP 2017



Note: The live biomass is estimated from census data (pigs, cattle and pet animals) and production data (poultry, fur animals and aquaculture). For poultry: the figures comprise only the biomass for the main production types (turkeys and broilers). The live biomass estimates for poultry, aquaculture and pet animals are based on 2012 data. The estimation procedures are described in Chapter 9, Materials and Methods.

an overall crude comparison of antimicrobial use in the veterinary and human sectors (Figure 4.1).

Since 2012, we have further presented 'defined animal daily dose' (DADD) and 'proportion of population in treatment per day' (DAPD) to monitor trends in antimicrobial consumption. These metrics are defined below, and for additional information on methodology, please refer to Chapter 9 and the web annex [www.Danmap.org].

DADD - Defined animal daily dose.
DADD is the average maintenance dose per day for the main indication of a drug in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species; and when appropriate, also age group. The DADDs have been specifically defined for use in DANMAP based on current knowledge (Chapter 9, Materials and Methods) and may vary from the prescribed daily dose or the recommended dosage in the Summaries of Product Characteristics (SPC) or in the VetStat database (Web Annex).

DAPD - proportion of population in treatment per day
Trends in antimicrobial usage for pigs are presented in DAPD, because this measure allows for comparison between sectors. DAPD=DADD per 1000 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 comparison between species with large differences in body-mass and life-span, but for DANMAP 2017, we have calculated treatment proportions for pigs only.

The estimated treatment proportion, DAPD, is a statistical measure that provides a rough estimate of the proportion of animals treated daily with a particular antimicrobial agent. For example, 10 DAPDs means that an estimated 1% of the pig population, on average, receives a certain treatment on a given day (Chapter 9, Materials and Methods). In principle, DAPD also allows comparisons with the antimicrobial consumption in the human sector, which is measured in defined daily dose per 1,000 inhabitants per day (DID), see Chapter 9 for a description of DID.

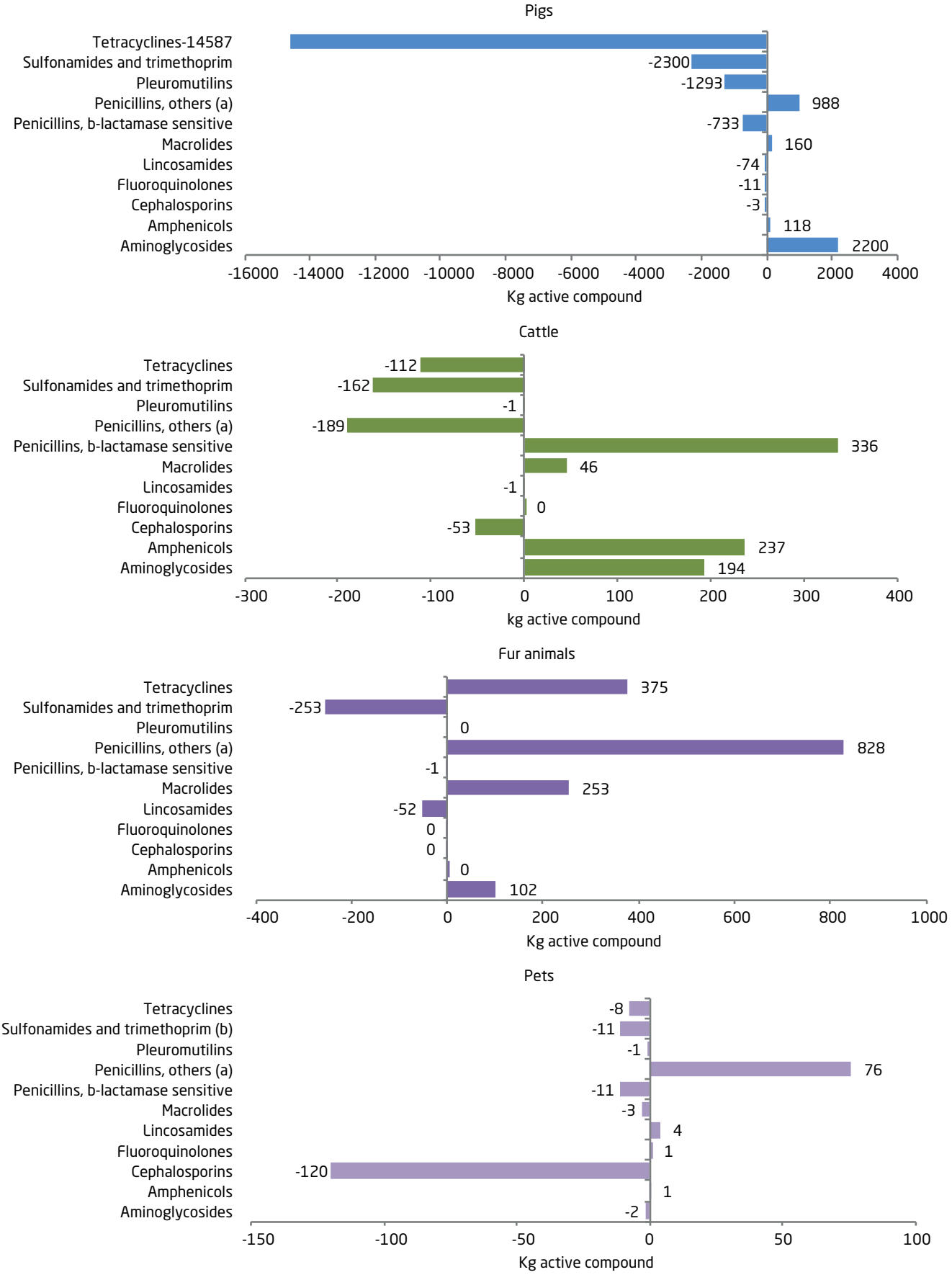
4.2 Total antimicrobial consumption in animals

Measured in kg active compound, the total use of antimicrobial agents used for all animals, amounted to 100.9 tonnes active compound, representing an overall 3% decrease compared with 2016, Figure 4.1 and Table 4.1.

In 2017, the antimicrobial use for pigs, cattle, fur animals and poultry comprised approximately 74%, and 12%, 6% and 1% of the total antimicrobial consumption for animals, respectively (Figure 4.2). The overall decrease in antimicrobial use for animals was mainly attributed to a 4% decrease in the amount used in the pig industry, which is the main driver of antimicrobial usage in animals in Denmark, due to the size of the production. Cattle and pigs comprise almost equal proportions of live biomass. However, the vast proportion of cattle biomass consists of dairy cows, which have very low consumption of antimicrobial agents compared with growing animals.

Figure 4.3 Change in antimicrobial use (kg active compound) in pigs, cattle, fur animals and pets (dogs and cats) 2013 - 2017, Denmark

DANMAP 2017



Note: The figure includes the antimicrobial agents registered for use in the particular animal species. Note the different scale on the x-axis. The use of antimicrobial agents for pets (dogs and cats) has been estimated as described in section 4.2.2. Poultry has not been included in the figure, since several serious disease outbreaks in 2014 and 2015 have caused considerable fluctuations in the use of antimicrobials during these years.
a) Penicillins with extended spectrum: amoxicillin/clavulanic acid, and also, ampicillin, amoxicillin and cloxacillin

Historically, the overall consumption, measured as kg active compound, was 51% lower in 2017 compared with 1994.). A major part of the decrease in consumption can be explained by the discontinued use of growth promoters from 1994 to 1999. In contrast, the total meat production increased by 12% during this period (Table 3.1 and Figure 4.1).

Between 2000 (start of VetStat) and 2009, when consumption was at its highest, the amount of kg active compound increased by 62% (Figure 4.1). This increase was driven mainly by consumption in pigs, and during this period the number of pigs produced went up by 23% (Table 3.1). At the same time, the proportion of exported live pigs (at approx. 30 kg) increased and thus resulted in a decrease in the overall biomass of the pig population. Since then, the proportions of exported live pigs has continued to increase. Overall, there has been a gradual decreasing trend in the use of antimicrobials for animals and in 2017 it was approximately 23% lower than in 2009.

Figure 4.3 illustrates the changes in antimicrobial usage in pigs, cattle, fur animals and pets over the past five years (in kg active compound). With the exception of fur animals, the changes in the antimicrobial use within the different animal species have been clearly affected by the different initiatives to reduce not only the total use of antimicrobials, but also the use of particular antimicrobial classes. Particularly, the use of tetracyclines for pigs has been reduced significantly over the past five years. The usage patterns in cattle has shifted towards more penicillins and less sulfonamides and trimethoprim, amphenicols and cephalosporins. In dogs and cats, the use has shifted away from the cephalosporins. For both pigs and cattle there has been an increase in the use of aminoglycosides and macrolides over the last five years.

4.3 Antimicrobial consumption by animal species

4.3.1 Antimicrobial consumption in pigs

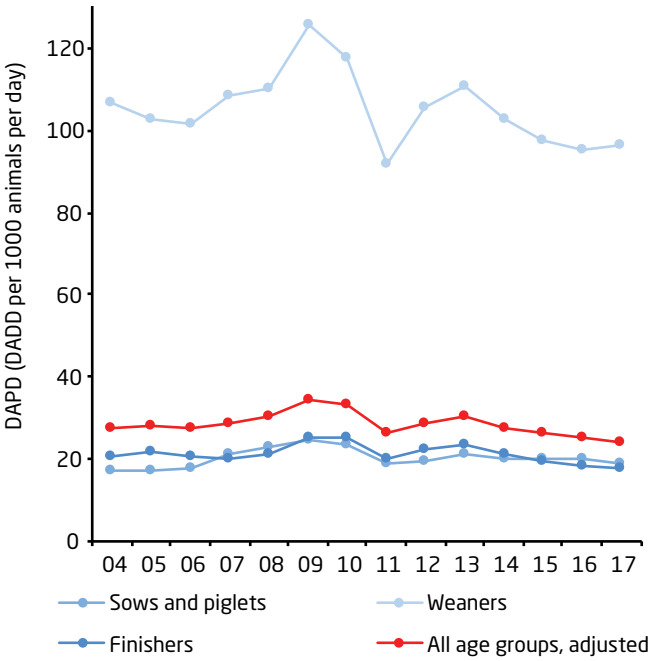
For this issue of DANMAP, updated data from 2016 and 2017 were extracted from VetStat and measures for the antimicrobial usage in pigs were calculated for 2016 and 2017, using the updated dataset.

In 2017, the total antimicrobial consumption in pigs (sows and piglets, weaners, finishers) was 74.8 tonnes active compound (Table 4.1), a decrease of 3.4 tonnes (4%) compared with 2016. The treatment proportions (DAPD) in the pig population overall and by age group are presented in Figure 4.4 and Figure 4.5 and the DADD's are shown in the in the web annex (Table A4.1 and in the DADD description).

The treatment proportion (DAPD) of the total population reflects the trends in selection pressure within the population. However, the treatment intensity is much higher in the weaning pigs than in finishers and sows (Figure 4.4). Furthermore, the biomass of the weaning pigs is also very small (7.5- 30 kg, 4 weeks), compared with the finishers (31-107 kg, 12 weeks) and the sows.

Figure 4.4 Antimicrobial consumption (a) in the pig production, and the distribution on age groups, Denmark

DANMAP 2017



Note: The" all age groups adjusted" is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in boars and piglets pre-weaning
a) The DAPD is 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)

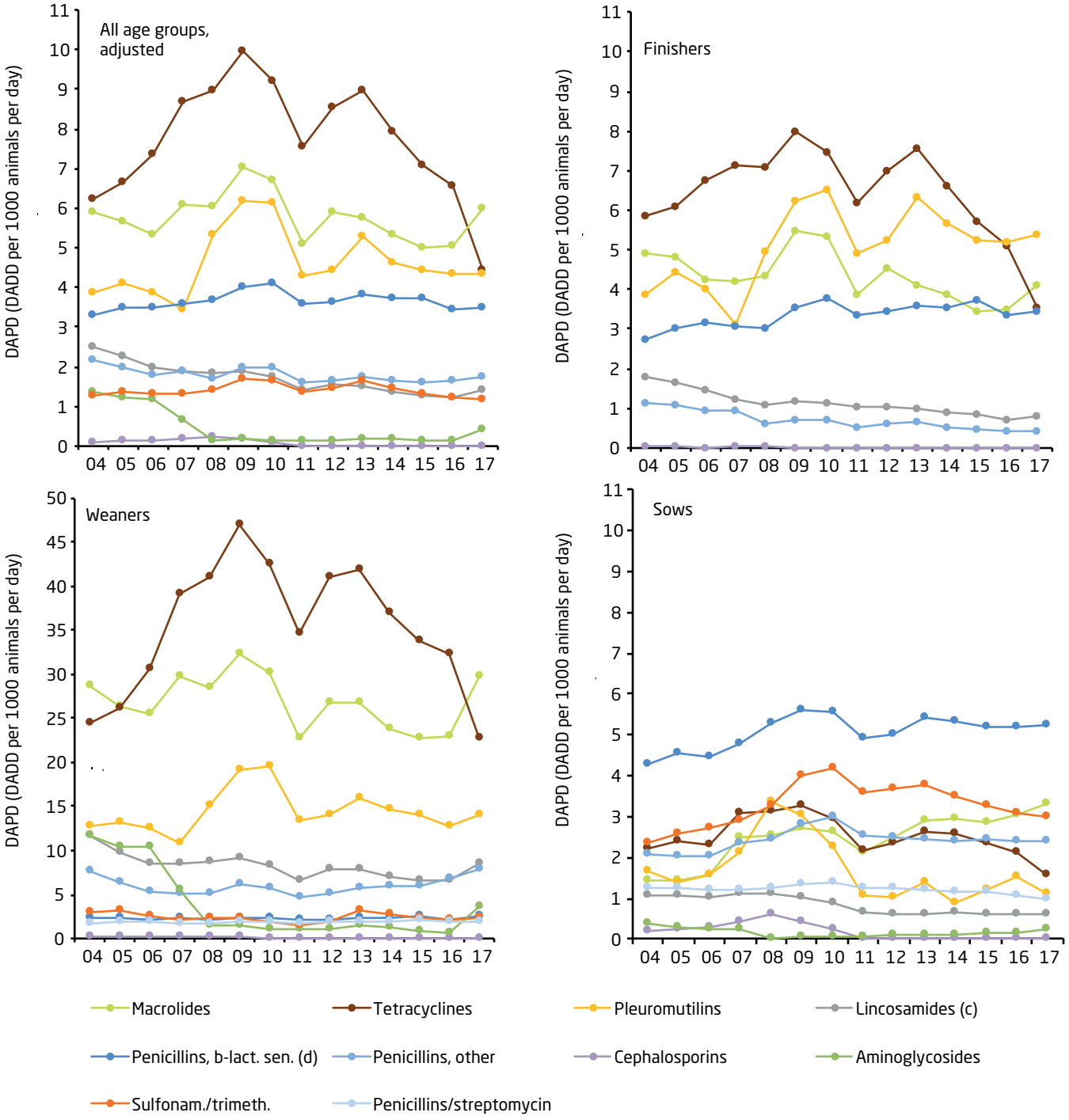
The large differences in DAPDs between age groups affects the DAPD of the total population and trends are influenced by changes in population structure. Thus, export or productivity need to be accounted for, before interpreting the antimicrobial consumption patterns and selection pressure in the pig production. As an example, increased export of live pigs right after weaning could lead to an increase in DAPD for the remaining population, since the exported pigs were only in the country, when the treatment proportion was highest.

Historically, the treatment proportion (DAPD) increased from 2004 to 2009, followed by a decrease in 2010 and 2011, which is considered a result of the "Yellow Card Initiative" (See DANMAP 2010).

In 2017, the antimicrobial consumption in pigs, measured in DAPD, decreased from approximately 25 DAPD to 24 DAPD (Figure 4.4) when adjusted for export. The number of exported live pigs continued to increase in 2017, while the total number of pigs slaughtered remained at approximately the same level (Table 3.1). Within the different age groups, the treatment proportions decreased slightly for sows and piglets and finishers, but increased for weaners (Figure 4.4). Thus, on a given day in

Figure 4.5 Antimicrobial consumption(a) in the total pig production(b), and in finishers, weaners, sows and piglets, Denmark

DANMAP 2017



Note: Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure.

a) The DAPD is 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)
b) The total is adjusted for the increasing export of pigs at 30 kg (see text). "Sows" includes treatment in boars and piglets pre-weaning
c) Lincosamide/spectinomycin combinations comprise 56% of this group
d) Beta-lactamase sensitive penicillins

2017, approximately 2% of sows and piglets, 1-2% of finisher pigs and 10% of weaner pigs were treated with antimicrobials. Also measured in DAPD, the antimicrobial use in pigs was 29% lower in 2017 than in 2009, when adjusted for changes in export (Figure 4.4).

Changes to the yellow card initiative were implemented during 2017, i.e. multiplication factors of 1.5 and 10 were applied to the use of tetracyclines and colistin, respectively, to promote further reduction (see Textbox 4.1).

The National MRSA Action plan aims to reduce the antimicrobial use in pigs by 15% in 2018, compared to 2014. In 2017, the overall use in the pig production was reduced by approximately 13%, both when measured in kg active compound and when measured in DAPD (adjusted for export).

Tetracyclines has been one of the most commonly used antimicrobials in the Danish pig production for more than a decade. It is almost exclusively administered orally, and is especially used for treatment of gastrointestinal disease in weaning pigs and finishers. The overall use of tetracyclines has decreased since 2013 and 2016 saw the lowest DAPD levels since 2005. In 2017, the use of tetracyclines was further sharply reduced, following the implementation of the higher multiplication factor in the Yellow Card Initiative. Measured in DAPD, the use of tetracyclines, for all age groups was reduced by 32% from 2016 to 2017 and has decreased 55% since 2009. The proportion of weaner pigs treated with tetracyclines on any given day has decreased from approximately 5% in 2009, to approximately 2% in 2017.In contrast, the use of other antimicrobials has increased, particularly the use of aminoglycosides (mainly neomycin), macrolides and pleuromutilins, see Figure 4.4

The use of colistin for pigs increased more than two-fold from 403 kg in 2009 to 864 kg in 2016, of which 752 kg were used for weaners. Since the implementation of a multiplication factor of 10 in the "Yellow Card" Initiative April 2017, the use of colistin has been close to zero (Textbox 4.2).

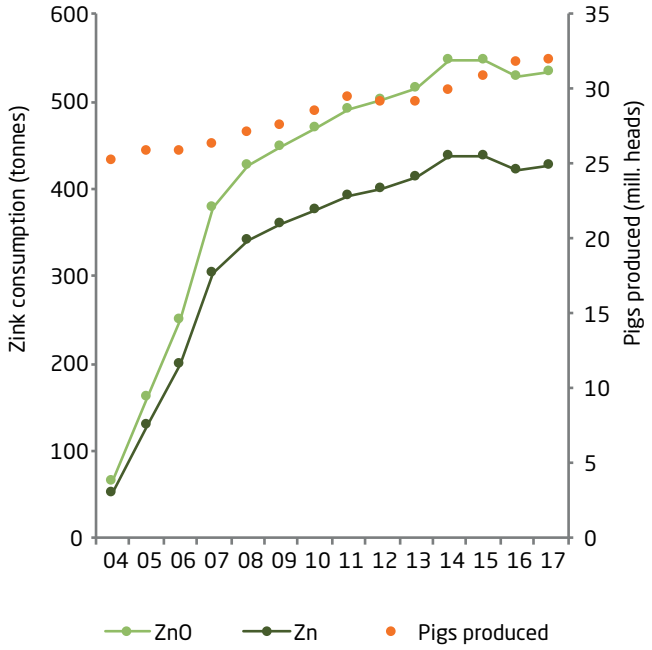
Use of the critically important antimicrobial agents, fluoroquinolones and cephalosporins was close to zero in 2017.

Use of medical zinc in pigs

In the latest issues of DANMAP, we have presented the use of medical zinc for pigs (Figure 4.6). This is relevant in the context of DANMAP, because its use may select for antimicrobial resistance in some bacteria, including MRSA. Medical zinc, in the form of zinc oxide, is fed to piglets after weaning to prevent or treat diarrhoea.

For more than a decade the use of zinc for pigs increased steadily, reaching a peak in 2015 and has since then fluctuated between approximately 527 and 548 tonnes. In 2017, the European Commission announced an EU wide ban on

Figure 4.6 Consumption (tonnes) of zinc oxide (ZnO) and zinc (Zn) in the pig production, Denmark DANMAP 2017



Note: The most commonly used product is zinc oxide (ZnO) which contains 80% zinc and which is largely insoluble in water

the use of medical zinc for pigs from June 2022. In February 2016, the Danish pig industry launched an action plan to help the pig producers reduce the use of medical zinc. The action plan, focusses on correct use/reduction of medical zinc and investigates alternative methods for preventing diarrhoea in weaner pigs.

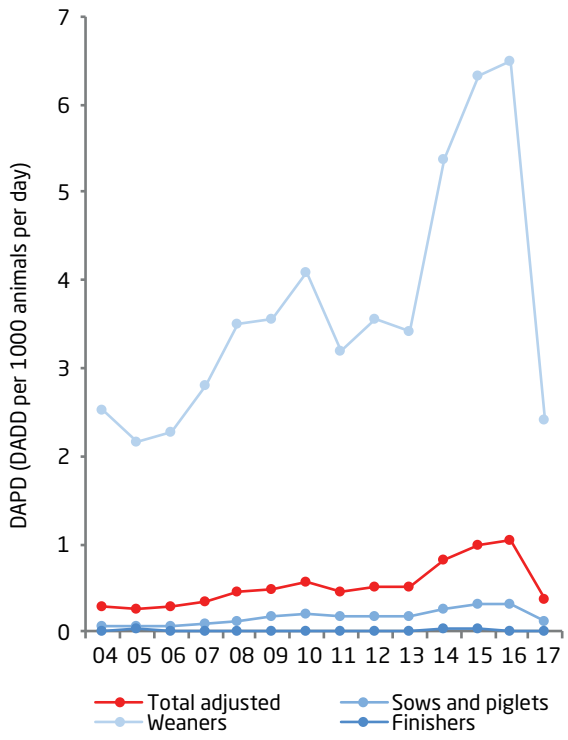
Use of colistin and neomycin

In Denmark, almost all the colistin prescribed for animals (882 kg in 2016 and 321 kg in 2017) is used in the pig production for treating gastrointestinal infections in weaners. Only smaller amounts are used for cattle and poultry. In 2010, colistin was introduced as one of the "first choice" antimicrobial agents for the treatment of gastroenteritis in the official treatment guidelines for pigs. Furthermore, in 2014, the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% and the steep increase in colistin use in 2014 and 2015 was probably a result of this. Since then, the policy has changed because of the emergence of new colistin resistance. As part of the differentiated 'Yellow Card' in April 2016, a multiplication factor of 10 was applied to colistin (see Textbox 4.2) and as a consequence, the use of colistin dropped significantly in 2017. An overview of colistin consumption in production animals 2004-2016 is shown in Figure 4.7.

As the use colistin has been phased out, the use of neomycin has been introduced. In VetStat only very little or zero use of neomycin has been recorded since 2008. In 2017, a new neomycin product

Figure 4.7 Total use of colistin in production animals, Denmark

DANMAP 2017



registered for pigs was introduced on the market and subsequently a total of 2,283 kg neomycin was used for pigs, mainly for weaners.

4.3.2 Antimicrobial consumption in cattle

In 2017, the overall consumption of antimicrobials in cattle decreased by approximately 600 kg compared to the previous two years, mainly due to a decrease in usage for cows and bulls (excl. intramammaries). The production of veal and beef decreased by approximately 5% from 2016 to 2017, while milk production continued to increase (Table 3.1). As shown in Figure 4.3, the usage pattern appears to have shifted away from the use of tetracyclines, sulfonamide/trimethoprim, extended spectrum penicillins and cephalosporins and towards an increased use of beta-lactamase sensitive penicillins, macrolides, amphenicols and aminoglycosides.

The use of fluoroquinolones in cattle has been close to zero for the last decade. Fluoroquinolones may only be prescribed to food producing animals, as a last line drug, based on microbiological and resistance testing in an accredited laboratory. Use of fluoroquinolones for food producing animals is also notifiable to the DVFA.

The use of cephalosporins (all generations) used for systemic treatment (orally and parenterally) was reduced to 9 kg in 2017. This represents a 69% decrease since 2014 (29 kg), when the cattle

Table 4.2 Use of antimicrobial agents for intramammary application in cattle in DADD's (1000s), Denmark

DANMAP 2017

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Doses per antimicrobial class	DADDs (1000's)												
Penicillins[a]	201	211	211	236	282	314	318	324	311	317	275	262	216
Aminoglycoside-benzylpenicillin combinations[b]	130	104	101	101	110	93	48	47	58	90	143	154	206
Cephalosporins, 1st generation	103	98	89	85	89	89	99	105	111	113	96	89	86
Cephalosporins, 3rd and 4th generation	110	124	127	112	76	51	34	30	24	21	10	6	3
Others[c]	21	20	16	15	14	12	9	8	0	0	11	10	11
Total	566	558	544	549	570	559	508	514	504	541	535	521	522
Total DADD per cow per year	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	0.9	1.0	1.0	0.9	0.9

Note: For intramammary treatment, 1 DADD is defined as the dose to treat two teats for 24 hours
a) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations (QJ51CE, QJ51CF, QJ51RC)
b) Mainly dihydrostreptomycin-benzylpenicillin combinations; includes also combinations of penicillin/aminoglycoside with bacitracin or nafcillin (QJ51RC)
c) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

Table 4.3 Number of treatments with antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2017

	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Total doses per indication (a)	DADDs (1000's)												
Drying off treatment (4 teats)	73	75	71	76	82	99	97	117	125	140	154	160	168
Therapeutic treatment (2 teats)	420	408	388	377	378	350	307	279	253	259	227	202	186

Note: Includes data for intramammaries registered for use in cattle. For intramammary therapeutic treatment, 1 DADD is defined as the dose to treat two teats for 24 hours. For drying off treatment, 1 DADD is defined as the dose to treat 4 teats.

industry decided to phase out its use, and an 85% reduction since 2008 (60 kg), when cephalosporin consumption was at its peak. The use of 3rd and 4th generation cephalosporins is low in cattle and mostly used for systemic treatment.

The majority of antimicrobials administered parenterally to cattle are used for dairy cows and prescribed mainly for mastitis. The overall level of intramammary treatment remained unchanged from 2016 to 2017. The antimicrobial use for intramammary application measured in DADDs is shown in Table 4.2. Since 2013, the use of penicillins and cephalosporins (all generations) has been reduced while the use of aminoglycoside-benzylpenicillin combinations has increased.

The board of Danish dairy and beef producers has recently renewed its strategy for good udder health. The goals are a 20% reduction in use of antimicrobials for treatment of mastitis and other cattle diseases as well as lowering geometric mean bulk tank cell counts to 150.000 by the year 2020. In addition, the dairy industry will promote use of dry cow therapy and mastitis treatment with simple penicillins. Since 2009, the number of antibiotic treatments at drying-off has increased and the relative proportion of drying-off treatment versus therapeutic treatment has shifted markedly from 22% versus 78% in 2010 to 48% versus 52% in 2017(Table 4.3). During the same period the use of 3rd and 4th generation cepahlosporins has decreased markedly.

4.3.3 Antimicrobial consumption in poultry

In Denmark, poultry production comprises mainly broiler production, followed by egg layers and turkey production. In addition, there is a small production of ducks, geese, and game birds.

Danish broiler farms have a very high level of biosecurity and the antimicrobial consumption in broiler production is generally low compared with other species. Accordingly, a few disease outbreaks in some farms can markedly affect and cause considerable fluctuations in the national statistics on antimicrobial usage. This was the case in late 2014 and throughout 2015. In 2016, use of antimicrobials for poultry (excl. turkeys) decreased sharply again and in 2017 the usage was further reduced to 848 kg. For broilers, amoxicillin has been the most commonly used antimicrobial agent for more than a decade, but in both 2016 and 2017 tetracycline was the most commonly used antimicrobial (Table 4.1). Cephalosporines have not been used in the poultry industry for more than a decade.

VetStat does not allow differentiation of the use of antimicrobials between different sectors of the poultry production. However, the consumption for turkeys was identified by combining information from the Central Husbandry Register with information provided by poultry veterinarians and the industry (personal communication: S. Astrup, PoultryVet, and M. Nielsen Blom, Danish Agriculture and Food Council) and the information in VetStat.

The annual usage in turkeys can also be notably affected by disease outbreaks in few flocks. In 2017, the antimicrobial use was approximately at the same level as in 2016 (Table. 4.1), almost half (45%) of which was tetracyclines (Table. 4.1).

4.3.4 Antimicrobial consumption in aquaculture, fur animals and companion animals

The antimicrobial consumption in aquaculture continued to decrease from 2016 to 2017 by a further 23% to 1,697 kg (Table 4.1). This is the lowest level of antimicrobial use for aquaculture ever recorded in VetStat. The antimicrobial consumption is mainly three compounds; sulphonamide/trimethoprim comprised 40%, quinolones 38% and amphenicols 21%, when measured in kg active compound.

Antimicrobial consumption in aquaculture is mostly influenced by the summer temperatures, because diseases are more likely to occur in warmer waters. In recent years, the aquaculture industry has developed new and better vaccines and improved vaccination strategies to reduce the risk of diseases that may require antibiotic treatment. A combination of favourable weather conditions (lower temperatures during summer – particularly in 2017) and a positive effect of the revised vaccination strategies may explain the reduced consumption seen over the last few years [personal communication: N. H. Henriksen, Danish Aquaculture].

The use of antimicrobial in mink has a distinct seasonal variation, with high use from the spring when the mink kits are born and again when they are weaned, furthermore there is usually an increase in antimicrobial use again in the autumn. The production of mink has increased significantly over the last decade, peaking at 17.8 million in 2015. In 2017, 17.1 million mink were produced in Denmark (Source: Kopenhagen Fur).

With the exception of 2013 and 2014, the use of antimicrobial agents in mink production has increased every year for more than a decade, from less than two tonnes in 2004 to more than six tonnes in 2017 yielding a treatment intensity of approximately 43 DADD per 1000 animals per day, i.e. on a given day in 2017, approximately 4% of Danish the mink were being treated with an antimicrobial. However, in 2017 there was no apparent increase in diagnostic submissions to provide an explanation for the increased use (National Veterinary Institute, DTU). This suggests that diagnostics are increasingly done by the veterinarians in private practice, and since these results are not collected systematically, there is a lack of information on which diseases are being treated in the Danish mink production [Dansk Veterinær Tidsskrift (in Danish), 5, 2018, pager 45] . It is particularly the use of tetracyclines, penicillins with extended spectrum and combinations penicillins that have increased over the past five years (Figure 4.3). Use of fluoroquinolones and cephalosporins in fur animal production has been close to zero for more than a decade.

A voluntary action plan to reduce the use of antimicrobials in mink is under development. The plan, which will include

restrictions on the use of antimicrobials and require increased diagnostic submissions from mink, will be implemented in 2018.

The use of prescribed medical zinc (zinc oxide) in mink production has fluctuated remarkably over the past three years. It seems that in 2016 an unusually high level of use occurred (1,045 kg)where as in 2014, 2015 and 2017, only approximately 500 kg was prescribed. In the mink production, medical zinc is applied topically on pre-weaning mink kits in the nest boxes.

The information available on antimicrobial consumption in companion animals is not as complete as for production animals, and a substantial amount of the antimicrobials used for companion animals are entered into VetStat without defining animal species. In Tables 4.4 and 4.5, all antimicrobial agents registered for use in dogs, cats and horses have been includ-

Table 4.4 Estimated use of antimicrobial agents for horses measured in kg active compound, Denmark

DANMAP 2017

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others[a]	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2012	1	0	0	0	0	0	0	<1	14	0	0	1000	3	1018
2013	1	0	0	0	0	1	0	<1	13	0	0	893	5	914
2014	1	0	0	0	0	0	0	<1	15	0	0	1024	6	1047
2015	3	0	0	0	0	0	0	<1	10	0	0	1049	4	1067
2016	1	0	0	0	<1	0	0	<1	8	0	<1	1117	5	1131
2017[b]	1	0	0	0	<1	0	0	<1	9	0	<1	1172	3	1184

Note: Data for 2017 and 2016 were extracted from VetStat 6th August 2018. The estimates include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in horses. Furthermore, antimicrobial agents, where no animal species is given, were allocated to horses based on relevant type of preparation (eg. oral paste) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included

a) Penicillins with extended spectrum and combination penicillins, incl. b-lactamase inhibitors

Table 4.5 Estimated use of antimicrobial agents for dogs and cats measured in kg active compound, Denmark

DANMAP 2017

	Aminoglycosides	Amphenicols	Cephalosporins	Fluoroquinolones	Lincosamides	Macrolides	Other AB	Other quinolones	Penicillin's, b-lactamase sensitive	Penicillin's, others[a]	Pleuromutilins	Sulfonamides and trimethoprim	Tetracyclines	Total
2012	22	<1	272	13	67	7	49	0	42	651	<1	306	51	1483
2013	19	<1	231	12	63	5	42	0	31	642	<1	292	45	1383
2014	21	<1	213	12	69	6	34	1	31	653	<1	300	35	1376
2015	18	<1	157	13	68	4	32	0	25	655	1	235	39	1249
2016	17	<1	137	14	69	3	30	0	20	718	<1	276	40	1326
2017[b]	18	1	111	13	67	2	29	0	19	718	<1	280	38	1296

Note: Data from 2017 was extracted from VetStat on 6th August 2018. Data include all antimicrobial agents registered, by the either the pharmacy or veterinarians for use in pets. Furthermore, antimicrobial agents, where no animal species is given, were allocated to pets based on relevant type of preparation (eg. tablets, eye- or eardrops) or registration. Antimicrobials administered parenterally - with no information on animal species - are not included

a) Penicillins with extended spectrum and combination penicilins, incl. b-lactamase inhibitors
b) In 2016, approximately 222 kg of the sulfonamides and trimethoprim registered for pets are products (oral paste) typically used for horses

ed. Furthermore, it is assumed that a substantial part of the antimicrobial agents where no animal species is given, have been used for companion animals. We have estimated this proportion using similar principles as described in DANMAP 2016, Textbox 4.4 and is briefly described in table 4.5.

Measured in kg active compound, the overall antimicrobial consumption appears to have increased for slightly for horses, while it has been reduced for pets.

A large proportion of antimicrobials used for companion animals are prescribed for treatment of chronic or recurrent disease, mainly dermatitis. Due to the close contact between owners and their pets, the repeated use of critically important antimicrobials may pose a risk to the owners.

In 2017, the use of fluoroquinolones for use in pets was 13 kg, which comprised the majority of fluoroquinolones used in animals in 2017. Similarly, the pets accounted for a significant proportion (111 kg or 62%) of the use of cephalosporins used in animals.

However, over the past years, since the treatment guidelines by Danish Veterinary Association (November 2012) were first published, the use of cephalosporins has been reduced by 59%, which is in line with the treatment guidelines, recommending that use of critically important antimicrobials should be reduced as much as possible. Thus, antimicrobial use in pets appears to be shifting away from the use of cephalosporins, sulfonamides and trimethoprim and towards broad spectrum penicillins, in particular amoxicillin with betalactamase inhibitor.

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Textbox 4.1

Political agreement on the veterinary strategy, 2018 - 2021

In December 2017, all members of the Danish parliament reached a joint political agreement on theveterinary strategy in the years 2018-2021. The agreement is called "Veterinærforlig III" and replaces "Veterinærforlig II" covering the previous four years.

The agreement includes a large number of goals and initiatives covering antimicrobial resistance, livestock-MRSA, the Danish animal disease contingency plans, animal welfare, as well as new research in the field. 302.6 million DKK (40.7 million €) has been set aside over the next four years to fund the different initiatives.

The core of the agreement is that healthy production animals is 1) the foundation of a low antimicrobial use and a low occurrence and development of resistance, 2) a significant contribution to increased animal welfare and 3) a prerequisite for an efficient and sustainable production with good economy and increased export. Hence, the main goal of the agreement is producing healthy production animals.

The largest project in the political agreement is "Healthy Animals". This project covers continued efforts in monitoring and regulating antimicrobial use, an upgrade to the VetStat database, improved biosecurity, continued surveillance and monitoring for antimicrobial resistance, and the creation of an expert council on veterinary medicines.

As one of its remits, the council will support the Danish Veterinary and Food Administration in decisions regarding the regulation of antimicrobial use. The council will consist of 9 members from both veterinary and human health sector, since the use of antimicrobials in production animals and the emergence of antimicrobial resistance needs to be addressed in a One Health perspective.

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Textbox 4.2

Effects of the differentiated Yellow Card initiative from 2016

The Yellow Card initiative was introduced in 2010 to reduce the use of antimicrobials in pig production in Denmark. By targeting the farms with highest consumption of antimicrobials, the Yellow Card initiative works as an incentive for pig producers to contribute towards the goal of reducing the overall use of antimicrobials (see DANMAP 2010).

In 2016, the Danish Veterinary and Food Administration (DVFA) further developed the Yellow Card by including a multiplication factors to adjust the amount used of some of the antimicrobial agents. The multiplication factors were determined by the DVFA and are used as risk mitigation tools for each class of antimicrobials.

Fluoroquinolones and cephalosporins are classified as critically important in treatment of humans and have been allocated the highest multiplication factor of ten. Fluoroquinolones are also under further restrictions by Danish law and the Danish pig industry has since 2010 voluntarily phased out the use of 3rd and 4th generation cephalosporins.

At first tetracyclines were allocated the multiplication factor of 1.2. This reduced the use of tetracyclines in the following months. In January 2017, the factor for tetracyclines was increased to 1.5 to promote a further reduction and the use continued to decline.

In 2016, the European Medicines Agency (EMA) recommended that colistin should only be used as a second line treatment in animals. Although Denmark was well below the threshold suggested by EMA, the Danish government increased the multiplication factor for colistin from one to ten as a precautionary measure. The use of colistin for pigs has consequently dropped to almost zero.

The differentiated Yellow Card has proven to be an efficient tool to promote prudent overall antimicrobial use in pig herds and to discourage use of certain critically important antimicrobials.

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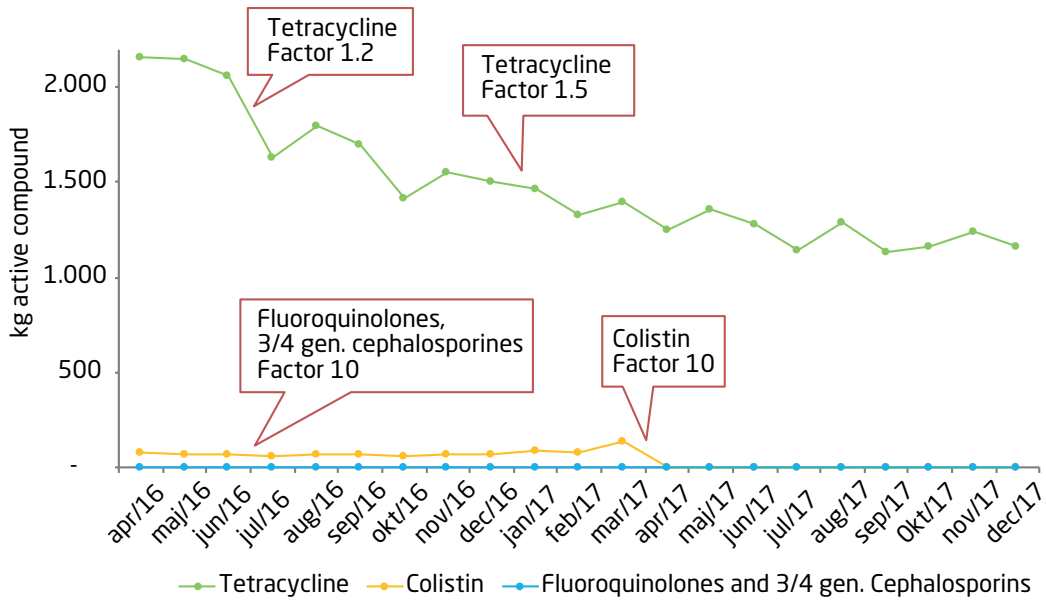
Figure 1 Use of selected antimicrobial agents in pigs per month (from april 2016), Denmark

DANMAP 2017



Note: Data was extracted from VetStat in May 2018. The data from the database is dynamic and the numbers above can change over time due to retrospective corrections. The usage of fluoroquinolones, 3rd and 4th generation cephalosporines does not show in the figure as the use is close to zero. The same applies to the use of colistin since April 2017.

5

ANTIMICROBIAL CONSUMPTION IN HUMANS

5. Antimicrobial consumption in humans

Highlights: In 2017, the total consumption of antimicrobials in humans was 17.55 defined daily doses per 1000 inhabitants per day (DID), lower than the consumption in 2016 (18.45 DID) and a decade ago in 2008 (18.09 DID). Overall, the consumption of antimicrobials increased from its first registrations in the DANMAP report 1996 (13.60 DID) until 2011 (19.80 DID) and has since levelled off.

Penicillins: In 2017, penicillins remained the most frequently consumed antimicrobial agents in both primary health care (67%) and hospital care (53%), but within the past decade marked changes within the group of penicillins were observed. Thus, the consumption of beta-lactamase penicillins in primary health care decreased with 27% from 5.31 DID in 2008 to 3.88 DID in 2017. In 2017, they accounted for 25 % of the total and 38% of all the penicillins consumed. Simultaneously, the consumption of the three other groups of penicillins increased markedly; in 2017, beta-lactamase resistant penicillins accounted for 1.56 DID (9.8%), penicillins with extended spectrum for 3.67 DID (24%) and combination penicillins for 1.18 DID (7.7%) of the total in primary health care, respectively. At hospitals, the combination penicillins and penicillins with extended spectrum were the biggest antimicrobial drug groups consumed in 2017 (16% and 19%, respectively).

Antimicrobials of critical interest: In Denmark, fluoroquinolones, cephalosporins and carbapenems are defined as antimicrobials of critical interest, cephalosporins and carbapenems being used solely at hospitals. In 2017, the consumption of the three drug classes constituted altogether 23% of the consumption at hospitals, a slight increase from 22% observed the year before, but a decrease from 31% in 2008. The increase in 2017 was observed for all five regions of Denmark and was mainly driven by a slighter increase in the use of carbapenems from 4.06 DBD to 4.26 DBD (6.0%) and a more marked increase of cephalosporins from 10.58 DBD to 12.49 DBD (18 %). These increases are probably linked to a longlasting shortage of piperacillin with tazobactam, where the consumption fell from 9.32 DBD to 7.75 DBD (- 17%).

Fluoroquinolones continued the decreasing trend observed since 2010, but with more pronounced decreases for the past two years. In 2016, fluoroquinolones accounted for a consumption of 8.37 DBD, corresponding to 8.2% of the total consumption at hospitals, in 2017 this had decreased to 8.06 DBD, corresponding to 7.3% of the consumption at hospitals. In primary care, fluoroquinolones constituted with 0.44 DID 2.9% of the total consumption.

In 2017, the total antimicrobial consumption at hospitals was measured at 110.28 DBD and 323.53 defined daily doses per 100 admissions (DAD), respectively, a rise from 104.21 DBD and 310.68 DAD the previous year. From 2008 to 2017, the total consumption at hospitals increased with 43% and 2.8%, when measured in DBD and DAD, respectively.

5.1 Introduction

In Denmark, all consumption of human medicine including antimicrobials is recorded through the Register of Medicinal Product Statistics at the Danish Health Data Authority. The primary sector has submitted antimicrobial sales data since 1994, whereas the hospital sector has submitted data since 1997.

Recording of the consumption in the primary sector covers all antimicrobials on prescription from general practitioners, medical specialists and dentists as well as prescriptions written to patients at hospitals upon discharge. No over-the-counter sale takes place, all sale is through pharmacies and based on prescription only. This enables an almost complete surveillance of all systemic antimicrobials used in Denmark. For the hospital sector, only data from public somatic hospitals is included – data from psychiatric hospitals, private hospitals, hospices and rehabilitation centers were omitted since they contribute with only a low consumption of antimicrobials (2.1% in 2017), and in many ways differ from the patient population at public somatic hospitals. For more detailed information on data reporting and registration, please see chapter 9, materials and methods.

In this chapter, the term 'antimicrobial agents' covers all systemic antibacterial agents for human use listed in the Anatomical Therapeutic Chemical (ATC) Classification under the code *J01*. The only other antimicrobials included are metronidazole (ATC code P01AB01) and vancomycin (ATC code A07AA09), since these constitute important systemic antibacterial treatments as well. Their consumption has been included in DANMAP since 2014. Tuberculostatica, antiviral and antifungal drugs are not included, but textbox 5.2 deals with the consumption of antifungal compounds and resistance patterns of human invasive isolates of *Candida* and *Aspergillus*.

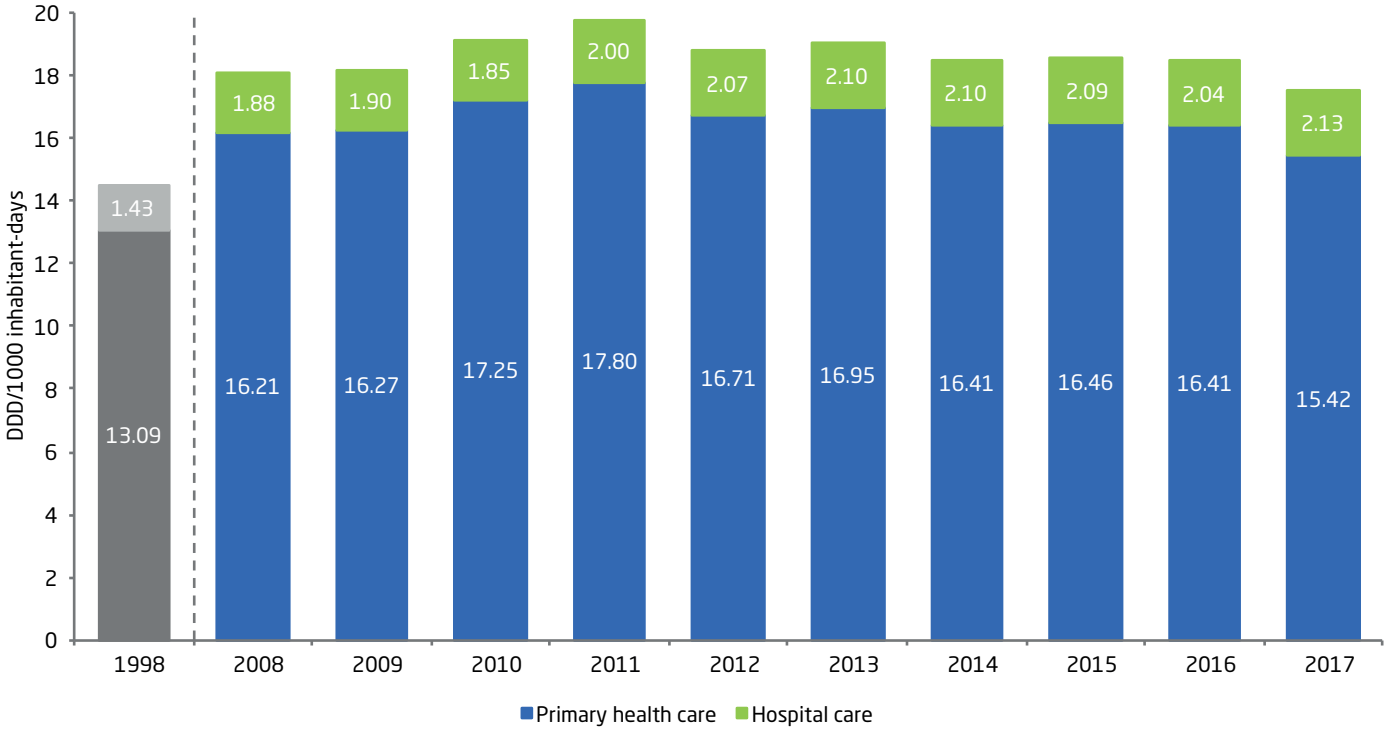
In DANMAP 2017, all numbers in the figures and tables were updated 10 years retrospectively, to correct for possible changes that may have happened in the data registers after data extraction for the report. Thus, minor changes may exist between the present and former reports.

Further information and further numbers on the use of antimicrobials in Denmark can be found at www.medstat.dk and http://esundhed.dk/sundhedsregistre/LSR/ANT/Sider/ANT.aspx.

The Danish healthcare system has undergone big changes since the DANMAP collaboration began in 1995. Most notably are the establishment of a more centralized hospital system, building on gathering highly specialized functions and skills in few tertiary care hospitals, paralleled by a reduction in the number of secondary care hospitals. Thus during the last two decades the number of hospitals in Denmark offering 24 hour acute care has diminished from 80 public somatic hospitals in 1995 to 43 in 2004. For 2020, it is expected that all acut care function can be merged to 21 public hospitals. A new political plan for the Danish Health system from 2018 and onwards is focusing on enforcement of the primary sector by moving a substantial part of the ambulatory care and rehabilitation from the hospitals back to the municipalities. This demands a restructuring and the strengthening of collaboration between all sectors.

Figure 5.1 Total consumption of systemic antimicrobial agents in humans in primary health care vs. hospital care, (DID), Denmark

DANMAP 2017



5.2 Total consumption (Primary Health Care and Hospital Care Sectors)

In 2017, the total consumption of antimicrobials in Denmark was 17.55 defined daily doses per 1000 inhabitants per day (DID), which is 4.8% less than the consumption in 2016 (18.45 DID) and 3.0% less than the consumption a decade ago in 2008 (18.09 DID), (Figure 5.1). The total consumption in 2017 corresponds to 50,925 kg active compound consumed (Table A5.2.1 in web annex).

Overall, the consumption of antimicrobials showed no significant trends during the first five years of registration from 1996 (13.40 DID) until 2000 (13.63 DID), but increased steadily and markedly until 2011 (19.80 DID) and has since decreased slightly but continuously. The recent decreases are solely due to decreases in consumption in the primary health sector, which accounts for approximately 90% of the total consumption and thus has a significant impact on the overall consumption patterns (Figure 5.1).

According to the annual reports from the European Center for Disease Control (ECDC) the European average in antimicrobial consumption in 2016 was approximately 22 DID; for the other Nordic countries (Iceland, Finland, Norway and Sweden) the average in 2016 was 16.19 DID, which is comparably close to the Danish average. Denmark has, together with Sweden the highest proportion of beta-lactamase sensitive penicillins consumed compared to the total antimicrobial consumption (> 25%). Regarding the consumption of combination penicillins, the Nordic countries are less similar, reflecting that in spite of comparable health care systems and a tradition of a generally restrictive use of antimicrobials, the Nordic countries probably still differ in recommendations on the use of specified antimicrobial drug classes. [https://ecdc.europa.eu/en/antimicrobial-consumption/database/quality-indicators].

5.3 Primary Health Care

5.3.1 Total consumption in Primary Health Care in DID

In 2017, the total consumption of antimicrobials in primary care was 15.42 DID, a decline of 6.0% from 2016 (16.41 DID). This is the first significant annual decline since 2012 (Table 5.1). Since 1998, the consumption has increased 17% from 13.09 DID , (Figure 5.1).

Beta-lactamase sensitive penicillins continued to be the biggest group consumed with 3.88 DID (accounting for 25% of the total consumption in primary care). They were followed closely by penicillins with extended spectrum with a consumption of 3.67 DID (corresponding to 24% of the total consumption). Macrolides remained the third biggest group consumed with 1.62 DID (accounting for 11% of the total consumption).

In total, the group of penicillins accounted for 10.30 DID, 67% of the total antimicrobials consumed. A decade ago in 2008, they accounted for 9.98 DID, 62% of the total antimicrobials consumed that year.

A distribution of the different antimicrobial classes between primary care and hospital care is shown in Figure A5.2.1 in web annex.

Table 5.1 Consumption of antimicrobial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

DANMAP 2017

ATC group[a]	Therapeutic group	Year									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	1.55	1.62	1.70	1.74	1.76	1.96	1.66	1.61	1.61	1.42
J01CA	Penicillins with extended spectrum	3.28	3.31	3.48	3.57	3.40	3.48	3.53	3.61	3.62	3.67
J01CE	Beta-lactamase sensitive penicillins	5.31	5.13	5.26	5.29	4.67	4.65	4.39	4.33	4.15	3.88
J01CF	Beta-lactamase resistant penicillins	1.13	1.14	1.17	1.22	1.20	1.30	1.36	1.38	1.47	1.56
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.27	0.45	0.68	0.89	1.04	1.22	1.30	1.42	1.42	1.18
J01D	Cephalosporins and other β-lactam antibiotics	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.49	0.48	0.51	0.5	0.52	0.53	0.55	0.56	0.56	0.56
J01EB	Short-acting sulfonamides	0.28	0.27	0.26	0.24	0.22	0.22	0.21	0.18	0.16	0.15
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.29	2.21	2.44	2.6	2.19	1.94	1.79	1.77	1.82	1.62
J01FF	Lincosamides	0.03	0.03	0.04	0.04	0.04	0.05	0.05	0.05	0.06	0.06
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.52	0.52	0.57	0.57	0.55	0.52	0.5	0.49	0.48	0.44
J01XC	Steroid antibacterials (combination fusidic acid)	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.47	0.49	0.51	0.5	0.49	0.49	0.48	0.45	0.43	0.26
J01XX	Other antibacterials (methenamine >99%)	0.27	0.26	0.27	0.26	0.25	0.24	0.24	0.25	0.27	0.28
J01XD and P01AB*	Nitroimidazole derivatives (metronidazole)	0.24	0.26	0.27	0.28	0.28	0.28	0.28	0.28	0.28	0.25
J01+P01AB	Antibacterial agents for systemic use (total)	16.21	16.26	17.25	17.80	16.71	16.95	16.41	16.46	16.41	15.42

a) From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system
*) all metronidazole preparations, formerly only listed as J01XD, 10 years retrospective data included in the DANMAP report since 2014

5.3.2 Trends in consumption of the leading antimicrobials in DID

Penicillins. In Denmark, penicillins are the only beta-lactams used in primary care; other beta-lactams such as cephalosporins, monobactams and carbapenems are solely used in hospital care and primarily at somatic hospitals with surgical or acute care functions. Although the total consumption of penicillins has changed only slightly over the years, considerable changes have been observed within the group of penicillins during the past decade. Since 2008, the consumption of beta-lactamase sensitive penicillins has decreased almost continuously with 27%, paralleled by a 29% decrease of macrolides (from 5.31 DID to 3.88 DID and from 2.29 DID to 1.62 DID, respectively), (Figure 5.2a, 5.2b and Table 5.1). For both drug classes, this is probably the result of a more restrictive use of antimicrobials in primary care in general, since the decrease in DID is followed by an overall decline in the number of treated patients and the number of redeemed antimicrobial prescriptions (Table 5.2 and 5.3). In Denmark, these two antimicrobial classes are the main drugs used for treatment of upper airway infections. Recommendations regarding overuse of antibiotics in general will thus have the biggest impact on beta-lactamase sensitive penicillins and macrolides.

For the same period from 2008 to 2017, continuous increases in the consumption of the three other penicillin groups' were observed: Penicillins with extended spectrum increased with 12%, the beta-lactamase resistant penicillins with 38% and the combination penicillins including beta-lactamase inhibitors with > 300% (Figure 5.2a and 5.2b). Thus, while the beta-lactamase sensitive penicillins constituted 53% of the group of penicillins consumed in 2008, in 2017 they constituted only 38%, (not shown).

The proportion of the main atnimicrobials used in primary healthcare is shown in Figure 5.3. The most remarkable change for the year 2017 was the marked decline in the consumption of combination penicillins including beta-lactamase inhibitors (represented solely by amoxicillin with clavulanic acid), which after years of steady increase (from 0.27 DID in 2008 to 1.42 DID in 2015 and 2016) suddenly decreased to 1.18 DID in 2017, (Figure 5.2a and Table 5.1). As for the beta-lactamase sensitive penicillins and macrolides these changes are probably related to initiatives on a more prudent use, as they also were followed by a reduced number of patients treated and reduced number of prescriptions redeemed (Table 5.2 and 5.3). The combination penicillins are a popular choice in the treatment of upper respiratory tract infections - but their main use is in the treatment of patients suffering from COLD and severe bronchitis or pneumonia. For these a clear decrease in the number of prescriptions was noted.

The increases described for the penicillins with extended spectrum from 3.28 DID in 2008 to 3.67 DID in 2017 are primarily due to increases in the consumption of pivmecillinam, accounting for about two thirds of this drug class (Table 5.1 and Figure 5.4). While pivmecillinam increased with 1.2% from 2016 to 2017 and with 70% since 2008, pivampicillin decreased with 9.1% from 2016 to 2017 and with 63% from 2008 to 2017.

Figure 5.2a Consumption of leading antimicrobial groups for systemic use in primary health care, 2008-2017, Denmark DANMAP 2017

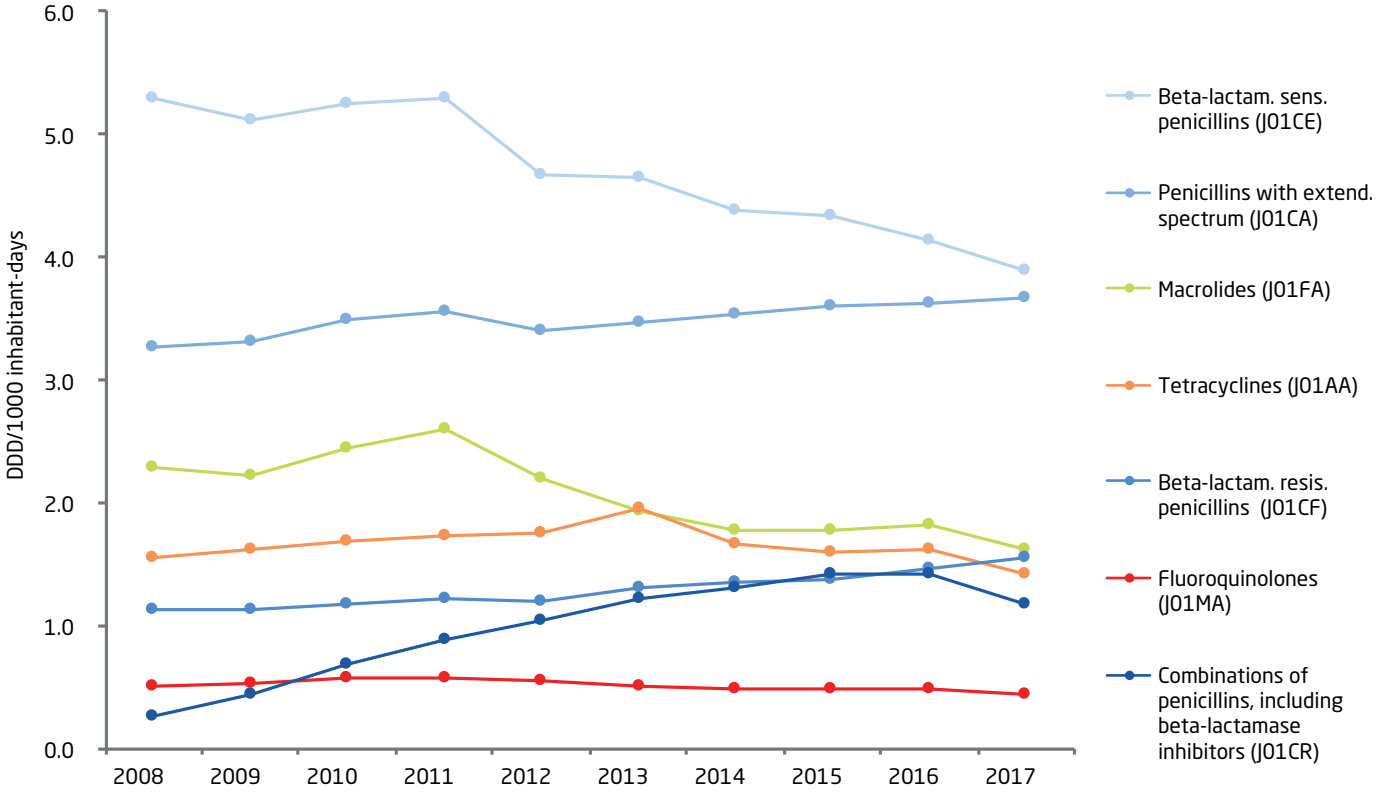


Figure 5.2b Changes in the consumption (DID) by leading groups of antimicrobial agents (J01) in the primary sector, 2008-2017, Denmark

DANMAP 2017

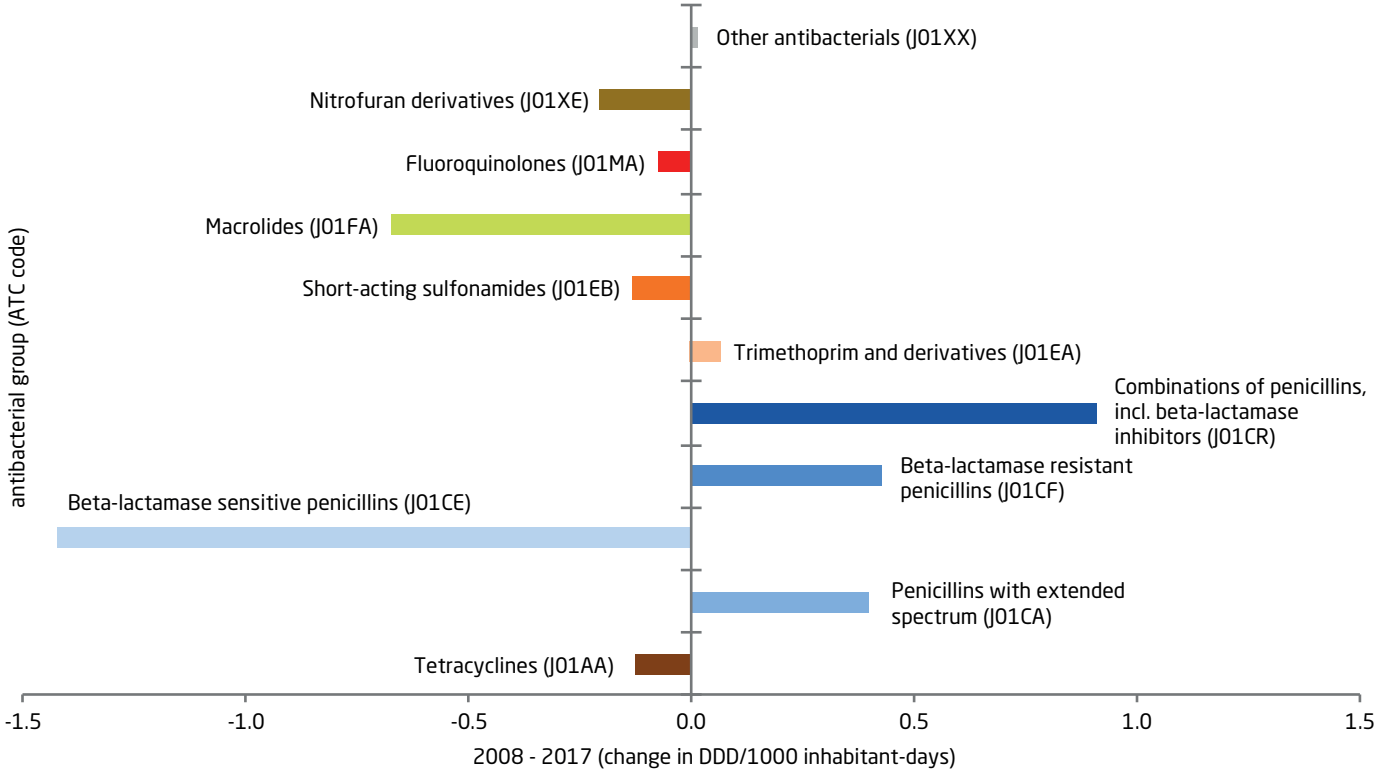
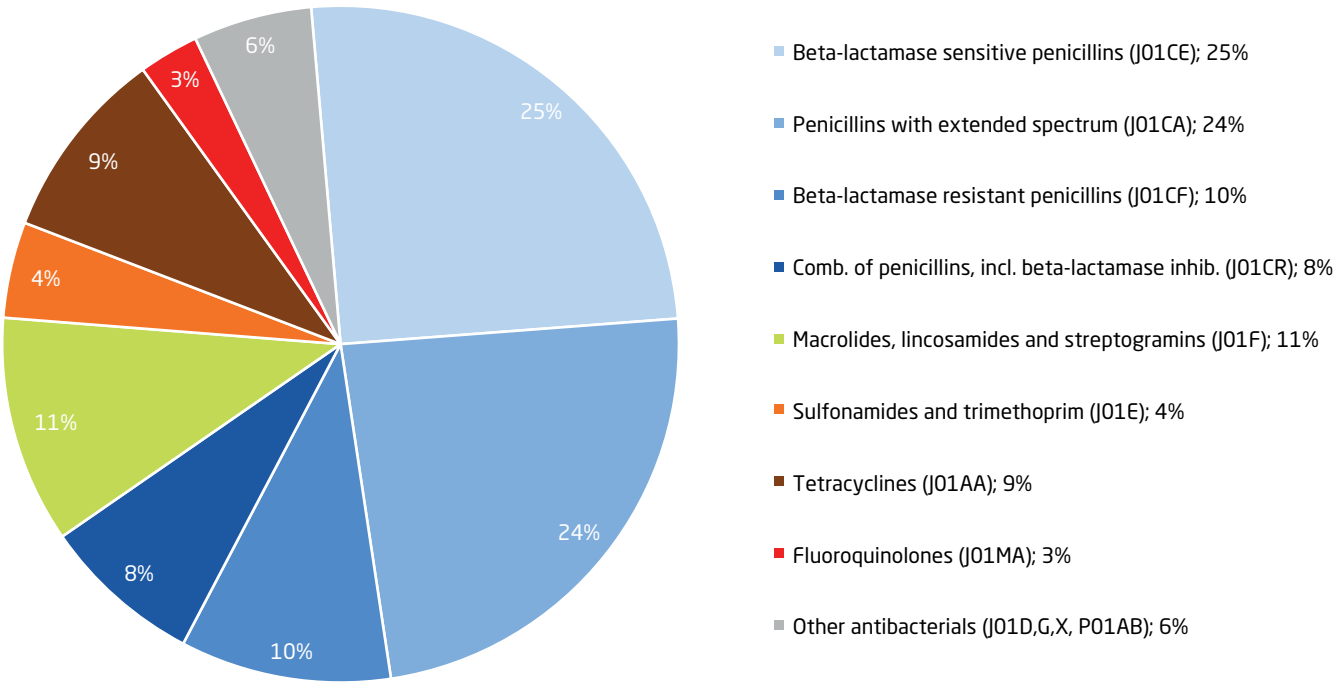


Figure 5.3 Distribution of the total consumption of antimicrobial agents in primary health care based on DDD, Denmark

DANMAP 2017



Amoxicillin increased with 3.7% from 2016 to 2017, but has decreased from 2008 to 2017 with 27%. In 2017, pivmecillinam accounted for 2.49 DID, amoxicillin for 0.95 DID and pivampicillin for 0.18 DID.

The increased consumption of beta-lactamase resistant penicillins from 1.13 DID in 2008 to 1.56 DID in 2017 is paralleled by an increased consumption at hospitals as well and follows the increased occurrence of staphylococcal infections observed in recent years (see section 8.7).

Tetracyclines are the fifth biggest group of antimicrobials consumed in Denmark. In 2017, they accounted for 1.42 DID, corresponding to 9.2% of the total consumption in primary health care. During the last decade, the consumption has decreased by 8.2% from 1.55 DID in 2008. In 2013, the consumption peaked unexpectedly at 1.96 DID but has since shown continuing decreases. Tetracyclines are used by all age groups above 12 years and by both genders, (Figures 5.6a and 5.7a).

Fluoroquinolones represent the smallest drug class among the leading antimicrobials. In 2017, they accounted for 0.44 DID, corresponding to 2.9% of the total consumption in primary care. From 2008 (0.52 DID) until 2010 and 2011 (peaking at 0.57 DID) the consumption of fluoroquinolones followed the general increasing trends in the total consumption and has since been decreasing, resulting in an overall decrease of 15% from 2008 to 2017, (Figure 5.2a and Figure 5.3). In Denmark, fluoroquinolones are designated as "antimicrobials of special, critical interest" by the National Health Authority and they are mentioned in the national recommendations on use of antibiotics issued in 2012. According to these, fluoroquinolones are to be solely used for treatment of very few specific infections, where they are considered the drug of choice (e.g. exacerbation in a patient with chronic obstructive lung disease and known penicillin allergy). They are also recommended in the case of infection with multidrug resistant bacteria, where microbiological results point towards a fluoroquinolone to be the best or only choice.

5.3.3 Measures at user level

In this and the following sections, the consumption of antibiotics is described at user level in either the number of prescriptions per 1000 inhabitants or the number of treated patients per 1000 inhabitants. The measures are thus based on information available through the sales to an individual and do not include the amount of antibiotics, mainly penicillins, sold to clinics, dentists and doctors on call.

In 2017, the total number of prescriptions was 489.49 per 1000 inhabitants, a 6.3% reduction from the 522.19 prescriptions per 1000 inhabitants in 2016 and a 19% reduction compared to the 606.26 prescriptions per 1000 inhabitants in 2008 (Table 5.2). In 2017, the average number of prescriptions redeemed per patient was 1.92, (not shown). In 2008, the number was 1.95. The number of treated patients in 2017 was 255.40 per 1000 inhabitants, a decrease of 18% compared to the 311 treated patients per 1000 inhabitants in 2008, (Table 5.3).

Trends in the number of prescriptions and treated patients for the different antimicrobial classes followed mainly the trends already described for the consumed DIDs. Most pronounced for the ten year period were the decreases in the number of prescriptions per 1000 inhabitants for beta-lactamase sensitive

Figure 5.4 Consumption of leading penicillins in primary health care, Denmark DANMAP 2017

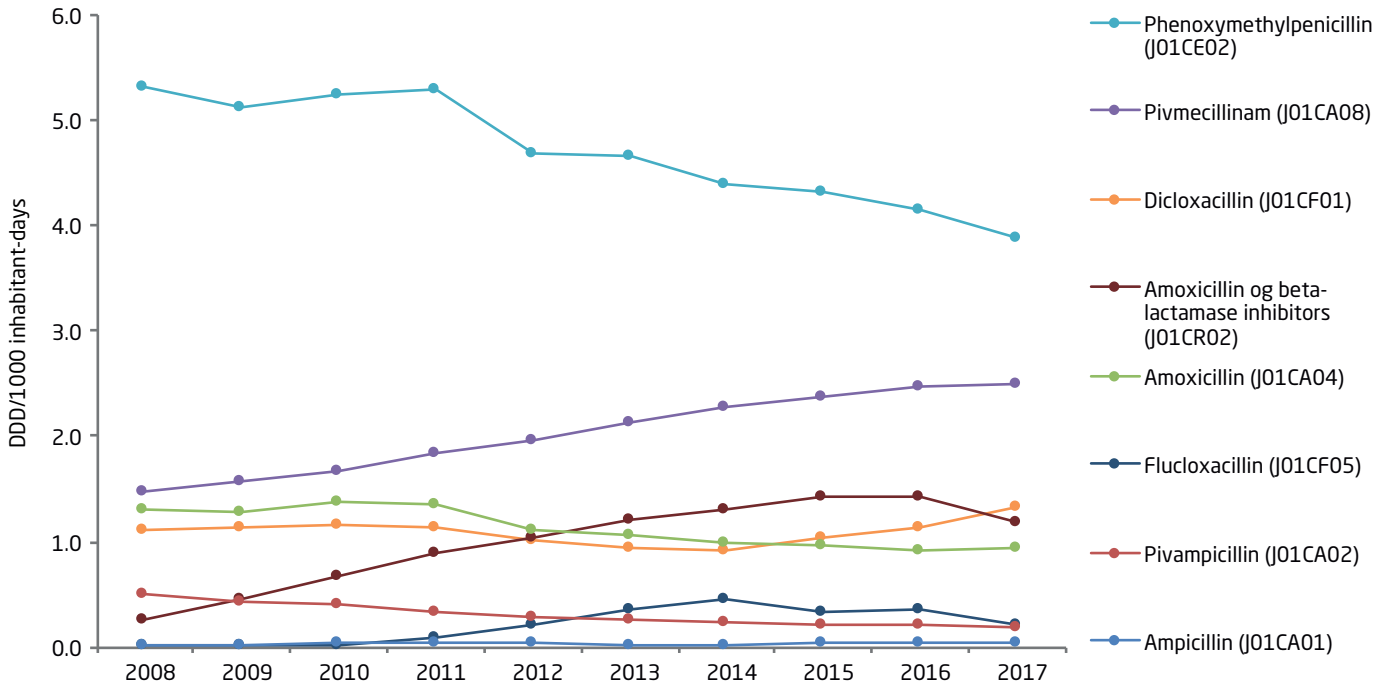


Table 5.2 Number of prescriptions per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark DANMAP 2017

ATC group[a]	Therapeutic group	Year									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	20.92	21.62	22.49	22.7	22.55	22.89	20.01	17.90	17.18	15.87
J01CA	Penicillins with extended spectrum	120.31	119.28	127.23	125.17	115.89	114.28	113.85	113.54	113.16	114.24
J01CE	Beta-lactamase sensitive penicillins	216.09	205.85	212.19	213.32	186.88	180.51	170.73	163.11	157.13	148.34
J01CF	Beta-lactamase resistant penicillins	42.22	42.1	42.32	42.75	40.41	41.24	41.04	40.82	41.87	41.82
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	7.05	11.15	16.53	21.11	24.71	28.01	29.02	30.73	31.13	27.06
J01E	Sulphonamides and trimethoprim	48.89	47.17	47.35	45.05	43.85	43.53	41.52	38.39	36.41	34.25
J01FA	Macrolides	91.47	87.24	97.34	104.22	85.87	74.50	68.02	68.01	68.85	59.93
J01MA	Fluoroquinolones	22.07	21.71	23.69	23.15	22.14	20.64	19.67	19.51	18.74	17.34
J01X	Other antibacterials (methenamine >99%)	17.34	17.93	17.49	18.24	18.03	17.41	16.73	16.28	15.82	10.17
P01AB	Nitroimidazole derivatives (metronidazole)	18.00	19.02	19.67	19.69	19.67	19.26	19.06	19.16	18.63	17.23
J01 (incl. P01)	Antibacterial agents for systemic use (total)	606.26	595.28	628.78	638.08	582.69	565.16	542.59	530.63	522.19	489.49

a) From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Numbers used in this table is based on registred sales to individuals

penicillins (-31%), sulphonamides (-30%), macrolides (-35%), tetracyclines (-24%) and penicillins with extended spectrum (-5.1%). Similar decreases were noted for the decade when measured in the number of patients treated with beta-lactamase sensitive penicillins (-28%), sulphonamides (-35%) and macrolides (-31%). Also for penicillins with extended spectrum and for tetracyclines, decreases were observed, (-9.0% and -19%, respectively).

Fluoroquinolones decreased with 21% in both the number of prescriptions per 1000 inhabitants and the number of treated patients. A comparison of the different indicators of consumption is presented in Figure 5.5.

In 2017, the average DDD/prescription remained with 11.08 at the same level as in 2016 (11.09), an increase of 18% compared to the 9.42 DDD/prescription in 2008 (Figure 5.5).

5.3.4 Consumption of antimicrobials in children

The total consumption in children of all age groups continued the decreases observed for the past decade, regardless of the indicator used (Figures 5.6a and 5.6b). In 2017, altogether 7.35 DID were consumed by children and young from 0 to 19 years, corresponding to 202.84 treated patients per 1000 inhabitants (children) and 325.73 prescriptions issued per 1000 inhabitants (not shown).

Figure 5.5 Indicators of antimicrobial consumption (J01, P01AB01) in primary health care, Denmark DANMAP 2017

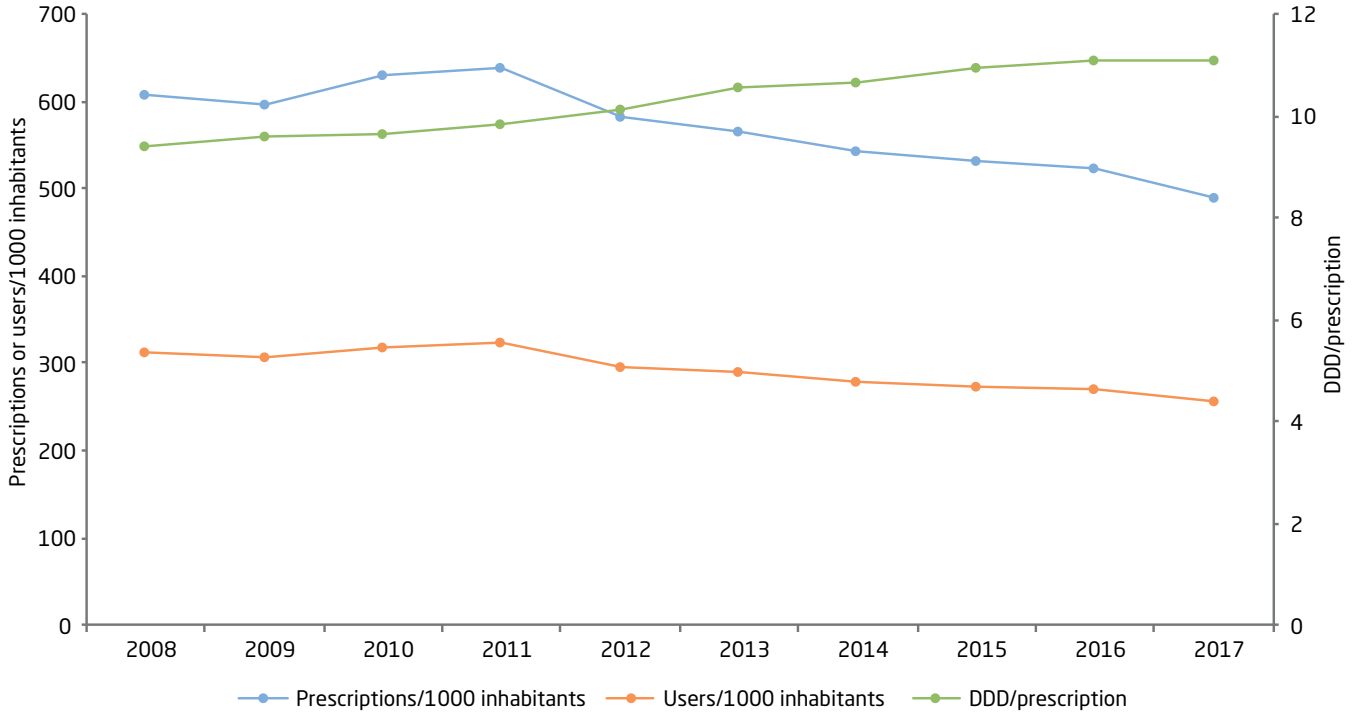


Table 5.3 Number of treated patients per 1000 inhabitants for leading antimicrobial agents in primary health care, Denmark

DANMAP 2017

ATC group[a]	Therapeutic group	Year									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	12.73	13.02	13.44	13.66	13.53	13.85	12.2	11.32	11.04	10.33
J01CA	Penicillins with extended spectrum	81.27	81.07	85.04	84.19	77.29	76.09	75.32	74.87	74.05	73.95
J01CE	Beta-lactamase sensitive penicillins	164.38	158.72	162.81	164.34	145.5	142.16	134.79	130.06	125.69	119.17
J01CF	Beta-lactamase resistant penicillins	29.89	29.87	30.02	30.34	28.5	29.07	29.24	28.85	29.70	29.92
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	4.95	8.02	11.7	14.95	17.31	19.71	20.52	22.03	22.17	19.87
J01E	Sulphonamides and trimethoprim	30.49	29.51	29.31	27.63	26.47	26.15	24.65	22.45	21.17	19.85
J01FA	Macrolides	66.84	64.44	72.67	78.75	64.72	56.15	51.38	51.75	53.21	45.95
J01MA	Fluoroquinolones	17.05	16.87	18.45	18.10	17.24	16.04	15.30	15.04	14.37	13.35
J01X	Other antibacterials (methenamine >99%)	7.43	7.67	7.53	7.74	7.54	7.48	7.16	7.35	7.47	5.00
P01AB01	Nitroimidazole derivatives (metronidazole)	16.28	16.28	16.73	16.9	16.86	16.51	16.31	16.47	16.03	14.82
J01 (incl. P01AB01)	Number of treated patients in total	311.37	306.41	318.69	324.91	296.32	289.47	278.62	273.49	269.72	255.40

a) From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system
Numbers used in this table is based on registred sales to individuals

It is important to note that measuring the consumption in children in Defined Daily Doses is problematic, since the system of Defined Dailys Doses was developed based on the "maintenance dose per day for its main indication in adults" (https://www.whocc.no/ddd/definition_and_general_considera/). For children, different pharmacodynamics and -kinetics apply and especially dosing in the younger classes is based on doses per bodyweight in kg. Still, assuming that dosage regimens did not vary considerably within the last decade, it is possible to compare the consumption in each age group with itself over time. Thus, the consumption of DIDs in the different age groups show a clear tendency to reductions for especially penicillins and macrolides, a trend that can also be observed in the number of prescriptions on these drugs issued to children, (Figure 5.6a and b).

From 2008 to 2017, the total DIDs consumed in 0 to 4 year olds decreased with 33%, in 5 to 9 year olds with 14%, in 10 to 14 year olds with 13% and in the young adults (15-19 year olds) with 15%. The corresponding number of prescriptions redeemed for all young age groups in total (0 to 19 year olds) decreased from 478.89 to 325.73 prescriptions per 1000 inhabitants (-32%) and from 276.43 to 202.84 treated patients per 1000 inhabitants (-27%) for the decade, respectively. Differences in reduction varied from a decrease of 39% in the number of prescriptions for the youngest (0 - 4 years old) to -24% for the oldest, (15 to 19 year olds). When measured in the number of treated patients, the decreases varied from - 30% in the youngest to -20% in the adolescents.

In the youngest age group of 0 to 4 year olds, the boys received on average 12% more prescriptions than the girls – a trend that has been quite stable. Thus in 2008, they received 883.81 versus 778.17 prescriptions per 1000 inhabitants (children) and in 2017, 535.89 versus 476.69 prescriptions per 1000 inhabitants, respectively (not shown). For the 5 to 19 year olds, opposite trends with girls receiving 18% more prescriptions on average were observed.

In general, children are more often treated with antibiotics compared to other age groups, since they are more prone to infections. Many of these will be viral or quickly passing bacterial infections that do not demand antibiotic treatment. Also, antibiotic treatment in the young may have pronounced and prolonged effects not only on the existing but also on the development of the normal flora of their mucosa. Finally, working with young – and their parents – on their perception of infections and the need for treatment is assumed to have a beneficial effect on the consumption of antibiotics in the future. Thus, paving the ground for a rational use of antimicrobials in the young may be of great interest to both the individual and the community as a whole, which makes it a core focus in antibiotic campaigns in Denmark and a central element in the National Action Plan on the reduction of antibiotics from 2017.

As for the general population, penicillins are the main antibiotics used in the treatment of bacterial infections in children. Beta-lactamase sensitive penicillins account for approximately 25-30% of the consumption. Penicillins with extended spectrum, primarily amoxicillin take the lead in the treatment of upper respiratory infections in small children (age 0 to 4 years). The decreases observed in the consumption of amoxicillin when measured in DID are mirrored in a reduced number of children treated with the drug (Figure 5.6a and 5.6b).

Macrolides play an important role in the treatment of infections in children and the young. They are the drug of choice for respiratory tract infections with *Mycoplasma pneumoniae,* and in young school aged children the consumption of macrolides will often mirror *Mycoplasma* epidemics. No epidemic occurred in the winter of 2016 or 2017. Macrolides are also used in the adolescents for the treatment of sexually acquired infections, e.g. *Chlamydia*. This is probably the reason for the relatively high consumption of macrolides in the 15 to 19 year olds (64.79 prescriptions per 1000 inhabitants per year compared to 17.85 prescriptions for the 10 to 14 year olds, 21.19 prescriptions for the 5-9 year olds and

Figure 5.6a Consumption of leading antimicrobials in children/adolescents aged 0 - 19, DID, Denmark DANMAP 2017

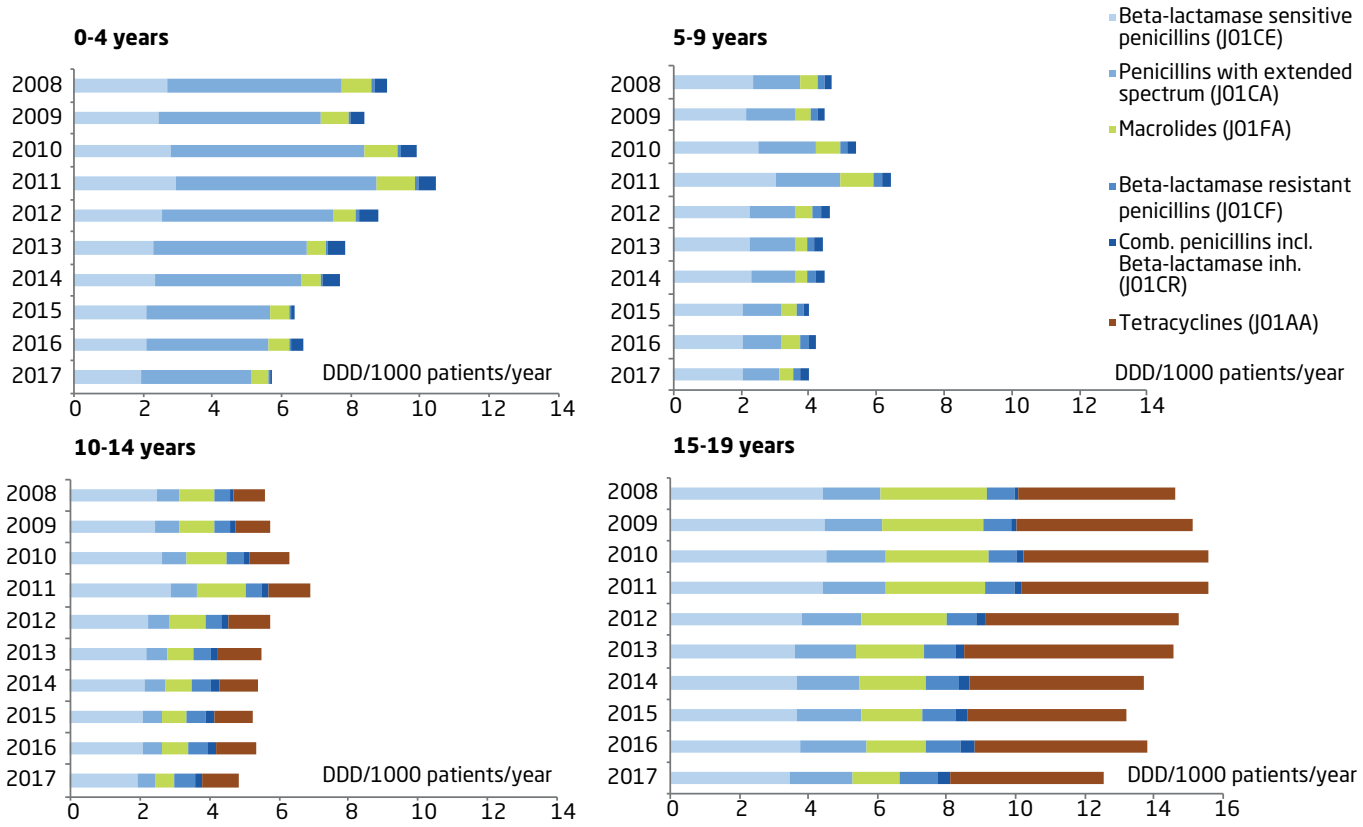
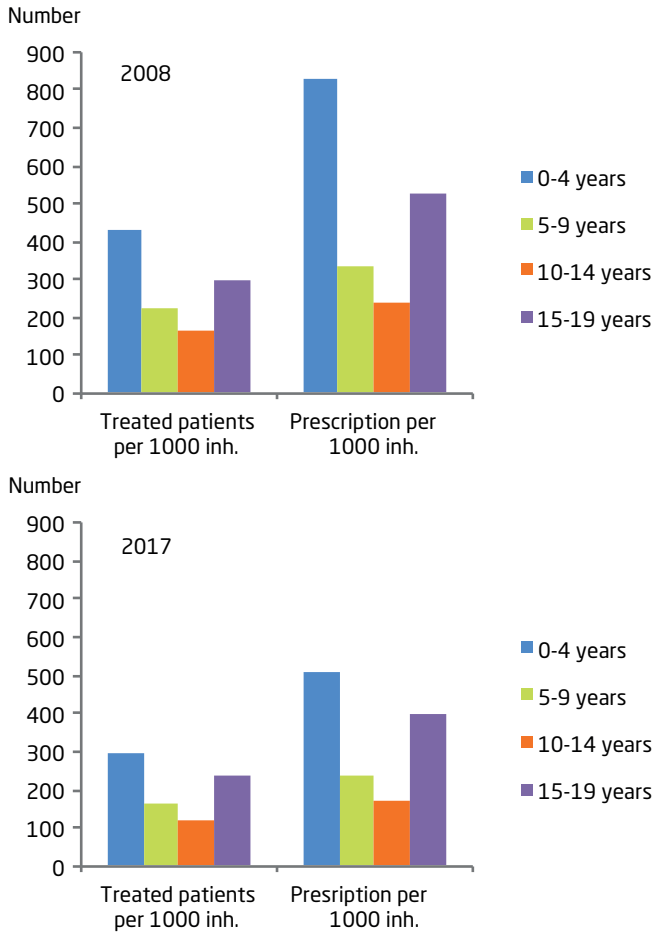


Figure 5.6b Number of prescriptions and treated patients per 1000 inhabitants aged 0-19 in 2008 and 2017, Denmark DANMAP 2017

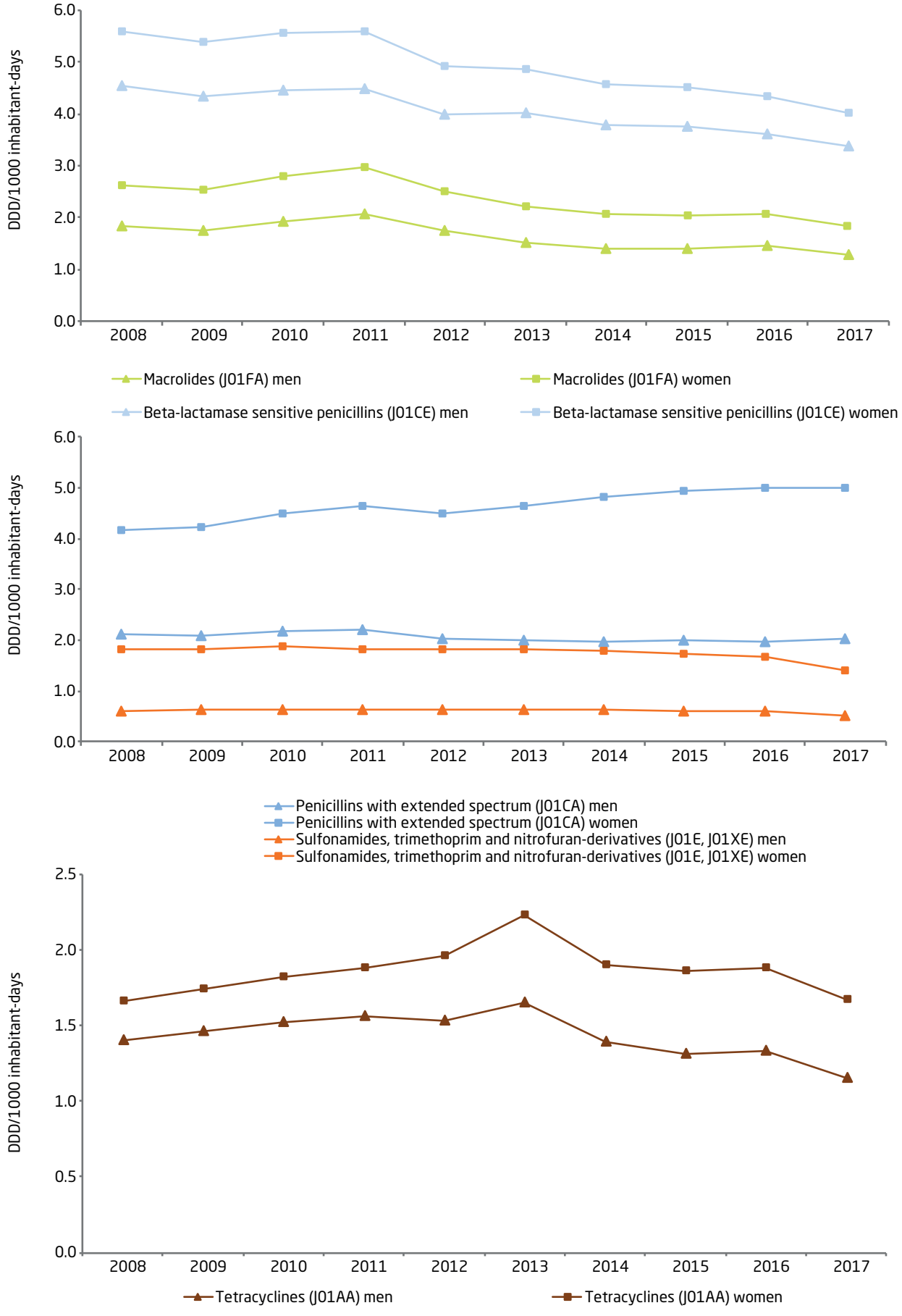


38.93 prescriptions for the 0-4 year olds for 2017, not shown). After an overall increase in the number of prescriptions on macrolides issued to children and adolescents from 2008 (60.64 prescriptions per 1000 inhabitants) to 2011 (73.47 prescriptions per 1000 inhabitants), the number of prescriptions has since decreased to 35.97 prescriptions per 1000 inhabitants in 2017. Decreases were noted for all, but were most obvious in the 15 to 19 year olds (from 111.19 prescriptions per 1000 inhabitants in 2008, corresponding to a decrease of 42%). This decrease is encouraging and may be a result of educational campaigns from the Danish Health Authority on sexually transmitted infections.

Tetracyclines account for a considerable part of the consumption of antimicrobials among adolescents due to the treatment of acne. Treatments last long (up to six months) and in addition may be repeated in a situation of relapse in patients, who may be suffering from the condition for years. Furthermore, within the same family/at the same family doctor, there may be the tendency to treat younger siblings if a treatment course in an elder brother or sister has been of success. Both genders are affected, but there exist clear differences in prescription habits between boys and girls. Thus, among girls the treatment periods are longer and extend into the young adults of 20 to 24 years, while boys primarily are treated in shorter periods at the age of 15 to 19 years. In 2008, 15 to 19 year old boys received 69.99 prescriptions per 1000 inhabitants per year on average, whereas girls received 50.63, corresponding to 31.93 versus 27.76 patients treated per 1000 inhabitants. In 2017, the num-

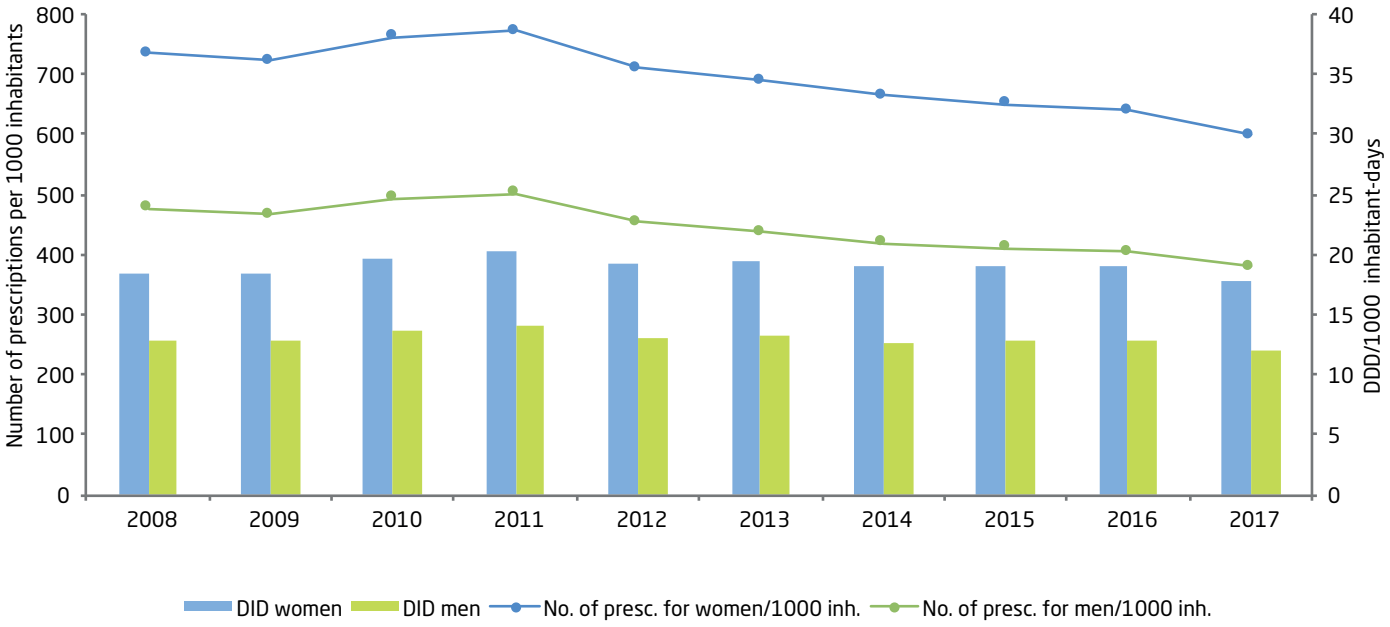
Figure 5.7a Consumption of leading antimicrobials (DDD/1000 inhabitants/day) in males and females, 2008-2017, Denmark

DANMAP 2017



Figur 5.7b Number of treated men/women and of prescriptions per gender per 1000 inhabitants, 2008-2017

DANMAP 2017



ber of 15-19 year old boys receiving treatment had declined to 25.52 treated patients (corresponding to 44.27 prescriptions) per 1000 inhabitants, while the number of 15 to 19 year old girls had increased to 30.86 treated patients (corresponding to 51.10 prescriptions redeemed) per 1000 inhabitants.

5.3.5 Consumption of antimicrobials according to gender

Differences between the genders regarding consumption of antimicrobials are well known. In general, women receive more treatment – a trend driven by a much higher consumption of antimicrobials used for the treatment of urinary tract infections. Thus, the consumption of sulphonamides, trimethoprim and nitrofurantoin is three times higher for women than for men. Moreover, the consumption of pivmecillinam in women doubles the consumption in men. Also for beta-lactamase sensitive penicillins and macrolides the differences in consumption, especially when measured in DID, are substantial.

For tetracyclines, there are less significant differences in gender and for the consumption of fluoroquinolones, no differences have been observed through the years, (Figure 5.7a and 5.7b).

From 2008 to 2017, the number of treated women per 1000 inhabitants decreased from 360.20 to 301.75 (-16%) and the number of treated men per 1000 inhabitants from 261.64 to 208.60 (-20%). During the same period, the amount of DDD/prescription increased for women from 9.16 to 10.81 (18%), and for men from 9.83 to 11.50 (17%), (not shown). Altogether the consumption in women decreased from 18.43 DID to 17.75 (-3.7%), and in men from 12.83 DID to 11.94 (- 6.9%).

Drugs for the treatment of upper respiratory tract infections.

For both women and men a decrease in the consumption of beta-lactamase sensitive penicillins and macrolides was observed. For women the consumption decreased for the beta-lactamase sensi-

Table 5.4 Consumption of antimicrobial agents for systemic use in primary health care at regional level, Denmark DANMAP 2017

Region	Indicator	Year					
		2012	2013	2014	2015	2016	2017
Capital Region	DDD/1000 inhabitants/day	16.85	16.93	16.28	16.14	15.96	14.97
	Prescriptions/1000 inhabitants	599.16	576.83	549.62	533.71	519.29	488.57
Region Zealand	DDD/1000 inhabitants/day	16.72	16.89	16.51	16.90	17.19	16.23
	Prescriptions/1000 inhabitants	618.59	601.42	579.82	575.42	574.97	539.01
Region of Southern Denmark	DDD/1000 inhabitants/day	16.16	16.51	15.84	15.82	15.78	14.81
	Prescriptions/1000 inhabitants	598.44	588.83	556.51	540.26	530.29	496.63
Central Denmark Region	DDD/1000 inhabitants/day	15.31	15.48	15.13	15.18	15.12	14.11
	Prescriptions/1000 inhabitants	531.91	512.72	499.96	494.41	487.31	457.81
North Denmark Region	DDD/1000 inhabitants/day	15.23	15.42	15.13	15.16	15.34	14.19
	Prescriptions/1000 inhabitants	557.63	541.29	525.41	510.30	509.18	472.23
Denmark (total)	DDD/1000 inhabitants/day	16.17	16.36	15.87	15.89	15.87	14.86
	Prescriptions/1000 inhabitants	582.69	565.16	542.59	530.63	522.20	489.49

Numbers used in this table is based on registred sales to individuals

tive penicillins from 5.58 DID in 2008 to 4.03 DID in 2017 and for the macrolides from 2.63 DID in 2008 to 1.85 DID in 2017. For men the changes were from 4.53 DID to 3.38 DID and from 1.82 DID to 1.28 DID, respectively, in the same decade, (Figure 5.7a).

Drugs for the treatment of urinary tract infection. From 2008 to 2017 the consumption of pivmecillinam in women increased continuously, from 4.18 DID to 5.01 DID, while it remained more stable in men. Since urinary tract infection (UTI) is a common condition in many women and contributes significantly to the number of antimicrobial treatments, several Danish studies have investigated in better diagnostic tests. Especially in elderly women, it can be difficult to distinguish unspecific symptoms from an actual urinary tract infection, not least due to transient asymptomatic bacteriuria. In 2016, the national antibiotic campaign focused on reducing the amount of antimicrobials consumed in the treatment of UTIs in women using two different approaches: one broadcasting an educating movie on the social media targeted young women, the other directed at health personnel at nursing homes dealing with confused or dement elderly women with unspecific signs of UTI. No significant decreases were observed for neither pivmecillinam nor the other three "urinary drugs" (nitrofurantoin, sulfamethizol or trimethoprim) for 2017, but a decreasing trend in the consumption of especially nitrofurantoin was noted, (Figure 5.7a).

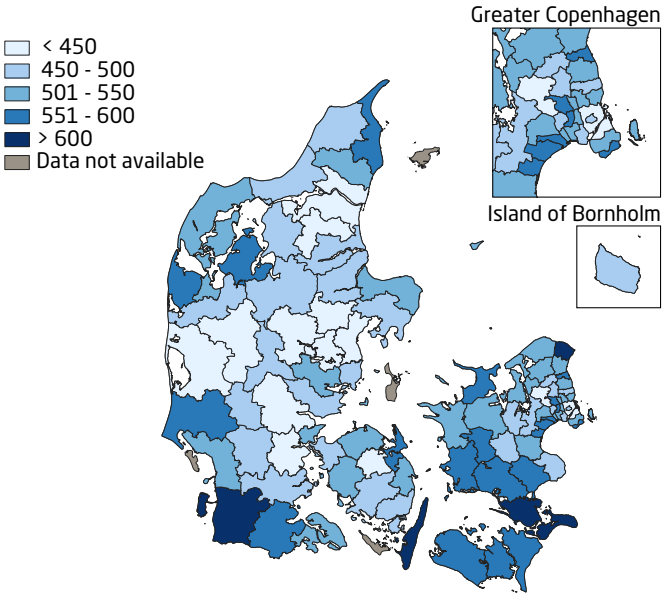
Tetracyclines. As mentioned in section 5.3.4, the treatment of acne in adolescence is the main driver of consumption and contributes considerably to the total consumption of tetracyclines with both DIDs and the number of redeemed prescriptions and patients treated as well. While the number of DIDs consumed did not change much in women (primarily used by girls from 15 to 24 years) from 1.66 DID in 2008 to 1.67 DID in 2017, it decreased slightly in men (primarily used by boys from 15 to 19 years) – from 1.41 DID in 2008 to 1.15 DID in 2017 (Figure 5.7a). Increases in the occurrence of sexually transmitted infections and changes in the treatment recommendations for these may challenge the consumption of tetracyclines in the future.

5.3.6 Prescribing activity in primary health care

Although Denmark has a very homogenous population with relatively small geographic and socioeconomic variations, considerable differences in the prescription habits among medical doctors are frequently observed. In 2017, the Central Denmark Region had the lowest prescribing activity, when compared to the other four, with 14.11 DID and 457.81 prescriptions per 1000 inhabitants, (Table 5.4). The Region of Zealand had the highest prescribing activity with 16.23 DID and 539.01 prescriptions per 1000 inhabitants. For all regions, significant decreases in the DIDs and number of prescriptions issued (on average 8.1% in DID and 16% in the number of prescriptions per 1000 inhabitants) for the six years shown were observed, (Table 5.4).

There may be several reasons to the differences in the number of prescriptions redeemed, e.g. the density of the population and number of general practitioners as well as the proportion of elderly or chronically ill in a given geographic area. Due to differing

Figure 5.8 Number of primary health care prescriptions/1000 inhabitants in Danish municipalities, 2017 DANMAP 2017



organization of general practitioners and clinical practices across the country comparison of prescribing habits based on the individual clinical praxis is difficult. A clinical praxis can be based on a single physician working solo but can also be a collaboration of up to seven physicians sharing facilities and staff. In addition, due to the lack of general practitioners in some areas, several new models of "health houses" served by physicians and other health staff are being established these years. General practitioners can follow their own prescription habits through the website www.ordiprax.dk, a closed IT system that collects all prescribed data and enables comparison with other practice on a regional level.

Support of the general practitioners regarding their prescribing habits is in general provided through regional medicine consultants, who also have access to Ordiprax on each clinic level, thus being able to monitor consumption and give individual advice. From 2018, the general practitioners in defined geographical areas are being joined in "quality clusters" for mutual support.

In Figure 5.8, the number of prescriptions on municipality level is shown, spanning from 394 to 648 prescriptions per 1000 inhabitants. In 2017, most municipalities lay within the range of 450 to 570 prescriptions per 1000 inhabitants. From the 98 municipalities in Denmark, four were excluded from the figure due to very small populations (typically islands). At web annex more maps and tables describing the prescribing activities on municipality level are shown.

As mentioned in the introduction, consumption in the primary sector includes prescriptions issued from hospital doctors upon discharge of a patient. In the past decade, the number of prescriptions issued through hospital doctors increased notably, probably due to changes in hospital work flow with shortening of bed days and increasing activity in ambulatory care. In 2017, hospital doctors accounted for 62.63 prescriptions per 1000 inhabitants (13% of the antimicrobials sold at pharmacies). In 2008, it was 38 prescriptions per 1000 inhabitants (corresponding to 6% of sales), (not shown).

Textbox 5.1

University of Copenhagen Research Centre for Control of Antibiotic Resistance (UC-CARE) – a multidisciplinary One Health approach.

Introduction: UC-Care is an initiative based on a One Health or One Medicine-concept grown from the increasing evidence indicating that antimicrobial resistance can be successfully combated only through intersectorial collaboration. UC-Care was established in 2013 as a four-year center of excellence at University of Copenhagen.

Although we to some extent understand the molecular biology of antibiotic resistance and know some of the factors contributing to spread of resistant bacteria, still several questions remain unanswered. Meanwhile immediate solutions are limited by the lack of new antibiotics, the long and expensive development process necessary to bring new drug leads from discovery to market, and not least conflicting rationales commonly encountered in current antibiotic use.

Drug discovery and development alone is thus not sufficient to mitigate antibiotic resistance problems neither on the short nor on the long term. A holistic and multidisciplinary approach is urgently warranted to qualify antibacterial interventions and to preserve the clinical efficacy of antibiotics through infection control and rational antibiotic use in both human and veterinary medicine.

By acknowledging the very complex nature of the problem, University of Copenhagen supported a multidisciplinary initiative by 4.3 million € and thereby enabled a synergistic combination of a very broad array of topics, merging research within veterinary and medical sciences with life sciences, social sciences and humanities. As such, UC-Care was a unique and holistic initiative contributing with a truly novel approach alleviating some of the major negative effects from antibiotic resistance.

The beauty of breadth and depth: UC-Care initially counted researchers from a total of 14 Departments at the Health and Medical Sciences, Faculty of Sciences, Faculty of Social Sciences and Faculty of Humanities, respectively. The center focused on six major, vastly different, areas all considered critical for future advancement in the fight against antibiotic resistance: 1) Research on new drug principles to revert antibiotic resistance or to inhibit horizontal gene transfer. Investigations aiming at enhancing *in vivo* efficacy of synthetic antimicrobial peptides and antisense peptide nucleic acids, both headed by Professor Fredrik Björkling. 2) Development of new interventions to lower antibiotic use in animals including novel bacterial vaccine platforms headed by Professor Anders Miki Bojesen. 3) Optimized treatment regimens and formulations against biofilm-related infections and intracellular pathogens headed by Professor Niels Høiby. 4) Definition of evidence-based diagnostic protocols allowing rational antibiotic use headed by Professor Lars Bjerrum. 5) Sustainability assessment of intervention strategies in livestock production headed by Professor Jørgen Dejgård Jensen. 6) To understand how societal factors influence antibiotic use headed by Assoc. Professor Carsten Strøby Jensen.

Several additional senior scientists along with 21 PhD-students and 12 Postdocs were associated with the research centre. Most junior scientists had supervisors at different Departments or even Faculties to stimulate a more holistic approach to the research questions.

Major outputs and future perspectives: UC-Care aimed at providing solutions – some on a short term, while others would not be achieved within the lifetime of the centre. Still, already some discoveries hold great promise and may turn into high impact interventions to the benefit of society in general. More than 120 peer-reviewed papers have been published within the centre as well as more than 250 mDKK have been generated in external funding by the projects principle investigators.

Revival of known antimicrobials: Amongst the most significant discoveries, we have developed and validated a high-throughput screening assay designed to detect bacterial envelope-permeabilizing helper drugs. This new tool has been exploited to enhance antimicrobial drug penetration by significantly increasing cell envelope permeability, ultimately making bacteria susceptible to antibiotics to which they were intrinsically resistant.

Prevention by smart vaccines: Two novel vaccine platform technologies have been developed and demonstrated to induce serotype-independent immunity, a rather unique invention in the bacterial field where several vaccines currently are needed for ensuring adequate protection. If one or few vaccines can provide a high level of protection without the need for antibiotics it will truly revolutionize vaccinology. Vaccine production has to happen at a very low cost, the sales price being decisive for their later application and use, since pig and poultry productions are characterized by very low profit margins and antibiotics generally are cheap.

Improved diagnostics allow lowered use: Antibiotic treatment in relation to common diseases like urinary tract infections is a challenge, since large quantities of antibiotics are prescribed for humans, pets and some food producing animals, e.g. pigs. Analyzes of the results of a substantial number of cases covering each area confirm that prescription of antibiotics based only on symptoms (which by far is the most common practice) cause up to 40% overuse of antibiotics. This can be largely counteracted by increased use of available point-of-care diagnostics.

Development of a new research field: Being truly interdisciplinary, UC-Care also focused on how antibiotic resistance affects societal areas besides the traditional fields, as human health and veterinary sciences. The research area looked at application of social scientific knowledge in ensuring the most effective or rational way of introducing important actions in the control of resistant bacteria, without simultaneously introducing stigma processes on defined populations. An example of investigation could be persons employed in the food producing industry, working at the interface between interests from the consumer and ethical questions. Raising pigs demands the use of antibiotics, no matter how rational the application is, thus contributing to the pressure from the use of antimicrobials. In Denmark, the production of pigs is also associated with an increased risk of being carrier of livestock associated MRSA (LA-MRSA), thereby posing a risk to the public due to possible transmission of LA-MRSA. A project thus focused on interrogating key professions, like veterinarians, pig farmers and farm workers on their perception on the situation, and investigate in their willingness but also resistance to supporting different measures of LA-MRSA control. This is an important, but not very developed research agenda that has appeared through the project activities.

Common One-Health course for veterinary and medical students: As a direct outcome of the UC-Care activities, a common course for veterinary and medical students including a broad range of topics associated with antimicrobial resistance has now become a compulsory part of the curricula at University of Copenhagen. This means that more than 400 students will engage in the one health antibiotic resistance agenda on an annual basis. The course has been completed twice already and appears to be well received by the students and represent a good opportunity for teachers to meet.

Inspiring multidisciplinarity and barriers to be broken: Although the intentions were good and the overall goal was quite clear, a considerable amount of energy still had to be diverted towards creating a fruitful research environment across fields with little or no tradition for collaboration. If not clear from the beginning, all participants in the UC-Care consortium soon realized that combatting antimicrobial resistance requires a highly heterogeneous approach at several levels to comprehend the complexity of the field. Performing research on a platform with a very high level of diversity, while aiming at placing most activities in perspective of each other, appeared to be an ambitious, yet very rewarding task. Clearly, most research fields are relatively narrow in their natural conformation so although most researchers had lots of experience from collaboration with people from different disciplines, it was rather challenging to combine basic research originating in life science with basic research rooted in humanities. Traditions in scientific approach and research communication differ substantially and from a practical point, this might give rise to difficulties at publishing common papers/books while maintaining a clear incentive for all involved. UC-Care remains as a non-mural center, yet no longer receives funding for the coordinating activities. This means that particularly fields like social science and humanities have difficulties at keeping up their engagement and ultimately will result in losses of some of the multidisciplinary input, we found so important. The challenges experienced at research level reflect very well what can be witnessed in the surrounding society. This underlines a high level of communication being crucial to ensure a good base for a mutual understanding required for any advancement.

UC-Care coordinator and Professor Anders Miki Bojesen, miki@sund.ku.dk

UC-Care

generates knowledge and tools to combat antimicrobial resistance in humans and animals

5.4 Hospital Care

5.4.1 Introduction

Antimicrobial consumption at hospitals is reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between the hospital and different medical suppliers. In case of shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency. These special deliverances are reported separately to DANMAP. For surveillance purposes, it has to be assumed that the amount of delivered antimicrobials is identical to the consumption at the different departments. But in reality, antimicrobials may be exchanged between different specialties and departments belonging to the same trust, which makes precise calculations of the consumption on specialty level difficult. In DANMAP, reporting of data on hospital consumption is therefore kept at a national or regional level. Data on hospital level can be supplied upon request.

Information on consumption at individual patient level is still lacking for the hospital sector. This information is expected to be available through the future national "Hospital Medicine Register", which is currently being developed.

DANMAP 2017 covers the total sales on systemic antimicrobials (all ATC code J01 as well as ATC code P01AB01 and A07AA09) reported from all Danish hospitals. Consumption at private hospitals and psychiatric departments is excluded, in 2017 accounting for approximately 2.1% of the total hospital consumption.

The consumption of antimicrobial agents in hospital care is presented as DDD per 100 occupied bed-days (DBD) and as DDD per 100 admissions (DAD) to account for hospital activity. Moreover, data are presented as DID to enable comparison with primary health care.

Table 5.5 Activity in somatic hospitals, Denmark DANMAP 2017

Region	No. bed-days somatic hospitals[a]	No. admissions somatic hospitals[a]
The Capital Region of Denmark	1,477,131	485,597
The Sealand Region	598,676	234,083
Region of Southern Denmark	778,641	257,042
Central Denmark Region	814,234	289,816
North Denmark Region	377,433	112,575
Denmark[b]	4,046,115	1,379,113

Source: The Danish Health Data Authority (www.sds.dk)
a) Excluding private hospitals, psychiatric hospitals, specialised clinics, rehabilitation centres and hospices
b) Compared to 2016 no. bed-days has decreased by 0.8% and no. admissions increased på 0.6%

As mentioned, during the past decade the hospitalization patterns in Denmark changed notably – the shortening of bed days at hospitals and the increasing ambulatory care function, including increased surgical activity, cause increased pressure on the health system at municipality level. There is thus an increased demand for "acute care beds" for patients dismissed from hospital, but not yet ready for continuing treatment at home. For these sector-crossing patients it is important to ensure continuation of antibiotic treatment, including the possibility to change from intravenous to per oral treatment. In 2017, fifty-three different antibiotics were used at Danish hospitals. When divided into groups according to routes of administration, 20 could be used parenterally and orally, 18 could be used only parenterally and 10 only orally. The remaining five antibiotics could be administered either rectal or through inhalation. For an overview of formulations available, please see Figure A5.4.3 at webannex.

The increasing number of invasive infections and infections at other sites also induces pressure into the system, increasing the demand for proper antibiotics (see section 8.0 introduction). Since selection pressure for the emergence of antimicrobial resistance increases with increasing hospital activity, the selection pressure has increased considerably from 2008 to 2017, see Figure A5.4.2. Table 5.5 presents data on regional hospital activity for 2017.

In 2017, the number of admissions at Danish hospitals was 1,379,113, while the number of bed-days was 4,046,115 (data from the Danish National Patient Register, June 2018). The annual proportion of occupied hospital beds was in 2017 between 80 and 86%, but especially the Central Denmark Region and the Capital Region experienced longer periods with overcrowding of patients. Since 2008, the number of bed-days decreased with altogether 17%, while the number of admissions increased with 15%. When measured in bed-days and admissions per 1000 inhabitants, the changes were -24% and + 10%, respectively. During the decade, activity in ambulatory care incerased with 26%. On average, the number of bed-days decreased with 2.1% yearly, while the number of admissions on average increased with 1.6% per year, (Figure A5.4.2 in web annex).

5.4.2 Somatic hospitals – DDD per 100 occupied bed days (DBD)

In 2017, the consumption of antimicrobial agents in somatic hospitals was 110.28 DBD, 5.3% higher than the 104.41 DBD in 2016 and 43% higher than the consumption measured a decade ago in 2008 (76.89 DBD). This is the highest consumption measured this decade, (Table 5.6).

The four penicillin groups accounted for altogether 58.32 DBD, corresponding to 53% (Table 5.6, Figure 5.9). In 2017, penicillins with extended spectrum constituted 20.39 DBD of the consumption in somatic hospital, a 14% increase from 2016 (17.91 DBD), making it the biggest group consumed (19%). Combination penicillins constituted 17.17 DBD, decreasing 7.8% from 2016, making them the second largest group

consumed in 2017 (16%). Beta-lactamase sensitive penicillins accounted for 11.32 DBD (10%) and beta-lactamase resistant penicillins for 9.44 DBD (9%).

Trends for the consumption of penicillins for the last decade were comparable to the trends observed for the primary sector. The combination penicillins increased steeply by 13.16 DBD (328%), the penicillins with extended spectrum and beta-lactamase resistant penicillins less markedly, but still continuously with 6.07 DBD (42%) and 2.30 DBD (32%), respectively, (Figure 5.10 and 5.11).

The consumption of beta-lactamase sensitive penicillins had shown continuous increases from 8.02 DBD in 1997 to its peak of 12.17 DBD in 2005. For the past decade it remained relatively stable oscillating between 9.14 and 10.36 DBD. The increase from 2016 to 2017 was the first marked change since 2005, increasing with 1.02 DBD, (Table 5.6).

Notable trends for other antimicrobials for the past decade were increases observed for tetracyclines, for combinations of sulfonamides and trimethoprim and for macrolides. Although tetracyclines only account for a minor part of the antimicrobials consumed at hospitals, the drug class has been continuously increasing during the past decade; in 2008 they accounted for 0.87 DBD, while in 2016 and 2017 the consumption had increased to 2.17 DBD. In 2017, the proportion of tigecyclin constituted close to zero of the tetracyclines consumed. Consumption of combinations of sulfonamides and trimethoprim, (not shown). increased from 2.47 DBD in 2008 to 5.42 DBD in 2017, a total increase of 119% for the decade. Finally, a rise in macrolides was observed from 3.15 DBD in 2008 to 6.02 DBD in 2017 (91%), (Table 5.6 and Figure 5.11).

In 2017, the consumption of the groups of antimicrobials used as first line treatment for the leading infections at hospitals increased again, a trend following the described increasing

Table 5.6 Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark DANMAP 2017

ATC group(a)	Therapeutic group	Year									
		2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	0.87	1.12	1.10	1.17	1.64	1.55	1.74	1.88	2.17	2.17
J01CA	Penicillins with extended spectrum	14.32	14.89	14.01	13.97	15.24	15.37	16.48	17.19	17.91	20.39
J01CE	Beta-lactamase sensitive penicillins	10.32	9.63	9.14	10.01	10.34	10.34	10.36	10.14	10.30	11.32
J01CF	Beta-lactamase resistant penicillins	7.14	7.24	7.52	8.40	8.66	9.25	9.61	9.91	9.34	9.44
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	4.01	5.30	6.63	8.67	11.89	13.92	16.11	17.99	18.61	17.17
J01DB	First-generation cephalosporins	0.16	0.12	0.12	0.12	0.12	0.11	0.06	0.04	0.04	0.04
J01DC	Second-generation cephalosporins	11.00	11.89	12.01	15.21	14.14	12.57	11.73	10.48	9.52	11.12
J01DD	Third-generation cephalosporins	1.17	1.18	1.02	1.10	1.06	1.10	1.03	1.07	1.06	1.37
J01DF	Monobactams	0.00	0.00	0.03	0.14	0.14	0.14	0.06	0.03	0.01	0.01
J01DH	Carbapenems	2.05	2.19	2.44	3.55	3.80	4.10	2.82	4.15	4.02	4.26
J01EA	Trimethoprim and derivatives	0.53	0.51	0.39	0.35	0.39	0.41	0.52	0.46	0.43	0.48
J01EB	Short-acting sulfonamides	0.47	0.43	0.34	0.26	0.21	0.19	0.18	0.15	0.13	0.13
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	2.47	2.26	1.86	2.92	3.28	4.30	4.70	5.09	5.22	5.42
J01FA	Macrolides	3.15	3.30	3.40	3.49	3.58	3.46	3.89	4.61	4.99	6.02
J01FF	Lincosamides	0.42	0.47	0.44	0.49	0.61	0.65	0.65	0.58	0.63	0.65
J01GB	Aminoglycosides	1.71	1.52	1.63	1.90	2.13	2.18	1.62	1.70	2.01	2.32
J01MA	Fluoroquinolones	9.65	10.11	9.78	9.98	9.93	9.98	9.93	9.40	8.37	8.06
J01XA	Glycopeptides	0.68	0.93	0.98	1.22	1.26	1.32	1.16	1.08	1.09	1.32
J01XB	Polymyxins	0.05	0.07	0.09	0.08	0.09	0.17	0.27	0.26	0.30	0.29
J01XC	Steroid antibacterials (fusidic acid)	0.26	0.29	0.32	0.25	0.23	0.22	0.23	0.17	0.11	0.07
J01XD	Imidazole derivatives	3.33	3.63	3.68	3.87	4.09	4.16	4.51	4.30	4.60	4.74
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.34	0.38	0.33	0.35	0.38	0.39	0.37	0.33	0.29	0.30
J01XX05	Methenamine	0.13	0.10	0.09	0.12	0.10	0.09	0.07	0.10	0.09	0.08
J01XX08	Linezolid	0.21	0.20	0.20	0.29	0.31	0.36	0.34	0.44	0.37	0.37
J01XX09	Daptomycin	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.04	0.05	0.08
P01AB01	Nitroimidazole derivatives (metronidazole)	2.35	2.56	2.53	2.50	2.42	2.33	2.05	2.08	2.26	2.12
A07AA09	Intestinal antiinfectives (vancomycin)	0.10	0.18	0.26	0.41	0.48	0.50	0.52	0.48	0.49	0.53
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin	76.89	80.52	80.39	90.84	96.53	99.20	101.05	104.12	104.41	110.28

a) From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.9 Distribution of the total consumption of antimicrobial agents in somatic hospitals, Denmark DANMAP 2017

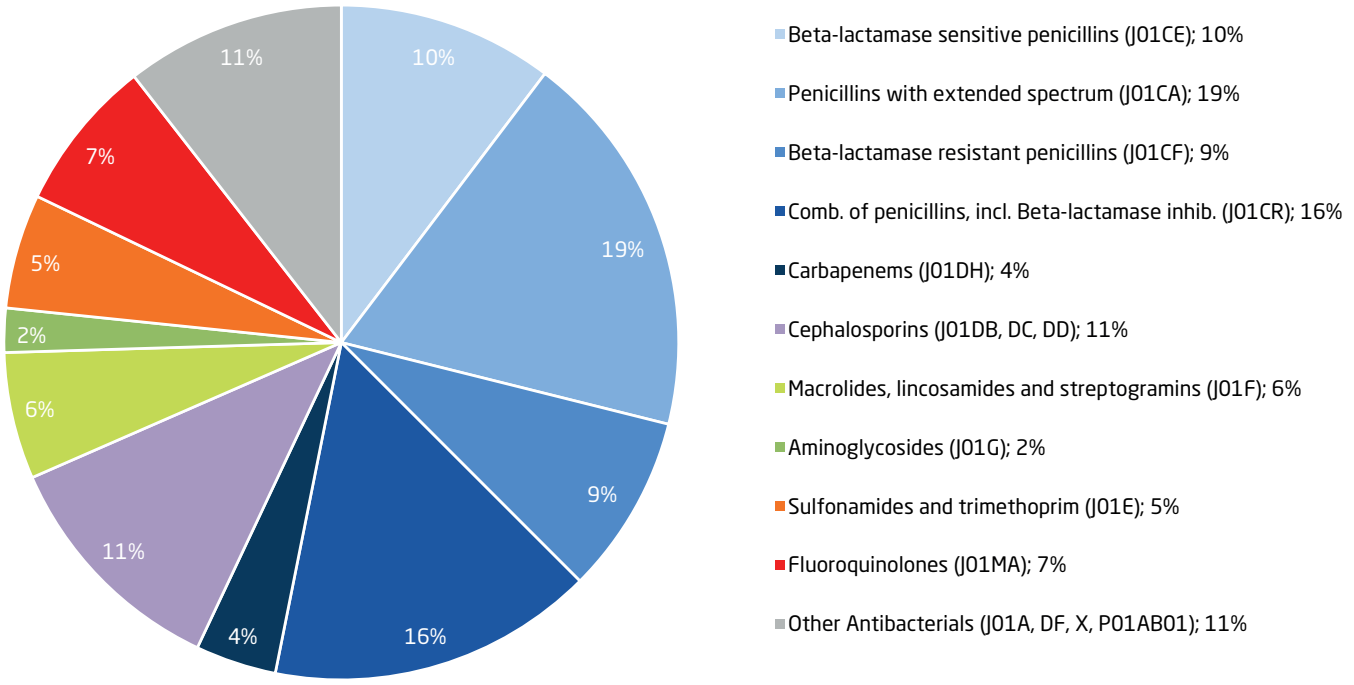
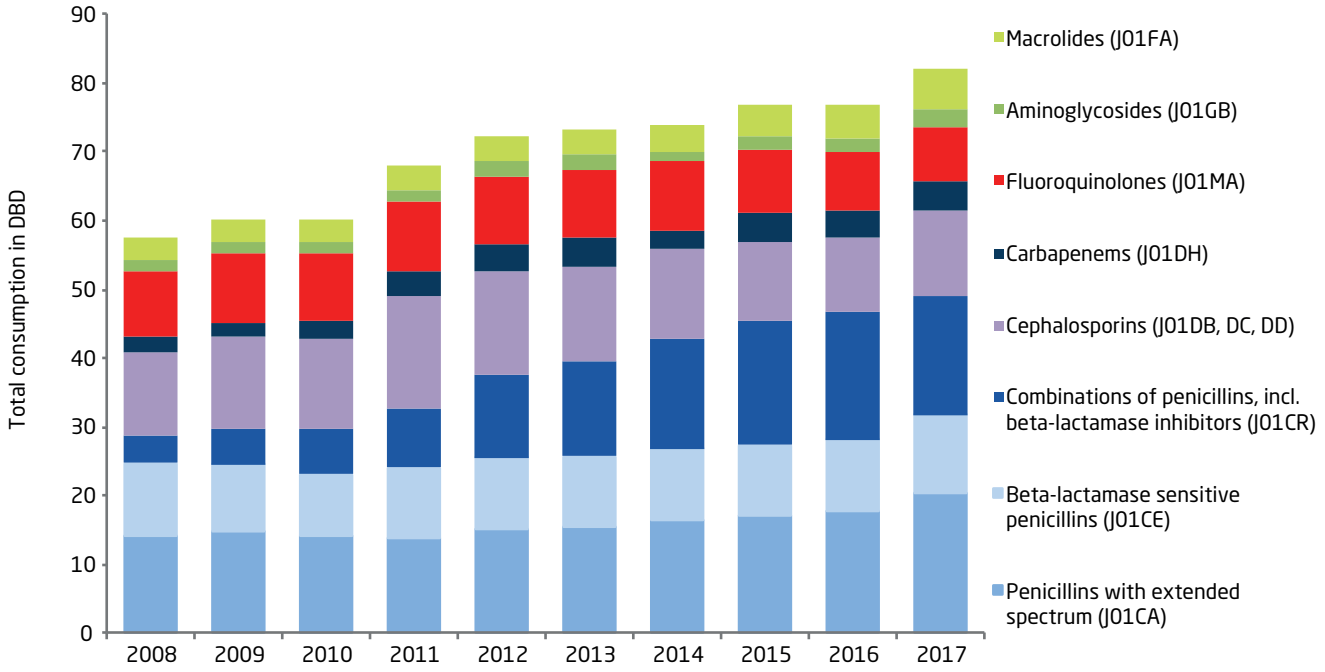


Figure 5.10 Total somatic hospital consumption (DBD) by leading groups of antimicrobial agents (J01), 2008-2017, Denmark DANMAP 2017



trends for the number of invasive isolates, (Figure 5.10 and Figure 8.0.2). In 2017, the leading antimicrobials constituted 82.08 DBD of the total consumption of 110.28 DBD (74%). In 2016 it was 76.83 DBD of a total of 104.41 DBD (74%).

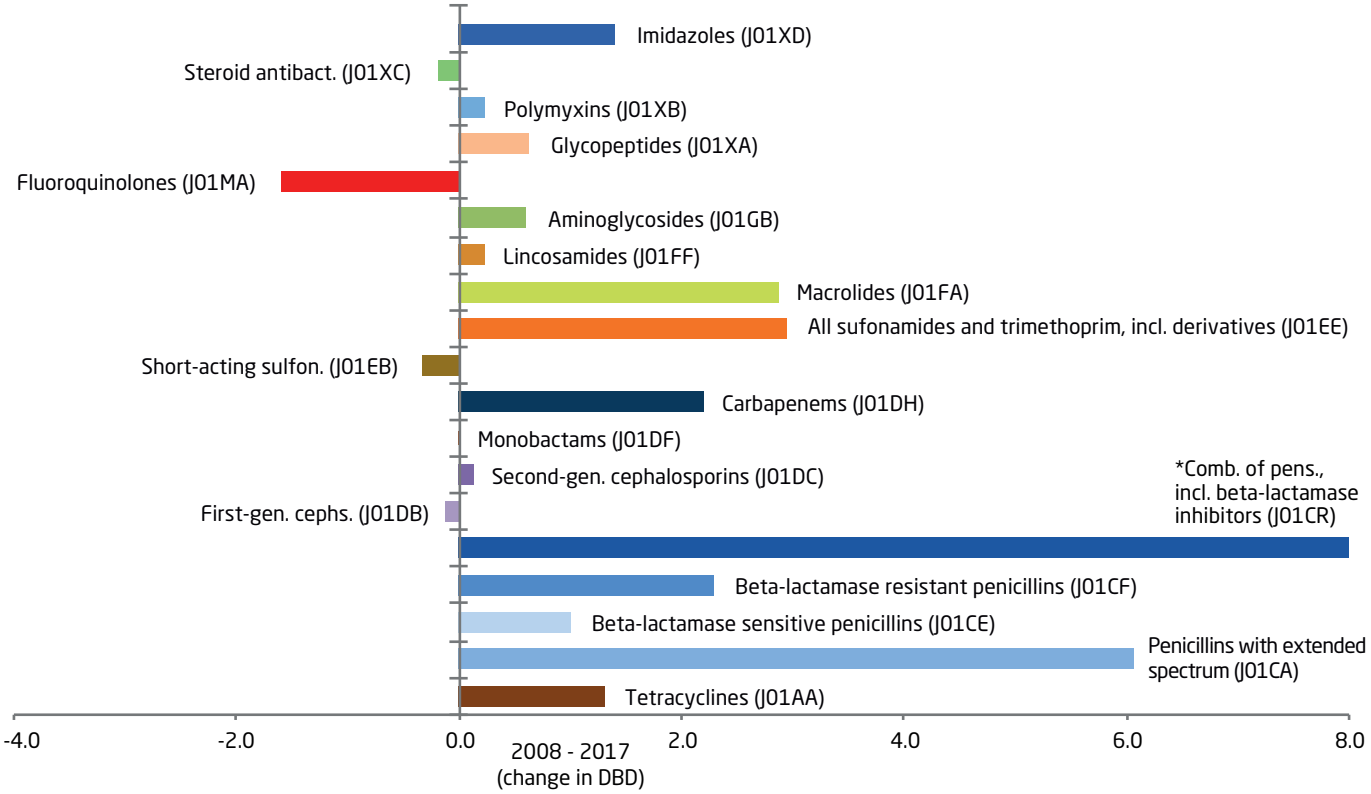
5.4.3 Other measures of consumption at somatic hospitals - DDD per 100 admissions (DAD)

The consumption of antimicrobials at hospitals can also be measured in relation to hospital activity calculated in number of patients "passing through", i.e. DDD per 100 admissions (DAD).

In 2017, the total consumption was 323.53 DAD, a 4.0% increase from the 310.68 DAD in 2016 and 2.7% increase from 314.64 DAD in 2008. The highest peak observed through the past decade was that of 2017, (Table 5.7). The trends in DAD reflect for most antimicrobials the trends observed in DBD. Yet differences in the number

Figure 5.11 Changes in the consumption (DBD) by leading groups of antimicrobial agents (J01) in the hospital sector, 2008-2017, Denmark

DANMAP 2017



*Comb. of penicillins, incl. beta-lactamase inhibitors (J01CR) increased with more than 8.0 DBD (13.16 DBD)

of patients treated with the specific drug class and the use of the individual antimicrobials in the treatment of acutely or chronically ill patients may exist compared to hospital activity. The observed rates of increases were more marked, when measured in DBD than in DAD for all the antimicrobial classes, (Tables 5.6 and 5.7).

At regional level, the hospital activity mirrors the density of the population, (Table 5.5). Trends in consumption showed similar, parallel increases for the past decade and are shown in DBD and DAD, respectively, (Figure 5.12).

A comparison of the usage of antimicrobials of the different antimicrobial classes in animals and humans, respectively measured in kg. active substance is presented in Table A5.2.1 in web annex. For comparison of consumption at hospitals with the consumption in the primary sector measured in DID go to Table 5.1 and Table A5.4.1 and Figure A5.2.1 in web annex.

5.4.4 Changes in consumption of antimicrobials of critical interest

In 2017, the three groups of antimicrobials of critical interest in Denmark (cephalosporins, fluoroquinolones and carbapenems) constituted together 23% of the total consumption at hospitals. In 2016, it was 22% and ten years ago, in 2008, it was 31%, (not shown). Cephalosporins accounted with altogether 12.54 DBD for 11% of the total consumption, a slight increase from the 10% in 2016. Second generation cephalosporins are the most used at hospitals and accounted for 11.12 DBD. Fluoroquinolones accounted for 8.06 DBD, a 3.7% reduction from 8.37 DBD in 2016 (Table 5.6). The consumption of fluoroquino- lones peaked in the years of 2009 to 2013 and has since shown slight declines. Carbapenems accounted in 2017 for 4.26 DBD, a 6% increase from the 4.02 DBD in 2016, (Table 5.6). A large decrease in the consumption of carbapenems was observed from 2013 to 2014 (4.10 to 2.82 DBD).

Trends in the consumption of cephalosporins, fluoroquinolones and carbapenems are shown in Figure 5.10 and 5.11 and on regional level in Figure 5.13. From 2012 to 2016, The Region of Southern Denmark and The Capital Region of Denmark showed clear and continuing declines in the consumption, while the three other regions more or less maintained their levels. For all five regions, increases in the consumption of the three critical important antimicrobials were observed from 2016 to 2017, (Figure 5.13).

The consumption of the three critical antimicrobial groups will be monitored closely in the future due to several local, regional and national initiatives, probably the most important one being reductions aimed at by the "National Quality and Learning Teams", an initiative spanning all Danish regions. These work on applying principles of antibiotic stewardship in many of the acute care hospitals, at emergency departments, and in medical departments with a relatively high number of acute patients. The regional initiatives are supported by the implementation of the third measurable goal in the National Action Plan on antibiotics from 2017, aiming at a 10% reduction in the consumption of cephalosporins, fluoroquinolones and carbapenems from 2016 to 2020.

Table 5.7 Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

DANMAP 2017

ATC group(a)	Therapeutic group	Year									
		2008[b]	2009	2010	2011	2012	2013	2014	2015	2016	2017
J01AA	Tetracyclines	3.55	4.21	3.88	3.95	5.35	4.98	5.39	5.70	6.47	6.36
J01CA	Penicillins with extended spectrum	58.60	55.90	49.40	47.31	49.7	49.41	51.15	52.28	53.28	59.82
J01CE	Beta-lactamase sensitive penicillins	42.22	36.17	32.24	33.91	33.74	33.24	32.17	30.83	30.65	33.22
J01CF	Beta-lactamase resistant penicillins	29.23	27.16	26.53	28.44	28.24	29.74	29.85	30.12	27.78	27.69
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	16.41	19.91	23.38	29.37	38.77	44.74	50.01	54.69	55.38	50.37
J01DB	First-generation cephalosporins	0.64	0.47	0.44	0.40	0.40	0.37	0.20	0.14	0.13	0.13
J01DC	Second-generation cephalosporins	45.00	44.65	42.37	51.50	46.11	40.40	36.43	31.85	28.32	32.64
J01DD	Third-generation cephalosporins	4.77	4.44	3.61	3.72	3.45	3.55	3.19	3.25	3.16	4.03
J01DF	Monobactams	0.01	0.00	0.12	0.49	0.47	0.46	0.20	0.08	0.03	0.02
J01DH	Carbapenems	8.41	8.22	8.60	12.01	12.38	13.19	8.77	12.62	11.95	12.49
J01EA	Trimethoprim and derivatives	2.18	1.91	1.38	1.17	1.28	1.33	1.63	1.41	1.29	1.40
J01EB	Short-acting sulfonamides	1.94	1.61	1.21	0.88	0.70	0.62	0.55	0.47	0.38	0.38
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	10.11	8.47	6.58	9.89	10.68	13.81	14.59	15.48	15.54	15.90
J01FA	Macrolides	12.88	12.39	11.98	11.81	11.68	11.12	12.09	14.02	14.83	17.66
J01FF	Lincosamides	1.72	1.77	1.55	1.66	1.99	2.09	2.03	1.77	1.88	1.91
J01GB	Aminoglycosides	6.98	5.70	5.73	6.43	6.96	6.99	5.03	5.16	5.98	6.81
J01MA	Fluoroquinolones	39.48	37.95	34.51	33.80	32.38	32.06	30.82	28.56	24.91	23.64
J01XA	Glycopeptides	2.78	3.48	3.47	4.14	4.12	4.23	3.59	3.29	3.25	3.86
J01XB	Polymyxins	0.21	0.25	0.32	0.28	0.30	0.54	0.85	0.78	0.88	0.86
J01XC	Steroid antibacterials (fusidic acid)	1.06	1.10	1.12	0.85	0.75	0.72	0.72	0.50	0.34	0.20
J01XD	Imidazole derivatives	13.63	13.64	12.99	13.10	13.34	13.37	13.99	13.06	13.69	13.92
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.38	1.42	1.18	1.20	1.22	1.26	1.14	0.99	0.85	0.87
J01XX05	Methenamine	0.53	0.36	0.30	0.39	0.34	0.30	0.23	0.30	0.28	0.25
J01XX08	Linezolid	0.84	0.76	0.72	0.99	1.01	1.14	1.06	1.33	1.09	1.09
J01XX09	Daptomycin	0.06	0.06	0.07	0.05	0.06	0.07	0.10	0.12	0.15	0.24
P01AB01	Nitroimidazole derivatives (metronidazole)	9.61	9.62	8.92	8.45	7.88	7.49	6.36	6.31	6.73	6.23
A07AA09	Intestinal antiinfectives (vancomycin)	0.42	0.66	0.91	1.39	1.55	1.60	1.62	1.44	1.46	1.55
J01, P01AB01, A07AA09	Antibacterial agents for systemic use, including metronidazole and vancomycin (total)	314.64	302.29	283.53	307.57	314.88	318.83	313.74	316.56	310.68	323.53

a) From the 2018 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) The number of admissions was affectedly low in 2008 due to a major hospital strike

Additionally, extra attention will be paid to the consumption of cephalosporins next year. Due to shortages of piperacillin with tazobactam in 2017, it may have been necessary to reintroduce cephalosporins in the treatment of acutely ill, septic patients at several hospitals. Thus, an increase of the overall consumption of cephalosporins was expected to occur and was observed, moving from 10.63 DBD in 2016 to 12.54 DBD in 2017. Simultaneously a decrease in the consumption of piperacillin with tazobactam was observed (Figure 5.14).

5.4.5 National initiatives on continued reductions of the antimicrobial consumption

The National Action Plan on the reduction of antibiotics in humans launched in July 2017 uses two measurable goals directed at the consumption trends in the primary care. The first goal aims at a continued general reduction in the number of prescriptions issued in Denmark (from 462 prescriptions per 1000 inhabitants among general practitioners, medical specialists and dentists in 2016 to 350 prescriptions per 1000 inhabitants in 2020). The second goal focuses on the more prudent choice of antimicrobials emphasizing the importance of continued use of beta-lactamase sensitive penicillins as the drug of choice in many common infections, especially in respiratory infections. The mentioned third goal aiming at a reduced consumption of the three critical antimicrobials focuses on the prudent use of antibiotics at hospitals. The goal is challenged through shortages like the mentioned failure in deliverance of piperacillin/tazobactam. It is woth considering how delivery of important small(er) spectrum antibiotics can be ensured also in the future.

The National Action Plan was issued by the Danish Health Ministry and supported by the National antibiotic council representing all relevant health institutions, organizations and specialties working with the prevention, control and treatment of infections in Denmark.

Together with the National Action Plan, a One Health Strategy was published, building on the National Action Plan on controlling the development of antimicrobial resistance from 2010. Both are available at the Danish Ministry of Health's homepage at www.SUM.dk

Reducing the amount of antimicrobials consumed can only be achieved through parallel actions on the continued improvement of diagnostics and through infection control measures. `Central Unit for Infectious Disease Prevention & Hygiene´ at Statens Serums Institut supports many of the National antibiotic initiatives through recommendation guidelines aimed at hospitals and health care settings. These are available at https://www.ssi.dk/Smitteberedskab/Infektionshygiejne/Retningslinjer/NIR.aspx (only available in Danish).

Karoline Skjold Selle Pedersen and Ute Wolff Sönksen
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Figure 5.12 Consumption of antimicrobials used at hospitals, regional levels, 2012-2017, Denmark DANMAP 2017

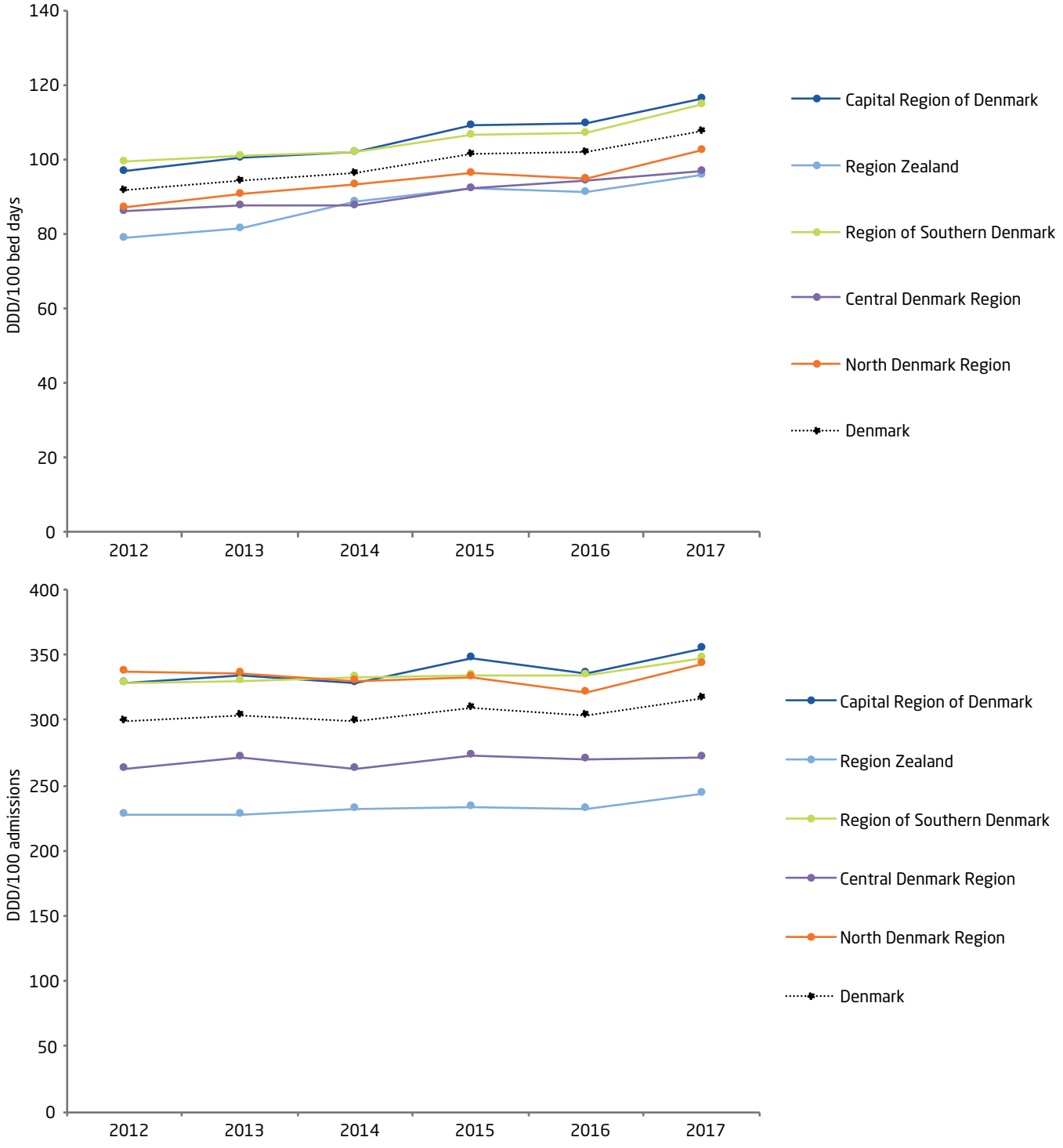


Figure 5.13 Antimicrobials of special critical interest, 2012-2017, Denmark DANMAP 2017

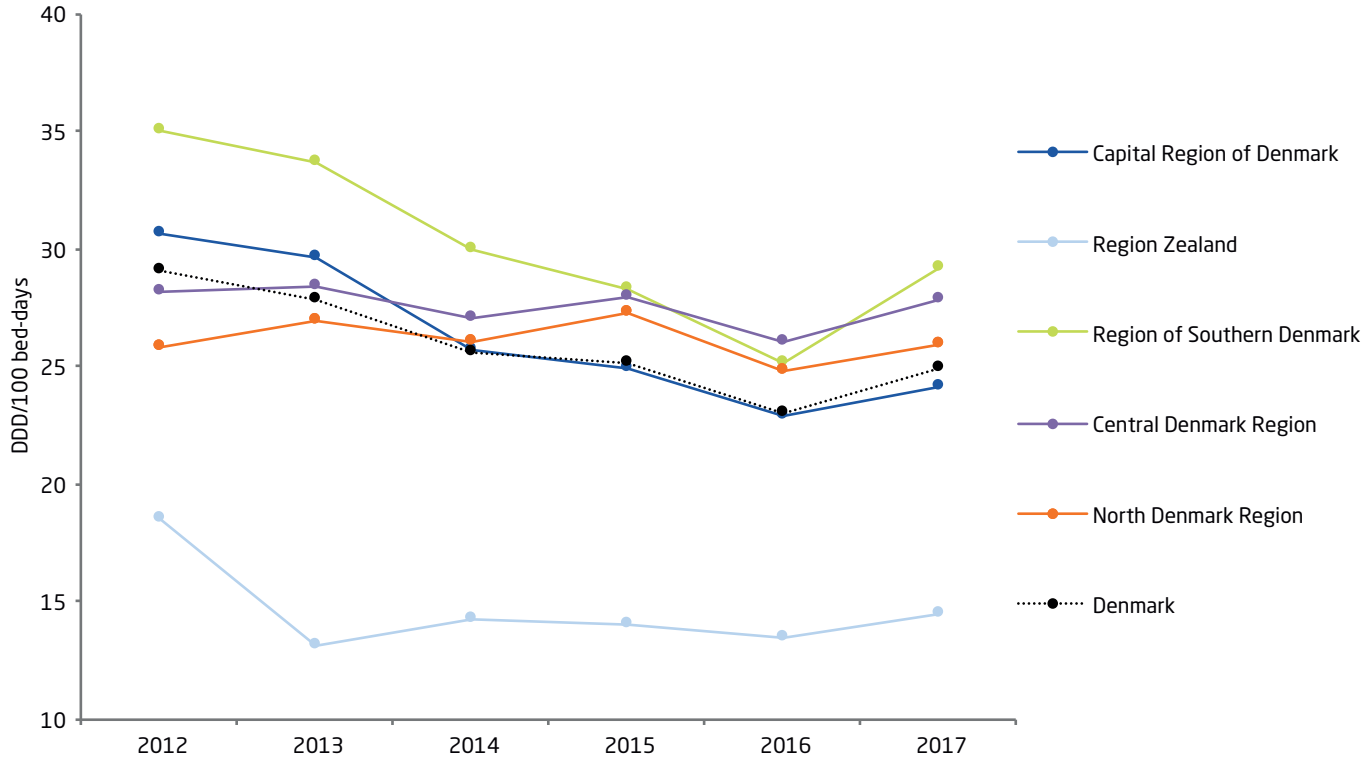
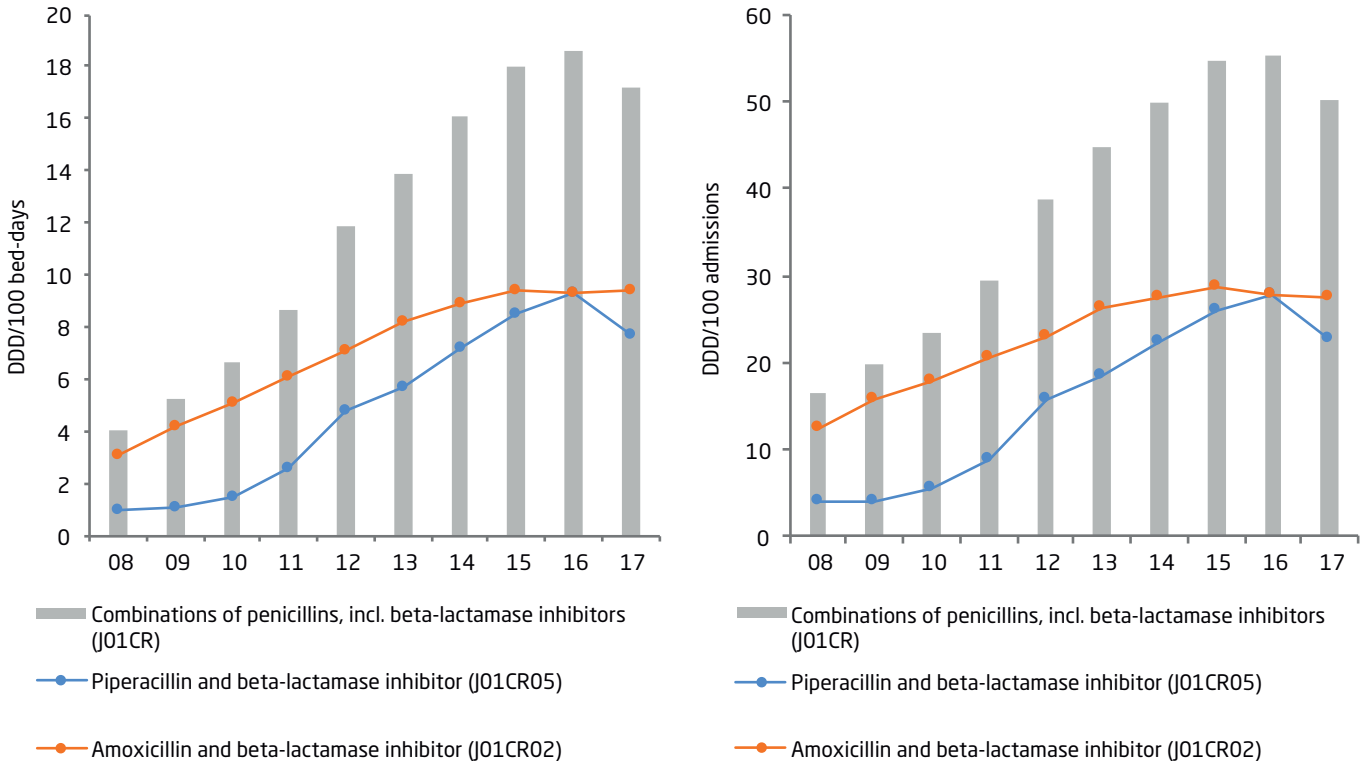


Figure 5.14 Consumption of combination penicillins (J01CR), 2012-2017, Denmark (DAD and DBD) DANMAP 2017



Textbox 5.2

Consumption of antifungal compounds and resistance patterns of human invasive isolates of *Candida*

Invasive candidiasis including candidaemia is the most severe manifestation of *Candida* infection. The Candidaemia diagnosis is made by detecting the yeast in the blood stream directly by culturing the blood. Candidaemia is a serious condition with an overall mortality as high as 30-40% despite advances in antifungal therapy [1]. There are several risk factors and risk groups associated with candidaemia such as: critically ill patients with long stay at intensive care departments, patients who undergo abdominal surgery, patients with haematologic malignancies or cancer, transplant recipients, patients treated with broad-spectrum antibiotics, low birth weight neonates and preterm infants and patients receiving parenteral nutrition or with a central vascular catheter in place [2].

There were few data on the epidemiology of fungaemia in Denmark until 2003 and therefore a surveillance programme covering 53% of the Danish population was initiated in 2003 [3]. The surveillance program was extended in 2004 to cover 64% of the Danish population [4] and subsequently the entire population [5, 6, 7].

The surveillance programme has the purpose of reporting the epidemiological trends of *Candida* species isolated from blood including susceptibility patterns and to report on antifungal consumption, in the primary health care sector and hospitals. Demographic data such as age, gender and geography are included.

A steady increasing incidence has been reported until it peaked in 2011 at 10.1/100,000 inhabitants [6, 7]. This number is higher than in any other Nordic countries and higher than most other countries worldwide. Subsequently, the average incidence has stabilized at 8.4/100,000 inhabitants in 2012 to 2015. The highest incidence was observed in the elderly (patients over 50 years) with a significant increase among males between 80-89 years in 2012-2015 and the incidence was generally higher for males than for females [7].

Across the 12 years of surveillance (2004-2015), the number of *Candida albicans,* the predominant species, decreased, while *C. glabrata*, the second most frequent species, increased significantly (Fig 1). In addition, *C. glabrata* was increasingly found in elderly and in women compared to men in all 10-year intervals above age 40 years [7].

A decreased susceptibility to fluconazole was observed with significantly fewer isolates susceptible to fluconazole from 2012 to 2015 (60.6%) compared with 2008-2011 (65.2%) and 2004-2007 (68.5%). Simultaneaously the proportion of *C. glabrata* increased. *C. glabrata* has a reduced intrinsic susceptibility to azoles and the increasing proportion of *C. glabrata* was observed concomitantly with an increasing and high use of azoles both in the primary and in the hospital sector, suggesting that a selection pressure has played a role in the changing species distribution. Systemic azoles are extensively used in Danish primary health care sector. For example, fluconazole is used as treatment of vaginitis, despite the fact that topical azoles can be used, and itraconazole is used for skin and nail infections, though terbinafine is the recommended first line therapy, when systemic treatment is required.

There are some *Candida* species, which are intrinsically less susceptible to echinocandins, i.e. *Candida parapsilosis, Candida fermentati* and *Candida guilliermondii.* A feared emerging pathogen, *Candida auris* is uniformly resistant to fluconazole, and a variable proportion concomitantly resistant to echinocandins and/or amphotericin B, thus being multi-drug resistant. *C. auris* has caused hospital outbreaks in UK and Spain, but it has not been found in Denmark yet. Of note, acquired echinocandin resistance emerged among *Candida* isolates from 0% in 2004-2007 to 0.6% in 2008-2011 and to 1.7% in 2012-2015. Although still uncommon, this increase in acquired echinocandin resistance is concerning, particularly as an acquired resistance is most commonly found in *C. glabrata* rendering this species multidrug resistant. On the contrary resistance to Amphotericin B remained low [7].

The antifungal consumption in Denmark per 1,000 inhabitants (790 DDDs in 2015) is higher than in the other Nordic countries (512, 321 and 762 DDDs, for Norway, Sweden and Finland, respectively) (Fig 2). Denmark witnessed an increase in antifungal use from 2004 to 2013-2014 with a stabilization in 2014-2015 for amphotericin B, echinocandins, voriconazole, terbinafine, fluconazole, itraconazole and ketoconazole, while the last four antifungals were predominantly used in the primary health care system from 2004 to 2015 (99.8%, 69.9%, 94.7% and 87.9%, respectively) [6,7]. Denmark had a higher consumption of azoles specifically (237 DDDs) compared to 87, 109, and 164 DDDs in Norway, Sweden and Finland, respectively) [7].

Textbox 5.2 continued...

In conclusion, the epidemiology of candidaemia differentiates from that of most other countries by a high incidence particularly in the older age groups and in men, and by an increasing proportion of the fluconazole resistant species *C. glabrata*, now exceeding 30%. Exposure to azoles for ≥ 7 days alters the colonizing flora, from where invasive infections arise [1]. Therefore, unnecessary systemic azole use should be limited in order to revert the continued increase of invasive *C. glabrata* infections [7].

We would like to thank our colleagues at the reference laboratory for *Candida* and *Aspergillus* for their great work and efforts.

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Figure 1 Species distribution 2004-2015 DANMAP 2017

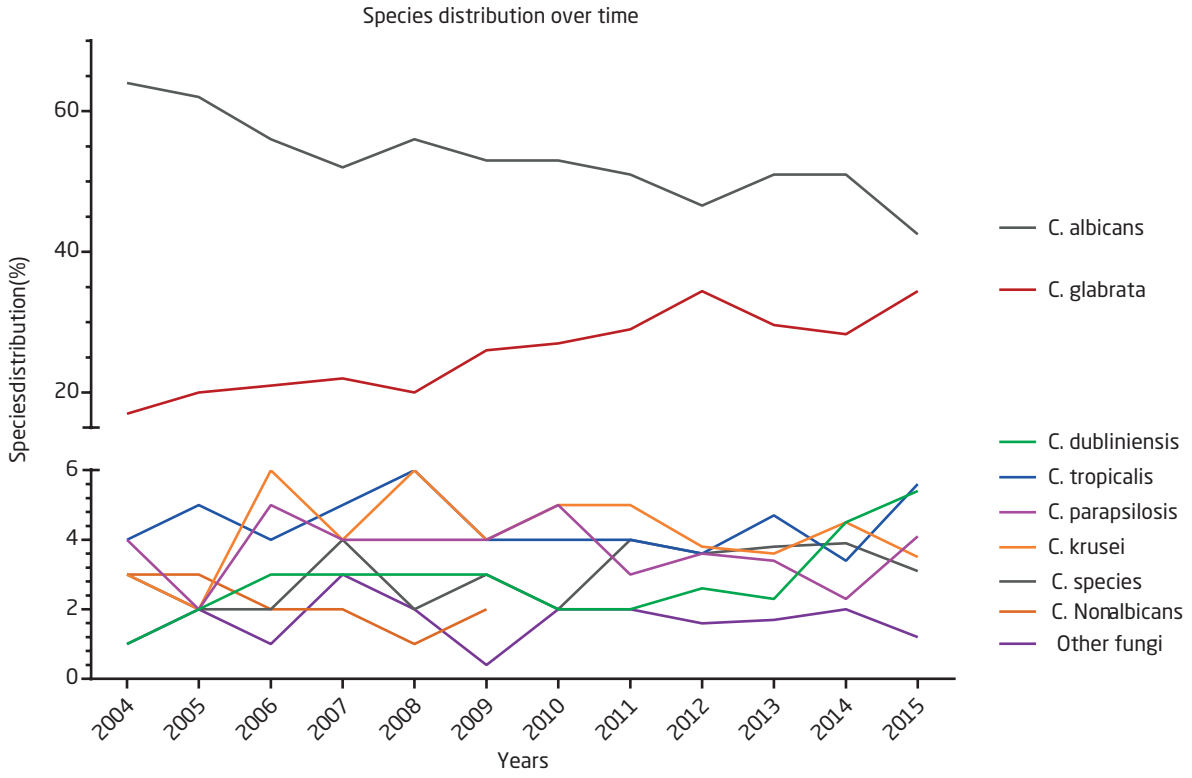

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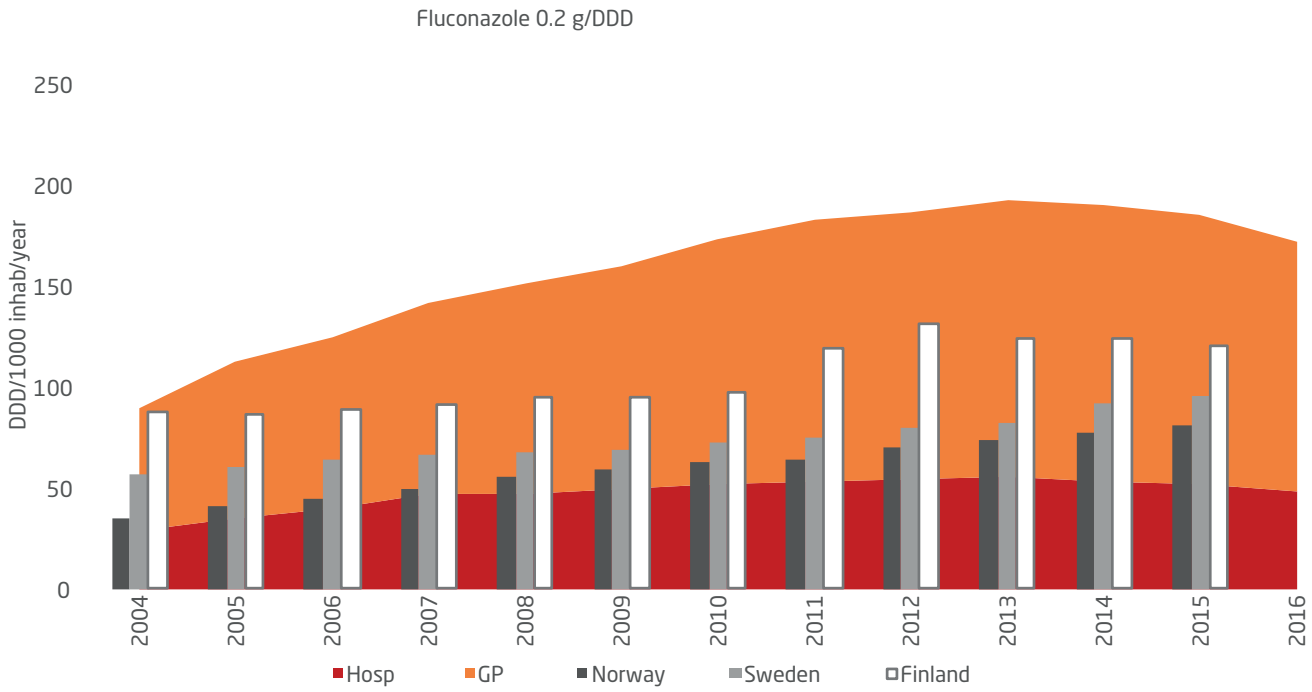
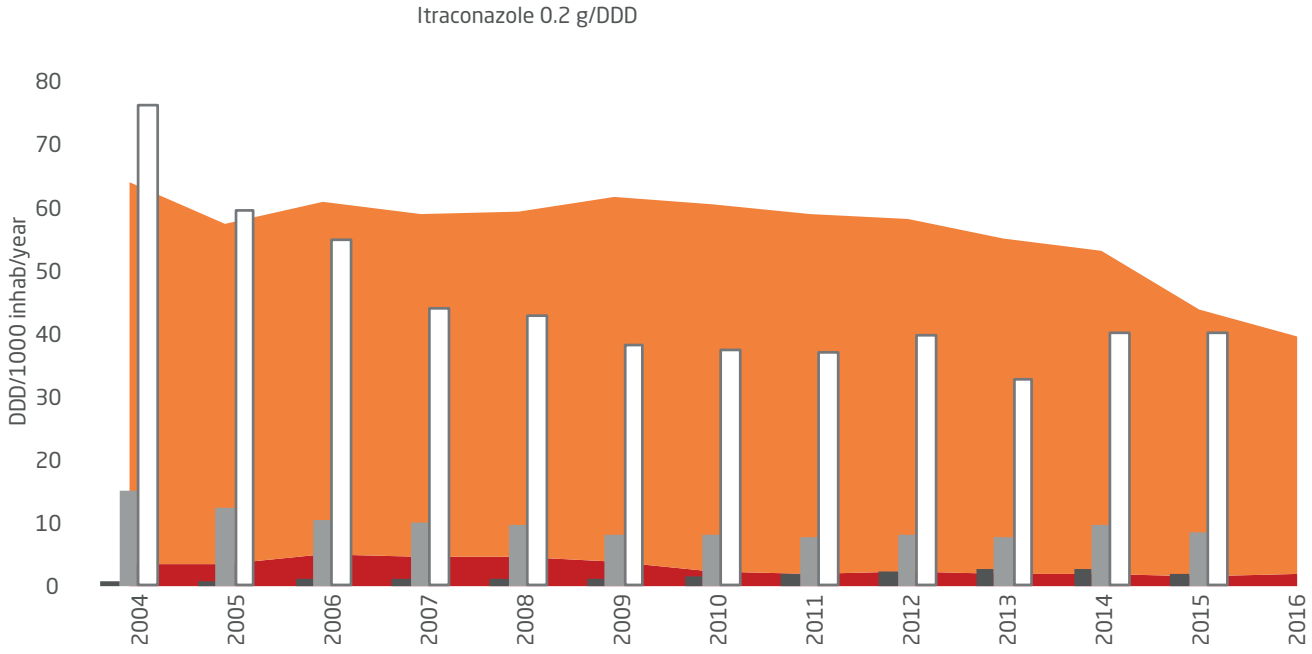
Figure 2 Annual consumption of systemic fluconazole (A) and itraconazole (B) in DDDs/1,000 inhabitants in 2004 to 2015 [7]
DANMAP 2017





Textbox 5.2 continued...

Consumption of antifungal compounds and resistance patterns of human invasive isolates of *Aspergillus*

Epidemiology: *Aspergillus* is a spore producing mould, ubiquitously dispersed in the air, allowing an airborne route of infection (1). It causes a number of serious acute and chronic infections (1). Overall, invasive aspergillosis (IA), covering pulmonary aspergillosis, infections of other organs and the central nervous system, has an estimated burden of 3-500,000 cases worldwide (1). High risk of IA has primarily been restricted to immunocompromised (neutropenic) patients and allogeneic stem cell transplant (HSCT) recipients, but influenza among critically ill - but otherwise immunocompetent - patients has increasingly been highlighted as a significant risk factor (2). The attributable mortality rates of IA is 30-50% and the applicability of sensitive and specific diagnostics methods as well as targeted treatment of infections play a key role in the management (3).

In Denmark, detailed knowledge on the overall epidemiology and burden of IA is still lacking. A previous three-month laboratory based study (January-April 2010) covering approximately one third of the Danish population but the majority of critically ill patients, investigated 11,368 airway samples for *Aspergillus* in order to understand the burden of chronic and invasive aspergillosis (4). Overall, 151 patients had positive *Aspergillus* cultures (>90% A. *fumigatus*). Among these, 9% had proven or probable invasive aspergillosis (following internationally defined criteria (5)), 3% had chronic allergic bronchopulmonary aspergillosis (ABPA) and the remaining 88% were considered colonised. overall this led to a conservative estimate of 50-60 cases/year or 0.9-1.1/100,000 inhabitants, assuming that no cases of IA occurred in the rest of Denmark (4). Another study based on previous epidemiological studies and rough estimates gave a population based approximation of 294 IA cases/year, resulting in an incidence of 5.3/100,000 inhabitants among 5.6 million Danes. This estimate is five times higher than the first study, primarily driven by the assumption that 1.3% of admitted chronic obstructive pulmonary disease (COPD) cases develop IA. While this may be an overestimation, several IA cases are probably never diagnosed, illustrated by a recent autopsy study (6). Thus the actual incidence may be between 1-5/100,000 inhabitants. This uncertainty illustrates the need for surveillance of invasive fungal infections in Denmark to clarify the actual prevalence and possible consequences associated with the disease.

Antifungal (azole) resistance: The antifungal drug class used for prophylaxis and first-line treatment of IA is triazoles with only two less efficacious and i.v.-only alternatives (3,7). Indeed, the emerging azole resistance constitutes one of the most serious threats for IA patients and is responsible for increased mortality rates to 80-100% (3,8). Two routes of resistance development are described in the primary pathogen A. *fumigatus*;

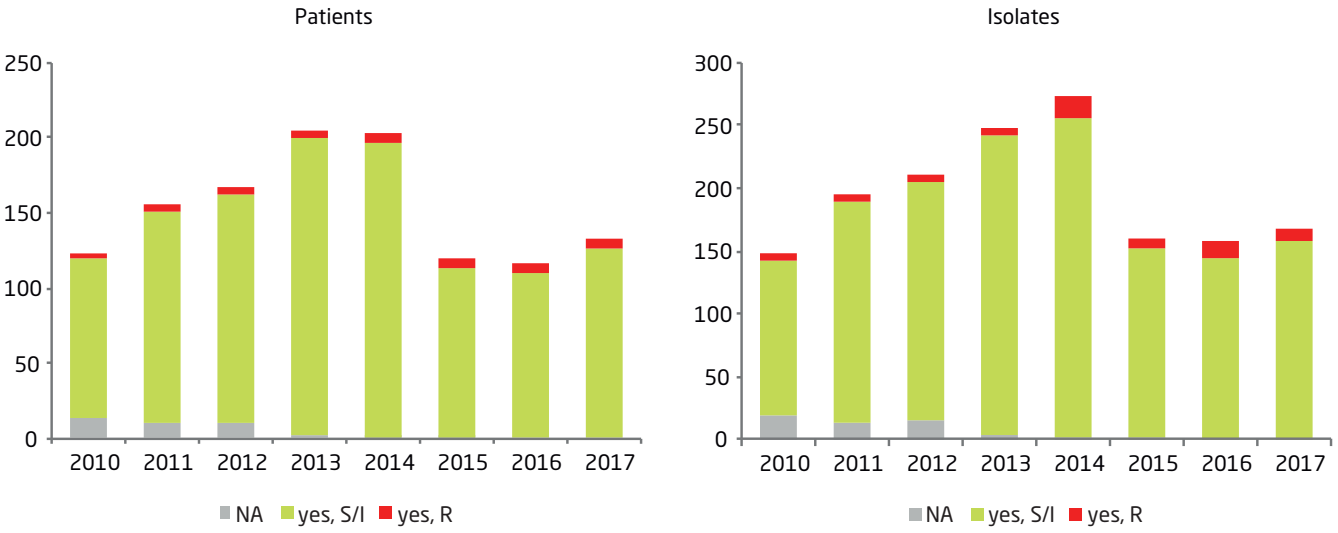
1) *in vivo* selection of resistance during long-term azole treatment
2) *ex vivo* in the environment, as a consequence of extensive azole fungicide used in the agriculture (9,10).

Azole resistance mechanisms are primarily structural changes or upregulation of the azole target lanosterol 14 α-demethylase encoded by *cyp51A* (3). Generally, azole resistance evolved *in vivo* is linked to non-synonymous mutations in *cyp51A* leading to single amino acid changes in hot-spots of the encoded protein, e.g. G54, G138, M220 and G448 (3). On the other hand, azole-resistant isolates obtained from environmental samples (air and soil) has almost exclusively been attributed to a tandem repeat (TR), 34, 46 or 53 base pairs in length in the promoter of *cyp51A* and with or without specific mutations in *cyp51A*, i.e. TR_{34}/L98H, TR_{46}/Y121F/T289A or TR_{53} (3). The latter situation is aggravated by several conditions. First, environmental resistance-mechanisms confer cross-resistance to medical triazoles; second, both azole exposed and azole naïve patients worldwide are continuously being diagnosed with azole resistant A. *fumigatus* infections; third, azole resistance is continuously being detected in environmental A. *fumigatus* isolates; and fourth, in some countries, environmental azole resistance accounts for the majority of azole-resistant infections (3,9). In the Netherlands, which has a vast tulip production and associated azole fungicide use, 90% of the reported clinical azole resistance cases of IA is due to the TR_{34}/L98H or TR_{46}/Y121F/T289A genotypes (11). As a consequence, European Center for Disease Control (ECDC) made a risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species (10). Conversely, large centres for chronic pulmonary aspergillosis in the UK, may explain a majority of *in vivo* selected azole resistance (12).

Due to the lack of a surveillance programme for mould infections in Denmark, a recent attempt to clarify azole resistance rates was made as a laboratory based study, which has a considerable drawback, as no clinical background information was included (13). The study covered 1162 clinical respiratory A. *fumigatus* isolates collected from 2010-2014 and presented around 4% and 6% azole resistance among patients and isolates, respectively. Importantly, around 50% azole resistance was due to the environmental mechanisms, TR_{34}/L98H or TR46/Y121F/T289A (13). The data now includes isolates from recent years 2015-2017 (unpublished data). Dividing the entire period 2010-2017 in two four-year periods, the incidence of azole resistance was 2.8% (17/613) in 2010-2013 and 4.3% (24/568) in 2014-2017, while the proportion of azole-resistant isolates were 3.5% (26/754) and 6.3% (48/757), respectively (Figure 3).

Figure 3 Number of patients (left) and A. fumigatus isolates (right). Grey indicates available susceptibility data and red indicates azole-resistance.

DANMAP 2017



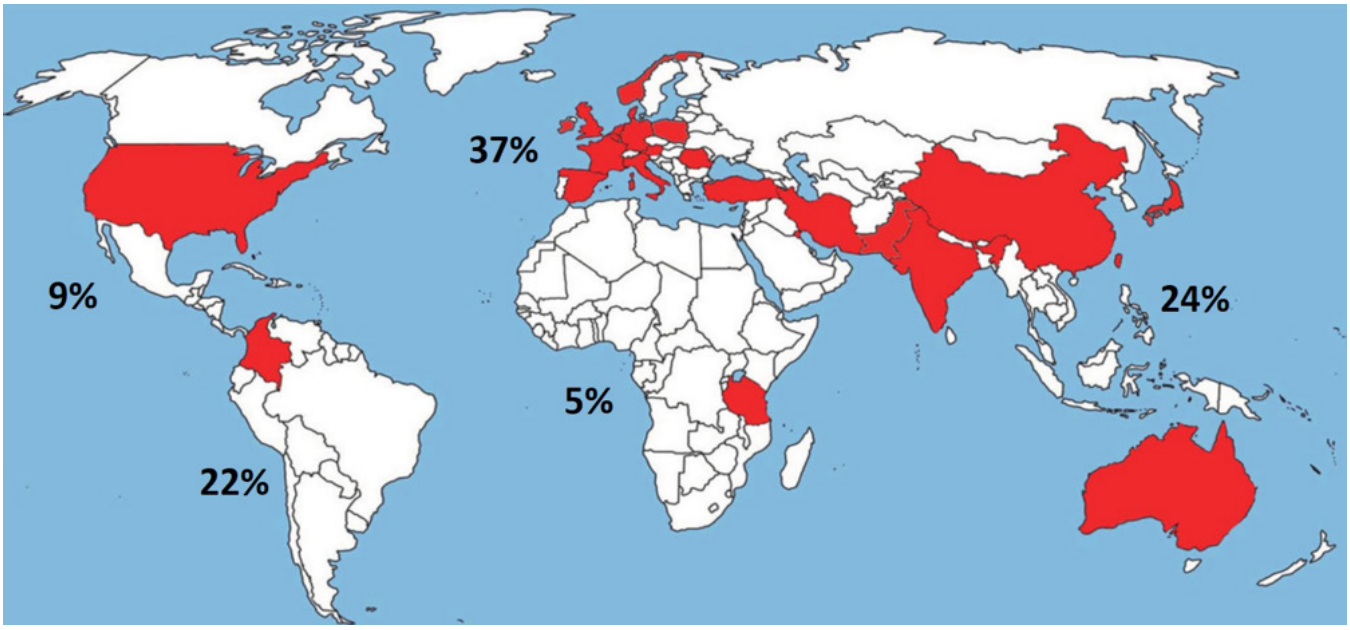
Using the updated numbers, environmental azole-resistance is responsible for 43% and 45% azole-resistant isolates and patients respectively.

The high proportion of environmental azole-resistance among Danish clinical isolates has not been correlated to Danish environmental studies (8). Despite several sampling periods involving both soil and air samples and a total of 215 A. *fumigatus* isolates recovered, only four isolates from 2009 displayed azole resistance with the TR_{34}/L98H resistance mechanism (8,13,14). This paradox, discussed by Astvad et al., could be seasonal variation and suboptimal sampling periods (8). Recently, a collaboration between SSI and a group from the Department of Agroecology, Aarhus University, Denmark was initiated. Collectively, several hundred air samples have been collected with a Burkard airsampler from 2012-2016, allowing DNA extracted from spores obtained each day during the summer period through several years. A recently developed real-time PCR assay enabled direct detection of the primary environmental resistance mechanism (TR_{34}/L98H) detected in Denmark (unpublished data). However, due to low sensitivity in this assay, requiring the specific amplification of *cyp51A*, among 826 samples, only 141 (17%) had a positive detection of A. *fumigatus cyp51A*. Samples were from 11 crop fields scattered mainly on Lolland-Falster but also near the Agroecology Department at Flakkebjerg, Slagelse. Of 141 samples, two samples (1.4%) both obtained in 2016 were positive for TR_{34}/L98H, constituting 5.6% (1/18) or 5.9% (1/17), when only considering the two involved crop fields. Despite, a high uncertainty with this finding, it underlines the fact that environmental azole resistance is present, probably in small numbers, but limitations of such method, involving a low number of spores, could potentially underestimate the actual burden.

Figure 4 illustrates the worldwide spread of environmental azole resistance and the agricultural fungicide use (by continent). Although the specification of specific azoles used is lacking, a large overall consumption in Western Europe correlates well with a high occurrence of environmental azole-resistant A. *fumigatus* isolates (15).

Textbox 5.2 continued...

Figure 4 Illustration of the global market share of azole fungicides as well as coloured countries where environmental azole resistance (TR34/L98H, TR46/Y121F/T289A, TR53) has been found in clinical and/or environmental A. fumigatus isolates. A large share is being used in Western Europe, which is considered an epi-center for especially the TR34/L98H resistance mechanism (3,15). DANMAP 2017



In conclusion, azole resistance is emerging and driven by long-term azole therapy in patients as well as in the environment due to the extensive use of azole fungicides in the agriculture. The latter is a critical concern as environmentally derived resistance is detected on a global scale and because azole resistance is continuously detected in both clinical and environmental A. *fumigatus* isolates. This constitutes a significant threat, especially to patients suffering from the critical IA disease responsible for elevated mortality rates up to 100%. Further knowledge of the Danish epidemiology of aspergillosis as well as a more exact clarification of azole resistance rates are demanded. Improved and collaborative research within clinical and agricultural science may help understand the origin of environmental resistance and may help manage this external threat.

We would like to thank our colleagues at the reference laboratory for Candida and *Aspergillus* for their great work and efforts.

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Textbox 5.3

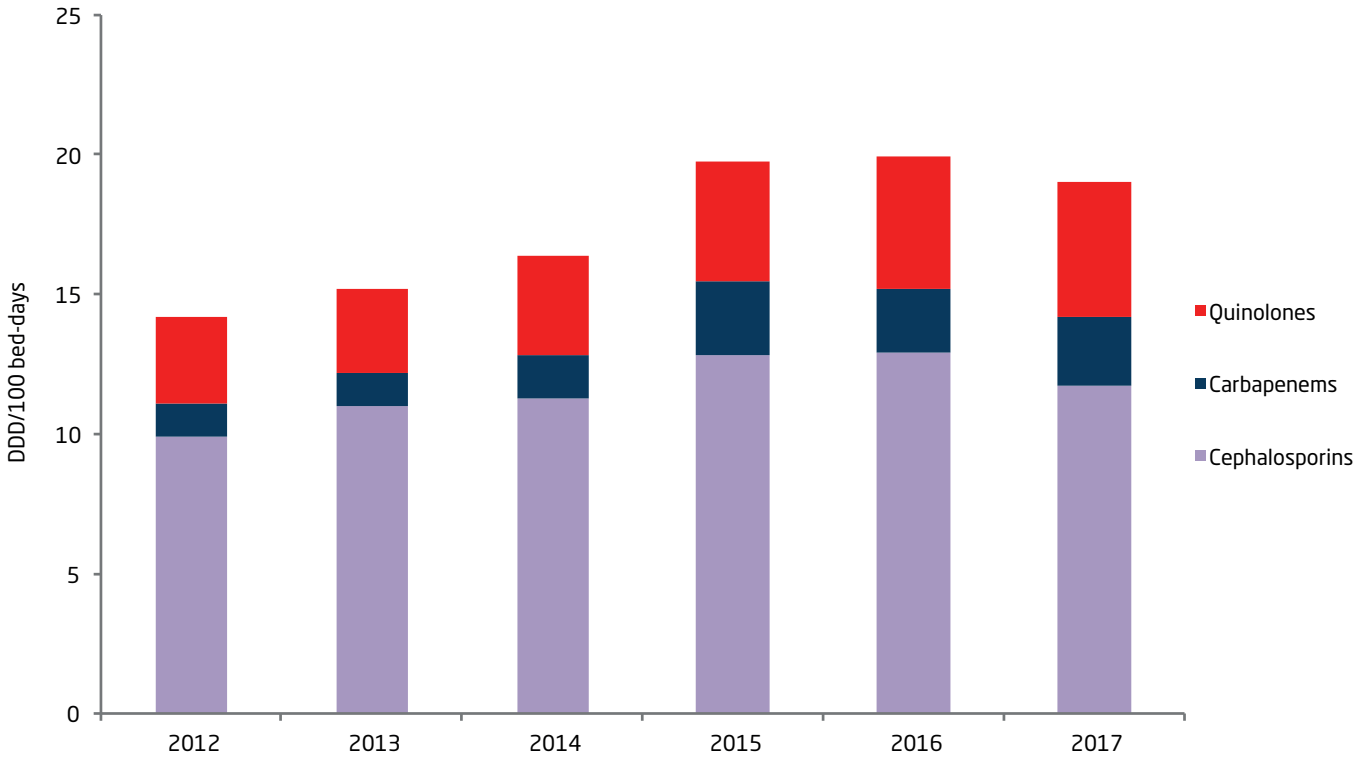
Antimicrobial resistance and consumption of antimicrobials in the Faroe Islands

Background: The Faroe Islands (FI) consist of 18 islands inhabited by approx. 49,000 inhabitants, 19,000 of whom live in the capital Tórshavn. The main hospital (Landssjúkrahúsið, LS, with 120 beds), is located in Tórshavn, and there are two smaller hospitals in Klaksvik (22 beds) and Súðuroy (22 beds). The Faroese healthcare system is comparable to the Danish healthcare system with general practitioners responsible for primary care and hospitals providing secondary care. LS has a local as well as a centralised function. In the case of specific diseases, demanding highly specialised care, patients are referred to hospitals in Denmark or other foreign hospitals.

Data and data sources: Data on antimicrobial consumption for FI and for the hospitals were supplied by the Chief Pharmaceutical Office. Data on MRSA and other resistant bacteria were obtained from LS, as were bed-days.

Resistant microorganisms: MRSA and ESBL-producing Enterobacteriales (*Escherichia coli* and *Klebsiella pneumoniae*) are continuously surveyed and screening is performed according to guidelines. Since April 2015, vancomycin-resistant enterococci (VRE) have been an increasing problem – especially at LS. Throughout 2015, systematic periodic screening of VRE was performed in the wards. From 2016 and onwards, screening was mainly performed after transfer of patients from hospitals abroad (including Denmark). Thus, the VRE data are based on results from screening and clinical samples. Since the beginning of surveillance, a total of 56 patients were colonised or infected with MRSA (surveillance period 2004-2017), 70 with ESBL-producing Enterobacteriales (2006-2017), and 162 with VRE (2015-2017).

Figure 1 Consumption of selected broad-spectrum antibiotics at LS, 2012-2017 DANMAP 2017

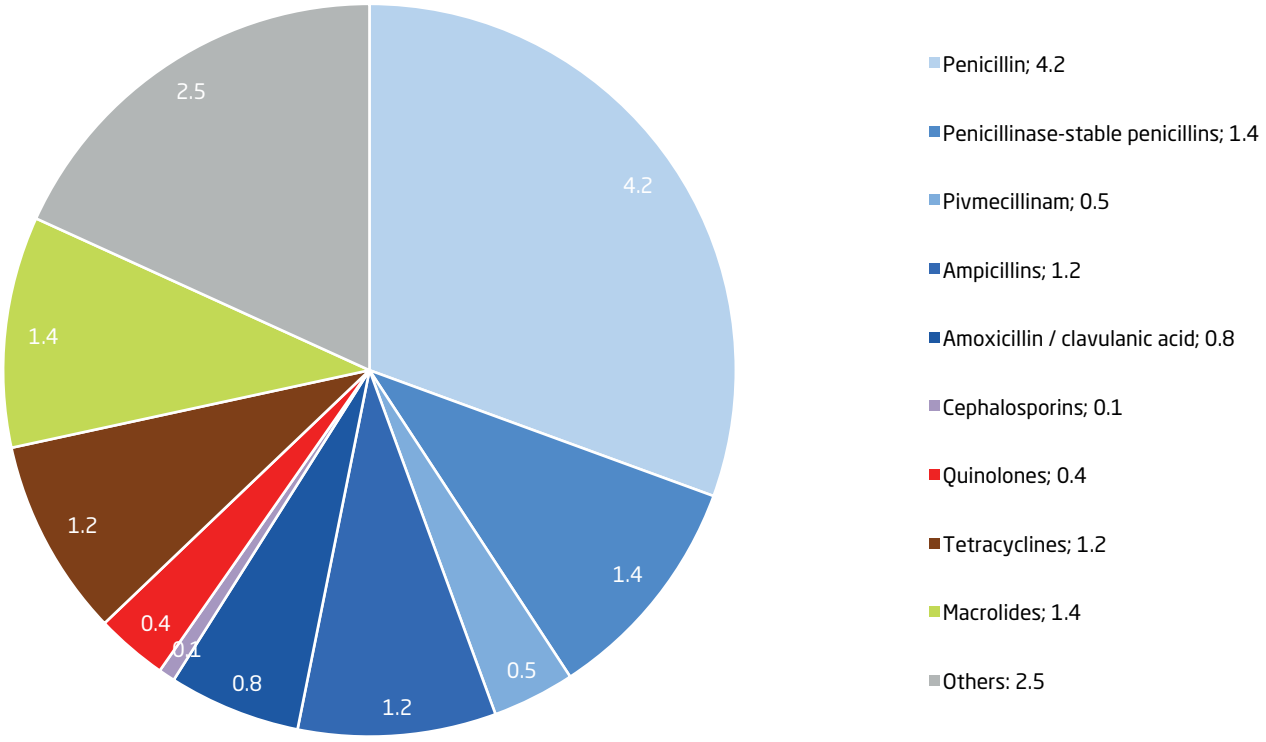


Antimicrobial consumption at Landssjúkrahúsið: The total antimicrobial consumption was 56.13 DDD/100 bed-days (DBD), representing a decrease compared to 2016. Special attention to three broad-spectrum antimicrobials, cephalosporins, carbapenems and fluoroquinolones is still required: In 2017, the consumption of cefuroxime was 10.79 DBD, a decrease of 12% from 12.27 DBD in 2016, still constituting 19% of the total antibiotic consumption at LS. Moreover, the consumption of carbapenems increased from 2.27 DBD in 2016 to 2.45 DBD in 2017 (8% increase) and that of ciprofloxacin increased from 3.18 DBD to 3.85 DBD (21% increase) (Fig. 1). Consumption of mecillinams (pivmecillinam and mecillinam) continued to increase from 0.43 DBD in 2012 to 4.52 in 2017, with a significant increase of 27% from 2016 to 2017. The use of penicillinase-stable penicillins increased as well, from 3.63 DBD in 2016 to 5.62 in 2017, (not shown).

Antimicrobial consumption in primary healthcare: In 2017, the total antimicrobial consumption in primary healthcare was 13.85 DDD/1,000 inhabitants/day (DID) – similar to that of 2016. The distribution of antimicrobial consumption is shown in Fig. 2. Remarkable are the proportions of ampicillins, penicillinase-stable penicillins and mecillinams (with increases of 13%, 8% and 13% respectively, compared to 2016), whereas the consumption of ciprofloxacin remained at almost the same level as in 2016 (3% increase). As this probably reflects a somewhat changed practice in treatment of urinary tract infections (UTI) (Fig. 3), it also stresses the continued need to screen patients for carnitine transporter gene defect (CTD), a gene defect common in the Faeroe island population. This is especially relevant for the elderly, which are more at risk of acquiring a UTI and therefore may undergo antibiotic treatment, where pivmecillinam can be safely used only in patients without the gene defect.

Antimicrobial consumption in primary vs. secondary healthcare: The consumption at the three hospitals was based on purchase data and constituted 11% of the total antimicrobial human consumption, while the consumption in primary healthcare was based on prescription data and constituted 89%.

Figure 2 Consumption of antimicrobials in primary healthcare, 2017, (DID) DANMAP 2017



Textbox 5.3 continued...

Conclusion: In 2017, the antimicrobial consumption decreased at LS and remained at the same level in primary healthcare.The consumption of cefuroxime finally seems to be reduced, a positive trend which to some extent probably is due to a shift to penicillinase-stable penicillins in the treatment of infections caused by staphylococci. Unfortunately, the consumption of carbapenems as well as ciprofloxacin seems to continue their increase at LS. It is, however, encouraging to observe a continued increase in the use of mecillinams, both at LS and in primary healthcare, a tendency that might be enhanced by screening of elderly for CTD. Further implementation of antibiotic stewardship and a continued focus on adherence to general infection control precautions are the necessary steps in the effort to reduce development and spread of antimicrobial resistance in LS and in primary healthcare.

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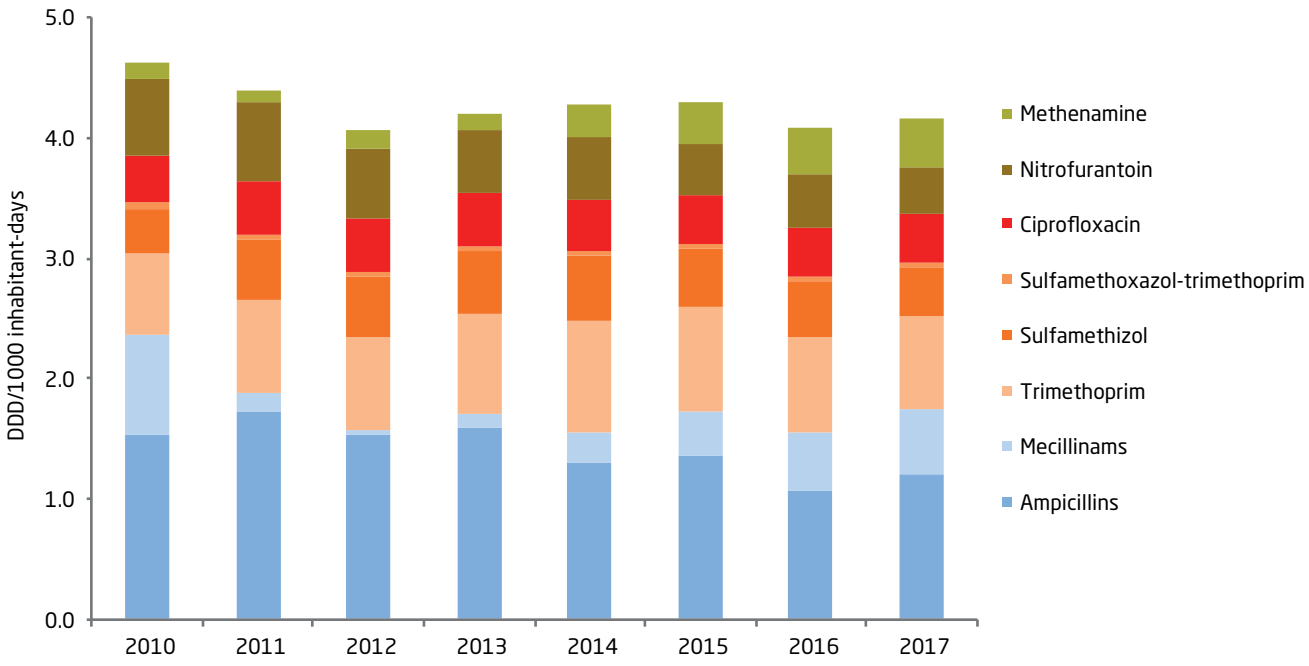
Figure 3 Consumption of selected typical UTI antibiotics in primary healthcare, 2010-2017 DANMAP 2017



6

RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Highlights: *Salmonella* Typhimurium remained the most prevalent *Salmonella* serotype isolated from Danish pigs and pork. Monophasic S. Typhimurium variants represented about two thirds of these, which is similar to the pattern in human *Salmonella* serotypes. The dominance of monophasic *Salmonella* isolates influences the resistance patterns in all populations with high levels and increasing resistance to tetracycline, ampicillin and sulfonamides.

Resistance levels to critically important antibiotics are low and fluoroquinolone (ciprofloxacin) resistance has not been identified in *S. Typhimurium* from Danish pigs and pork since 2010 and 2007, respectively. Among human cases, resistance to quinolones remained higher among isolates from the travel-related cases than among cases acquired in Denmark. Resistance to 3rd generation cephalosporins and carbapenems was very low in S. Typhimurium from human cases with no travel history and not found in the *Salmonella* isolates from Danish pigs and pork.

Resistance to quinolones (nalidixic acid and ciprofloxacin) was the most common resistance in *Campylobacter jejuni* from all populations: broilers, cattle and humans. The resistance levels are increasing in all three populations. Around one third of all isolates from animal origin and domestically acquired human cases were resistant to ciprofloxacin, whereas almost all the travel associated human isolates were resistant.

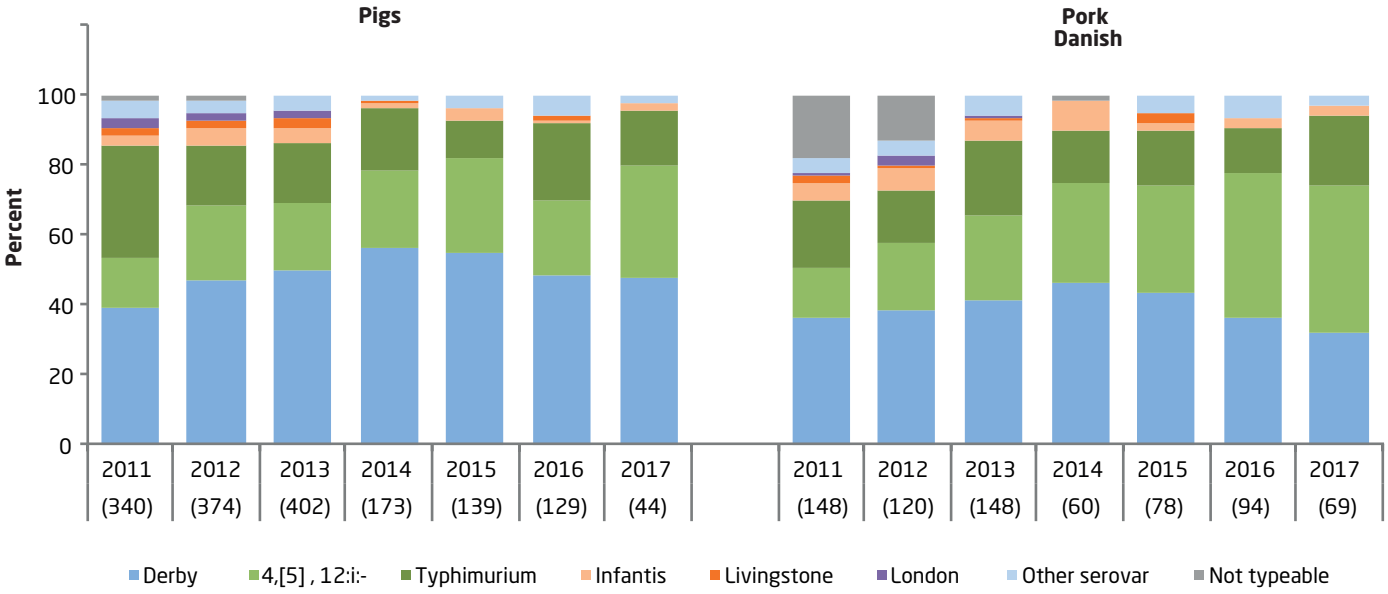
6.1 Introduction

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food or environment. Zoonotic bacteria, such as *Salmonella* and *Campylobacter*, can develop resistance towards antimicrobial agents, which subsequently may lead to limited treatment possibilities, prolonged illness and treatment failure of human infections. The development and spread of antimicrobial resistance is multifactorial and can happen in many ways, including antimicrobial treatment of animals and humans, transfer of genes between bacteria or dissemination of successful clones carrying resistance genes.

A more detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2017 [www.food.dtu.dk].

Salmonella Typhimurium, *Salmonella* Enteritidis, *Campylobacter jejuni* and *Campylobacter coli* have been included in the DANMAP programme since 1995, where isolates were recovered for susceptibility testing in samples from broilers, cattle and pigs as well as from human cases. Sampling of fresh meat was initiated from 1997. Since 2014, sampling and testing of *Salmonella* and *Campylobacter* have been done in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU].

Figure 6.1 Relative distribution (%) of *Salmonella* serotypes from pigs and Danish pork, Denmark DANMAP 2017



Note: Number of isolates included each year is presented in the parenthesis

6.2 Salmonella

Salmonella is the second most frequent zoonotic bacterial pathogen in humans in Denmark and can have a severe impact on both animal production and human health [Annual Report on Zoonoses in Denmark 2017].

In Denmark, *S.* Enteritidis and *S.* Typhimurium are the serotypes that most frequently are associated with human illness. Human cases caused by *S.* Enteritidis are frequently associated with consumption of contaminated eggs, whereas *S.* Typhimurium cases often are associated with contaminated pork, beef and poultry meat.

Salmonella isolates for DANMAP 2017 were derived from national surveillance and control programmes. Pig isolates originate from slaughterhouses, where representative samples from healthy pigs (caecum) and pork (carcass swabs) are collected each year. Salmonellosis is a notifiable disease in humans and isolates from nearly all reported *S.* Typhimurium cases are susceptibility tested. Only one isolate per farm, meat sample or human case was included in this report. For further details see Chapter 9, Materials and Methods.

The occurrence of *Salmonella* in domestic broilers, layers and cattle as well as some other types of Danish and imported meat are monitored in Denmark each year. However, these are not included in DANMAP 2017, as only few isolates were found and thus, fall below the inclusion threshold for DANMAP of 15 isolates per population. The data are however reported to EFSA, and are included in the European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017.

The DANMAP report highlights the resistance in *S.* Typhimurium, because they are the most important serotype in public health. However, resistance in other *Salmonella* serotypes from pigs and pork is also monitored for 2011 and onwards, which is the year Denmark started to susceptibility test all serotypes according to EU legislation.

In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas *S.* 4, [5],12:i:- as recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826].

The antimicrobials recommended by EFSA were used for susceptibility testing. MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.1 - A6.5).

6.2.1 *Salmonella* in pigs and domestically produced pork - all serotypes

From 295 representative pig caeca and 11,166 pig carcass swabs (pork) sampled on Danish slaughterhouses 119 Salmonella isolates were obtained. A total of 113 of these were tested for antimicrobial resistance. As in the previous years, *S.* Typhimurium (including the monophasic variants) and *S.* Derby were the most common serotypes, representing 95% of all the isolates (Figure 6.1). Last year *S.* Typhimurium overtook *S.* Derby as the most prevalent serotype from domestically produced pigs and pork. This trend continued in 2017, with only 38% *S.* Derby and 57% *S.* Typhimurium. Interestingly, the proportions of *S.* Derby and *S.* Typhimurium isolates in pigs arriving at the slaughterhouse are similar (48% vs. 48%), but in the meat *S.* Typhimurium is more frequent than *S.* Derby (62 % vs. 32%).

Monophasic *S.* Typhimurium were the most common isolates in pork (42%), which will affect the resistance patterns in meat in 2017, because monophasic *S.* Typhimurium strains tend to be more resistant than *S.* Derby.

6.2.2 S. Typhimurium in pigs and domestically produced pork

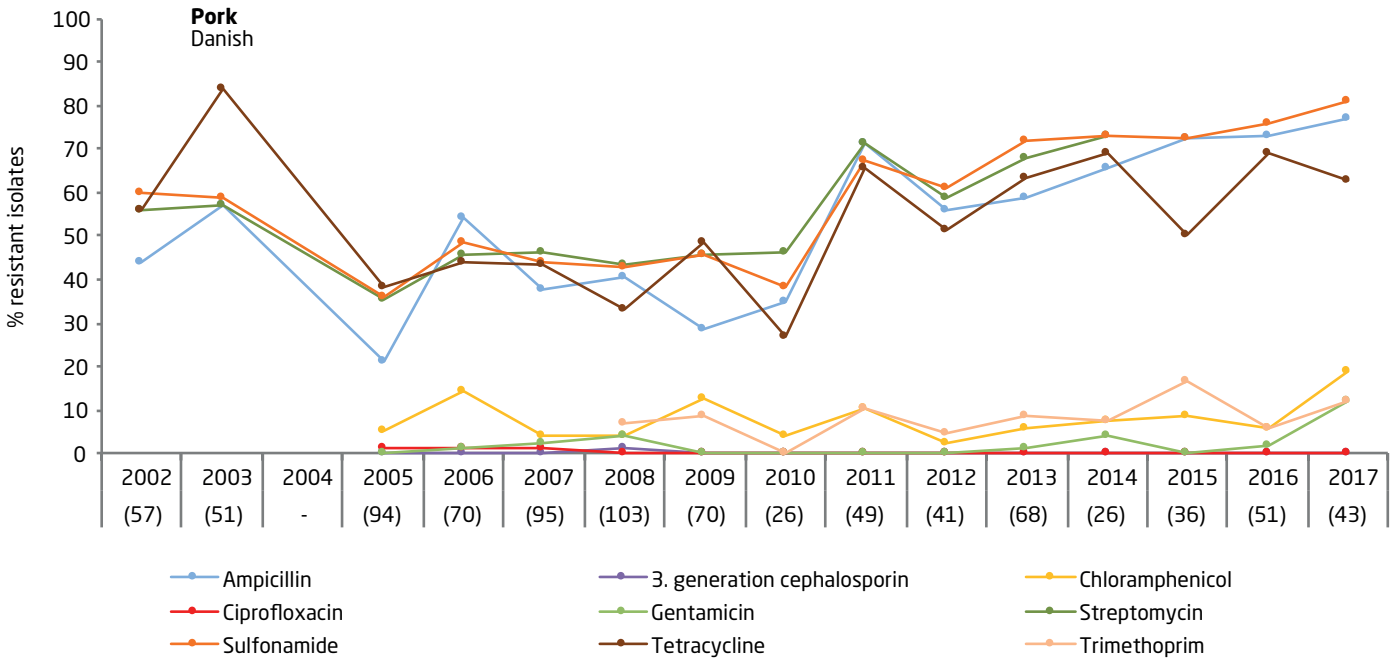
S. Typhimurium remains the most important zoonotic serotype originating from pigs. A total of 64 *S.* Typhimurium (21 diphasic and 43 monophasic variants) were isolated from Danish pigs and pork in 2017. The level of fully susceptible *S.* Typhimurium isolates in pigs (19%) and from domestically produced pork (14%) remained similar to 2016 (15% and 12%) (Table 6.1).

As in the previous years, resistance towards ampicillin, tetracycline and sulfonamide were common in *S.* Typhimurium isolates. Sulfonamide resistance was more frequent in isolates from pork than from pigs, probably reflecting the larger proportion of monophasic variants in pork.

Resistance to ampicillin declined significantly in pigs from 68% in 2016 to 48% in 2017. A slight decline in sulfonamide resistance and a small increase in tetracycline resistance were observed, but because of the small number of isolates (21), we are only 65% certain that these observations are different from last years. The decrease in ampicillin resistance was mainly driven by the fact that no resistance was found in the seven diphasic *S.* Typhimurium isolates. The proportion of sulfonamide and ampicillin resistant monophasic *S.* Typhimurium remained high at 71%.

In domestically produced pork, the resistance trends were more stable most likely due to a larger sample size. A decline was observed in tetracycline resistance, but this was not a sig-

Figure 6.2 Resistance (%) in *Salmonella* Typhimurium[a] in pork, Denmark DANMAP 2017



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs were not available or less than 25 isolates were available

a) Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-

nificant change. Gentamycin resistance increased to 12% from 2% in 2016 (Figure 6.2). This year, 56% of the *S.* Typhimurium isolates carried the ASuT resistance profile and 68% were multi-resistant. Two monophasic *Salmonella* isolates were sensitive to all antibiotics and seven only resistant to tetracycline. One monophasic isolate from pork was resistant to azithromycin and sulfonamide.

None of the *S.* Typhimurium isolates from pigs or domestic pork were resistant to quinolones (ciprofloxacin and nalidixic acid), cephalosporins (cefotaxime and ceftazidime) or carbapenems (meropenem), providing 95% confidence that these resistances are not present in more than 13% of *S.* Typhimurium isolates from pigs and 7% *S.* Typhimurium from pork (See Chapter 9, Materials and Methods).

Tetracyclines, macrolides (mainly Tylosin and Tilmicosin), pleuromutilins and beta-lactamase sensitive penicillins are the main antimicrobial agents used in pigs in Denmark (Figure 4.4). The distinct changes in usage of tetracycline over the last 4-5 years in pigs were not reflected in the 2017 levels of resistance in *S.* Typhimurium from pigs and domestically produced pork. The levels of resistance to tetracycline continued to increase, despite an almost 50% reduction in DAPD of tetracycline in Danish pigs since 2013. This is due to the continued increase of the proportion of monophasic Typhimurium variants, which dominates the resistance patterns and often are multi-resistant (Figure 6.2).

Table 6.1 Resistance (%) among *Salmonella* Typhimurium[a] isolates from pigs, Danish pork and human cases[b], Denmark DANMAP 2017

Antimicrobial agent	Pigs	Pork	Human cases		
	Danish %	Danish %	Domestically acquired %	Travel abroad reported %	Total %
Tetracycline	71	63	68	65	67
Tigecycline	0	0	<1	11	3
Chloramphenicol	5	19	17	11	13
Ampicillin	48	77	73	62	68
Cefotaxime	0	0	2	4	2
Ceftazidime	0	0	1	4	1
Meropenem	0	0	0	0	0
Trimethoprim	10	12	1	15	6
Sulfonamide	52	81	74	71	70
Azithromycin	0	2	0	5	1
Gentamicin	5	12	5	5	5
Ciprofloxacin	0	0	3	11	5
Nalidixic acid	0	0	<1	4	2
Colistin	0	0	<1	2	<1
Fully Sensitive (%)	19	14	22	24	22
Number of isolates	21	43	151	55	288

a) Include isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-
b) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease
c) All strains classified as tigecyline resistant had a MIC value that was one dilution step higher than the ECOFF

6.2.3 Resistance in other relevant *Salmonella* serotypes in pigs and pork

S. Derby was isolated from 21 slaughter pigs and from 22 Danish pork samples. *S.* Derby is common among pigs, but only few human cases (n=12) were reported in Denmark in 2017 [Annual Report on Zoonoses in Denmark 2017]. In 2015, 72% of *S.* Derby were sensitive to all antimicrobials tested, in 2016 the proportion was 63%, and in 2017 60% of *S.* Derby isolates were fully sensitive to all antimicrobials tested. Resistance to tetracycline, ampicillin, sulphonamides and trimethoprim were most common, either alone or in combination. Only 15% of isolates were multi-resistant, one isolate was resistant to azithromycin and two to chloramphenicol, but no resistance to any other antimicrobials was found.

6.2.4 Resistance in *Salmonella* in imported pork

A total of 153 samples were collected from imported pork, yielding 16 *Salmonella* isolates (11% positive). Half of these were monophasic *Salmonella*, five were diphasic *S.* Typhimurium, two *S.* Derby and the last one was a *S.* London isolate. One monophasic, both *S.* Derby isolates and the *S.* London isolate were sensitive to all antibiotics in the panel. Of all the isolates, 44% were multi-resistant. One *S.* Typhimurium isolate was resistant to nalidixic acid and ciprofloxacin and multi-resistant, but apart from that isolate all the other exhibited patterns similar to the Danish meat.

6.2.5 *Salmonella* in humans.

In 2017, *Salmonella* continued to be the second most frequent cause of bacterial intestinal infections in Denmark. A total of 1,067 human laboratory-confirmed cases of salmonellosis were reported (18.5 cases per 100,000 inhabitants). The most common serotypes were *S.* Typhimurium (including the monophasic variants) and *S.* Enteritidis with 5.0 and 3.9 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2017].

6.2.6 *S.* Typhimurium in humans

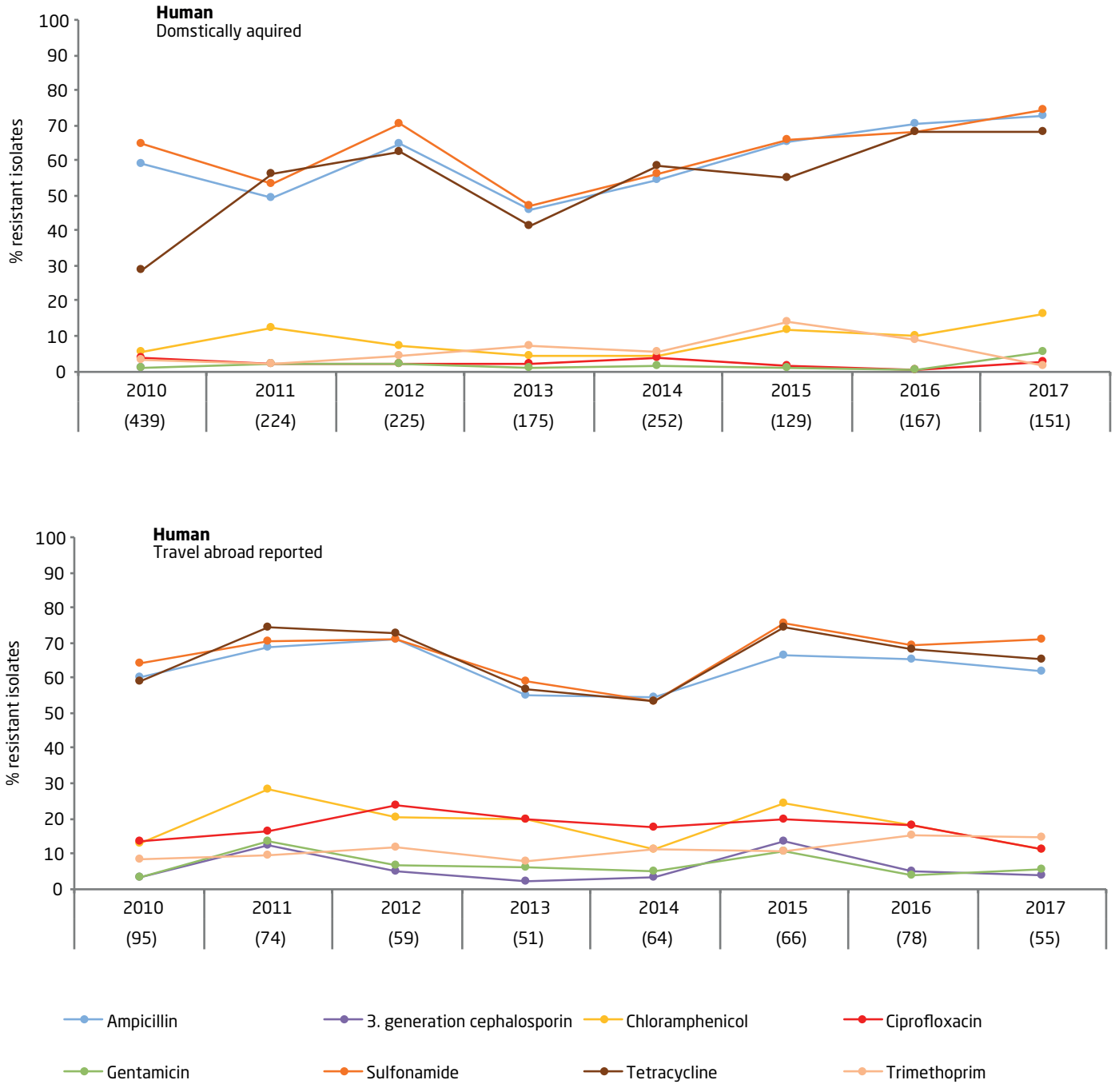
The serotypes of *Salmonella* were mainly established from whole genome DNA sequences. *Salmonella* Typhimurium, including the monophasic variants were most commonly identified among the human cases (290 cases) and MIC data from 288 of these isolates were included in this report. The monophasic variants represented approximately two thirds of the *S.* Typhimurium cases (174 monophasic and 114 diphasic). Information on patient travel history was available for 72% of the 288 case, 19% of the cases were categorised as travel associated, 52% were likely acquired in Denmark, and the remaining cases had unknown travel status (Table 6.1). A total of 80 human cases were considered 'outbreak-related'

and originated from 11 outbreaks of which 7 were caused by monophasic *S.* Typhimurium. The largest outbreak included 16 patients. None of the outbreaks were associated with travel.

As in the previous years, high levels (67-70%) of resistance to ampicillin, sulfonamide, and tetracycline were observed (Table 6.1). Isolates that exclusively exhibited resistance towards the three antimicrobials were especially common among monophasic *Salmonella*, and 62% of all monophasic variants (n = 108) harboured this resistance profile.

The levels of resistance in strains from domestically acquired cases are at the same levels as in the previous years with two minor deviations (Figure 6.3). From 2016 to 2017, an increase from 10% to 16% was observed for chloramphenicol resistance and a significant decrease from 9% to 1% was observed for trimethoprim. The increase in chloramphenicol resistance can be explained by two outbreaks that were caused by chloramphenicol resistant strains. There is no obvious explanation for the decrease in trimethoprim resistance, but trimethoprim resistance was not observed among the 80 outbreak strains tested.

Figure 6.3 Resistance (%) in *Salmonella* Typhimurium[a] in human cases[b], Denmark DANMAP 2017



a) Include isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-

b) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

The levels of resistance in isolates from human cases associated with travel are also in line with the observed levels in the previous years. The level of fluoroquinolone resistance (ciprofloxacin) decreased from 18% to 11% from 2016 to 2017, but the level is still significantly higher in travel-associated isolates compared to isolates from domestic cases (Figure 6.3).

Resistance to colistin was observed in isolates from both domestically acquired (<1%) and travel associated human cases (2%). The level of cephalosporin resistance was 2% for cefotaxime and 1% in ceftazidime. Carbapenem resistance (meropenem) was not observed in any of the tested strains.

6.3 *Campylobacter*

Thermotolerant *Campylobacter* spp. are the most commonly reported causes of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union [EU Summary Report 2014, ECDC/EFSA 2015]. In Denmark, 85-95% of the human campylobacteriosis cases are caused by *C. jejuni*.

Campylobacter are widespread in nature and the most important reservoirs are the alimentary tract of wild and domesticated birds and mammals. Among sporadic human cases, broilers have been identified as the primary source of infection, though other sources such as untreated water and other infected animals are also important.

In 2017, most of the *Campylobacter* isolates for DANMAP were obtained by sampling of randomly selected broilers and cattle at slaughter (caecum). In humans, Campylobacteriosis is a notifiable disease, but only a selection of isolates from reported human *C. jejuni* cases are susceptibility tested. Only one isolate per farm or human case was included. For details see Chapter 9, Materials and Methods. The susceptibility methods follow EFSAs recommendations and MIC distributions for *C. jejuni* from broilers, cattle and humans are presented in the web annex (Tables A6.6 - A6.7).

6.3.1 *C. jejuni* in broilers

A total of 43 *C. jejuni* isolates were derived from 163 broiler flocks sampled throughout the year. The level of fully sensitive isolates from broilers was 74%, which was similar to previous years.

Resistance to ciprofloxacin (26%) and nalidixic acid (26%) were most frequently observed and always in the same isolates (Table 6.2). Since 2007, a slow increase in proportion of ciprofloxacin resistant isolates has been observed from approximately 10% in 2006 to 26% in 2017, despite little or no use of quinolones in the poultry industry since 2009. The Danish increasing trend of resistance to quinolones is similar to the increasing trend of ciprofloxacin resistance in the EU, but the levels in Denmark still remain much lower than in the EU, where overall 62-66% of isolates were resistant to quinolones [EFSA 2018. EFSA Journal 16(2): 5182]. All ciprofloxacin resistant isolates were also resistant to nalidixic acid suggesting a chromosomal resistance mechanism.

Tetracycline resistance increased slightly from 12% to 16% in broilers after a steady increase peaking in 2013 followed by a drop back to 2008 level. All tetracycline resistant isolates were also resistant to ciprofloxacin and nalidixic acid. Thus, there is no clear relationship between resistance and the use of tetracycline in broilers, which increased sharply from 2012 to 2015 and decreased in 2016.

No resistance to erythromycin, gentamycin or streptomycin was observed in 2017, suggesting that resistance to these antimicrobials are only present in 7% or less of the *Campylobacter* isolates from broilers (See Chapter 9, Materials and Methods).

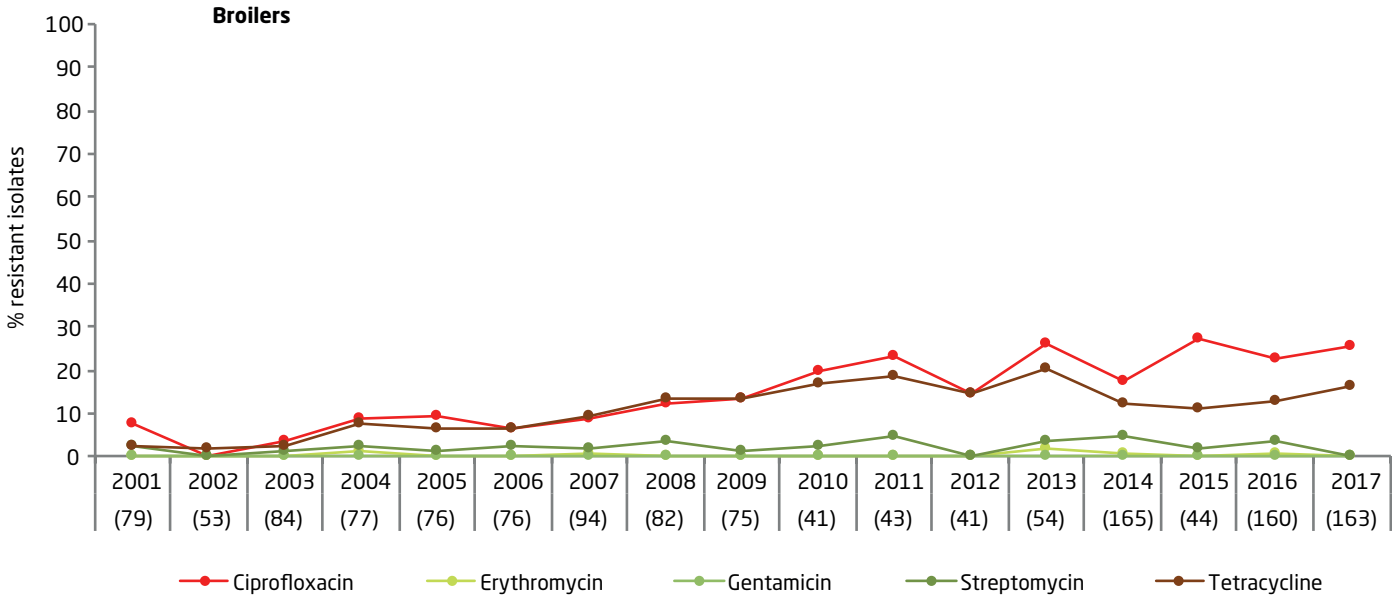
Table 6.2 Resistance (%) in *Campylobacter jejuni* isolates from animals, meat of Danish and imported origin and human cases[a], Denmark

DANMAP 2017

Antimicrobial agent	Cattle	Broilers	Humans		
	Danish %	Danish %	Domestically acquired %	Travel abroad %	Total %
Tetracycline	7	16	22	68	32
Erythromycin	<1	0	1	6	2
Streptomycin	<1	0	0	-	-
Gentamicin	0	0	1	3	1
Ciprofloxacin	30	26	37	92	49
Nalidixic acid	30	26	37	92	49
Fully sensitive (%)	67	74	59	8	48
Number of isolates	236	43	252	79	397

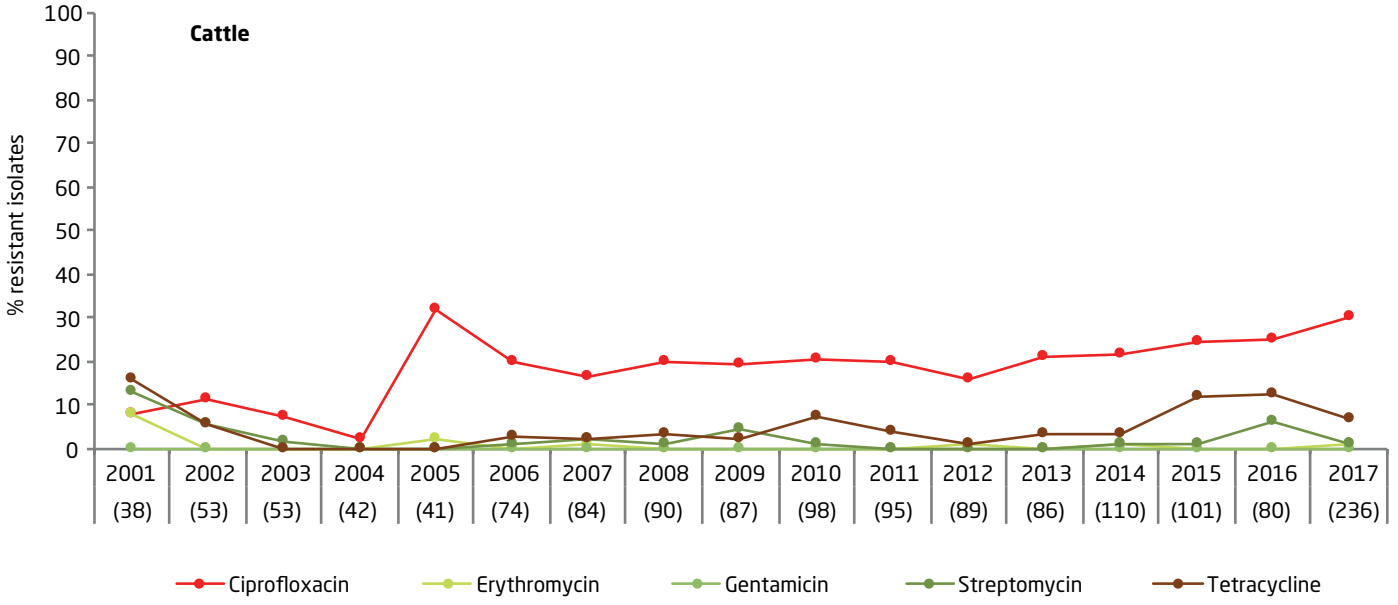
a) An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Figure 6.4 Resistance (%) among *Campylobacter jejuni* from broilers, Denmark DANMAP 2017



Note: Number of isolates included each year is presented in the parenthesis

Figure 6.5 Resistance (%) among *Campylobacter jejuni* from cattle, Denmark DANMAP 2017



Note: Number of isolates included each year is presented in the parenthesis

6.3.2 *C. jejuni* in cattle

A total of 236 *C. jejuni* isolates were isolated from 297 cattle caeca taken at slaughter from all over Denmark. All isolates were susceptibility tested. Most of the isolates (67%) were fully sensitive and the remaining isolates were resistant to ciprofloxacin, tetracycline, streptomycin and erythromycin (30%, 7% 1% and 1% of all isolates, respectively) and to various combinations of these (Table 6.2).

Resistance to ciprofloxacin continued to increase, although the consumption of fluoroquinolones by cattle in Denmark is almost none (Figure 6.5). The epidemiology of these resistant strains is unknown, but the co-resistance with nalidixic acid suggested chromosomal resistance rather than plasmid borne. Resistance to streptomycin increased in 2016, but the levels are back to 1% in 2017, suggesting the peak may have been a result of the small sample size last year. A reduction in resistance to tetracycline was also observed after two years of high prevalence. The use of tetracycline in cattle has decreased over the last 5 years, but it is uncertain whether the decline in antimicrobial resistance is directly associated to less use.

6.3.3 *Campylobacter* in humans

Campylobacter, and in particular *C. jejuni*, continued to be the most frequent cause of bacterial intestinal infections in Denmark in 2017 with a total of 4,257 reported human laboratory confirmed cases of campylobacteriosis (73,9 per 100,000 inhabitants) [Annual Report on Zoonoses in Denmark 2017].

A representative selection of *Campylobacter* isolates submitted to Statens Serum Institut (SSI) by regional clinical microbiological laboratories were identified to species level and susceptibility testing was performed on 397 isolates of *C. jejuni*. A total of 252 of these isolates were from patients with no history of travel abroad, 79 isolates were from travel-associated cases and 66 isolates were from patients with no information on travel.

Among the domestically acquired infections, 148/252 (59%) of isolates were fully sensitive to all the antimicrobials tested. The proportion was significantly lower for isolates from travel-associated cases (6/73, 8%) (Table 6.2).

In isolates from domestically acquired cases, the most frequent resistance profile was the combination of ciprofloxacin and tetracycline, which was found in 44% of the resistant isolates. Resistance to erythromycin and gentamicin were observed in 1% of the isolates (Table 6.2). The levels of resistance in isolates from domestically acquired cases were similar to previous years (Figure 6.6).

In line with previous years, the level of resistance in isolates from travel-associated cases was higher than the levels in isolates from domestically acquired cases. The difference was statistically significant for ciprofloxacin, tetracycline, and erythromycin. From 2016 to 2017, a significant increase in ciprofloxacin resistance was observed and it reached an all-time high with 92% in isolates from patients with a travel history in 2017.

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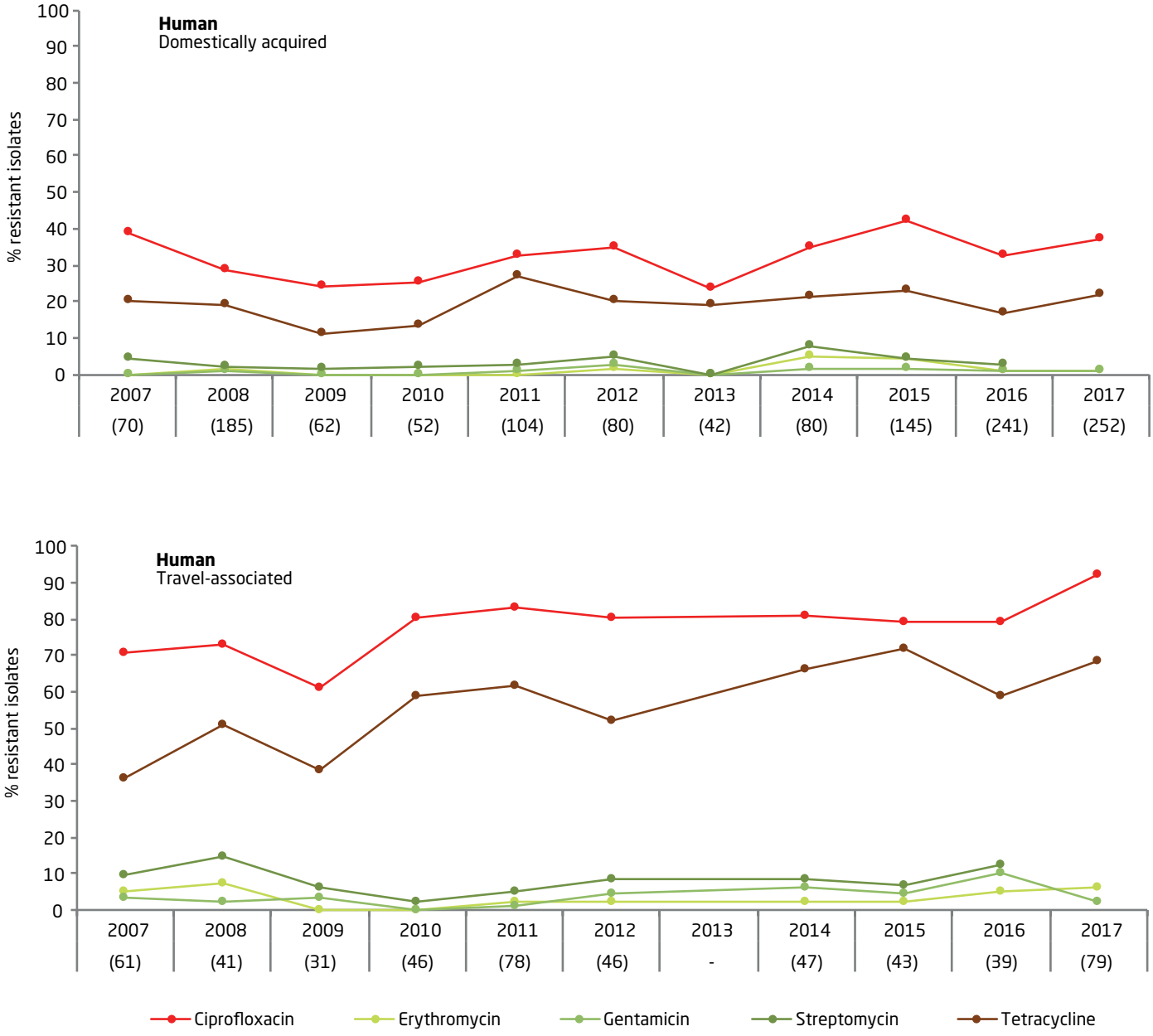
Figure 6.6 Resistance (%) among *Campylobacter jejuni* from humans[a], Denmark DANMAP 2017



Note: Number of isolates included each year is presented in the parenthesis. The '-' indicate data for application of 2016 cut-offs not available or less than 25 isolates were available

a) An isolate was categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

Textbox 6.1

Resistance in bacteria from diagnostic submissions from pigs

Data on susceptibility of three important veterinary pathogens *Escherichia coli* O149, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae* were obtained from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. The number of isolates belonging to other bacterial species was too small to be included in this overview.
The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Since approved clinical breakpoints are not available for most of the drug-bacterium combinations, the results are presented both as MIC distributions, which allows for the reader's own interpretation, and as % resistant isolated according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases.

*E. coli O*149

Enterotoxigenic *E. coli* (ETEC) in combination with *Brachyspira pilosicoli* and *Lawsonia intracellularis* are the most prevalent causes of bacterial diarrhoea in Danish pigs. Most cases of diarrhoea that require treatment occur during the weaning period and tetracyclines, neomycin or aminopenicillins are the compunds of choice. The most virulent ETEC strains belong to serovars O138, O139, O141, and O149, are haemolytic and positive for enterotoxin and for F4 or F18 fimbrial adhesins, which are used for attachment to the intestinal mucosa. In general, the F18 positive strains belong to the serovars O138, O139 and O141, while serovar O149 carry the F4 fimbriae. The MIC distributions and resistance data for the 72 serovar O149 isolates from 2017 are shown in Table 1. High resistance levels were recorded for ampicillin, streptomycin, sulphonamides, tetracyclines, trimethoprim, and spectinomycin. Numerically, tetracycline, ampicillin, and sulphonamides resistance decreased compared to 2016, whereas resistance to trimethoprim, streptomycin, and spectinomycin increased. It is uncertain whether the decrease in tetracycline resistance is due to natural variation or whether it is a trend following the decrease in tetracycline usage. An increase was also noted for florfenicol, now mounting 18% compared to 10% in 2016 and 2015, but 3% in 2012 and zero in 2011. The reasons for this apparent steady increase need further investigation. Isolates that were resistant to florfenicol were also resistant to chloramphenicol, but resistance levels to chloramphenicol did not increase. Fourteen isolates (19%) were resistant to nalidixic acid compared to 10% in 2016. These isolates also had increased MIC values to ciprofloxacin, but all remained under the breakpoint. This finding is surprising, since no quinolones are used in Danish pig production. Notably, resistance to colistin remains at zero. The relatively high resistance levels to many compounds increase the benefits of susceptibility testing before treatment. The resistance profile of the serovar O139 isolates deviated somewhat from that of O149, and in general O139 shows less resistance. Among 76 O139 isolates from 2016 and 2017 combined, only 3 (4%) were resistant to florfenicol, while no isolates were resistant to nalidixic acid or neomycin, and resistance to other compounds were lower or at the same level as for O149 isolates (data not shown). In Denmark, the serovar O139 is almost exclusively responsible for edema disease and carries F18 fimbriae and verotoxin 2e, but no enterotoxins. Prophylaxis of edema disease is often performed effectively by vaccination, while this is still not the case for diarrhoea caused by the other serovars of *E. coli*.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumonia causes pleuropneumonia in pigs. Severity differs with serotype, but common clinical signs are fever, coughing, depression, loss of appetite, and bloody discharge from the nose. Outbreaks need rapid onset of treatment to minimize losses, but fortunately, *A. pleuropneumoniae* remains to have a predictable resistance pattern and low resistance to most compounds, including florfenicol and macrolides like tilmicosin, tildipirosin and tulathromycin, which are often used for treatment. MIC distributions and percent resistance are shown in Table 2. All 135 isolates were resistant to erythromycin, but almost fully susceptible to all other compounds, including to other macrolides. There are several O-serotypes, the far most prevalent ones among the clinical isolates being O2 and O6, but resistance patterns did not differ between the two serotypes.

Streptococcus suis

Streptococcus suis may cause several different infectious conditions in pigs, such as meningitis, otitis media, arthritis, pneumonia, and septicaemia, and causes losses to the farmers due to increased mortality and veterinary costs. MIC distributions and percent resistant isolates are shown in Table 3. Resistance in the 152 isolates was highest to macrolides (erythromycin), streptomycin, and tetracyclines, although resistance to tetracyclines had decreased from 51% in 2016 to 40% in 2017. All isolates were susceptible to both penicillin and florfenicol. Several serotypes of *S. suis* exist and in Danish pig production the serotypes 1, 2, 7, and 9 are the most often isolated. There seems to be differences in resistance patterns between serotypes, however, this needs further investigation.

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continued ... Textbox 6.1

Table 1 Distribution of MICs and resistance (%) in clinical *Escherichia coli* O149 from pigs (n=72), Denmark DANMAP 2017

Antimicrobial agent	Clinical breakpoint µg/ml	% Resistant	95% Confidence interval	Distribution (isolates) of MICs																
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	>1024
Tetracycline	>8	62.5	[51.6-73.4]								36.1	1.4			2.8	59.7					
Chloramphenicol	>16	23.6	[14-33.3]									50.0	23.6	2.8	4.2	1.4	18.1				
Florfenicol	>16	18.1	[9.2-26.9]								4.2	51.4	26.4				18.1				
Ampicillin	>16	45.8	[34.6-57]							6.9	43.1	4.2			1.4	44.4					
Cefotaxime	>0.25	2.8	[0-7.2]				97.2						2.8								
Sulfonamide	>256	61.1	[50.1-72.1]													38.9			1.4	59.7	
Trimethoprim	>8	55.6	[44.4-66.7]							44.4						55.6					
Apramycin	>16	9.7	[2.7-16.7]									87.5	2.8		9.7						
Gentamicin	>4	9.7	[2.7-16.7]						80.6	9.7			4.2	4.2	1.4						
Neomycin	>8	13.9	[5.9-21.9]								86.1				1.4	12.5					
Streptomycin	>16	77.8	[68.3-87.2]										20.8	1.4	16.7	8.3	16.7	36.1			
Ciprofloxacin	>2	0.0	[0-2.5]	69.4	9.7	1.4		15.3	4.2												
Nalidixic acid	>16	19.4	[10.4-28.5]									77.8	2.8			19.4					
Colistin	>8	0.0	[0-2.5]							98.6			1.4								
Spectinomycin	>64	56.9	[45.8-68.1]											37.5	1.4	4.2		20.8	36.1		

Note: Interpreted by clinical break-points for *E. coli* isolate from pigs as applied by DTU National Veterinary Institute and The Danish Pig Research Centers Laboratory for Pig Diseases. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals. Vertical solid lines indicate EUCAST epidemiological cut-off values. Breakpoints are indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table 2 Distribution of MICs and resistance (%) in clinical *Actinobacillus pleuropneumoniae* from pigs (n=135), Denmark DANMAP 2017

Antimicrobial agent	Clinical breakpoint µg/ml	% Resistant	95% Confidence interval	Distribution (isolates) of MICs															
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256
Tetracycline	>4	2.2	[0-5]					12.6	54.1	29.6	0.7	0.7	2.2						
Florfenicol	>4	0.0	[0-1.4]				3.0	88.9	7.4	0.7									
Ampicillin	>2	0.7	[0-2.7]						99.3						0.7				
Penicillin	>2	0.7	[0-2.7]	0.7	0.7	3.0	23.0	42.2	26.7	3.0					0.7				
Ceftiofur	>4	0.0	[0-1.4]		96.3	3.0	0.7												
Sulfa-trimethoprim	>2	0.0	[0-1.4]		45.9	48.1	5.9												
Erythromycin	>0.5	100.0	[100-100]								2.2	28.9	65.9	3.0					
Tulathromycin	>8	1.5	[0-3.9]									2.2	50.4	45.9	0.7	0.7			
Tilmicosin	>16	0.0	[0-1.4]									2.2	37.8	59.3	0.7				
Tiamulin	>16	0.0	[0-1.4]								0.7	0.7	24.4	60.7	13.3				
Spectinomycin	>64	0.0	[0-1.4]											2.2	34.1	63.7			

Note: Interpreted by clinical break-points for *Actinobacillus* isolates from pigs as applied by DTU National Veterinary Institute and The Danish Pig Research Centers Laboratory for Pig Diseases. Breakpoints are indicated as vertical dotted lines. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals. White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table 3 Distribution of MICs and resistance (%) in clinical *Streptococcus suis* from pigs (n=152), Denmark DANMAP 2017

Antimicrobial agent	Clinical breakpoint µg/ml	% Resistant	95% Confidence interval	Distribution (isolates) of MICs														
				0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512
Tetracycline	>4	40.1	[32.4-47.8]						8.6	16.4	15.8	19.1	0.7	0.7	11.2	27.6		
Chloramphenicol	>8	0.0	[0-1.2]								15.1	75.7	9.2					
Florfenicol	>16	0.0	[0-1.2]							25.0	73.7	1.3						
Penicillin	>2	0.0	[0-1.2]			89.5	2.6	3.9	1.3	2.6								
Sulfa-trimethoprim	>2	8.6	[4-13.1]			80.3	1.3	3.9	5.9	3.3	1.3	2.0	2.0					
Trimethoprim	>8	9.9	[5.1-14.7]				75.5	0.7	0.7	8.6	4.6		1.3	8.6				
Erythromycin	>0.5	48.7	[40.8-56.5]			50.7	0.7	0.7		0.7	0.7	1.3	45.4					
Streptomycin	>16	47.4	[39.5-55.2]							9.2	26.3	17.1	16.4	3.3	27.6			
Tiamulin	>16	23.7	[17-30.4]			5.9	7.9	21.1	28.3	5.9	4.6	2.6	13.8	9.9				
Spectinomycin	>64	25.7	[18.8-32.5]									45.4	28.9			2.0	23.7	

Note: Interpreted by clinical break-points for *Streptococcus* isolates from pigs as applied by DTU National Veterinary Institute and The Danish Pig Research Centers Laboratory for Pig Diseases. Breakpoints are indicated as vertical dotted lines. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals. White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Textbox 6.2

MRSA contamination of human volunteers after short time visit in MRSA positive pig farms

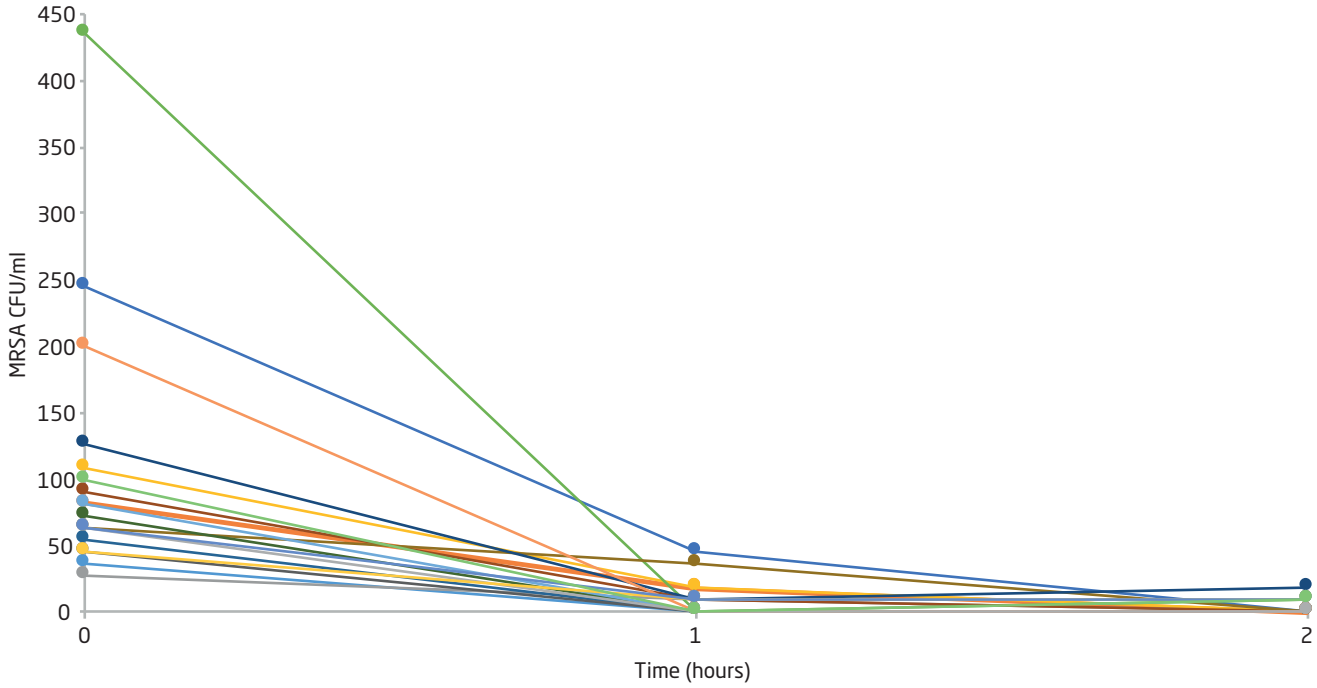
Spread of MRSA from pig farms to the community and ultimately to the hospitals is a major concern. In 2017, people with contact to pigs comprised 84% (n = 1,019) of all new LA-MRSA cases, while the remaining 16% (n = 193) of the new LA-MRSA cases did not report any contact to pigs and thus may have been colonized or infected due to secondary transmissions. Spread of LA-MRSA from pig farms most likely occurs by humans carrying MRSA out of the farms. Knowledge of the extent to which this happens is therefore pivotal for designing initiatives to prevent spread of LA-MRSA.

In 2016, a study of four trials was conducted to investigate the frequency and duration of MRSA carriage in humans after a short-term exposure at a methicillin-resistant *Staphylococcus aureus* (MRSA) positive pig farm.

The group of altogether 34 volunteers stayed for 1 hour at the pig farm. In two of the trials, the influence of farm work with pig contact was studied. In these trials, the volunteers were allocated into an active group (collecting nasal and skin swabs from the pigs) and a passive group (standing in the row between the pens). In order to diminish individual factors for MRSA carriage, a crossover study design was chosen, meaning that the volunteers changed group after a three-week washout period. In the two other trials, all volunteers were passive. The quantities of MRSA in nasal swabs, throat swabs, and air samples were measured at different time points and analyzed in relation to relevant covariates. After the visits in the pig farm, 94% of the volunteers had acquired MRSA with no significant differences between volunteers in the active or passive group. Two hours after the volunteers left the stable, the nasal MRSA count had declined to unquantifiable levels in 95% of the samples. After 48 hours, 94% of the volunteers were MRSA-negative. One volunteer was MRSA positive at day 7 but negative at day 14. An example of the MRSA decrease in nasal specimens of the volunteers after leaving the farm is shown in Figure 1.

Figure 1 Nasal MRSA count (CFU/ml swab fluid) of human volunteers 0, 1 and 2 hours after leaving the stable. One example from the four trials is shown.

DANMAP 2017



All volunteers carried personal air samplers to measure their individual exposure to MRSA. These experiments showed a positive correlation between the nasal MRSA level immediately after leaving the stable and personal exposure to airborne MRSA. Being in the active group resulted in the highest level of personal exposure and nasal MRSA counts. Nasal MRSA carriage was therefore positively correlated to personal exposure to airborne MRSA and farm work involving pig contact but no association was observed between MRSA carriage and face touching behavior, nasal methicillin-susceptible *Staphylococcus aureus* (MSSA) carriage, age, or gender (1).

continued ... Textbox 6.2

The increase in human MRSA carriage among the volunteers with pig contact therefore seems to depend on the increased concentration of airborne MRSA during work and not directly on physical contact with pigs. MRSA was not detected in any of the throat samples.

In conclusion, the short-term exposure to airborne MRSA poses a substantial risk for farm visitors to become nasal carriers, but the carriage is typically cleared within hours to a few days. The risk for short-time visitors to cause secondary transmissions of MRSA is most likely negligible due to the observed decrease to unquantifiable levels in 95% of the nasal samples after only 2 hours. Interventions to reduce the level of airborne MRSA or the use of facemasks might consequently reduce nasal contamination.

In a different experiment in 2017, the effect of wearing P2 facemasks (normal dust masks) to prevent MRSA contamination during a short-term visit was investigated. A total of 118 human volunteers from five agricultural colleges were randomly allocated into a mask-wearing group and into a control group. After a one-hour stay on a MRSA positive pig farm, on average 9% of the participants wearing masks were MRSA-positive compared to 62% of the participants not wearing masks. An odds ratio of 18.9 (CI: 6.4-56.2) for being MRSA-positive was found for those not wearing masks compared to those wearing masks.

Introducing masks to short-time visitors in MRSA positive pig farms may therefore protect against MRSA contamination during the farm stay. Due to the fast clearance of MRSA from the nose, the implications for secondary transmission by short-time visitors however seems negligible. Working with P2 facemasks is only allowed for 3 hours daily and thus cannot be used as protection for farmworkers staying at the farms for longer periods. Use of facemasks as protection against MRSA colonization could however be of interest for people that are repeatedly exposed to MRSA at pig farms for shorter periods, example veterinarians.

Both studies were part of the One Health LA-MRSA program (OHLAM) sponsored by the Ministry of Environment and Food. The first study was conducted by Statens Serum Institut in collaboration with the National Research Centre for the Working Environment (Angen et al. 2017). The second study was conducted by Statens Serum Institut in collaboration with SEGES.

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References:

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7

RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Highlights: In 2017, resistance to erythromycin and tetracycline was observed in 55% and 78% of the *Enterococcus faecalis* isolates from Danish pigs, respectively. A few gentamicin-resistant isolates (non-HLGR) were observed, where as resistance to other antimicrobial agents critical to human medicine was not detected.

The proportion of fully susceptible indicator *E. coli* increased slightly in poultry and pigs, and decreased slightly in cattle in 2017 compared to 2016. Resistance patterns and levels in indicator *E. coli* from poultry, pigs and cattle were overall similar to previous years and no resistance to colistin, meropenem and tigecycline was detected.

ESBL/AmpC-producing *E. coli* were recovered from 25% and 7% of the samples from Danish pigs and cattle, from 1% and 4% of the samples from domestically produced pork and beef, and from 14% and 3% of the samples from imported pork and beef. The ESBL/AmpC occurrence in *E. coli* from the sources monitored in 2017 was comparable to the levels observed in 2015. ESBL/AmpC genotypes were determined for isolates from meat only. CTX-M-1 was the most common ESBL in *E. coli* from domestically produced pork and imported pork and beef. CTX-M-14, CTX-M-15, and CTX-M-1 were equally prevalent in domestically produced beef isolates. No plasmid-mediated AmpCs such as CMY was detected.

7.1 Introduction

Enterococci (*E. faecium* and *E. faecalis*) and *Escherichia coli* are included in the DANMAP programme to monitor antimicrobial resistance in Gram-positive and Gram-negative bacteria, respectively. These bacterial species were selected as indicators of occurrence of antimicrobial resistance in different reservoirs through the food chain for several reasons. They are ubiquitous and present as commensals in the gut microbiota of both animal and human reservoirs, they can acquire antimicrobial resistance and resistance genes may be maintained as a response to antimicrobial selective pressure, and finally they have the potential both to cause infections in humans and to transfer antimicrobial resistance to pathogenic bacteria of the same or other species.

Extended-spectrum beta-lactamase-producing (ESBL/AmpC) bacteria exhibiting resistance to third-generation cephalosporins are one of the fastest spreading antimicrobial resistance problems in both humans and production animals worldwide. Recently, several studies have found similar ESBL/AmpC genes, plasmids and/or clones of *E. coli* isolates in animals and meat thereof and in human infections, which suggests a zoonotic link. Carbapenemase-producing Enterobactericeae (CPE) are an even greater threat to human health, since carbapenems are the last-line antimicrobial agents for treatment of infections caused by multidrug-resistant Gram-negative bacteria.

Since 2014, sampling and testing of *Enterococcus* spp., indicator *E. coli* and ESBL/AmpC-producing *E. coli* have been performed according to the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU]. In DANMAP 2017, indicator bacteria and ESBL/AmpC producing *E. coli* originate from caeca of pigs and cattle at slaughter. ESBL/AmpC producing *E. coli* from and in pork and beef at retail were also included in the EU mandatory sampling.

Table 7.1 Resistance (%) among *Enterococcus faecalis* from pigs, Denmark DANMAP 2017

Antimicrobial agent	Pigs Danish %
Tetracycline	78
Tigecycline	0
Chloramphenicol	24
Ampicillin	0
Erythromycin	55
Gentamicin	7
Ciprofloxacin	0
Vancomycin	0
Teicoplanin	0
Linezolid	0
Daptomycin	0
Fully sensitive (%)	22
Number of isolates	55

7.2 Enterococci

DANMAP 2017 *Enterococcus faecalis* isolates originate from randomly collected caecal samples from healthy fattening pigs at slaughter. The antimicrobials recommended by EFSA were used for MIC testing.

MIC distributions and occurrence of resistance among *E. faecalis* from pigs are presented in the web annex (Table A7.1). *E. faecalis* is considered intrinsically (i.e. naturally) resistant to streptogramin A and B (quinupristin/dalfopristin), and interpretation of the MIC testing for this antimicrobial agent was not evaluated in DANMAP.

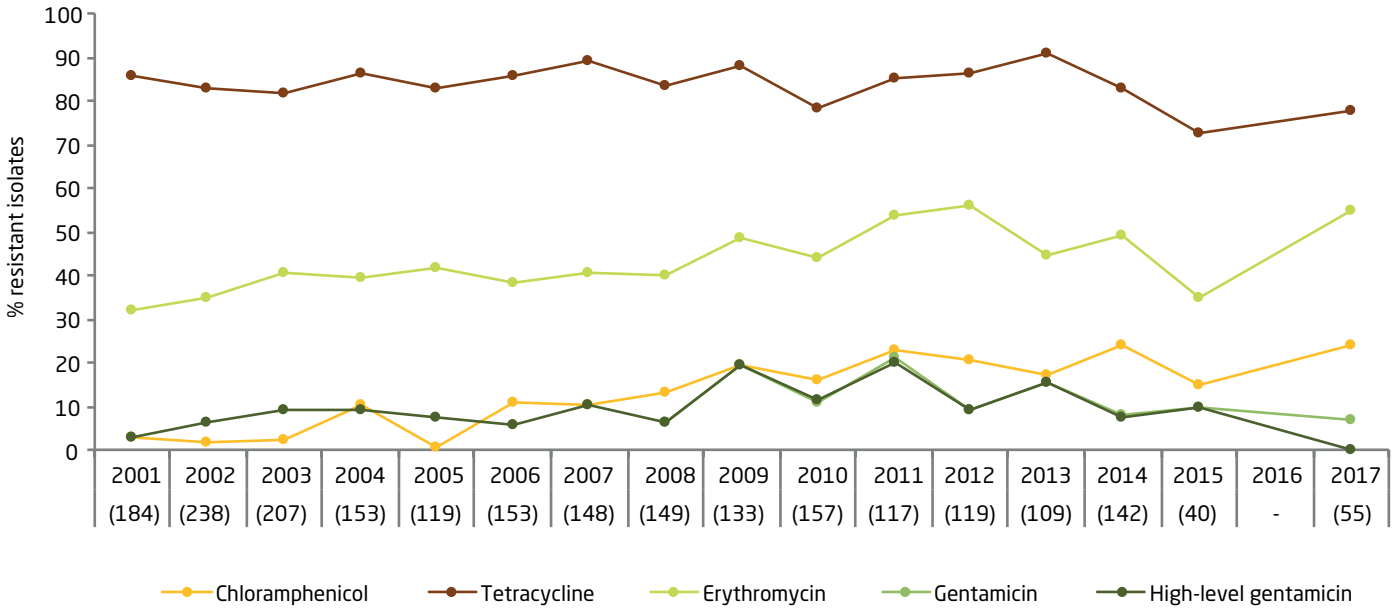
7.2.1 *E. faecalis* in pigs

A total of 55 *E. faecalis* isolates were derived from 295 pig caeca from samples taken from all over Denmark. In 2017, the majority of *E. faecalis* from Danish pigs were resistant to tetracycline (78%) and half of the isolates were resistant to erythromycin (55%, Table 7.1). Tetracyclines and macrolides (e.g. erythromycin) are the most commonly used antibiotics in the Danish pig production (Figure 4.4). All isolates resistant to erythromycin were also resistant to tetracycline (n=30). Chloramphenicol resistance was also common (24%), and all chloramphenicol resistant isolates was also co-resistant to both erythromycin and tetracycline (n=13). Twelve isolates (22%) were susceptible to all antimicrobials tested.

From 2004 to 2012, increasing resistance to erythromycin (from approximately 30% to 56%) and chloramphenicol (from less than 5% to around 20%) was reported in *E. faecalis* from pigs (Figure 7.1). However, over the last five years, the observed levels of resistance have varied more or less within the same ranges, where the reduced number of available isolates probably is a contributing factor.

Tetracycline resistance varied between 80% and 90% during 2004-2012, not reflecting the visible increase in use of tetracycline in the Danish pig production during the same period (Figure 4.4). Since 2013, the consumption of tetracycline in pigs is reduced around 50% (Figure 4.4; all age groups, adjusted) and the observed levels of tetracycline resistance in 2015 (73%) and 2017 (78%) was significantly lower than in 2013 (91%). More samples are needed to verify whether the reduction in use will lead to a reduction in tetracycline resistance.

Figure 7.1 Resistance (%) among *Enterococcus faecalis* from pigs, Denmark DANMAP 2017



Note: Number of isolates included each year is presented i the parenthesis. No isolates were collected in 2016

Resistance to gentamicin was observed in four isolates, all in combination with resistance to erythromycin, tetracycline and chloramphenicol. None of the isolates displayed high-level resistance to gentamicin, defined as resistance to MIC values >128 mg/L. From 2004-2015, high-level resistance to gentamicin was observed in 6% to 20% of the *E. faecalis* isolates from Danish pigs (Figure 7.1).

Resistance to ampicillin, ciprofloxacin, daptomycin, linezolid, tigecycline and vancomycin was absent, providing 95% certainty that resistance to these compounds are presented in less than 5% of *E. faecalis* from pigs in 2017. Resistance to ampicillin, vancomycin, tigecycline and linezolid have not been observed since these compounds were included in the test panel in 2005. A few isolates resistant to ciprofloxacin and daptomycin have been observed since 2005.

7.2.2 Perspectives

Enterococci are commensal bacteria in the intestine in both animals and humans; however both pathogenic strains of *E. faecalis* and *E. faecium* can cause human disease (chapter 8.5). Since 2012, an increase in vancomycin resistant *E. faecium* has been observed in Danish hospitals, whereas only few vancomycin resistant *E. faecalis* have been observed [Textbox 8.3].

Multi-locus sequence typing (MLST) have shown that *E. faecalis* from hospital outbreaks often belongs to specific MLST clonal complexes, however some of these sequencetypes have also been observed in food and animals. In Denmark (2001-2002), the same *E. faecalis* ST16 with high-level resistance to gentamicin (HLGR) were found in patients with endocarditis (2 of 20 HLGR isolates), from pigs (18 of 19 HLGR isolates) and from pork (1 of 1 HLGR isolate) [Larsen et al. 2010. Emerg Infect Dis. 16(4):682]. Since then, the presence of this HLGR ST16 type in patients and Danish pigs have not been investigated further, however none of the 55 isolates were high-level resistant to gentamicin, providing 95% certainty that resistance to these compounds are presented in less than 5% of *E. faecalis* from pigs in 2017. In comparison, 3.3% of the *E. faecalis* collected from pigs in 2001 displayed high-level resistance to gentamicin.

7.3 Indicator *Escherichia coli*

All isolates originated from caecal samples randomly collected from healthy pigs, broilers and cattle at slaughter. The antimicrobials recommended by EFSA were used for MIC determination. Only one isolate per farm was included. MIC distributions and occurrence of resistance among indicator *E. coli* are presented in the web annex (Table A7.2). These results were obtained using the non-selective isolation procedure. Results obtained by using selective procedures for detection of cefotaxime-resistant *E. coli* are reported in section 7.4.

7.3.2 Indicator *E. coli* from broilers

A total of 115 indicator *E. coli* isolates from broilers were tested for antimicrobial susceptibility out of 135 samples processed (Table 7.2). More than half (63%) of indicator *E. coli* were susceptible to all antimicrobials tested. Moderate (12-17%) occurrence of resistance to ampicillin, nalidixic acid, ciprofloxacin, sulfonamide, tetracycline and trimethoprim was observed. Low (3-6%) occurrence of chloramphenicol and gentamicin resistance and no occurrence of resistance to the other compounds tested were observed (Table 7.2). Occurrence of resistance to nalidixic acid and ciprofloxacin, was significantly higher in *E. coli* from broilers compared to *E. coli* from pigs and cattle.

A total of 17 resistance profiles were detected among the 42 resistant isolates. These profiles ranged from resistance to one antimicrobial (or two antimicrobials from the same class) in the majority of resistant isolates (nalidixic acid/ciprofloxacin, 24%; tetracycline, 10%; ampicillin, sulphamethoxazole or trimethoprim 5%) to resistance to compounds from four antimicrobial classes in 9% of resistant isolates. Resistance to nalidixic acid/ciprofloxacin was noticeably observed in 33% of resistant isolates mainly alone (71%) but also in combination with resistance to gentamicin (29%), which is also a critically important antimicrobial for human medicine. Co-resistance to ampicillin, sulfonamide and trimethoprim (ASuTm resistance profile) was the most common profile of resistance to antimicrobials from different classes and was found in 33% of the resistant isolates mostly in combination tetracycline and/or chloramphenicol resistance.

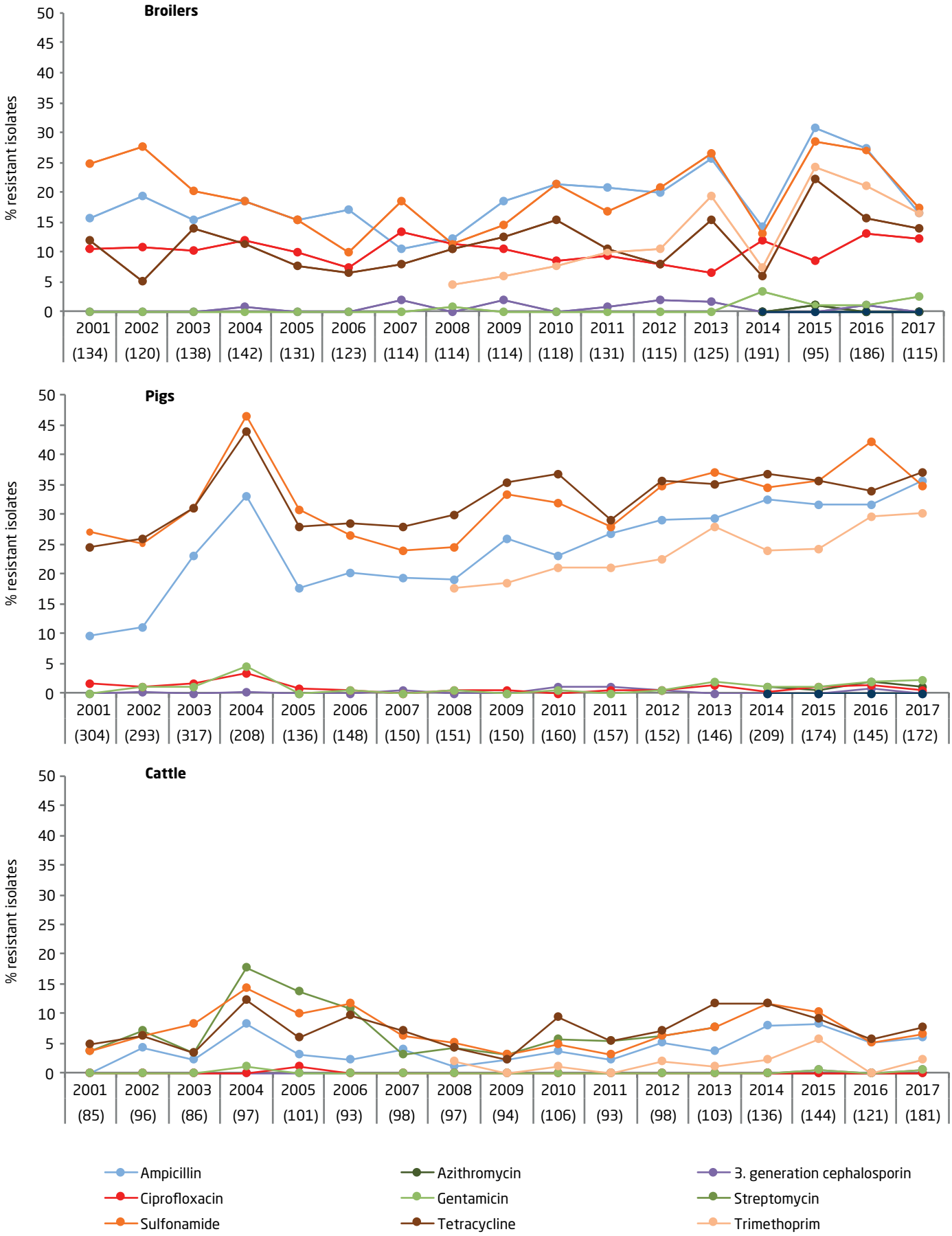
Table 7.2 Resistance (%) among *Escherichia coli* from animals, Denmark DANMAP 2017

Antimicrobial agent	Broilers	Cattle	Pigs
	Danish %	Danish %	Danish %
Tetracycline	14	8	37
Tigecycline	0	0	0
Chloramphenicol	6	6	6
Ampicillin	17	6	36
Cefotaxime	0	<1	0
Ceftazidime	0	<1	0
Meropenem	0	0	0
Trimethoprim	17	2	30
Sulfonamide	17	7	35
Azithromycin	0	0	1
Gentamicin	3	<1	2
Ciprofloxacin	12	0	<1
Nalidixic acid	12	0	<1
Colistin	0	0	0
Fully sensitive (%)	63	90	49
Number of isolates	115	181	172

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panels

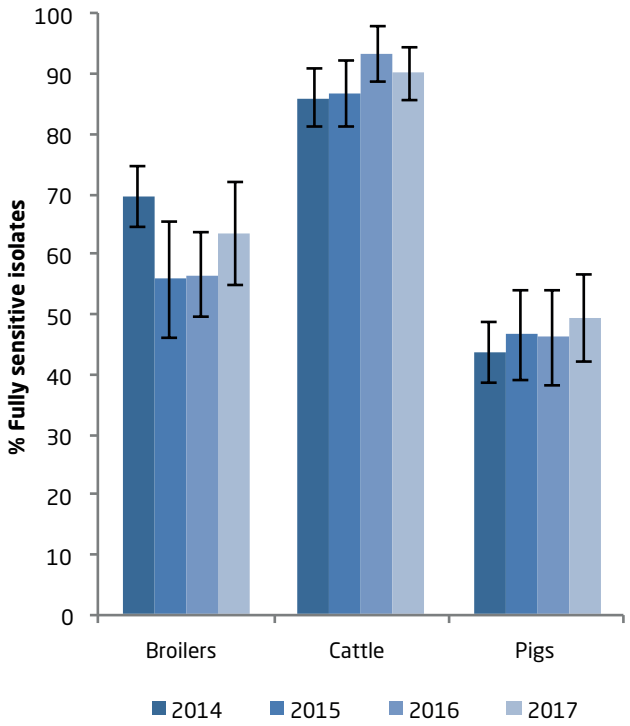
Figure 7.2 Resistance (%) among *E. coli* from animals, Denmark

DANMAP 2017



Note: The number of isolates included each year is shown in parentheses

Figure 7.3 Proportion of fully susceptible *Escherichia coli* isolates from broilers, cattle and pigs, Denmark DANMAP 2017



Note: An isolate is considered fully susceptible if susceptible to all antimicrobial agents included in the test panels. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals

Compared to 2016, occurrence of resistance to any compound except gentamicin declined (Figure 7.2). The decline in ampicillin resistance was statistically significant. The percentage of fully susceptible isolates was the second highest after 2014, which is the year when the current antimicrobial panels were introduced (Figure 7.3).

7.3.2 Indicator *E. coli* from pigs

A total of 172 indicator *E. coli* isolates from 193 pig caeca were tested for antimicrobial susceptibility (Table 7.2). Approximately half (49%) of the isolates from pigs were susceptible to all antimicrobials tested. High (30-37%) occurrence of resistance to ampicillin, sulfonamide, tetracycline and trimethoprim and low (2-6%) occurrence of resistance to gentamicin and chloramphenicol were observed. Occurrence of resistance to the remaining antimicrobials tested was very low (≤ 1%) or not detected (Table 7.2). Occurrence of resistance to ampicillin, sulfonamide, tetracycline and trimethoprim was significantly higher in *E. coli* from pigs compared to *E. coli* from broilers, which is noteworthy even though none of these compounds is classified among the critically important antimicrobials for human medicine.

A total of 23 resistance profiles were detected among the 87 resistant isolates. These profiles ranged from resistance to one antimicrobial in a moderate proportion of resistant isolates (tetracycline, 16% and trimethoprim, 2%) to resistance to compounds of four and even five antimicrobial classes in 8% and 1% of resistant isolates, respectively. Co-resistance to ampicillin, sulfonamide and trimethoprim (ASuTm resistance profile) was the most common profile of resistance to antimicrobials from different classes found in 53% of the resistant isolates. The majority of isolates with this profile exhibited additional resistance to tetracycline alone or in combination with resistance to other compounds including critically important antimicrobials such as azithromycin and nalidixic acid/ciprofloxacin (1% of resistant isolates), azithromycin (1%) and gentamicin (1%). Gentamicin resistance was also observed in combination with ampicillin and tetracycline resistance (1%) and tetracycline resistance (1%).

Compared to 2016, occurrence of resistance underwent minor fluctuations that were not statistically significant for any antimicrobial (Figure 7.2). The percentage of fully susceptible isolates was the highest since 2014, when the current antimicrobial panels were introduced (Figure 7.3).

7.3.3 Indicator *E. coli* from cattle

A total of 181 indicator *E. coli* isolates from 190 cattle caeca were tested for antimicrobial susceptibility (Table 7.2). The vast majority of isolates (90%) was susceptible to all tested antimicrobials. Low (2-8%) occurrence of ampicillin, chloramphenicol, sulphonamide, trimethoprim and tetracycline resistance was observed. Occurrence of resistance to the remaining antimicrobials was very low (<1%) or not detected (Table 7.2). This included also resistance to cefotaxime and ceftazidime (3rd generation cephalosporins) which was found only in one isolate that could not be classified as ESBL or AmpC phenotype and was defined as "other phenotype" based on current interpretive criteria (See chapter 9, Materials and methods).

Twelve resistance profiles were detected in the 18 resistant isolates, varying from resistance to one antimicrobial class in a moderate proportion of resistant isolates (tetracycline, 11% and chloramphenicol or ampicillin/ertapenem, 5%) to resistance to compounds of four antimicrobial classes in 22% of the resistant isolates. The latter consisted of resistance to ampicillin, chloramphenicol, sulfamethoxazole (and trimethoprim in one isolate) and tetracycline.

Compared to 2016, there was an increase in occurrence of all resistances observed, though not statistically significant (Figure 7.2). The percentage of fully susceptible isolates was the second highest after 2016 in the 4-year period since the introduction of the current antimicrobial panels (Figure 7.3).

7.3.4 Perspectives

In 2017, the most common resistance patterns described in indicator *E. coli* from broilers, pigs and cattle included resistance to ampicillin, sulfamethoxazole, trimethoprim and/or tetracycline, which is similar to what has been described in previous DANMAP reports and, more broadly, at EU level [EUSAMR report 2016, EFSA/ECDC 2017]. These resistances occurred in different

Table 7.3 Antimicrobial resistance (%) and classification of the beta-lactam resistance phenotype (%) of ESBL/AmpC-producing Escherichia coli from pigs and cattle and meat thereof, Denmark DANMAP 2017

Antimicrobial agent	Pigs	Pork		Cattle	Beef	
	Danish %	Danish %	Imported	Danish %	Danish %	Imported %
Tetracycline	53	0	75	23	43	33
Tigecycline	0	0	0	0	0	0
Chloramphenicol	11	0	25	5	29	0
Ampicillin	100	100	100	100	100	100
Cefoxitin	73	0	25	46	0	0
Cefotaxime	100	100	100	100	100	100
Ceftazidime	99	100	100	68	57	100
Cefepime	36	100	75	55	100	100
Meropenem	0	0	0	0	0	0
Ertapenem	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0
Trimethoprim	37	100	25	9	29	67
Sulfonamide	52	100	50	23	43	67
Azithromycin	4	33	0	0	0	0
Gentamicin	1	0	25	9	29	0
Ciprofloxacin	3	0	25	0	29	0
Nalidixic acid	1	0	25	0	0	0
Colistin	0	0	0	0	0	0
CPE phenotypes	0	0	0	0	0	0
ESBL phenotypes	27	100	75	50	100	100
AmpC phenotypes	73	0	25	45	0	0
Other phenotypes	0	0	0	5	0	0
Number of isolates	73	3	4	22	7	3
Number of samples	295	248	29	297	170	120

Note: Isolates recovered by the selective enrichment methods described in the EURL-AR laboratory protocol (January 2017)

prevalence in the various animal populations likely reflecting the current and past usage of antimicrobials in animals. The determinants of resistance to these antimicrobials are often genetically linked and use of one compound can select for resistance to different antimicrobials.

The antimicrobial resistance phenotypes detected in animal-origin indicator *E. coli* of most relevance to human health were ciprofloxacin resistance in *E. coli* from broilers and azithromycin resistance in *E. coli* from pigs. The ciprofloxacin resistance phenotype occurred in a moderate but noticeable proportion of broiler isolates (12%). Although the molecular bases of ciprofloxacin resistance were not investigated, the phenotype was indicative of chromosomal mutations and, as a consequence, the main risk to human health is linked to the potential of these strains to cause disease. The azithromycin resistance phenotype was detected in a very low proportion of pig isolates (1%) and thus the potential human risk, which might derive by infections with these strains and/or transfer of azithromycin resistance to pathogenic strains appears to be very low.

Resistance to other antimicrobials relevant for human medicine such as colistin, cefotaxime, ceftazidime, meropenem and tigecycline was not detected, which indicates that the true prevalence of these resistance phenotypes was below 2% in *E. coli* from pigs and cattle and below 3% in *E. coli* from broilers (see Chapter 9, Materials and Methods). However, cefotaxime- and ceftazidime-resistant *E. coli* were detected when using a highly sensitive method (selective enrichment) as detailed in the following paragraph.

7.4 Extended-spectrum beta-lactamase (ESBL)-, AmpC- and carbapenemase-producing *E. coli*

DANMAP 2017 includes ESBL/AmpC- and carbapenemase-producing *E. coli* from caeca of pigs and cattle at slaughter and from Danish and imported pork and beef at retail. Samples were collected randomly and cultured directly in a selective enrichment for detection of cefotaxime-resistant *E. coli*

Table 7.4 Number of ESBL/AmpC and CPE enzymes detected in *E. coli* isolates from beef and pork of Danish and imported origin, Denmark

DANMAP 2017

Enzymes	Pork				Beef			
	Danish		Imported		Danish		Imported	
	2015	2017	2015	2017	2015	2017	2015	2017
CTX-M-1	2	3	0	2	0	2	3	2
CTX-M-14	0	0	0	1	2	3	0	0
CTX-M-15	1	0	1	0	0	2	2	0
CTX-M-32	0	0	0	0	0	0	1	0
TEM-52C	0	0	0	0	0	0	0	1
Chromosomal AmpC	2	0	0	1	0	0	0	0
Number of ESC positive samples	4	3	1	4	2	7	6	3
Number of tested samples	239	248	50	29	149	170	166	120

Note: Among the isolates from meat of Danish origin from 2017, ESBL enzymes was detected in eight sequence types: CTX-M-1 in ST88, ST362, ST117 and ST1434; CTX-M-14 in ST34 and ST162; CTX-M-15 in ST58 and ST224. Isolates recovered by the selective enrichment method described in the EURL-AR laboratory protocol (January 2017)

and carbapenemase-producing *E. coli* (CPE, including strains producing OXA-48-like enzymes). Obtained *E. coli* isolates were then used for testing of MIC of the antimicrobials recommended by EFSA. Whole genome sequencing (WGS) and in silico bioinformatics tools were used to determine ESBL/AmpC/CPE-encoding genes of isolates from meat. MIC distributions and occurrence of resistance among ESBL/AmpC-producing *E. coli* isolates are presented in the web annex (Table A7.3-A7.4).

7.4.1 ESBL/AmpC and carbapenemase-producing *E. coli* from pigs and domestically produced pork

A total of 295 samples from pigs and 248 samples from domestically produced pork resulted in 73 (25%) and 3 (1%) isolates, respectively (Table 7.3). The number of investigated samples and occurrence of ESBL/AmpC-producing *E. coli* isolates from Danish pigs and pork were similar to those tested and identified in 2015 (Figure 7.4). Most samples were also examined for CPE. No CPE isolates were recovered, suggesting that we can be 95% certain that CPE isolates are only present in 1% or less of slaughter pigs and domestically produced pork.

Among the 73 ESBL/AmpC -producing *E. coli* isolates from pigs, 73% belonged to an AmpC phenotype and 27% to an ESBL phenotype displaying 100%, 97%, 73%, and 36% resistance to cefotaxime, ceftazidime (3rd generation cephalosporins), cefoxitin (2nd generation cephalosporin), and cefepime (4th generation cephalosporin), respectively (Table 7.3). In comparison to 2015, no significant changes were observed in the occurrence of samples with either AmpC or ESBL phenotypes (18% vs. 21% and 7% vs. 7%, Figure 7.4). The ESBL/AmpC genotypes of isolates from pigs were not determined.

The three ESBL/AmpC-producing *E. coli* isolated from domestically produced pork displayed an ESBL phenotype and all harboured the CTX-M-1-encoding gene. By multi-locus sequence typing (MLST), two of the isolates were ST117 and one was ST88. The CTX-M-1-encoding gene and the STs observed (ST117 and ST88) were also found among ESBL/AmpC-producing *E. coli* from domestically produced pork in 2015 (Table 7.4).

The 76 ESBL/AmpC-producing *E. coli* isolates from pigs and domestically produced pork exhibited varying levels of resistance to other antimicrobials. No resistance to tigecycline and colistin was detected. (Table 7.3) Resistance to quinolones was low, only 3% (n = 2) and 1% (n = 1) of the ESBL/AmpC-producing *E. coli* from pigs were resistant to ciprofloxacin and nalidixic acid, respectively, suggesting that the isolates harboured a plasmid-mediated quinolone resistance gene. Different combinations of multidrug-resistance were observed in five pig isolates primarily including ampicillin, chloramphenicol, tetracycline, sulphonamides, and trimethoprim. One pig isolate resistant to all these drugs was, in addition, resistant to both ciprofloxacin and nalidixic acid.

Resistance to gentamicin and azithromycin was observed in a few ESBL/AmpC-producing *E. coli* from pigs (n = 3) and domestically produced pork (n = 1). The ESBL/AmpC-producing isolates resistant to azithromycin were also resistant to ampicillin, ciprofloxacin, tetracycline and sulphonamides. Azithromycin belongs to the critically important antimicrobials for human medicine and resistance to it is increasing in isolates originating from Europe and globally.

Resistance to sulphonamides and trimethoprim was observed in the ESBL/AmpC -producing *E. coli* isolates from pigs and domestically produced pork, and 29 (38%) of the isolates were resistant to both sulphonamides and trimethoprim as well as to ampicillin and tetracycline. Co-resistance to sulphonamides and trimethoprim is likely attributable to the presence of class 1 integrons, which occasionally also harbour genes encoding resistance to ampicillin, tetracycline and chloramphenicol.

7.4.2 ESBL/AmpC and carbapenemase-producing *E. coli* from cattle and domestically produced beef

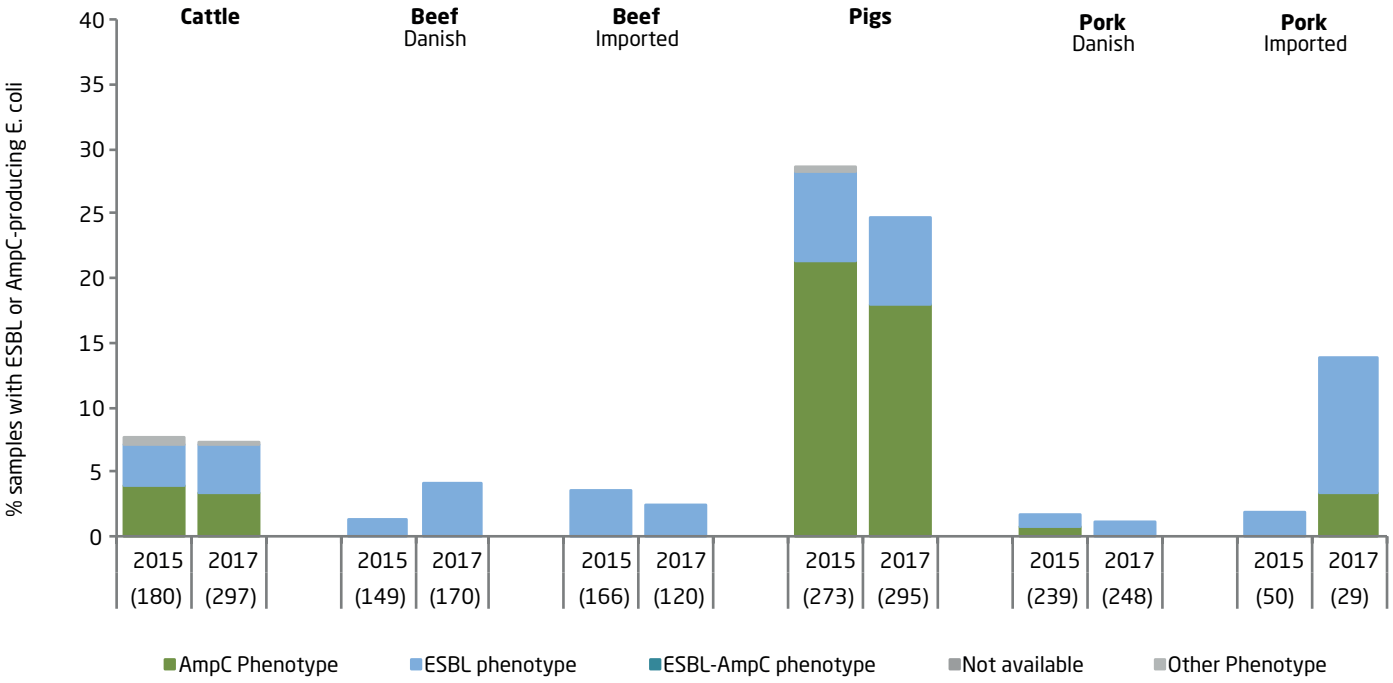
A total of 297 samples from cattle and 170 samples from domestically produced beef resulted in 22 (7%) and 7 (4%) isolates, respectively (Table 7.3). The number of investigated samples and occurrence of ESBL/AmpC-producing *E. coli* isolates from cattle and domestically produced beef were similar to those tested and identified in 2015 (Figure 7.4). Most samples were also examined for CPE and no CPE isolates were recovered, suggesting, with 95% confidence, that CPE isolates may be present in less than 1% of the samples from cattle and in less than 2% of the samples from domestically produced beef.

Among the 22 ESBL/AmpC-producing *E. coli* isolates from cattle, 5% displayed an ESBL phenotype and 45% an AmpC phenotype, with 100%, 68%, 46%, and 55% resistance to cefotaxime, ceftazidime, cefoxitin, and cefepime, respectively (Table 7.3). One isolate was classified as 'other phenotype'. This strain was susceptible to carbapenems and cefoxitin, resistant to all tested 3rd and 4th generation cephalosporins but with no synergy when the two 3rd generation cephalosporins were combined with clavulanic acid (see definitions in chapter 9, Materials and Methods). The ESBL/AmpC genotypes of isolates from cattle were not determined.

All seven ESBL/AmpC-producing *E. coli* isolates from domestically produced beef exhibited an ESBL phenotype (Table 7.3). By whole genome sequencing, three ESBL types were detected, and each isolate was positive for one ESBL type only. The seven isolates harboured CTX-M-14- (n = 3), CTX-M-1- (n = 2), and CTX-M-15- (n = 2) encoding genes. The three strains harbouring the CTX-M-14-encoding gene belonged to two STs by MLST: ST34 (n = 2) and ST162. The two CTX-M-1- and two CTX-M-15-producing strains belonged to different STs: ST362 and ST1434, and ST58 and ST224, respectively (Table 7.4). Only ST58 and ST362 were reported in 2015, both in association with the CTX-M-1-encoding gene.

The ESBL/AmpC-producing *E. coli* isolates from cattle and domestically produced beef exhibited, in general, a moderate level of resistance to chloramphenicol, sulphonamides, tetracycline and trimethoprim and no resistance to tigecycline, azithromycin and colistin (Table 7.3). Two ESBL/AmpC -producing *E. coli* isolates from domestically produced beef and one isolate from cattle exhibited a multidrug-resistance profile besides being ESBL/AmpC-producers. This profile included resistance to ampicillin, gentamicin, sulphonamides, tetracycline, and trimethoprim. No resistance to nalidixic acid was observed in domestically produced beef whereas two isolates (29%) exhibited resistance to ciprofloxacin, thus suggesting plasmid-mediated quinolone resistance. No resistance to fluoroquinolones was observed among the ESBL/AmpC -producing *E. coli* isolates from cattle.

Figure 7.4 Occurrence (%) of samples with ESBL/AmpC-producing *E. coli* from cattle, beef, pigs and pork, Denmark DANMAP 2017



Note: Number of samples tested each year is shown in the parentheses. Samples were processed according to the EURL-AR laboratory protocol (January 2017)

7.4.2 ESBL/AmpC and carbapenemase-producing *E. coli* from imported pork and beef

Samples from imported pork (29) and beef (120) yielded four (14%) and three (3%) ESBL/AmpC-producing isolates, respectively (Table 7.3). Most samples were also examined for CPE and no CPE isolates were recovered.

No statistically significant changes were observed in the occurrence of ESBL/AmpC-producing *E. coli* in these sources from 2015 to 2017. ESBL phenotypes were more prevalent compared to AmpC phenotypes. The AmpC phenotypes were observed in 3.4% of samples from imported pork from 2017 whereas they were not observed in the samples collected in 2015 (Figure 7.4).

The ESBL/AmpC-producing *E. coli* isolates from imported pork in general exhibited a moderate level of antimicrobial resistance and included one multi-resistant isolate that showed resistance to antimicrobials from five different classes besides being an ESBL producer. ESBL/AmpC -producing *E. coli* isolates from imported beef exhibited susceptibility to most of the antimicrobials tested. In both sources, no resistance to tigecycline, azithromycin and colistin was observed. In addition, in the isolates from imported beef, no resistance to chloramphenicol, fluoroquinolones and gentamicin were observed. In contrast, isolates from imported pork conferred exhibited resistance to these compounds and to sulphonamides, tetracycline and trimethoprim (Table 7.3).

Genotyping by whole genome sequencing of the four ESBL/AmpC-producing *E. coli* isolates from imported pork revealed i) CTX-M-1-encoding gene in *E. coli* ST117 (n=1) and ST2668 (n=1); ii) CTX-M-14 encoding gene in *E. coli* ST455 (n=1), and iii) upregulation of chromosomal *ampC* (by C42T mutation) in *E. coli* ST88 (n=1). Genotyping of the three ESBL/AmpC-producing *E. coli* isolates from imported beef revealed CTX-M-1-encoding gene in *E. coli* ST69 and ST362, and TEM-52-encoding gene in *E. coli* ST446 (Table 7.4). None of the STs found in 2017 were observed in 2015.

7.4.5 Perspectives

As in 2015, no carbapenemase-producing *E. coli* were detected in the approximately one-thousand samples from the domestic pig and cattle production examined during 2017. Carbapenems are critically important for treatment of severe infections caused by multidrug-resistant Gram-negative bacteria in human patients, thus it is important to continue monitoring for introduction of CPE in the Danish animal populations to take prompt action in case CPE emerges.

In 2017, ESBL/AmpC-producing *E. coli* occurred in samples from Danish pigs, cattle and domestically produced meat at levels comparable to those observed in 2015. In 2017, the most frequent beta-lactam resistance phenotype among ESBL/AmpC-producing *E. coli* from meat was the ESBL phenotype occurring in 1% and 4% of the samples from domestically produced pork and beef, respectively. Concerning imported meat, the EU harmonised sampling required the packages of meat to be selected independently of the country of origin and, as only few isolates from imported meat were available in 2017, it is not possible to draw conclusions on trends or associations of ESBL/AmpC types with specific countries. In 2015, the EU harmonised monitoring reported an overall EU-prevalence of ESBL-producing *E. coli* of 7% and 5% from pork and beef, respectively [EUSAMR report 2016, EFSA/ECDC 2017].

In textbox 7.1, ESBL/pAmpC-producing *E. coli* isolated from humans (bloodstream infection cases from 2017) and meat (isolates from 2016 and 2017) were compared at whole genome sequence level. Close phylogenetic relatedness of CMY-2-producing *E. coli* ST131 and CMY-2-producing *E. coli* ST429 from poultry and human origin indicated a possible zoonotic spread. Plasmid transfer of genes encoding ESBL/pAmpC enzymes was not investigated, and this, together with the occurrence and evolution of these *E. coli* ST/pAmpC combinations need to be monitored closely in the future to understand to what extent pAmpC *E. coli* infections in humans might be attributable to meat sources.

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Textbox 7.1

ESBL/pAmpC-producing *Escherichia coli* – comparison of isolates of animal origin with isolates obtained from human bloodstream infections

Background: ESBL/pAmpC-producing bacteria are widespread in both humans and production animals worldwide. Previous national and international studies have found similar ESBL/pAmpC producing *E. coli* in animals, meat and human infections, suggesting a zoonotic link. In this study, possible clonal zoonotic spread of ESBL/pAmpC producing *E. coli* was investigated by comparing whole-genome sequencing data from *E. coli* isolates of human and meat origin. Plasmid transfer of genes encoding ESBL/pAmpC enzymes was not investigated.

Materials and methods: ESBL/pAmpC-producing *E. coli* isolates from production animals and meat (section 7.4) obtained in 2016 and 2017 were compared with ESBL/pAmpC-producing *E. coli* isolates from human bloodstream infections (Textbox 8.1) obtained during 2017. Any possible clonal relationships between isolates sharing the same combination of ESBL/pAmpC genes and Multilocus Sequence Types (STs), were investigated by whole-genome based single-nucleotide polymorphism (SNP) analysis. The combination was further characterized if <100 SNPs were observed between isolates of animal and human origin.

Results: During 2016-2017, 85 samples from broiler meat, 24 samples from broilers, 10 samples from beef, and six samples from pork were tested positive for genes encoding ESBL/pAmpC enzymes. In 2017, 337 ESBL/pAmpC positive isolates were collected from human bloodstream infections.

When comparing ESBL/pAmpC and ST from the different origins, the same combinations of ESBL/pAmpC and STs were detected in *E. coli* of human and animal origin on seven occasions; CTX-M-1 in combination with ST69, ST88, ST117 and ST362, CMY-2 in combination with ST131 and ST429, and CTX-M-15 in combination with ST224. SNP-based comparisons were performed for each of the seven combinations. Only for ST131 with CMY-2 and ST429 with CMY less than 100 SNPs between the isolates were observed.

The ST131 CMY-2-producing *E. coli* encompassed three samples from imported broiler meat from 2016 and one isolate from a patient with bloodstream infection from 2017. Between the *E. coli* isolate of human origin and two of the three *E. coli* isolates from broiler meat, 39 and 40 SNPs were detected. For the last isolate from broiler meat 1.014 SNPs were detected (Web annex Figure A7.5).

The ST429 CMY-2-producing *E. coli* encompassed 11 isolates obtained from broiler meat from 2016 (three import and eight Danish), three isolates obtained from Danish broilers from 2016 and one isolate obtained from a patient from 2017. Between the *E. coli* isolate of human origin and the isolates of animal origin, 6-118 SNPs were detected (Web annex Figure A7.6).

For the combinations ST69 with CTX-M-1, ST88 with CTX-M-1, ST117 with CTX-M-1, ST224 with CTX-M-15, and ST362 with CTX-M-1, more than 100 SNPs were observed between the isolates of animal and human origin. These five combinations were not investigated further.

Discussion and conclusion: ST131 *E. coli* is the most frequently detected ST among ESBL/pAmpC-producing *E. coli* isolates from human bloodstream infections in Denmark (Textbox 8.1), whereas the pAmpC enzyme CMY-2 is less frequent detected among *E. coli* isolates from humans. The opposite is seen for *E. coli* of animal origin, where the pAmpC enzyme CMY-2 is often detected, especially among broilers, but very few of the *E. coli* isolates of animal origin belong to ST131. The combination of ST131 and CMY-2 is not commonly observed in Denmark; neither from *E. coli* of animal origin nor from human origin. In 2016, the first ST131 CMY-2-producing *E. coli* isolate causing bloodstream infection in a Danish patient was detected, and in 2017 another isolate was observed. During 2016-2017, three ST131 CMY-2-producing *E. coli* were detected from imported broiler meat by the DANMAP surveillance [DANMAP 2016, section 7.4]. Investigation of the clonal relationship of ST131 CMY-2-producing *E. coli* from human and animal origin indicates possible clonality between the isolates from these reservoirs, suggesting a potential zoonotic link between isolates obtained from imported broiler meat and the isolate obtained from a patient with bloodstream infections.

continued ... Textbox 7.1

In 2014-2017, ST429 CMY-2 producing *E. coli* was isolated 18 times from broilers (animals and meat). In 2016, the first ST429 CMY-2 producing *E. coli* isolate causing a bloodstream infection was observed. Investigation of the clonal relationship of ST429 CMY-2 producing *E. coli* from human and animal origin suggested a close zoonotic link, with only six SNP differences between the human isolate (O) and the closest broiler (animal) isolate (G). Additionally, among nine of the isolates of animal origin (including isolate G) 33 or less SNPs were detected, indicating high clonality between the isolates.

The observation of close phylogenetic relations in ST131 CMY-2-producing *E. coli* and ST429 CMY-2-producing *E. coli* from animal origin and human bloodstream infections indicating a possible zoonotic spread should be noted. Occurrence and evolution of these ST/pAmpC combinations need to be monitored closely in the future.

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Textbox 7.2

Whole genome sequence (WGS)-based prediction of antimicrobial resistance in clinical Escherichia coli from one day in Denmark

Background: Next-generation sequencing techniques allow us to generate data that can be analysed in great detail regarding antimicrobial resistance genes, virulence factors and other determinants. In a hospital setting, WGS data from clinical isolates could potentially provide accurate species identification, predict the antimicrobial resistance (AMR) profiles, and inform about their source, transmission and relation to other isolates infecting multiple patients. We hypothesise that WGS can aid or even replace traditional diagnostic methods and eventually be routinely used in clinical microbiology laboratories to guide clinical decision making including the adequate pharmacotherapy to be prescribed to the patients. The objective of this study was to determine the AMR genotype-phenotype correlation in contemporary *E. coli* from clinical sources in Denmark.

Materials and methods: We describe the antimicrobial resistance phenotypes and genotypes of 170 *E. coli* isolates recovered from the 11 Danish Departments of Clinical Microbiology (DCM) on one single day in January 2018. These isolates represent all *E. coli* found in a subset of 500 isolates randomly selected among the 2,024 bacterial cultures processed by the DCMs on that day. As such, they represent a non-biased snapshot of the *E. coli* available from that day in the clinical settings in Denmark. Of the 170 isolates, 148 (87.1%) were from urine, eight (4.7%) were from blood, and the remaining 14 (8.2%) were from other sources including urethral and tracheal swabs, pus, faeces and abscess. Antimicrobial susceptibility testing (AST) was performed by broth microdilution using GN3F panel (Sensititre™ Gram Negative MIC Plate) and a custom colistin panel (Sensititre™ Custom Plate). MIC interpretation was performed according to the EUCAST clinical breakpoints. WGS was performed by Illumina NextSeq and WGS data were analysed with CGE Bacterial Analysis Pipeline (https://cge.cbs.dtu.dk/services/cge/) and other public CGE services (https://cge.cbs.dtu.dk/services/).

Results: A total of 2,890 isolate-antimicrobial combinations were examined, including ampicillin, cefazolin, cefuroxime, cefoxitin, cefpodoxime, ceftazidime, ceftriaxone, ertapenem, meropenem, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, tobramycin, amikacin, tetracycline, tigecycline and colistin. According to the EUCAST clinical breakpoints[1] no isolates presented phenotypic resistance to meropenem, ertapenem, tigecycline and colistin and no genetic determinants of resistance to these antimicrobial agents were observed. A total of 91 isolates (53.5%) were fully susceptible to the antimicrobial agents considered and susceptibility was supported by the absence of genetic determinants conferring resistance to the antimicrobials tested. All remaining isolates (n=79, 46.5%) displayed antimicrobial resistance, with 15 (8.8%) being resistant to one antimicrobial agent, one (0.6%) being resistant to two antimicrobials of the same class (beta-lactam antimicrobials), 25 (14.7%) being resistant to two antimicrobial agents of different classes and 38 (22.4%) being resistant to three or more antimicrobial agents. Of the latter, 28 (16.5%) were resistant to antimicrobial agents of three or more different classes, eight (4.7%) were resistant to antimicrobial agents from two different classes and two (1.2%) were resistant to antimicrobials from the same class (in particular beta-lactam antimicrobials).

Overall, genotype-phenotype concordance was observed for all but 14 isolate-antimicrobial combinations (Table 1). There was limited diversity of the antimicrobial resistance genes detected especially regarding the genes mediating resistance to critically important antimicrobial agents such as 3rd generation cephalosporins and fluoroquinolones. The availability of WGS data allowed a quick screening of the *E. coli* diversity based on multilocus sequence typing (MLST). The most prevalent MLST sequence types (STs) were ST-73 (n=22; 12.9%), ST-69 (n=19; 11.2%), ST-131 (n=13; 7.6%) and ST-95 (n=10; 5.9%), with other STs represented by six or less isolates each. All bacteria belonging to ST-131 (n=13) had the serotype O25:H4. Seven (53.8%) of the 13 ST131 isolates were resistant to 3rd generation cephalosporins, attributed either to $bla_{\text{CTX-M-15}}$ (n=5) or $bla_{\text{CTX-M-27}}$ (n=2). These isolates correspond to 70% of all 3rd generation cephalosporin-resistant isolates observed in this study (n=10).

[1] EUCAST Clinical Breakpoints were used for all antimicrobials considered except:
- Cefazolin: No EUCAST clinical breakpoint nor ECOFF available. CLSI clinical breakpoint was used (R ≥ 32 mg/L).
- Cefoxitin: No EUCAST clinical breakpoint available. ECOFF was used (8 mg/L).
- Cefpodoxime EUCAST clinical breakpoint (1 mg/L) not present in panel range. ECOFF was used (2 mg/L).
- Ertapenem: EUCAST clinical breakpoint (1 mg/L) and ECOFF (0,064 mg/L) not present in panel range. CLSI clinical breakpoint was used (R ≥ 2 mg/L).
- Tetracycline: No EUCAST clinical breakpoint available. ECOFF was used (8 mg/L).

continued ... Textbox 7.2

Discussion and conclusion: WGS data were able to accurately predict 99.52% of resistant and susceptible phenotypic profiles for the 17 antimicrobials considered in a random and genetically diverse collection of 170 *E. coli* from clinical sources that presented various antimicrobial susceptibility profiles. Thus, WGS-based AMR prediction appears to be a promising antimicrobial susceptibility testing method for *E. coli*. Further work to assess the applicability of WGS-based AMR prediction to other clinically relevant bacteria as well as the impact of the obtained results on clinical decisions is ongoing.

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Table 1 Total number of isolates resistant to selected antimicrobials based on EUCAST clinical breakpoints and respective antimicrobial resistance determinants detected by WGS DANMAP 2017

Antimicrobial agents	Number of resistant isolates	Genes or mutations responsible for resistance in resistant isolates[a]	Number of resistant isolates with no responsible genes	Number of susceptible isolates with genes
AMP	66	bla_{TEM-1B} (n=45); $bla_{CTX-M-15}$ (n=6); bla_{TEM-1C} (n=4); $bla_{CTX-M-27}$ (n=2); bla_{OXA-1} (n=3); bla_{TEM-1A} (n=2); $bla_{CTX-M-14}$ (n=1); bla_{TEM-1D} (n=1); bla_{SHV-1} (n=1); bla_{DHA-1} (n=1)	0	1[b]
CZO	14	$bla_{CTX-M-15}$ (n=6); bla_{TEM-1B} (n=3); $bla_{CTX-M-27}$ (n=2); bla_{DHA-1} (n=1); $bla_{CTX-M-14}$ (n=1); bla_{TEM-1C} (n=1)	0	1[b]
CUR and/or CXI	14	$bla_{CTX-M-15}$ (n=6); $bla_{CTX-M-27}$ (n=2); bla_{OXA-1} (n=2); bla_{DHA-1} (n=1); $bla_{CTX-M-14}$ (n=1)	2[c]	2[bd]
CPO and/or CTZ and/or CTR	10	$bla_{CTX-M-15}$ (n=6); $bla_{CTX-M-27}$ (n=2); bla_{DHA-1} (n=1); $bla_{CTX-M-14}$ (n=1)	0	1[b]
MER and/or ERT	0	None	0	0
CIP	23	*parC S80I, gyrA S83L, gyrA D87N/D87H* [e] (n=17); *parC S80I, gyrA S83L, gyrA D87N* [e] *and aac(6')-Ib-cr* (n=5); *qnrS1* (n=1)	0	2[f]
TRS	37	*dfrA17* (n=18); *dfrA14* (n=5); *dfrA1* (n=3); *dfrA5* (n=4); *dfrA7* (n=2); *dfrA12* (n=2); *dfrA8* (n=1)	2[g]	2
GEN and/or TOB and/or AMI	12	*aac(6')-Ib-cr* (n=5); *aac(3)-IId* (n=3); *aac(3)-IIa* (n=1); *aac(3)-IVa* (n=1); *aac(6')-Ib-cr and aac(3)-IIa* (n=1)	1	0
TET and/or TIG	45	*tet*(A) (n=23); *tet*(B) (n=22)	0	0
COL	0	None	0	0

AMP: ampicillin, CZO: cefazolin, CUR: cefuroxime, CXI: cefoxitin, CPO: cefpodoxime, CTZ: ceftazidime, CTR: ceftriaxone, MER: meropenem, ERT: ertapenem, CIP: ciprofloxacin, TRS: trimethoprim/sulfamethoxazole, GEN: gentamicin, TOB: tobramycin, AMI: amikacin, TET: tetracycline, TIG: tigecycline, COL: colistin

a) In cases where several genes mediating the same phenotype were present, only one was included in the table. Discrepancies in phenotypic-genotypic results can be found in the two right hand columns.

b) The isolate is the same for all four cases and presents the genes $bla_{CTX-M-15}$ and bla_{OXA-1}

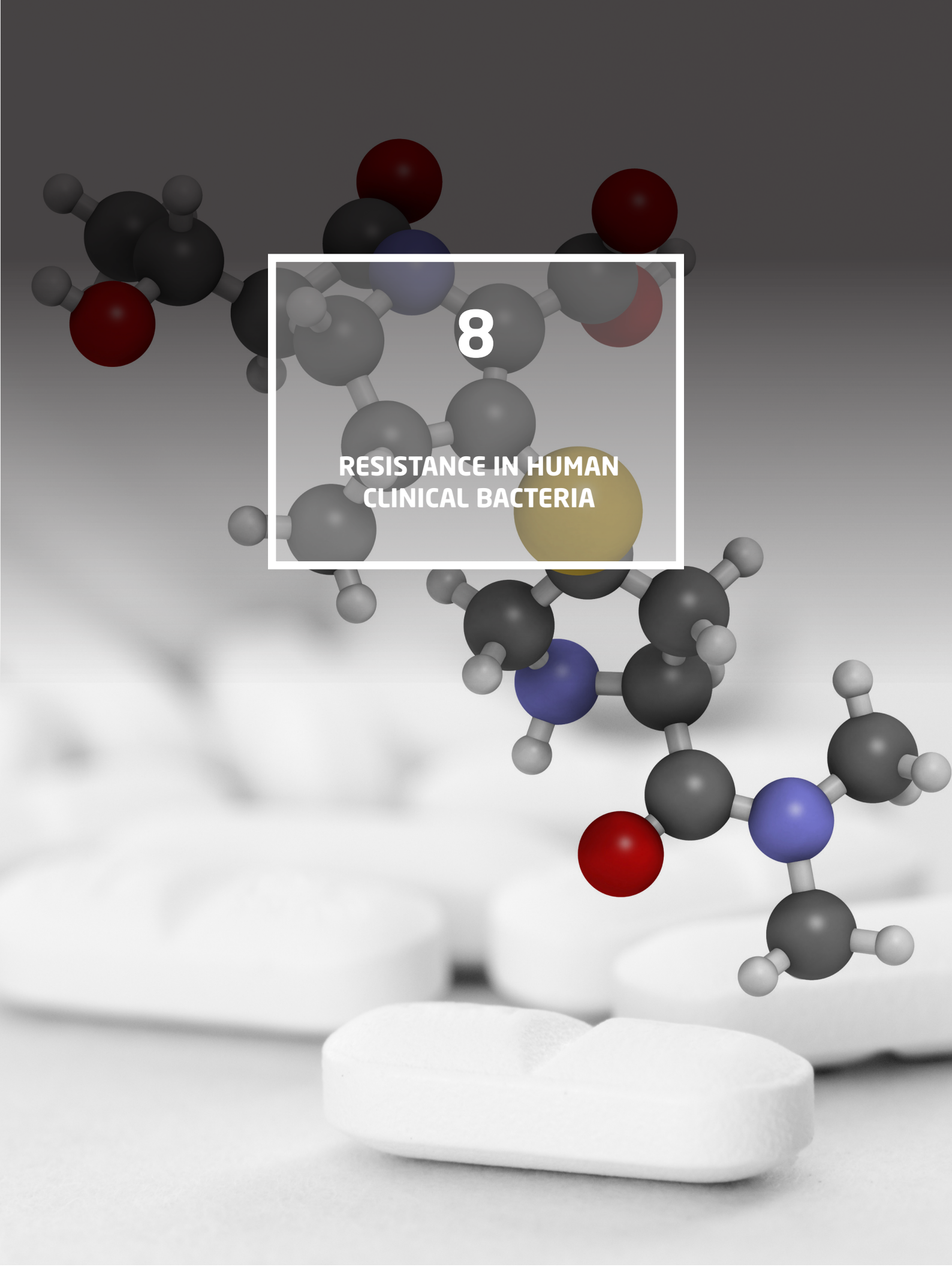
c) One isolate presents the bla_{TEM-1C} gene. One isolate does not present beta-lactam resistance determinants and is phenotypically susceptible to other cephalosporins.

d) One of the isolates is the one referred to in b. The other isolate presents a bla_{OXA-1} gene.

e) Isolates presented at least the three described point mutations simultaneously, with (n=21) or without (n=1) additional *parC* and/or *parE* point mutations.

f) One isolate presents the *aac(6')-Ib-cr* gene without co-occurrence of *gyrA*, *parC* or *parE* point mutations. The remaining isolate presents the *qnrB4* gene.

g) One isolate presents only *sul1* without trimethoprim resistance determinants. The remaining isolate harbors *sul1* and *sul2* genes without trimethoprim resistance genes.



8

RESISTANCE IN HUMAN CLINICAL BACTERIA

8. Resistance in human clinical bacteria

Highlights: DANMAP 2017 presents resistance data on invasive and other clinically important human infections reported from the Danish Departments of Clinical Microbiology (DCM) for the past decade. Overall, an increasing trend in the total number of blood isolates has been observed since 2009 and after a period with either decreasing or stable resistance rates in clinical *Escherichia coli* and *Klebsiella pneumoniae,* an upward going trend in resistance was observed for several antimicrobial classes in 2017. For multidrug-resistant species; methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and carbapenemase-producing organisms (CPO), the incidences/total number of isolates kept on increasing. (Textboxes 8.1, 8.2, 8.3 and section 8.7).

For ***Escherichia coli*** (section 8.1), resistance to ciprofloxacin and cefuroxime in isolates from invasive infections showed increases from 2016-2017 (from 11% to 13% and from 8.6% to 9.7%, respectively). For ciprofloxacin this is probably due to changes in clinical breakpoints for 2017. Cefuroxime was the only antimicrobial with continously increasing resistance rates measured over the past decade, (from 6.0 to 9.7% in invasive isolates and from 4.0 to 7.1% in isolates from hospital urines, respectively).

Decreases were observed for sulfonamide and ampicillin resistance. For sulfonamide resistance, an overall decrease from 38% til 29% has been observed since 2008. Likewise, the resistance rate for ampicillin from samples from primary health care (PHC) decreased, although less markedly from 41% to 38% since 2008.

Data on ***Klebsiella pneumoniae*** (section 8.2) were obtained from blood and urine samples as well. Although resistance rates in the invasive isolates in general have shown decreases since 2008, some tendencies of increase were observed in 2017. As for *E. coli, significant* increases were observed for resistance to ciprofloxacin, but also for piperacillin/tazobactam (from 5.4% to 9% and from 5.8% to 7.4%, respectively). Additionally, marked increases in mecillinam resistance and in sulfonamide resistance from 2016 to 2017 were observed for both hospital samples (from 8.9% to 16% for mecillinam and from 17% to 26% for sulfonamide) and PHC samples (from 9% to 17% for mecillinam and from 19% to 26% for sulfonamide).

The increasing resistance towards ciprofloxacin was also observed for ***Neisseria gonorrhoeae*** (section 8.8) – increasing from 18% in 2016 to 28% in 2017, though a significant, continuing decrease has been maintained since a peak of 75% in 2009.

Moreover, data on methicillin-resistant ***Staphylococcus aureus*** (MRSA, section 8.7) revealed a significant increase in the number of community-acquired infections since 2009. This increase in infections follows the increase in total number of newly diagnosed cases proportionally. Also the number of bacteraemias with *Staphylococcus aureus* increased after a stable period the past two years, reaching 2,104 isolates in 2017.

For the first time, DANMAP 2017 includes resistance data on ***Haemophilus influenzae*** (textbox 8.4). *H. influenzae* has been monitored since 2014, where a total of 128 cases of systemic inflections were reported. In 2017, 115 cases were reported. A slightly increasing resistance to ampicillin should be noted.

DANMAP 2017 also presents data on ***Mycoplasma genitalium*** (textbox 8.6), for which rapidly increasing resistances towards all antimicrobials available is worrisome.

8.0 Introduction

DANMAP receives national resistance data on invasive isolates from the 10 Departments of Clinical Microbiology (DCM) for all species causing bloodstream infections in humans in Denmark: *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa, Acinetobacter* species and *Staphylococcus aureus.* In addition, DANMAP also receives resistance data for *E. coli* and *K. pneumoniae* from urine samples from either hospitalised patients or patients seen at the general practitioner. Surveillance has been performed since 1995. In the very beginning, based on the reporting from two DCM, but quickly joined and supported by most clinical microbiological laboratories. From 2009 to 2014, DANMAP received data from all but one DCM resulting in a coverage of approx. 95% of the population. Since 2015, all DCM have participated in the program, thus representing the complete Danish population. Data are reported once yearly, submitting resistance on the first annual isolate per patient. For further information on the resistance testing and reporting see chapter nine, materials and methods.

DANMAP also receives resistance data from the reference laboratories at SSI, where strains from several species are submitted for descriptive and/or surveillance purposes. Thus, all invasive strains of *Staphylococcus aureus* have been submitted on a voluntary basis since 1957. Also voluntary is the submission of invasive beta-haemolytic streptococci, while invasive *Streptococcus pneumoniae* and *Haemophilus influenzae* are mandatory to submit. The detection of methicillin-resistant *S. aureus* (MRSA) and *Neisseria gonorrhoeae* from all clinical sites is notifiable and the submission of the isolated strains mandatory.

In addition, the DCM submit isolates from hospital samples of ESBL-producing *E. coli* from blood and vancomycin-resistant enterococci (VRE) and carbapenemase-producing organisms (CPO) from all clinical sites, irrespective of infection or colonisation, based on a mutual agreement to survey the development and spread of these often multi-resistant bacteria at Danish hospitals.

From 2009 to 2017, the total number of reported invasive isolates increased by 33% (from 8,277 to 10,975 isolates). The largest increase observed was for *E. faecium* (89%). The only species decreasing was *S. pneumoniae* (-27%). Table 8.0.1 presents the percentage distribution of invasive species in 2009 and 2017, respectively. The biggest changes were observed in *E. coli*, increasing its proportion by 3.9%, and *S. pneumoniae* decreasing by 5.4%.

In 2017, the number of isolates maintained the increasing trend with *E. coli* constituting close to 50% of the total (Figure 8.0.1).

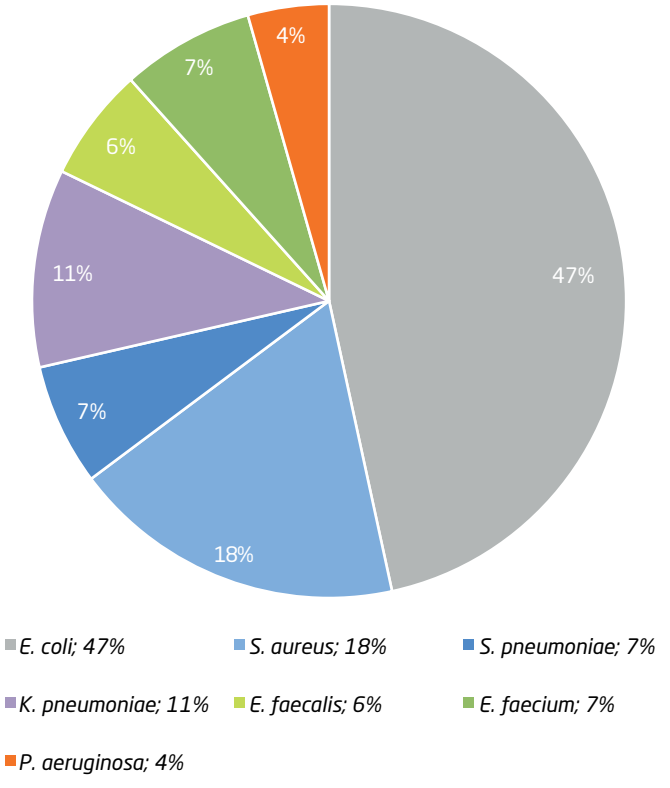
Figure 8.0.2 follows the absolute proportional changes for each species year by year.

The increase in the total number of isolates probably mirrors demographic changes with a growing population of elderly and of chronically ill or immunocompromised. These changes are paralleled by increasing activity at hospitals during the past decade, where the number of admissions increased with 10% and the number of treated patients in ambulatory care with 26%. In 2017, hospital activity amounted to 221 admissions and 647 bed days per 1000 inhabitants, combined with 1350 ambulatory treatments per 1000 inhabitants. The increasing number of invasive infections is of concern to a health care system that is under pressure. It demands fast and effective antibiotic treatment, while increasing the risk for the development of resistant infections and the spread of these in hospital environments with fragile patient populations. It also underlines the need for a health system with firmly established infection prevention and control and an understanding of the importance of proper diagnostics and a rational use of antibiotics, reserving the most broad-spectrum antibiotic classes to the few patients with the still rare, multi-resistant infections.

Table 8.0.1 Distribution of species in invasive isolates (based on total number of isolates) and the percentage change from 2009 to 2017, Denmark DANMAP 2017

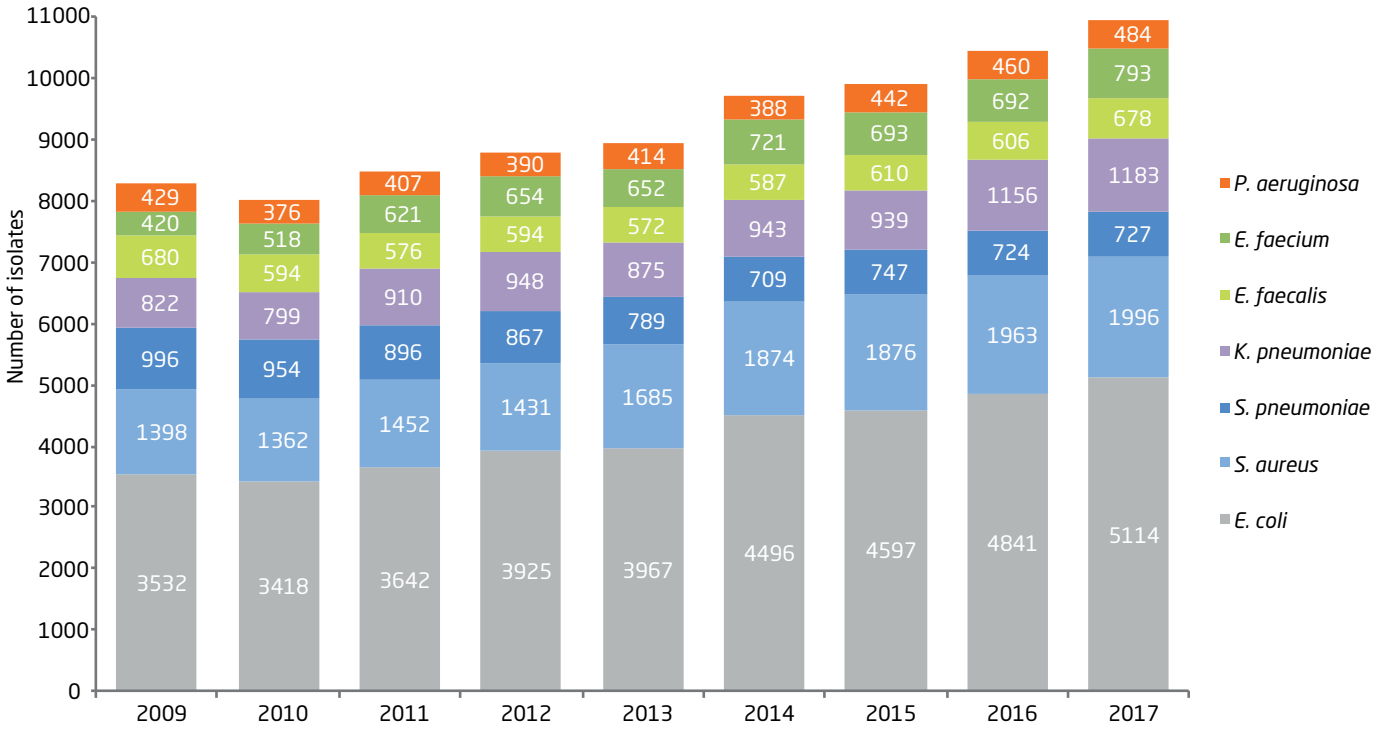
Species	2009	2017	% change
E. coli	42.67%	46.60%	↑ 3.92%
S. aureus	16.89%	18.19%	↑ 1.30%
S. pneumoniae	12.03%	6.62%	↓ -5.41%
K. pneumoniae	9.93%	10.78%	↑ 0.85%
E. faecium	5.07%	7.23%	↑ 2.15%
E. faecalis	8.22%	6.18%	↓ -2.04%
P. aeruginosa	5.18%	4.41%	↓ -0.77%

Figure 8.0.1 Distribution of species from invasive isolates, 2017, Denmark DANMAP 2017



This chapter describes the resistance rates along with sampling details and surveillance data for the above-mentioned bacteria along with a couple of other. In addition, this report presents data on *Haemophilus influenzae* for the first time.

Figure 8.0.2 Number of submitted invasive isolates (from 2009 to 2017) for each of the species under surveillance. DANMAP 2017



Test for trend in resistance

For the first time, this chapter contains results of trend analysis applied to reported resistance rates for *E. coli* and *K. pneumoniae*. Significance levels were calculated for the main seven antimicrobials.

The Cochran-Armitage test for trend in proportions was performed on susceptibility data through five and ten years respectively. This test was chosen because of its ability to uncover trends in binomial proportions explained by an ordinal variable.

One sided tests was performed and the respective significance levels have been reported by the p-value and marked by an arrow indicating probability of increase or decrease. The one-sided p-value for trend is computed as:

$$P_1 = \begin{cases} \text{Prob}(Z>T) & \text{if } T>0 \\ \text{Prob}(Z<T) & \text{if } T\leq 0 \end{cases}$$

The null-hypothesis shared by both computations assumes no trend. One-sided tests were chosen because of a preliminary expected direction in trend.

Most of the significant probabilities are commented and considered together with the graphs to highlight the situation of antimicrobial resistance.

Note that the significance levels are reported here to support the interpretations of the graphs and thus should be interpreted with caution.

8.1 *Escherichia coli*

Escherichia coli (*E. coli*) is the most frequent cause of community- and hospital acquired urinary tract infections and of bacteraemia in Denmark. It is part of the normal intestinal flora in both animals and humans, where it comes into close proximity to many other bacterial species. Transferred resistance mechanisms from other bacterial species to *E. coli* are frequently seen. Some *E. coli* contain virulence factors that dispose for gastrointestinal illnesses of varying severity, such as the often mild Traveller's diarrhoea or the severe gastrointestinal illness associated with the development of haemolytic uremic syndrome.

8.1.1 Blood isolates from hospitalised patients

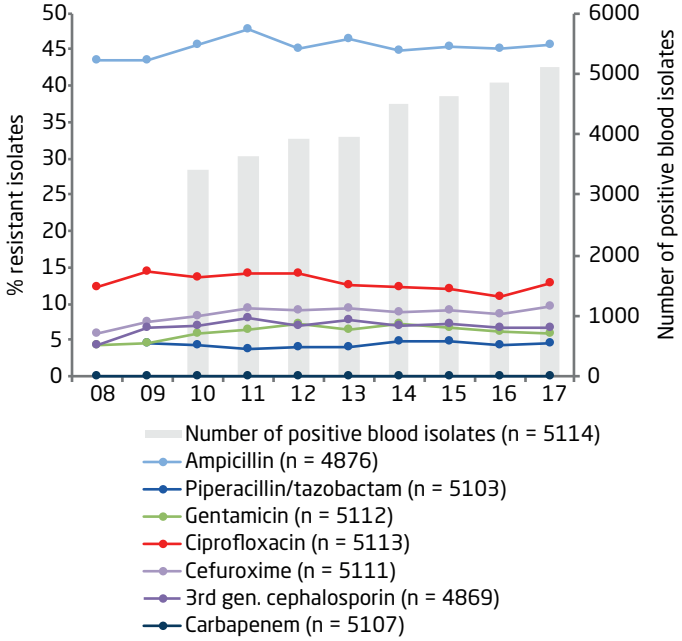
For 2017, DANMAP received data on the antibiotic susceptibility in 5,114 *E. coli* isolates from blood cultures from all 10 Departments of Clinical Microbiology (DCM) in Denmark. All 10 DCM routinely (>75% of isolates) tested for resistance to ampicillin, ciprofloxacin, piperacillin/tazobactam, gentamicin, cefuroxime, 3rd generation cephalosporin and carbapenems. Tested 3rd generation cephalosporins were either cefpodoxime, ceftazidime or cefotaxime, while the tested carbapenem was meropenem for all DCM in 2017. In addition, nine DCM routinely tested for mecillinam resistance and four routinely tested for resistance to amoxicillin/clavulanic acid. Resistance testing was mainly performed by disc diffusion. The presented data consist of the reported interpretation results, performed by the DCM, based on the S-I-R system.

Resistance results for 2017 for all tested antibiotics, presented as a national mean for each antibiotic class, are summarized in Table 8.1.1. In Figure 8.1.1, rates of resistance are shown for the past decade – here data are presented as a national mean, when at least six DCM performed routine testing. Time trends and significance levels of these, based on the resistance rates five and ten years back, respectively, are presented in Figure 8.1.1 c). Test results for mecillinam resistance in invasive *E. coli* has been excluded from Figure 8.1.1, since the S-I-R interpretation rules for the individual DCM differ and/or vary over time, making comparison of the results difficult and time trends unreliable.

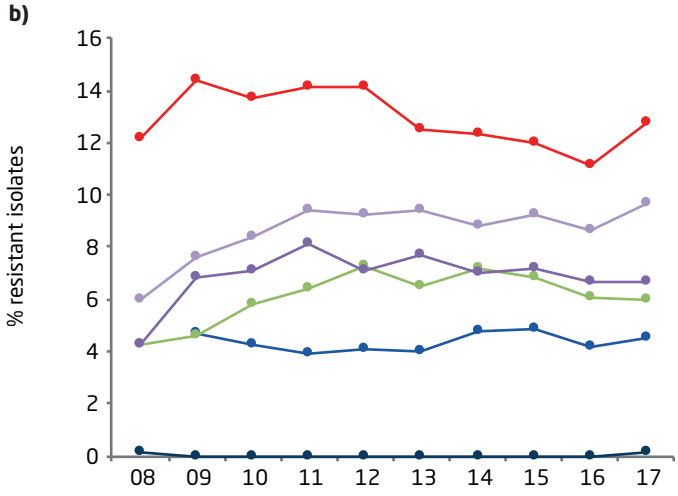
A continuous increase in the number of reported *E. coli* isolates from blood cultures was observed throughout the years, from 3,426 isolates in 2010 to 5,114 isolates in 2017 (a 49% increase), Figure 8.1.1 a). As discussed previously (see introduction to this chapter and DANMAP 2016) this may be due to several factors such as demographic changes, improved culturing methods, and changes in hospital workflow. When comparing these steep increases in positive blood cultures to

Figure 8.1.1 Resistance (%) in *Escherichia coli* invasive isolates from humans, 2008-2017, Denmark DANMAP 2017

a)



b)



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2008 - 2017)	5 years (2013 - 2017)
Ampicillin	↑ p = 0.1	↓ p = 0.31
Piperacillin/tazobactam	↓ p = 0.16*	↑ p = 0.42
Gentamicin	↑ p = 7.702e-06	↓ p = 0.02
Ciprofloxacin	↓ p = 0.01	↑ p = 0.47
Cefuroxime	↑ p = 1.881-07	↓ p = 0.27
3rd generation cephalosporins	↑ p = 0.32	↓ p = 0.03
Carbapenem	↓ p = 0.12	↑ p = 0.15

Note: Figure 8.1.1 a) Resistance results and the total number of positive blood isolates are presented. b) Resistance rates excluding ampicilin. c) Time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test). The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017. *For piperacillin/tazobactam a nine years test is performed, since data are not complete before 2009.

Table 8.1.1 Resistance (%) in *Escherichia coli* isolates from humans, Denmark, 2017 DANMAP 2017

Substance	Blood isolates, hospitals %	Urine isolates, hospitals %	Urine isolates, primary health care %
Ampicillin	46	42	38
Mecillinam	14	7.5	5.5
Piperacillin/tazobactam	4.5	3.7	3.1
Amoxicillin/clavulanic acid	17	14	8.3
Sulfonamide		31	29
Trimethoprim		23	23
Nitrofuratoin		1.2	1.1
Gentamicin	6.0	4.9	4.1
Ciprofloxacin	13	10	8.4
Cefuroxime	9.7	7.1	5.0
3rd generation cephalosporins	6.7	6.2	4.5
Carbapenem	<1	<1	<1
Max. number of isolates tested for resistance to the presented antibiotics	5113	46580	73478

All rates of resistance are presented for each antibiotic in each of the three groups as a mean of the resistance rates reported by the DCM. Included are all DCM that report testing for >75% of the isolates in each antibiotic/sample group.

the actual numbers of blood cultures taken, as registered in the Danish Microbiology Database, MiBa, a marked increase in the sampling number was also observed; from approximately 365,000 in 2010 to approximately 548,000 in 2017, a 50% increase (Data extracted from MiBa on all collected blood culturing samples with a unique sample ID, for the time period of January 1st 2010 to December 31st 2017). It seems reasonable to investigate for possible causal factors, including systematic typing of some of the strains to better understand potential clonal nosocomial outbreaks that may have passed unnoticed.

In 2017, the proportion of ciprofloxacin resistant strains increased markedly (12.8%) compared to 2016 (11.1%). But these recent changes in resistance rates may reflect a change in the interpretation of S-I-R more than a true epidemiologic change, since new EUCAST breakpoints for ciprofloxacin were implemented in most of the Danish DCM as for January 2017. During the last decade resistance to ciprofloxacin showed significant decreases in time trend analysis.

For cefuroxime and gentamicin in invasive *E. coli*, significant increasing trends in resistance were observed for the last decade, but reverting to a decreasing trend in resistance to gentamicin for the past five years. Also for 3rd generation cephalosporins in invasive *E. coli*, the resistance rate decreased during the last five years. (For more details see Figure 8.1.1 a), b) and c)).

The number of carbapenem resistant *E. coli* isolates remained continuously very low with three carbapenem resistant and two intermediary resistant *E. coli* isolates in 2017 – the same level as previous years. Although still at a low level, the risk of increasing levels of carbapenem resistance in the future is worrisome. The level of multi-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *E. coli* remained at around 2%, Table 8.1.2. No pan-resistant *E. coli* have been described in Denmark yet.

8.1.2 Urine isolates from hospitalised patients

For 2017, DANMAP received data on the antibiotic susceptibility in 46,884 *E. coli* isolates, cultured in urine samples from hospitalised patients from all 10 DCM in Denmark.

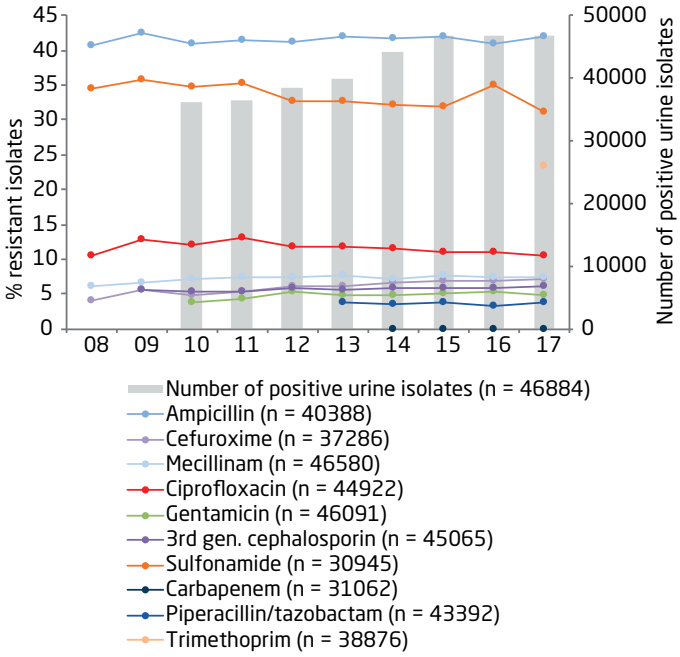
All 10 DCM routinely (>75% of isolates) tested for resistance to mecillinam and gentamicin; in addition nine DCM routinely tested for resistance to ampicillin, ciprofloxacin and 3rd generation cepha-

Table 8.1.2 Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in blood *E. coli* isolates from humans, Denmak DANMAP 2017

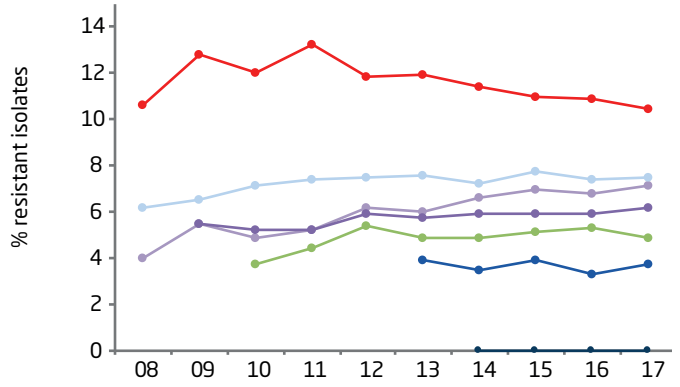
	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)
Resistance	1.8 (72)	2.3 (93)	1.8 (87)	1.8 (88)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	90 (4039)	88 (4071)	98 (4763)	95 (4883)
Total number of blood isolates	4495	4614	4841	5114

Figure 8.1.2 Resistance (%) in *Escherichia coli* urine isolates from humans in hospitals, Denmark DANMAP 2017

a)



b)



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2008 - 2017)	5 years (2013 - 2017)
Ampicillin	↑ p = 0.08	↓ p = 0.09
Mecillinam	↑ p < 2.2e-16	↑ p = 0.4
Piperacillin/tazobactam		↓ p = 0.08
Sulfonamide	↓ p = 0.08	↓ p = 0.09
Gentamicin	↑ p = 4.223e-12	↑ p = 0.18
Ciprofloxacin	↓ p < 2.2e-16	↓ p = 1.074e-13
Cefuroxime	↑ p < 2.2e-16	↑ p = 1.301e-08
3rd generation cephalosporins	↑ p = 6.469e-14	↑ p = 0.01

Note: Figure 8.1.2 a) Resistance results and total number of positive urine isolates are presented. b) Resistance rates excluding ampicilin, sulfonamide and trimethoprim. c) Time trends and significance levels for the past five and 10 years respectively. The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

losporins; eight DCM routinely tested for resistance to cefuroxime, piperacillin/tazobactam, and trimethoprim; seven DCM routinely tested for carbapenem resistance; six DCM routinely tested for sulfonamide resistance and five DCM routinely tested for resistance to nitrofurantoin and amoxicillin/clavulanic acid.

Resistance results for 2017 for all tested antibiotics are summarized together with the results from the invasive isolates as a national mean for each antibiotic in Table 8.1.1. In Figure 8.1.2, rates of resistance are presented as a national mean when at least six DCM performed routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.1.2 c).

Time trend analysis revealed that mecillinam gentamicin, cefuroxime and 3rd generation cephalosporins all had significantly increasing resistance rates looking back 10 years. Looking back five years, the increases were no longer significant for mecillinam and gentamicin. For ciprofloxacin the resistance rates was significantly decreasing looking at time trends both 10 and five years back. (For more details see Figure 8.1.2 a), b) and c)).

The level of carbapenem resistance remained continuously low with total reports of 11 resistant and 8 intermediary resistant *E. coli* urine isolates from hospitalised patients in 2017.

8.1.3 Urine isolates from primary health care

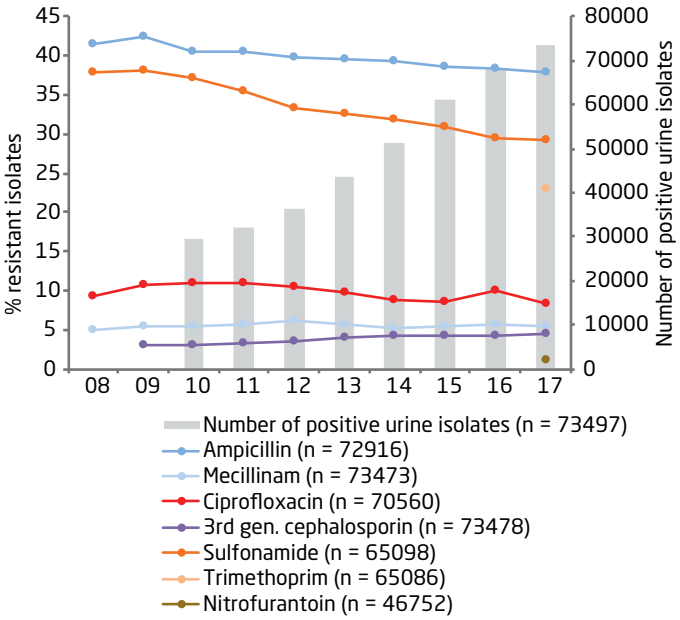
For 2017, DANMAP received data on the antibiotic susceptibility in 73,497 *E. coli* isolates, cultured in urine samples from primary health care, from nine DCM in Denmark. These nine DCM cover all samples from primary health care in Denmark, since one Danish DCM only handles hospital samples. In general, many GPs perform culturing of urine samples at their practice and thus only a selected number will be referred to a DCM. In the Capital Region of Denmark there is one private laboratory in addition, performing culturing of urine samples from primary care.

All nine DCM routinely (>75% of isolates) tested for resistance to ampicillin, mecillinam and 3rd generation cephalosporins; eight DCM routinely tested for resistance to sulphonamide, trimethoprim and ciprofloxacin; six DCM routinely tested for nitrofurantoin resistance; four DCM routinely tested for resistance to gentamicin and cefuroxime; three DCM routinely tested for carbapenem resistance and two DCM routinely tested for resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam.

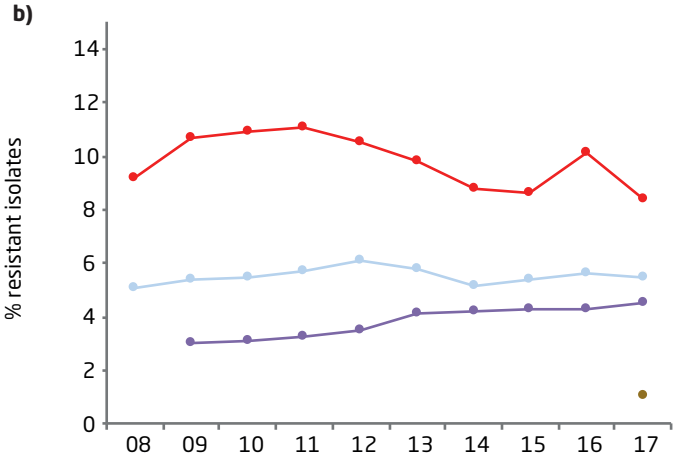
As for the results from invasive isolates and isolates from hospital urines, resistance results for 2017 for all tested antibiotics are shown as national means in Table 8.1.1. In Figure 8.1.3, rates of resistance are presented as a national mean when at least six DCM performed routine testing. Time trends and significance levels, based on the resistance rates for the past five and 10 years respectively, are presented in Figure 8.1.3 c).

Figure 8.1.3 Resistance (%) in *Escherichia coli* urine isolates from humans in primary health care, Denmark DANMAP 2017

a)



b)



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2008 - 2017)	5 years (2013 - 2017)
Ampicillin	↓ p < 2.2e-16	↓ p = 3.064e-11
Mecillinam	→ p = 0.5	↑ p = 8.329e-05
Sulfonamide	↓ p < 2.2e-16	↓ p < 2.2e-16
Ciprofloxacin	↓ p < 2.2e-16	↓ p = 0.0001
3rd generation cephalosporins	↑ p < 2.2e-16	↑ p = 0.0006

Note: Figure 8.1.3 a) Resistance results and total number of positive urine isolates are prensented. b) Resistance rates excluding ampicilin, sulfonamide and trimethoprim. c) Time trends and significance levels for the past five and 10 years respectively. The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

A very steep increase (150%) in reported *E. coli* isolates cultured from urine samples from primary health care has been observed since 2010. Data extractions from MiBa for the same period show a steep increase in the total number of submitted urines for culturing as well. In 2010 in between 164,000 and 206,000 urine samples were submitted to the DCM from the primary sector, compared to in between 430,000 and 439,000 urine samples in 2017, representing an increase in between 114% and 162%. The imprecision in the numbers extracted from MiBa is caused by, in some cases, difficulties in categorisation of hospital vs primary care urines.

Time trend analysis revealed that resistance to 3rd generation cephalosporins has increased significantly both in the past decade and in the past five years. Mecillinam resistance rates have increased in the past five years. Ciprofloxacin, sulfonamide and ampicillin resistance rates have decreased both in the past decade and in the past five years. (For more details see Figure 8.1.3 a), b) and c)).

In 2017, three carbapenem resistant and five intermediary resistant *E. coli* isolates from primary health care were reported.

Conclusion

A substantial increase in the total number of *E. coli* found in blood cultures and in urine samples submitted from the primary sector to the DCM were observed since 2010 (beginning of the observation period). In the same time period, a corresponding increase in the total numbers of registered blood cultures and urine samples from the primary sector occured. The numbers of *E. coli* found in urine samples from hospitals showed less increases as did the total numbers of registered urine samples from hospitals. It is reasonable to conclude that at least part of the increase in the number of *E. coli* isolates is due to an increased number of performed cultures. It remains to be answered whether these increases also could be due to changes in *E. coli* virulence.

Time trend analyses for all three categories of *E. coli* isolates revealed an increase in cephalosporin (cefuroxime and 3rd generation cephalosporins) resistance rates both for the past decade, and for resistance rates in urines also for the past five years. One exception was the resistance to 3rd generation cephalosporins in invasive *E. coli,* which showed decreasing resistance rates in invasive *E. coli* in the past five years.

The time trends for ciprofloxacin showed decreasing resistance in the past ten years for all categories, which is positive. The time trends for gentamicin resistance rates were decreasing for the past five years. In urine isolates, the time trends for sulfonamide resistance rates were decreasing and for mecillinam rather stable.

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8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae (*K. pneumoniae*) is capable of colonising the gastrointestinal and respiratory tract in humans, especially in hospitalised patients. It may cause infections such as urinary tract infections, severe pneumonia and blood stream infections – the latter especially in patients with indwelling devices - and may give rise to nosocomial outbreaks. *K. pneumoniae* rather easily acquires and is able to transfer plasmid borne resistance traits.

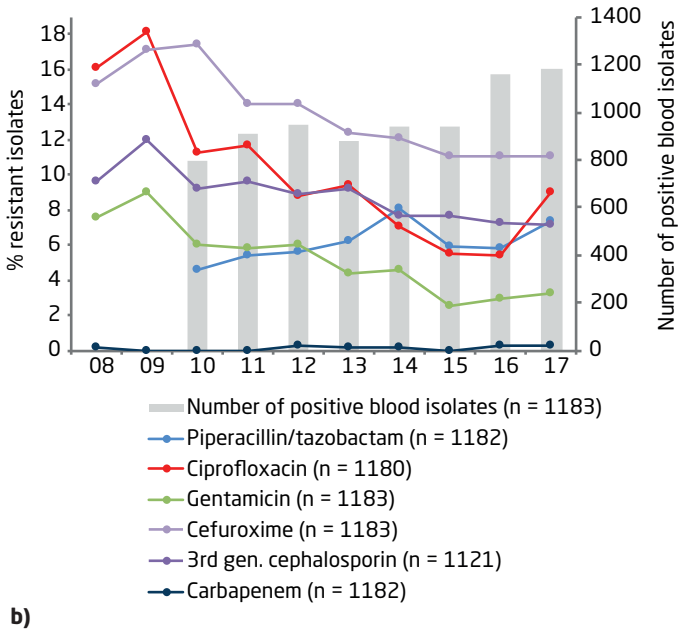
8.2.1 Blood isolates from hospital patients

For 2017, DANMAP received data on the antibiotic susceptibility in 1,183 *K. pneumoniae* isolates from blood cultures from all 10 DCM in Denmark. All 10 DCM routinely (>75% of isolates) tested for resistance to ciprofloxacin, piperacillin/tazobactam, gentamicin, cefuroxime, 3rd generation cephalosporin and carbapenem. Tested 3rd generation cephalosporins were either ceftazidime, ceftriaxone, cefotaxime or cefpodoxime and the tested carbapenem was meropenem. In addition, nine DCM routinely tested for mecillinam resistance and four DCM routinely tested for resistance to amoxicillin/clavulanic acid. Resistance testing was mainly performed by disc diffusion. The presented data consist of the reported interpretation results, performed by the DCM, based on the S-I-R system.

Resistance results for 2017 for all tested antibiotics, presented as a national mean for each antibiotic class, are summarized in Table 8.2.1. In Figure 8.2.1 rates of resistance are shown for the past decade – here data are presented as a national mean, when at least six DCM have performed routine testing. Time trends and significance levels, based on the resistance rates for the past five and ten years respectively, are presented in Figure 8.2.1 b). Test results for mecillinam resistance in invasive *K. pneumoniae* are excluded from Figure 8.2.1, since the S-I-R interpretation rules for the individual DCM differ and/or

Figure 8.2.1 Resistance (%) in *Klebsiella pneumoniae* blood isolates from humans, Denmark DANMAP 2017

a)



b)

Substance	Time trends (Cochran-Armitage test) 10 years (2008 - 2017)	5 years (2013 - 2017)
Piperacillin/tazobactam		↑ p = 0.47
Gentamicin	↓ p < 2.2e-16	↓ p = 0.03
Ciprofloxacin	↓ p < 2.2e-16	↓ p = 0.28
Cefuroxime	↓ p = 8.386e-07	↓ p = 0.13
3rd generation cephalosporins	↓ p = 3.111e-05	↓ p = 0.05
Carbapenem	↑ p = 0.12	↑ p = 0.29

Note: Figure 8.2.1 a) Resistance results and the total number of positive blood isolates are presented. b) Time trends and significance levels for the past five and 10 years, respectively (Cochran-Armitage test). The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

Table 8.2.1 Resistance (%) in *Klebsiella pneumoniae* isolates from humans, Denmark, 2017 DANMAP 2017

Substance	Blood isolates, hospitals	Urine isolates, hospitals	Urine isolates, primary health care
	%	%	%
Mecillinam	19	16	17
Piperacillin/tazobactam	7.4	6.7	7.6
Amoxicillin/clavulanic acid	19	16	14
Sulfonamide		26	25
Trimethoprim		26	27
Nitrofuratoin		13	15
Gentamicin	3.2	3.6	2.1
Ciprofloxacin	9.0	7.6	5.4
Cefuroxime	11	9.4	5.0
3rd generation cephalosporins	7	7.1	4.9
Carbapenem	<1	<1	<1
Max. number of isolates tested for resistance to the presented antibiotics	1183	8092	8939

All proportions of resistance are presented for each antibiotic in each of the three groups as a mean of the resistance rates reported by the DCM. Included are all DCM that repports testing for >75% of the isolates in each antibiotic/sample group.

Table 8.2.2 Combined resistance to 3rd generation cephalosporins, ciprofloxacin, and gentamicin (multiresistance) in *K. pneumoniae* blood isolates from humans, Denmark

DANMAP 2017

	2014 % (N)	2015 % (N)	2016 % (N)	2017 % (N)
Resistance	3.0 (26)	1.1 (9)	1.6 (18)	2.4 (27)
Percentage (no.) of isolates tested for combined resistance (multiresistance)	91 (859)	89 (840)	98 (1131)	95 (1122)
Total number of blood isolates	943	943	1156	1183

vary over time, making comparison of the results difficult and time trends unreliable.

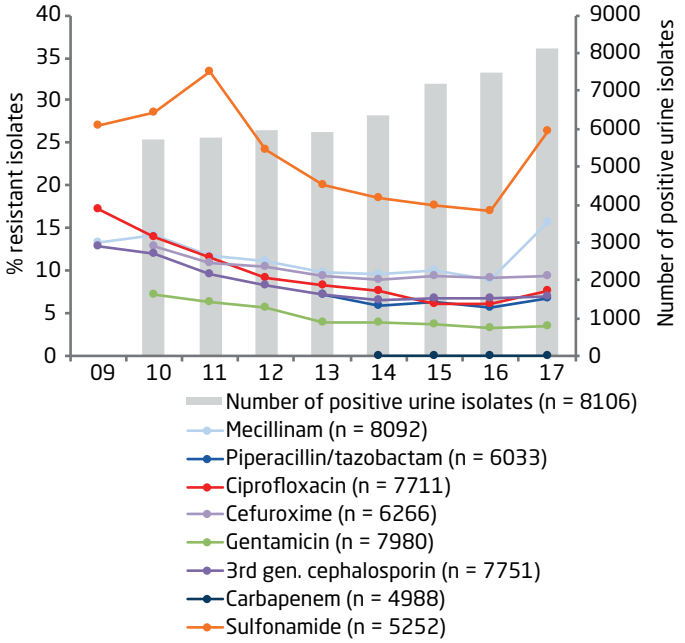
As for *E. coli* isolates from blood cultures, a continuous increase in the number of reported *K. pneumoniae* isolates from blood cultures was observed throughout the years, from 799 isolates in 2010 to 1,183 isolates in 2017 (a 48% increase), Figure 8.2.1 a). This matches a 50% increase in actual numbers of blood cultures taken in the same time period, as registered in the Danish Microbiology Database, MiBa, as described in section 8.1.1.

Curves and time trend analysis revealed that resistance rates have decreased markedly over the past 10 years for gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins, but with lesser or insignificant decreases in the past five years. (For more details see Figure 8.2.1 a) and b)). The increased ciprofloxacin resistance rate in 2017 compared to 2016 may reflect a change in interpretation of S-I-R more than a true epidemiologic change as described in section 8.1.1, *E. coli* blood isolates.

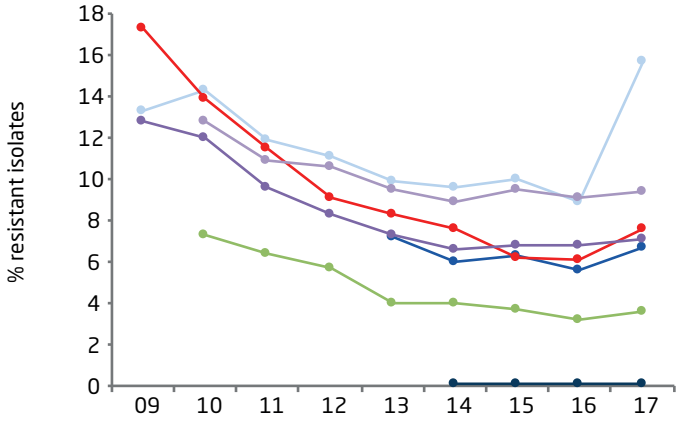
The number of carbapenem resistant isolates remained continuously very low with three carbapenem resistant and none intermediary resistant invasive *K. pneumoniae* isolates in 2017. Although still at a low level, the risk of increasing levels of carbapenem resistance in the future is worrisome. The level of multi-resistant (combined resistance to 3rd generation cephalosporins, ciprofloxacin and gentamicin) invasive *K. pneumoniae* remained at around 2%, Table 8.2.2. No pan-resistant *K. pneumoniae* have been described in Denmark yet.

Figure 8.2.2 Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark DANMAP 2017

a)



b)



c)

Substance	Time trends (Cochran-Armitage test) 10 years (2008 - 2017)	5 years (2013 - 2017)
Mecillinam	↓ p = 0.01	↑ p < 2.2e-16
Piperacillin/tazobactam		↓ p = 0.2
Gentamicin	↓ p < 2.2e-16	↓ p = 0.03
Ciprofloxacin	↓ p < 2.2e-16	↓ p = 0.008
Cefuroxime	↓ p = 4.316e-12	→ p = 0.5
3rd generation cephalosporins	↓ p < 2.2e-16	↓ p = 0.4

Note: Figure 8.2.2 a) Resistance results and total number of positive urine isolates are presented, b) Resistance rates excluding sulfonamide and the number of positive urine isolates. c) Time trends and significance levels for the past five and nine years respectively. The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

8.2.2 Urine isolates from hospitalised patients

For 2017, DANMAP received data on the antibiotic susceptibility in 8,106 *K. pneumoniae* isolates cultured in urine samples from hospitalised patients from all 10 DCM in Denmark. All 10 DCM routinely (>75% of isolates) tested for resistance to mecillinam and gentamicin; nine DCM routinely tested for resistance to ciprofloxacin and 3rd generation cephalosporins; eight DCM routinely tested for resistance to cefuroxime and trimethoprim; seven DCM routinely tested for resistance to sulfonamide, piperacillin/tazobactam and carbapenem and five DCM routinely tested for resistance to nitrofurantoin and amoxicillin/clavulanic acid.

Resistance results for 2017 for all tested antibiotics are summarized together with the results from the invasive isolates as a national mean for each antibiotic in Table 8.2.1. In Figure 8.2.2, rates of resistance are presented as a national mean when at least six DCM performed routine testing. Time trends and significance levels, based on the resistance rates for the past five and nine years, respectively, are presented in Figure 8.2.2 c).

As for the number of invasive isolates, an increase (41%) in reported *K. pneumoniae* isolates cultured from hospital urine samples has been observed since 2010. Data extractions from MiBa for the same period show a smaller increase of only 1.6% to 15% in the total number of submitted hospital urine cultures - the imprecision is due to uncertainty regarding the correct categorisation of some urines as hospital or primary care samples.

In 2017, a very steep increase in the resistance to mecillinam was observed in urine isolates from hospitals. The same steep increase was observed for sulfonamide resistance. Otherwise the trends are similar to the trends observed for invasive *K. pneumonia* with decreased resistance to gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins for the past 10 years and with a less pronounced or insignificant decrease for the past five years. (For more details see Figure 8.2.2 a), b) and c)).

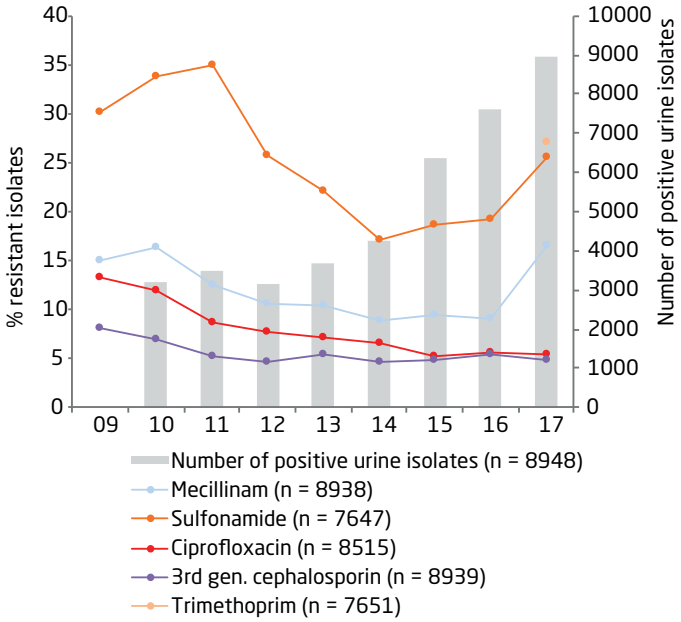
In 2017, 13 carbapenem resistant and six intermediary resistant *K. pneumonia* isolates from hospital urines were reported.

8.2.3 Urine isolates from primary health care

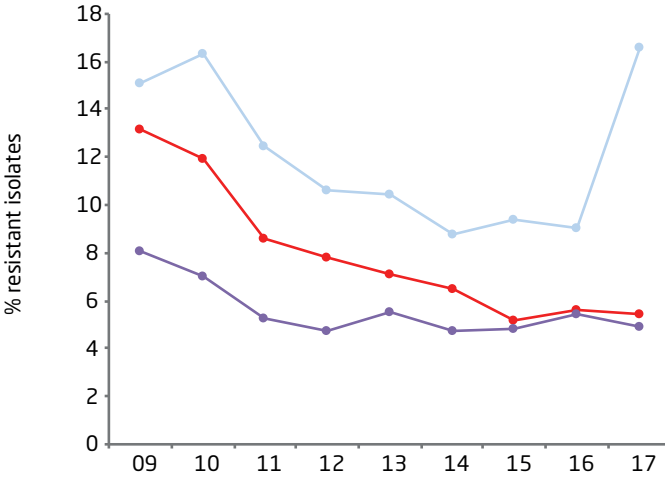
For 2017, DANMAP received data on the antibiotic susceptibility in 8,948 *K. pneumoniae* isolates cultured in urine samples from primary health care from nine DCM in Denmark. These nine DCM cover all samples submitted from the general practitioner to clinical laboratories in Denmark. In general, many GPs perform culturing of urine samples at their practice and thus only a selected number will be referred to a DCM. In the Capital Region of Denmark there is one private laboratory in addition, performing culturing of urine samples from primary care.

Figure 8.2.3 Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark DANMAP 2017

a)



b)



c)

Substance	Time trends (Cochran-Armitage test) 9 years (2008 - 2017)	5 years (2013 - 2017)
Mecillinam	↓ p = 0.06	↑ p < 2.2e-16
Sulfonamide	↓ p < 2.2e-16	↑ p = 6.048e-14
Ciprofloxacin	↓ p < 2.2e-16	↓ p = 0.0003
3rd generation cephalosporins	↓ p = 1.016e-05	↓ p = 0.13

Note: Figure 8.2.3 a) Resistance rates and total numbers of positive urine isolates are presented. b) Resistance rates excluding sulfonamide, trimethoprim. c) Time trends and significance levels for the past five and nine years, respectively (Cochran-Armitage test). The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

Resistance testing for mecillinam and 3rd generation cephalosporins was performed routinely at all nine DCM (>75% of isolates); eight DCM routinely tested for resistance to sulfonamide, trimethoprim and ciprofloxacin; five DCM routinely tested for nitrofurantoin resistance; four DCM routinely tested for resistance to gentamicin and cefuroxime; three DCM routinely tested for carbapenem resistance and two DCM routinely tested for resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam.

As for the results from invasive isolates and isolates from hospital urines resistance results for 2017 for all tested antibiotics are shown as national means in Table 8.2.1. In Figure 8.2.3, rates of resistance are presented as a national mean when at least six DCM performed routine testing. Time trends and significance levels, based on the resistance rates for the past five and nine years respectively, are presented in Figure 8.2.3 c).

Also the total number of *K. pneumoniae* isolates in urine samples from primary health care saw a very steep increase (180%) since 2010. As mentioned in section 8.1.3, *E. coli* in urine samples from primary health care, the increase in the total number of urine samples submitted to the DCM from the primary sector, was in-between 114% and 162%.

As for *K. pneumoniae* in urine samples from hospitals, a very steep increase in resistance to mecillinam and sulfonamides, was noted in 2017. (For more details see Figure 8.2.3 a), b) and c)).

Three carbapenem resistant and none intermediary resistant isolates were reported in 2017.

Conclusion

As for *E. coli*, the continuing increase in total numbers of isolates of *K. pneumoniae* is worrisome and explanations for this needs to be further investigated. In 2017, a very steep increase in resistance in *K. pneumoniae* to mecillinam and sulfonamides was observed, after a period with decreasing resistance rates. Decreased resistance to gentamicin, ciprofloxacin, cefuroxime and 3rd generation cephalosporins for the past 10 years and with a less or insignificant decrease in the past five years was observed. Despite the worrying tendencies, the proportions of resistance to the critically important antibiotics seemed tethered, which is encouraging.

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8.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen causing relatively rare but serious disease in humans. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes bloodstream infections. It is a relatively frequent coloniser of medical devices (e.g. indwelling catheters). *P. aeruginosa* infection is a serious problem in immunocompromised patients with e.g. cancer and in patients with cystic fibrosis. The case fatality rate in these patients is high. *P. aeruginosa* is intrinsically resistant to the majority of antimicrobial agents. The antimicrobial classes which can be used for treatment include some fluoroquinolones, some aminoglycosides, some beta-lactams (piperacillin-tazobactam, ceftazidime and carbapenems) and colistin.

8.3.1 Blood isolates from hospital patients

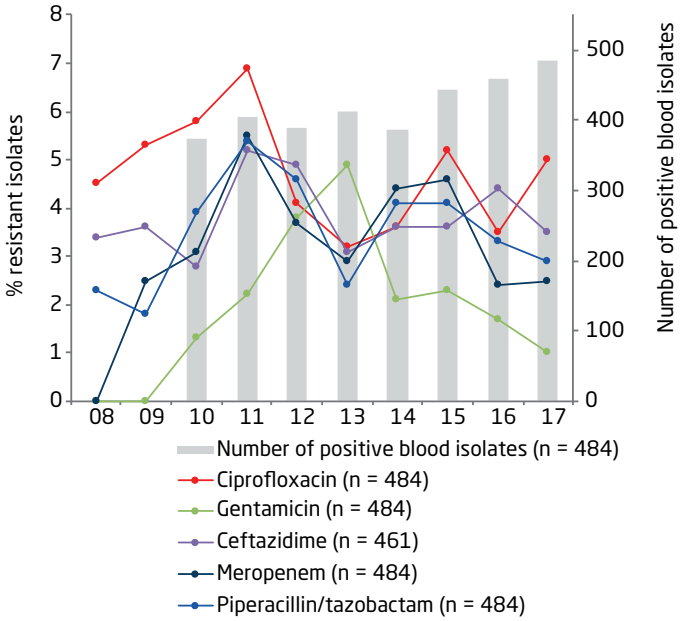
For 2017, DANMAP received data on the antibiotic susceptibility in 484 *P. aeruginosa* isolates from blood cultures from all 10 Departments of Clinical Microbiology (DCM) in Denmark. For all 10 DCM, resistance testing to ciprofloxacin, gentamicin, ceftazidime, meropenem and piperacillin/tazobactam was performed on a routine basis (>75% of isolates) using primarily disc diffusion or Etest. The presented data consist of the reported interpretation results, performed by the DCM, and are based on the S-I-R system.

Data are presented in Figure 8.3.1.

Resistance levels to all tested antimicrobial agents were not significantly different from the levels in 2016.

Figure 8.3.1 Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark

DANMAP 2017



Note: The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

Conclusion

10 years with surveillance of invasive *P. aeruginosa* indicate that the situation in Denmark is quite stable. EARS-Net 2016 reported an increasing trend in resistance in the EU/EEA population-weighted mean against ceftazidime in 2013-2016 (13.0% in 2016) and decreasing resistances in the EU/EEA population-weighted mean against fluoroquinolones (15.0% in 2016), ami-

noglycosides (10.0% in 2016) and carbapenems (15.0% in 2016), while resistance towards piperacillin/tazobactam (16.3% in 2016) did not change significantly. Denmark remained at or below 5% resistance proportions in invasive *P. aeruginosa* isolates in 2017.

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8.4 *Acinetobacter* species

The genus *Acinetobacter* includes several species and is found widespread in nature, in soil, water and/or animals and humans. In humans, *Acinetobacter* can colonise the skin and wounds but may also be the cause of hospital-acquired infections like central line-associated bloodstream infections, nosocomial pneumonia, urinary tract infections and wound infections. 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 the most clinically important. *Acinetobacter* species possess an inherent resistance to a broad range of antibiotics because of a low membrane permeability and constitutive expression of efflux systems. The antimicrobial classes which can be used for treatment include some fluoroquinolones, aminoglycosides, carbapenems and colistin. For especially *A. baumannii,* multiresistant clones are widespread in the hospital environment in many south- and east European countries, where they cause problems with outbreaks in fragile patient subpopulations at e.g. intensive care units. Of worldwide concern are severely war-wounded soldiers colonised or infected with multiresistant *A. baumannii.*

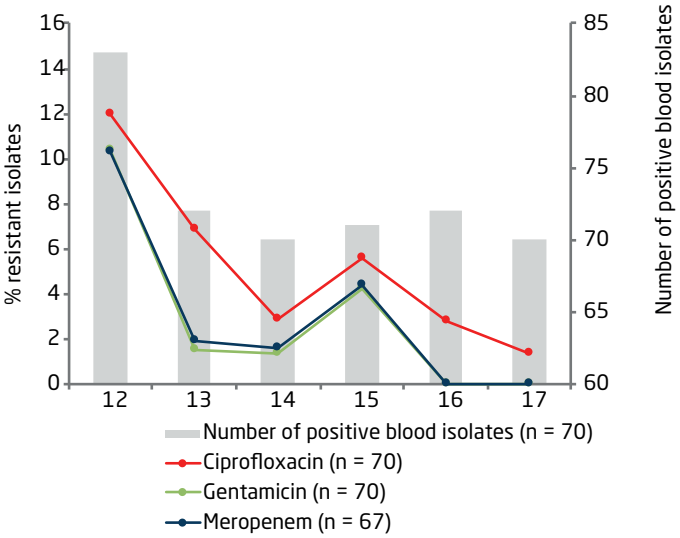
8.4.1 Blood isolates from hospital patients

For 2017, DANMAP received data on the antibiotic susceptibility in 70 *Acinetobacter* species isolated from blood cultures from all 10 Departments of Clinical Microbiology (DCM) in Denmark. All 10 DCM routinely (>75% of isolates) tested for resistance to ciprofloxacin and gentamicin and nine DCM routinely tested for meropenem resistance. Resistance testing was mainly performed by disc diffusion. The presented data consist of the reported interpretation results, performed by the DCM and are based on the S-I-R system.

Data are presented in Table 8.4.1 and in Figure 8.4.1.

Compared to 2016, no change in total isolate numbers of invasive *Acinetobacter* species or in resistance proportions was observed.

Figure 8.4.1 Resistance (%) in *Acinetobacter* species blood isolates from humans, Denmark
DANMAP 2017



Note: The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017.

Conclusion

In 2012, significantly more *Acinetobacter* isolates from blood were resistant towards ciprofloxacin, gentamicin and meropenem (resistance observed in 10-12% of the isolates) than the following years. In 2016 and 2017, resistance was only found in 0-3%. In EARS-Net, marked differences in resistance profiles across Europe have been reported with more than 50% of the isolates being resistant to at least one of the three surveyed antimicrobials (fluoroquinolones, aminoglycosides and carbapenems). Particularly the Baltic and southern and south-eastern countries of Europe reported on problems with high resistance, where the proportion of multiresistant *Acinetobacter* spp. for some countries even outnumbers the proportion of carbapenemase-producing enterobactereales (CPE). All northern countries reported below 2% combined resistance in 2016.

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Tabel 8.4.1 Invasive *Acinetobacter* spp, Denmark. Number of resistant isolates per year per antibiotic and number of tested isolates per year per antibiotic
DANMAP 2017

Substance	2012		2013		2014		2015		2016		2017	
	res.	n	res.	n	res.	n	res.	n	res.	n	res.	n
Ciprofloxacin	10	83	5	72	2	69	4	71	2	72	1	70
Gentamicin	8	77	1	65	1	70	3	71	0	70	0	70
Meropenem	6	58	1	52	1	62	3	68	0	69	0	67
Total number of blood isolates		84		72		72		71		72		70

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

Textbox 8.1

Characterization of ESBL/pAmpC- and carbapenemase-producing *Escherichia coli* from bloodstream infections, 2017 Denmark

Background: The resistance to third-generation cephalosporins in *Escherichia coli* most often occurs 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). Before 2007, the occurrence of third-generation cephalosporin-resistant *E. coli* (3GC-R Ec) isolated from bloodstream infections in Danish patients was low.
The aim of the present study was to characterize the resistance genes encoding 3GC-R or meropenem resistance and Multilocus Sequence Types (MLSTs) of ESBL, **pAmpC and carbapenemase**-producing *E. coli* from bloodstream infections reported in 2017 in Denmark.

Material and Methods: During January 2017 through December 2017, all Danish departments of clinical microbiology collected their 3GC-R (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime resistance) and carbapenemase-producing *E. coli* from bloodstream infections. The isolates were sent to Statens Serum Institut for further characterization. Only one isolate per patient/per year was included in the study.
The isolates were whole genome sequenced, assembled by SPAdes v. 3.11.0, and in *silico* analysed for acquired ESBL-, pAmpC-, and carbapenemase genes and MLST using The Bacterial Analysis Pipeline – Batch upload version 1.0 from Center of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/cge/). For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate cAmpC.

Results: In 2017, whole genome sequencing data were obtained from 354 *E. coli* isolates. Genes encoding ESBL-, pAmpC- and/or carbapenemase production were detected in 337 isolates, compared to 312 isolates in 2016. In 2017, 17 isolates were cAmpC hyper producers only; these isolates were not further investigated.
The regional distribution of the 337 isolates with ESBL-, pAmpC- and carbapenemase encoding genes was compared to data from previous years (Table 1 and Figure 1).
Demographic data was available for all 337 *E. coli* isolates in 2017; 209 (62%) of the patients were men and 128 (38%) were women, compared to 166 (53%) and 146 (47%), respectively, in 2016. Thus, a significant increase of men from 2016 to 2017 ($p = 0.023$) was observed. The average age at diagnosis was 70 years, ranging from below one to 98 years. Forty-eight patients (14%; 31 men and 17 women) of the 337 patients died within 30 days of diagnosis (average age at death was 74 years; ranging from 51 to 94 years).
From 2014 to 2017, the reported cases of 3GC- and carbapenem-resistant *E. coli* in bloodstream infections have changed from 245 to 337 per year. In The Capital Region, the number of reported cases was stable (110 cases in 2014 and 112 cases in 2017). Comparing the countrywide distribution, the number of cases decreased significantly from 45% to 33% ($p = 0.004$) through the years. In the regions of Southern Denmark and Central Denmark, the number of reported cases almost doubled from 2014 to 2017; from 43 to 76 cases in Southern Denmark and from 43 to 80 cases in Central Denmark. The distribution for the two regions changed from 18% in both regions to 23% and 24%, respectively, resulting in a total significant increase ($p = 0.007$) from 2014-2017 caused by the two regions. For the remaining two regions, Region Zealand and North Denmark Region, the number of cases varied during the four years, but the region-wide distribution returned in 2017 to the levels observed in 2014.
Among the 337 isolates, 21 different ESBL, pAmpC and carbapenemase-enzymes were detected (Table 2), including one novel CMY-variant (CMY-162). As in previous years, CTX-M-15 was the most prevalent enzyme (49%) followed by CTX-M-27 (15%), and CTX-M-14 (15%) (Table 2). Two isolates carried the carbapenemase enzyme OXA-181 together with CMY-2. In several *E. coli* isolates, more than one gene encoding ESBL/pAmpC and/or carbapenemases were detected (Table 2). Plasmid-mediated colistin resistance genes *(mcr-1 - mcr-5)*, were not observed in 2017.
In 2017, the 337 *E. coli* isolates belonged to 64 different MLSTs. In 2017, ST131 was still the most common sequence type (ST) with 52% belonging to this type. Other commonly observed sequence types were ST38 (6%), ST69 (6%), ST405 (3%), ST648 (2%), ST1193 (2%), ST410 (2%), and ST12 (2%), whereas the remaining isolates belonged to STs, which were only detected in 1-5 isolates (<1-2% per type) (Table 3).

Among the 175 *E. coli* isolates belonging to ST131, CTX-M-15 (55%) was most common, followed by CTX-M-27 (24%), CTX-M-14 (10%) and CTX-M-101 (5%). The carbapenemase producing OXA-181/CMY-2 isolates belonged to ST410.

Conclusion: Almost one half of the isolates carried CTX-M-15 (49%), a trend also observed in previous Danish studies of ESBL-producing *E. coli* from bloodstream infections [DANMAP 2016, Roer *et al.* JAC. 2016, Hansen *et al.* Microb. Drug Res. 2014], whereas only two isolates were carbapenemase producers (OXA-48 group). A minor part (3%) of the isolates carried CMY-variants, of which one novel pAmpC-encoding gene was observed (CMY-162).
The MLST distribution did not change according to previous years; the worldwide disseminated ST131 clone was still strongly represented in 2017 (52%). In addition, a large amount of other international STs related to spread of 3GC- and carbapenemase-resistance (e.g., ST38, ST69, ST405, ST648, ST1193, ST410 and ST12) was observed.

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Table 1 Distribution of ESBL and carbapenemase-producing *E. coli* from bloodstream infections DANMAP 2017

	2014		2015		2016		2017	
Region	Numbers	%	Numbers	%	Numbers	%	Numbers	%
The Capital Region of Denmark	110	45	116	42	111	36	112	33
The Zealand Region	27	11	14	5	36	12	38	11
Region of Southern Denmark	43	18	45	16	67	21	76	23
Central Denmark Region	43	18	59	21	66	21	80	24
North Denmark Region	22	9	41	15	32	10	31	9
Total Numbers	245		275		312		337	

Table 2 Most common ESBL enzymes and carbapenemases detected in *E. coli* from bloodstream infections, Denmark DANMAP 2017

	2014		2015		2016		2017	
Enzyme	Number	%	Number	%	Number	%	Number	%
CTX-M-15[1]	121	49	139	51	157	50	164	49
CTX-M-27[1]	25	10	33	12	44	14	52	15
CTX-M-14[1]	38	16	33	12	40	13	49	15
CTX-M-1	10	4	7	3	8	3	17	5
CTX-M-55[1]	8	3	14	5	6	2	13	4
CTX-M-101	12	5	15	5	14	4	9	3
CTX-M-3	4	2	4	1	7	2	8	2
CMY-2[1]	10	4	6	2	10	3	7	2
CTX-M-14b	5	2	5	2	9	3	3	< 1
Other CMY variants[1]	4	2	10	4	3	< 1	3	1
Other ESBL enzymes	14	6	16	6	27	9	18	5
OXA-48-group[1]	3	1	3	1	1	< 1	1	< 1

[1]In some isolates more than one enzyme was detected in 2017

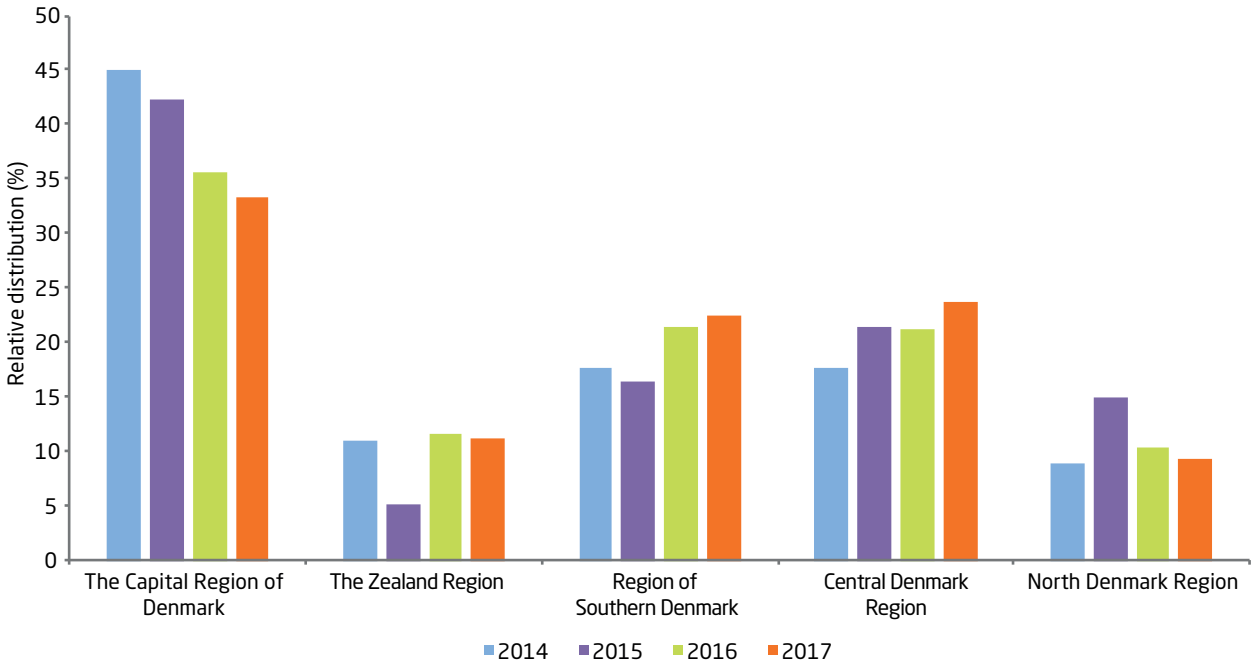
Textbox 8.1 continued...

Table 3 Distribution of MLSTs in *E. coli* from bloodstream infections, Denmark 2017 DANMAP 2017

	2014		2015		2016		2017	
MLST	Number	%	Number	%	Number	%	Number	%
ST31	121	49	139	51	157	50	164	49
ST38	25	10	33	12	44	14	52	15
ST405	38	16	33	12	40	13	49	15
ST410	10	4	7	3	8	3	17	5
ST69	8	3	14	5	6	2	13	4
ST648	12	5	15	5	14	4	9	3
ST12	4	2	4	1	7	2	8	2
ST88	10	4	6	2	10	3	7	2
ST1193	5	2	5	2	9	3	3	< 1
Other STs[1]	4	2	10	4	3	< 1	3	1

[1] less than 5 isolates per ST in 2017

Figure 1 Region-wide distribution of ESBL and carbapenemase-producing *E. coli* from bloodstream infections. DANMAP 2017



For each region, the annual percentage was calculated based on the total number of reported cases in Denmark in the corresponding year, and reported as the relative distribution.

Textbox 8.2

Carbapenemase producing bacteria in Denmark, 2017

Background: Carbapenems comprise one of the only classes of antimicrobial agents that can be used for treatment of infections with multi-resistant Gram-negatives 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 caused by the presence of various carbapenemases of which the most frequently occurring are *K. pneumoniae* carbapenemase (KPC), Oxacillinase (OXA), Verona integron-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM) and Imipenemase (IMP).

In recent years, Danish Departments of Clinical Microbiology (DCM) have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at Statens Serum Institut. The present textbox describes carbapenemase-producing *Enterobacterales* (CPE), *Pseudomonas* spp. and *Acinetobacter* spp.

Carbapenemase-producing organisms: During 2017, 123 carbapenemase-producing organisms (CPO) were detected from 115 patients compared with 115 CPO from 99 patients in 2016 leading to a 6% overall increase of submitted CPO isolates compared to 2016. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases.

Enterobacterales: In 2017, 104 CPE isolates were detected from 96 patients compared to 82 CPE from 72 patients in 2016 (Figure 1) leading to a 26% increase of submitted CPE isolates compared to 2016. In 2017, 27 of the patients had been travelling abroad prior to detection of the CPE; 13 of the patients had no history of recent travel and for the remaining 57 patients, travel information was unavailable (Figure 2).

Seven of the 104 CPE isolates produced both NDM and OXA-48 group enzymes, 71 produced OXA-48-like enzymes and 22 were NDM-producing (Figure 1). Furthermore, three KPC-producing isolates and one VIM-producing isolate were detected.

The NDM-1 producing *Citrobacter freundii* outbreak, which started in 2012 in the North Denmark Region, continued in 2017 (Table 1). Until the end of 2017, 19 patients had been involved in this outbreak in the period 2012-2017. None of these patients had a prior history of travel noted in their hospital records. The origin of the NDM-1 producing *C. freundii* was unknown. NDM-1 plasmid transfer to other CPE was also detected for samples from the patients involved in the outbreak [Hammerum *et al.* 2016, J. Antimicrob. Agents, 71:3117-3124].

Another large outbreak was detected in Zealand with spread of ST410 NDM-5/OXA-181 *E. coli*. The first patient with ST410 NDM-5/OXA-181 *E. coli* was hospitalised in the Capital Region in 2015 after hospitalisation in Egypt [Overballe-Petersen *et al.* 2018, Genome Announc.6(5): e01542-17]. The other patients with ST410 NDM-5/OXA-181 *E. coli* were hospitalised in the Region Zealand in 2016 and 2017. By the end of 2017, ten patients had been involved in this outbreak [Roer *et al.* 2018, mSphere. 2018 Jul 18;3(4). pii: e00337-18].

Spread of ST101 OXA-48 producing *K. pneumoniae* were detected between two patients. Furthermore, it seemed very likely that the increase in OXA-48 producing CPE was due to plasmid transfer, but this was not investigated further.

Finally, three patients had OXA-436 producing CPEs in 2017. Genomic analysis revealed that spread of OXA-436 producing *E. cloacae* had occurred between two patients. OXA-436 is a novel carbapenemase belonging to the OXA-48 enzyme group and has according to our knowledge only been detected in Danish patients [Samuelsen *et al.* 2017, Antimicrob. Agents and Chemother 62(1): e01260-17].

***Acinetobacter* spp:** In 2017, 15 carbapenemase producing *Acinetobacter* spp isolates were detected compared to 26 isolates in 2016. In 2017, 13 OXA-23 producing *A. baumannii* isolates were detected from 13 patients. Nine of these patients had been travelling prior to detection. A possible spread of OXA-23 producing *A. baumannii* was detected between two patients, of which the first had been travelling to Turkey. Furthermore, a possible spread of OXA-72 producing *Acinetobacter bereziniae* was detected between two patients without known travel history.

Textbox 8.2 continued...

***Pseudomonas* spp:** In 2017, two VIM-2 producing *P. aeruginosa* and two IMP-producing *P. putida* were detected. One of the patients with IMP-producing *P. putida* had been travelling, whereas no information was given for the other three patients.

Conclusion: The occurrence of carbapenemase-producing bacteria in Denmark is increasing, a trend worrisome to patients and clinicians. Especially the spread of CPE is of concern, since *Enterobacterales* can be carried in the intestine for a long time without any symptoms of infections, which makes outbreak control difficult.

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Figure 1 Numbers of carbapenemase-producing Enterobacterales (CPE), 2008-2017, Denmark DANMAP 2017

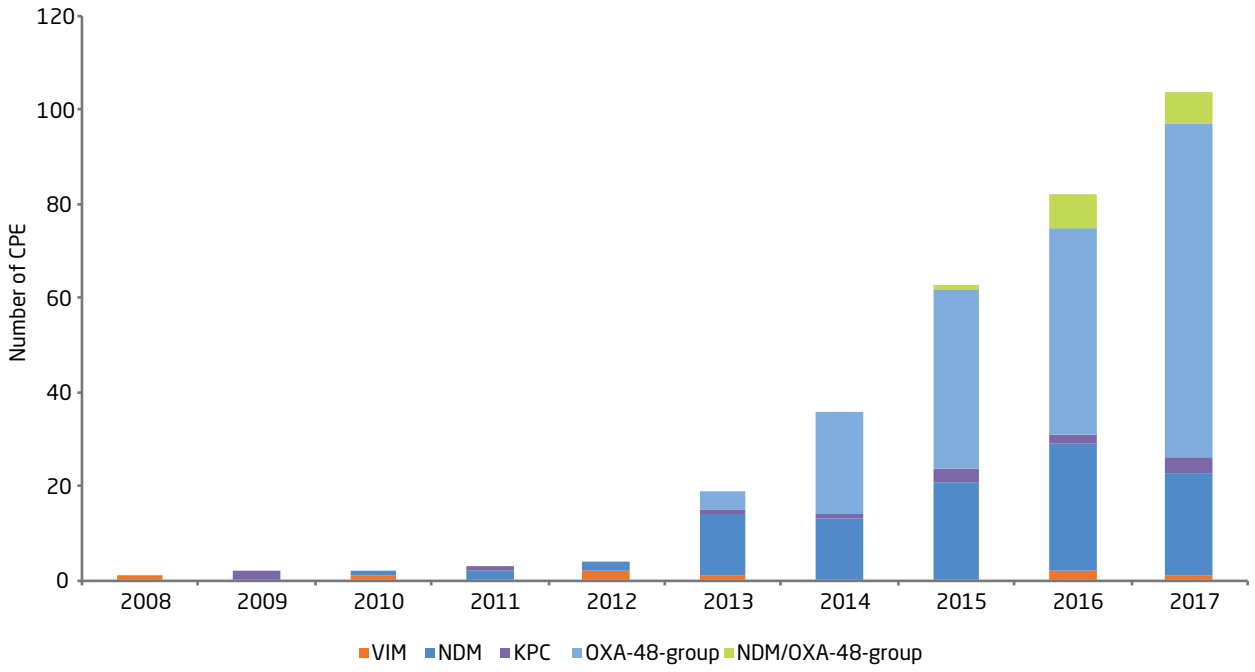
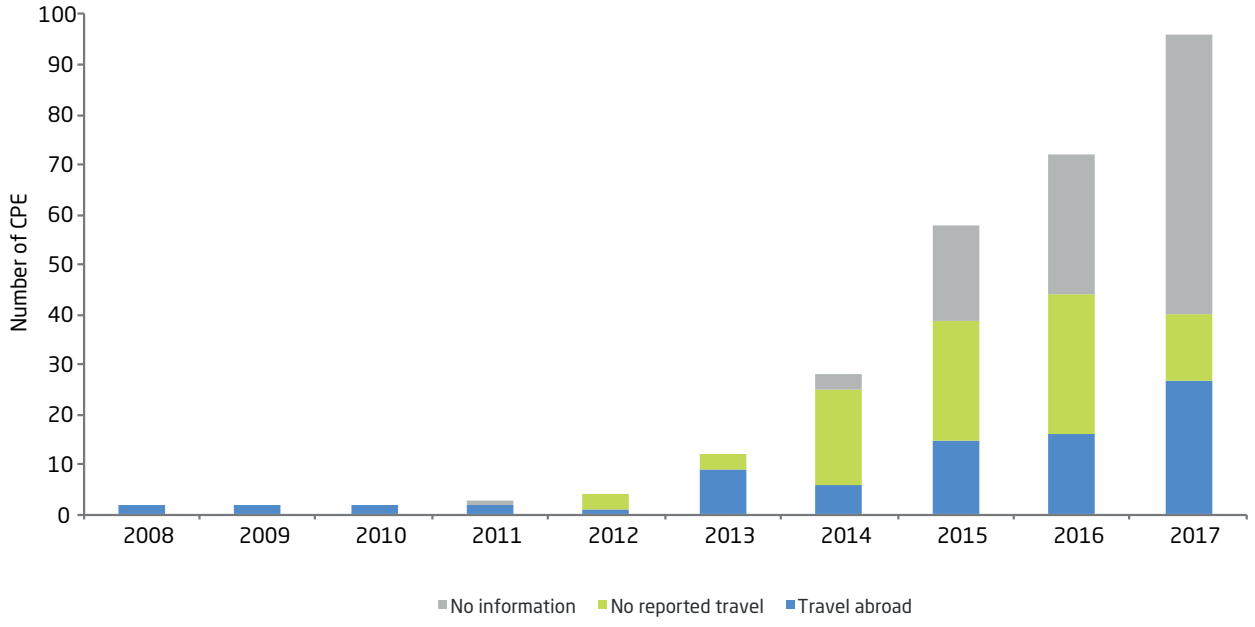


Figure 2 Travel information for patients with carbapenemase-producing Enterobacteriales (CPE) during 2008-2017 DANMAP 2017



8.5 Enterococci

Enterococci constitute a part of the normal intestinal microbiota in humans and animals and colonise the host in beneficial co-existence. More than 54 species belonging to the genus *Enterococcus* have been described, but the majority of the human infections are caused by *E. faecalis* and *E. faecium.* Enterococci are inherently resistant to many groups of antibiotics and thereby get a selective advantage in e.g. hospitalised patients under antibiotic treatment. Most common clinical infections caused by *Enterococcus* species include urinary tract infections, bacteraemia and bacterial endocarditis (an inflammation process of the inner tissues of the heart, usually the valves). Found in hospital environment, bacteria can lead to colonisation or infection of a hospitalised patient. The source of hospital infection is often associated with the use of medical supplies, such as catheters, as well as other instruments and medical devices. Use of antibioitics in these patients increases the risk for an enterococcal infection. Therapy of enterococcal infections is complicated and has limited options due to its high level of natural antimicrobial resistance. In severe cases, enterococcal infections are treated with vancomycin. Combinational therapy based on a synergistic effect of beta-lactam antibiotics (penicillin/ampicillin) with an aminoglycoside (most often gentamicin) or glycopeptide (vancomycin) is required in cases of endocarditis. More recent antibiotics, such as linezolid (oxazolidinone) and daptomycin (lipopeptide) are the only options for treatment of the multiresistant, vancomycin-resistant *Enterococcus* (VRE), which adapts to persist in the health care facilities. A new antibacterial from the oxazolidinone-class with restrictive indications for usage was introduced to the Danish market in 2015.

8.5.1 Blood isolates from hospital patients

For 2017, DANMAP received data on the antibiotic susceptibility in 678 *E. faecalis* and 793 *E. faecium* isolates from blood cultures from all 10 Departments of Clinical Microbiology (DCM) in Denmark. All 10 DCM routinely (>75% of isolates) tested for resistance to ampicillin and vancomycin in both species; six DCM routinely tested for linezolid resistance in *E. faecium* and three DCM routinely tested for linezolid resistance in *E. faecalis*. One DCM routinely tested for high-level resistance to gentamicin in both species. Resistance testing was mainly performed by disc diffusion. The presented data consist of the reported interpretation results, performed by the DCM and are based on the S-I-R system.

Resistance rates to all tested antibiotics for 2017 are presented as a national mean of the combined DCM reportings in Table 8.5.1. In Figure 8.5.1, total numbers of invasive isolates of *E. faecalis and E. faecium* and the rates of resistance to vancomycin in both for the past decade are shown.

Total number of invasive isolates

From 2016 to 2017, the total number of bacteraemia cases increased from 606 to 678 for *E. faecalis* and from 692 to 793 for *E. faecium*.

Ampicillin-resistance

No change was observed in 2017 compared to 2016.

Vancomycin-resistance

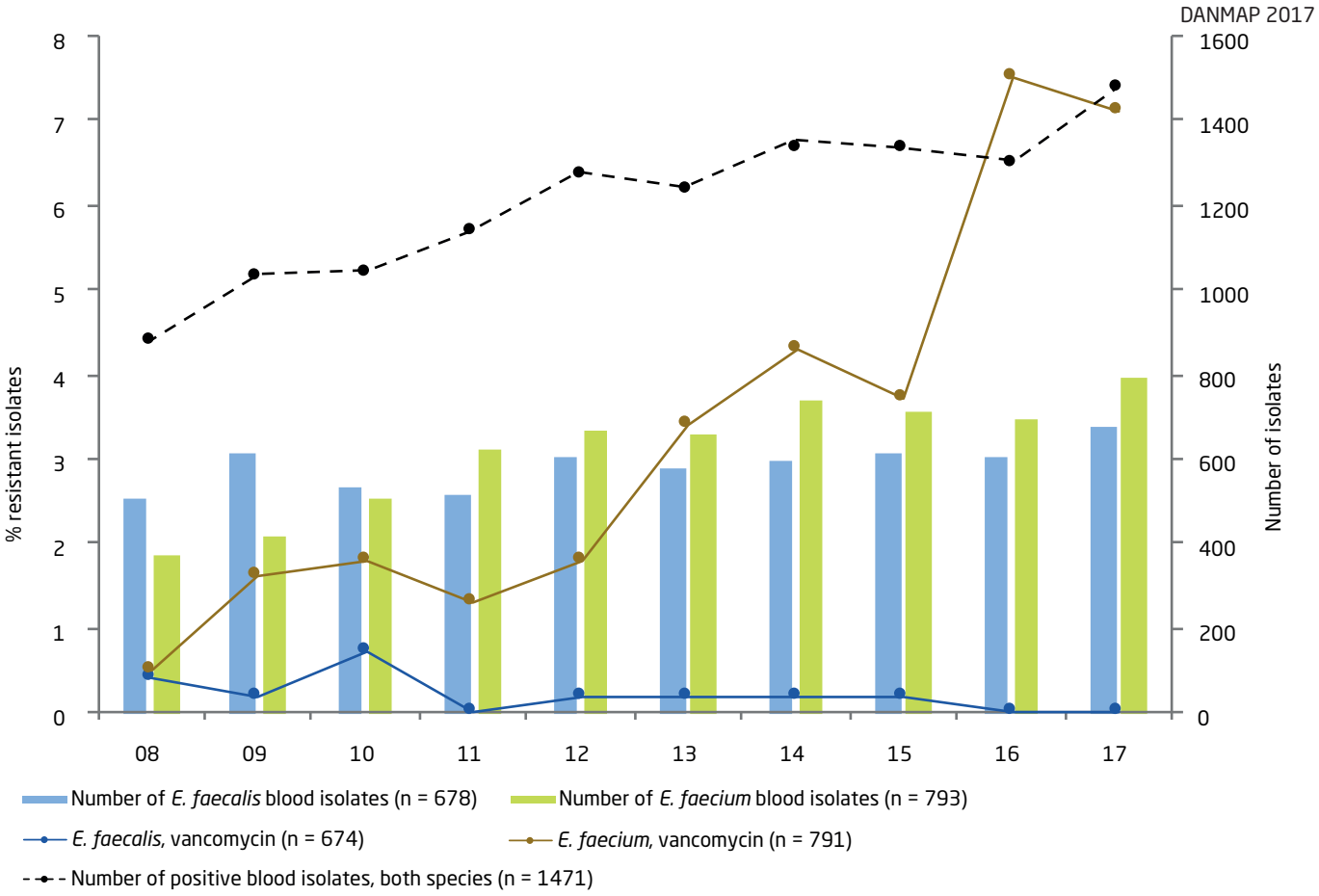
The proportion of invasive *E. faecium* with transferable vancomycin-resistance (7.1%) remained unchanged compared to 2016. Still, the proportion of invasive vancomycin-resistant *E. faecium* is high in Denmark , especially when compared to the other Nordic countries, France and Spain, all of which stay below 2.5% [EARS-Net 2016]. None vancomycin-resistant *E. faecalis* from bloodstream infections were reported in 2017.

Table 8.5.1 Resistance (%) in invasive *E. faecalis* and *E. faecium* isolates from humans, 2017 DANMAP 2017

Substance	*E. faecalis*	*E. faecium*	Number of tested isolates (number of DCM)	
	%	%	*E. faecalis*	*E. faecium*
Ampicillin	0.3	94	670 (10)	779 (10)
Vancomycin	0.0	7.1	674 (10)	791 (10)
Linezolid	2.3	1.6	264 (3)	491 (6)
High-level gentamicin	7.1	43	56 (1)	46 (1)

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

Figure 8.5.1 Number of isolates of *Enterococcus faecalis* and *Enterococcus faecium* and rates of resistance to vancomycin (%) in bloodstream isolates from humans, 2008-2017, Denmark



Note: The number (n) in parentheses represents either the total number of positive isolates or the individual numbers of isolates tested for that specific antibiotic in 2017. In 2008 and 2009 the presented data covers 75% of the Danish population. From 2010 to 2014 data covers 95% of the Danish population and from 2015 the total Danish population is covered.

High-level gentamicin resistance

The proportion of high-level gentamicin resistance (MIC > 128 mg/L) was reported from only one DCM (>75% of isolates tested) as in the previous 10 years, Table 8.5.1. Based on these rather sparse data, a decreasing trend in High-level gentamicin resistance in invasive *E. faecalis* has been observed over the decade, from 35% in the first years to 20% in 2016 and 7.1% in 2017. In *E. faecium* the level has been oscillating between 55% and 75% in the same time period, but in 2017 the resistance decreased to 43%.

Linezolid-resistance

In 2017, linezolid resistance was routinely examined in 264/678 invasive *E. faecalis* and 491/793 invasive *E. faecium* (according to reporting from three and six DCM, respectively). Resistance to linezolid was found in six cases of invasive *E. faecalis* and in eight cases of invasive *E. faecium,* corresponding to 2.3% and 1.6% of the tested isolates, respectively, (Table 8.5.1). This is worrisome, since in 2016 only one linezolid resistant case in each species was reported. But due to selective antibiotic testing concerning linezolid at most DCM, it is not possible to directly compare data from the two years.

In DANMAP 2016, we reported on a specific survey from one DCM regarding linezolid resistance in non-invasive *E. faecalis* and *E. faecium*. In these, resistance to linezolid was found in 1.3% and 4.2%, respectively.

Conclusion

An ongoing increase of invasive enterococci, mainly caused by an increase in invasive *E. faecium,* has been observed during the last 16 years (Figure 8.1.1 DANMAP 2015). The increase is combined with firstly, an increase in ampicillin resistant *E. faecium* (65% in 2002 and more than 90% since 2010) and since 2013, an increase in vancomycin resistant *E. faecium*. In 2017, no further increase in vancomycin resistant rates in invasive *E. faecium* was observed but the total number of VRE isolates still kept increasing as discussed in textbox 8.3 on whole-genome based surveillance of VRE. Due to the limited options of treatment of VRE-infections, the use of oxazolidinones and lipopeptides must be reserved to the treatment of these patients.

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Textbox 8.3

Emergence of clinical *vanA E. faecium* in Denmark, 2017

Background: *Enterococcus faecalis* and *Enterococcus faecium* are commensal bacteria in the human intestine and belong to the most common bacteria causing disease in humans (see section 8.0 and 8.5). During the last decade an increase in the occurrence of vancomycin-resistant enterococci (VRE) has been observed in Denmark and internationally, often found in outbreaks related to hospital care and the treatment of severely ill patients, causing concern and demanding intensification of efforts on infection control.

Surveillance of VRE: Since 2005, Danish Departments of Clinical Microbiology (DCM) have voluntarily submitted VRE for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut (SSI). Since 2013, an increase in clinical VRE isolates has been observed (Figure 1 and Figure 2), mainly driven by increases observed in the Capital Region.

To determine any underreporting in the submissions, the number of VRE submitted to SSI in 2016 and 2017 were compared to the number of clinical VRE reported by the DCMs to MiBa (the Danish Microbiology Database (https://miba.ssi.dk/Service/English.aspx)). From this comparison, it was discovered that the number of submitted VRE isolates were not complete, since 80 and 81 patients were missing from surveillance in 2016 and 2017, respectively (Figure 1). In 2017, 429 VRE isolates were submitted to SSI. Among these, two patients had both a vancomycin resistant *E. faecium* and a vancomycin resistant *E. faecalis*. By adding the 80 and 81 VRE isolates extracted from MiBa, this added up to 510 VRE isolates from altogether 508 patients in 2017 compared to 515 VRE isolates from 515 patients in 2016 (Figure 1).

In 2017, 66 of the VRE isolates were obtained from 66 patients with bloodstream infections, compared to 51 patients in 2016 and 27 patients in 2015. Twenty-nine of the 66 patients (44%), died within 30 days of diagnosis. Many of the VRE patients were chronically ill patients and the high mortality probably owes to this.

As for the former years, a geographic distribution was observed with the Capital Region contributing with the highest number. Of the 510 clinical VRE isolates detected in 2017, 348 (68%) were from hospitals located in the Capital Region (Figure 2).

Sequence analysis of the submitted strains: From 2015 through 2017, all clinical VRE isolates sent to SSI have been submitted for whole-genome sequencing (WGS). In total, 429 VRE were submitted to WGS in 2017. From the WGS data, MLSTs and van-genes were extracted *in silico*. Most of the clinical VRE (415) were *vanA E. faecium* (Figure 1).

Core genome MLST (cgMLST) analysis was performed on the 425 vancomycin-resistant *E. faecium* isolates (Table 1). The majority of the *E. faecium* isolates belonged to three sequence types, ST80, ST117 and ST203. The STs were all part of the CC17 complex, which are commonly detected in hospitals outside Denmark. cgMLST subdivided the 425 *vanA E. faecium* isolates into 58 complex types (CTs). The most prevalent cgMLSTs are shown in Table 1. As for the last two years, ST203-CT859 was the most prevalent sequence type, constituting 61% of the 425 WGS vancomycin-resistant *E. faecium* isolates (Table 1). Comparison to the cgMLST.org database, previous studies, and personal communications with neighboring countries revealed that the novel complex type ST203-CT859 emerged in December 2014 and spread to the South of Sweden and the Faroe Islands during 2015 [Hammerum *et al.* 2017 J. Antimicrobial. Chemother.]. One patient with ST203-CT859 *vanA E. faecium* was transferred from Denmark to Greenland in 2017, but the VRE strain did not spread to other patients in Greenland (Anne Kjerulf, personal communication).

Compared to 2015, the number of *vanA E. faecium* isolates belonging to ST80-CT14 has decreased in 2016 and 2017 (Table 1). This type was also detected in the Capital Region in 2013 (www.cgMLST.org), however the cgMLST database did not contain any non-Danish ST80-CT14 isolates.

Conclusion: The high number of VRE cases in 2017 in Denmark is worrying. VRE can be carried in the intestine for a long period without showing any symptoms. Moreover, they can persist in the hospital environment, which makes infection control difficult. Infection control should include proper cleaning, good hand hygiene, VRE screening and subsequent isolation of patients.

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Textbox 8.3 continued...

Figure 1 Numbers of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from clinical samples and *van* genes, Denmark

DANMAP 2017

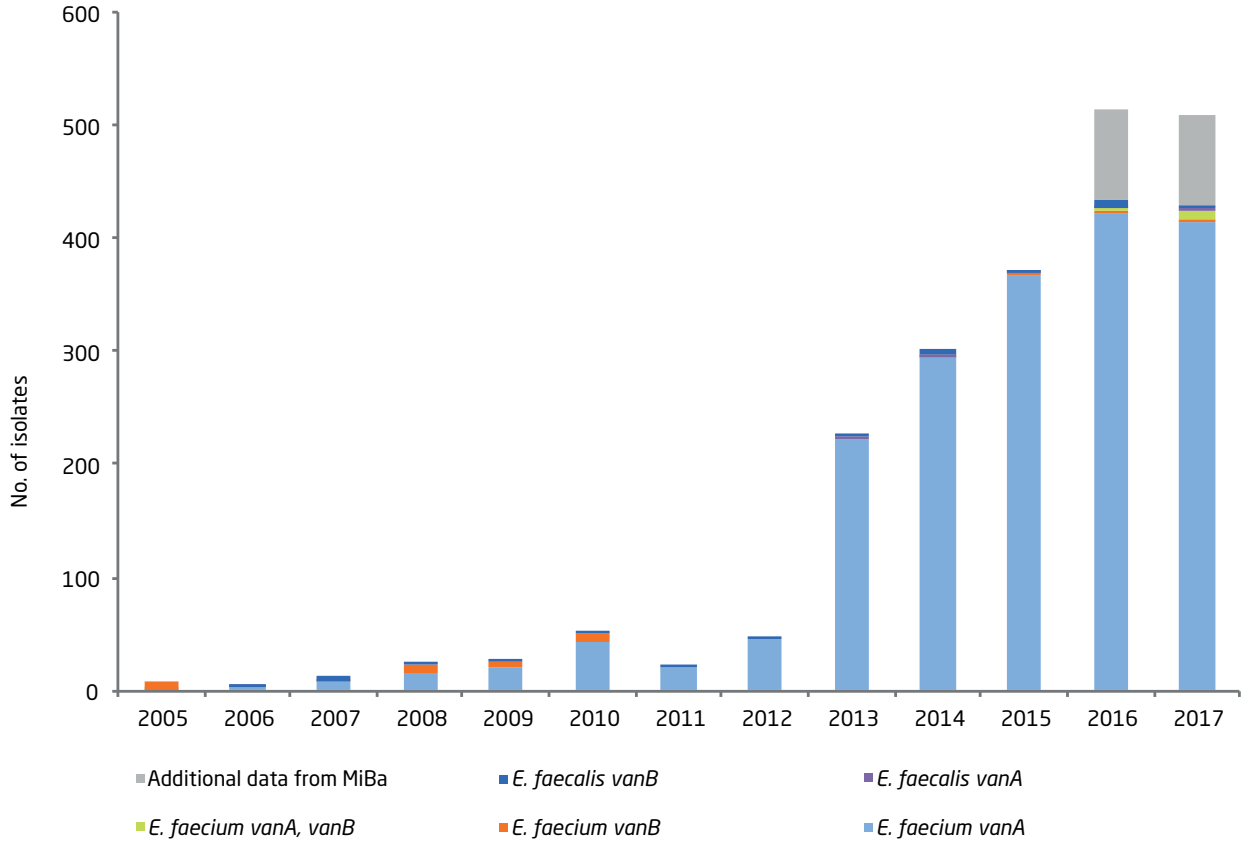

Figure 2 Distribution of the clinical VRE isolates according to the five Danish regions. Data were obtained from clinical samples submitted to SSI 2005-2017 together with data obtained from MiBa from 2016 and 2017.

DANMAP 2017

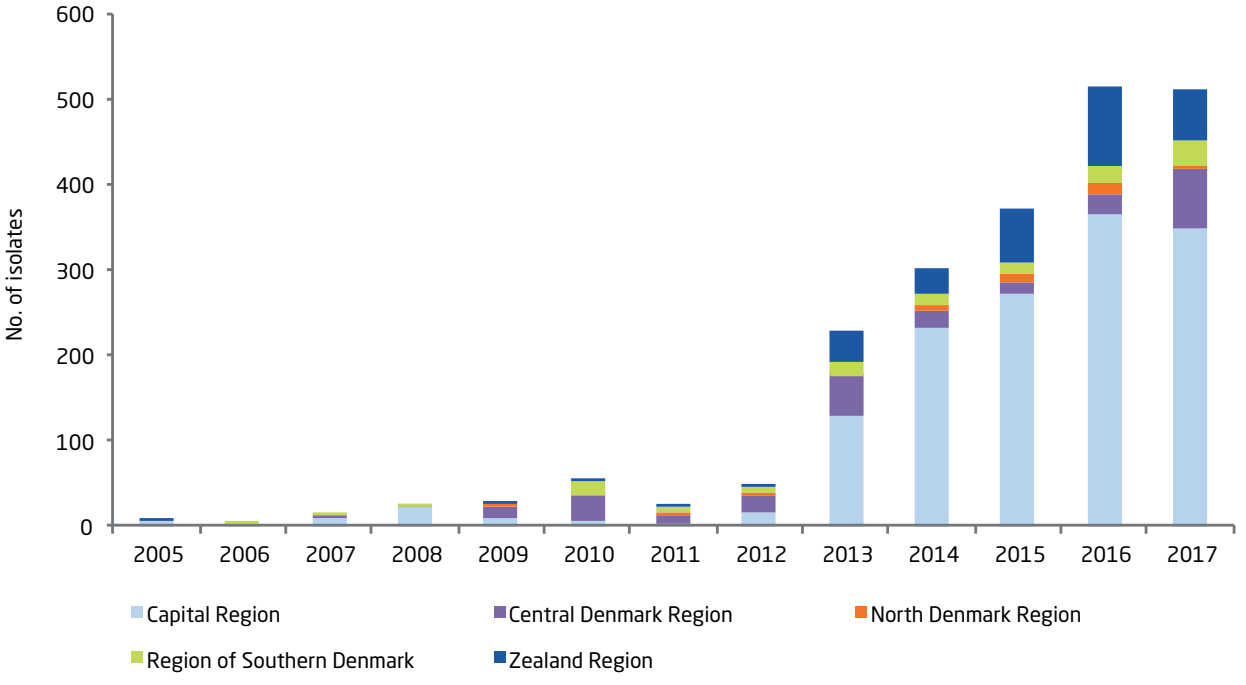

Table 1 Description of the most common types of vancomycin resistant *Enterococcus faecium* send to SSI according to multilocus sequence type (MLST) and coregenome MLST (cgMLST), 2015-2017, Denmark

DANMAP 2017

	2015	2016	2017
ST80-CT14	22%	9%	4%
ST80-CT24	6%	4%	3%
ST80-CT860	2%	3%	N.D.
ST80-CT866	4%	2%	2%
ST80-CT871	1%	1%	3%
ST80-CT993	N.D.	3%	2%
ST80-CT1064	N.D.	<1%	2%
ST80-CT1160	N.D.	N.D.	2%
ST117-CT873	1%	3%	N.D.
ST203-CT859	51%	63%	61%
ST1421-CT1134	N.D.	<1%	3%
Numbers of *E. faecium*	369	427	425

Vancomycin-variable enterococci (VVE)

Background: In recent years, *E. faecium* harboring the *vanA* gene complex, but being phenotypically vancomycin susceptible, has been reported from several countries including Denmark. These enterococci are referred to as vancomycin-variable enterococci (VVE). In Canada and Norway, VVE have caused nosocomial outbreaks and development of revertant mutants becoming vancomycin resistant *in vitro* and *in vivo* has been described. This makes the detection of VVE highly clinically relevant in order to avoid treatment failure with vancomycin. VVE can only be detected by molecular methods, and cannot be cultured on a selective vancomycin-containing media.

Surveillance of VVE: In 2015 and 2016, in the Capital Region of Denmark, sporadic VVE with different genetic background have been detected in relation to concurrent VRE-outbreaks [BJ Holzknecht, personal communication]. Due to this, in 2017 *E. faecium* isolates obtained from blood cultures were tested by *vanA* PCR for VVE in two of the three DCM in the Capital Region. VVE were detected in 4% and 7% of the tested *E. faecium* isolates, respectively. This increase was mainly due to the spread of one single clone, which displays variable vancomycin susceptibility due to a deletion in the *vanX* gene and belongs to ST1421-CT1134. [TA Hansen, J. Antimicrob. Agents, published online, 2018].

In the remaining Danish Regions, VVE have not been reported. With the exception of one DCM in the Region of Southern Denmark, systematic molecular testing has not been undertaken.

Conclusion: The detection of VVE is clinically relevant, although it requires molecular testing. The increase in VVE in Denmark is of concern, especially since VVE are likely to be underdiagnosed. Close surveillance of VVE is important in the future.

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8.6 Streptococci

Streptococci include *Streptococcus pneumoniae* (pneumococci), beta-haemolytic streptococci (BHS) and non-haemolytic streptococci (NHS). All streptococci are Gram-positive bacteria that may in varying degree and frequency be the cause of both common and severe infections. In the following section, the surveillance of pneumococci and beta-haemolytic streptococci causing invasive disease is presented.

8.6.1 Pneumococci

The surveillance of pneumococci causing invasive disease in Denmark happens through mandatory submission of clinical isolates to Statens Serum Institut (SSI). At SSI, the isolates are serotyped and tested for antimicrobial susceptibility.

771 cases of invasive pneumococcal disease (*Streptococcus pneumoniae*) were registered in Danish patients in 2017, and isolates were received from 741 of these cases. The isolates originated from either blood (694 isolates from bacteraemias, of which 14 patients also had a positive isolate from cerebrospinal fluid) or from cerebrospinal fluid alone (40 isolates). Seven isolates were moreover received from other, normally sterile sites (ascites, pleura, joint), but results from these are by tradition not included in this report. The 734 isolates from blood or cerebrospinal fluid belonged to 41 different serotypes, and 693 isolates were fully susceptible to both *penicillin and erythromycin* (94.4%).

For penicillin, 28 isolates (3.8%) were intermediary susceptible and none were resistant. For erythromycin, 26 isolates (3.5%) were resistant. For both antibiotics, the values of non-susceptibility in 2017 were lower than registered in the DANMAP report for 2016 (6.2% for penicillin and 4.8% for erythromycin). Moreover, they were lower than reported for the last six years, Figure 8.6.1.

When comparing to results from neighbouring countries, the levels of penicillin non-susceptibility reported in 2016 to EARS-Net were: Sweden (7.1%), Norway (4.4%) and Germany (4.0%), and the levels of erythromycin non-susceptibility were: Sweden (5.8%), Norway (9.5%) and Germany (7.8%). The results of non-susceptibility for invasive pneumococci from Denmark in 2017 were thus lower than the reported values from 2016 from both Denmark and the neighbouring countries.

For pneumococci, antibiotic susceptibility is closely connected to serotypes of which there are a minimum of 92 different known today. Vaccines and natural fluctuation influence the incidence of infections caused by different serotypes. Thus, the differences in serotype-distributions should be kept in mind, when comparing the percentages of non-sensitive isolates from different years.

The serotypes with more than 25% non-susceptibility to erythromycin were: 6B, 14, 15A, 19F and 25A, while serotypes with more than 25% non-susceptibility to penicillin were: 6B, 14, 15A, 23B and 25A. All isolates belonging to 22 different serotypes were fully susceptible to both antibiotics (n = 230)**,** (Figure 8.6.2 and Table 8.6.1).

The five most dominant serotypes in 2017 were: 8, 12F, 22F, 3 and 9N (n = 432), and the non-susceptibility data for these were 0.23% for penicillin and 0.69% for erythromycin. The childhood vaccine contains 13 serotypes (n = 91). For these, resistance was 5.5% penicillin and 6.6% to erythromycin.

In summary, the levels of non-susceptibility to penicillin and erythromycin in invasive pneumococci show a high degree of variation through the years. However, the levels in 2017 were found to be slightly lower than for the last six years.

8.6.2 Beta-haemolytic streptococci

Beta-haemolytic streptococci (BHS) are divided into serological groups according to antigenic properties of their 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 any BHS group.

Figure 8.6.1 Non-susceptibility (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark DANMAP 2017

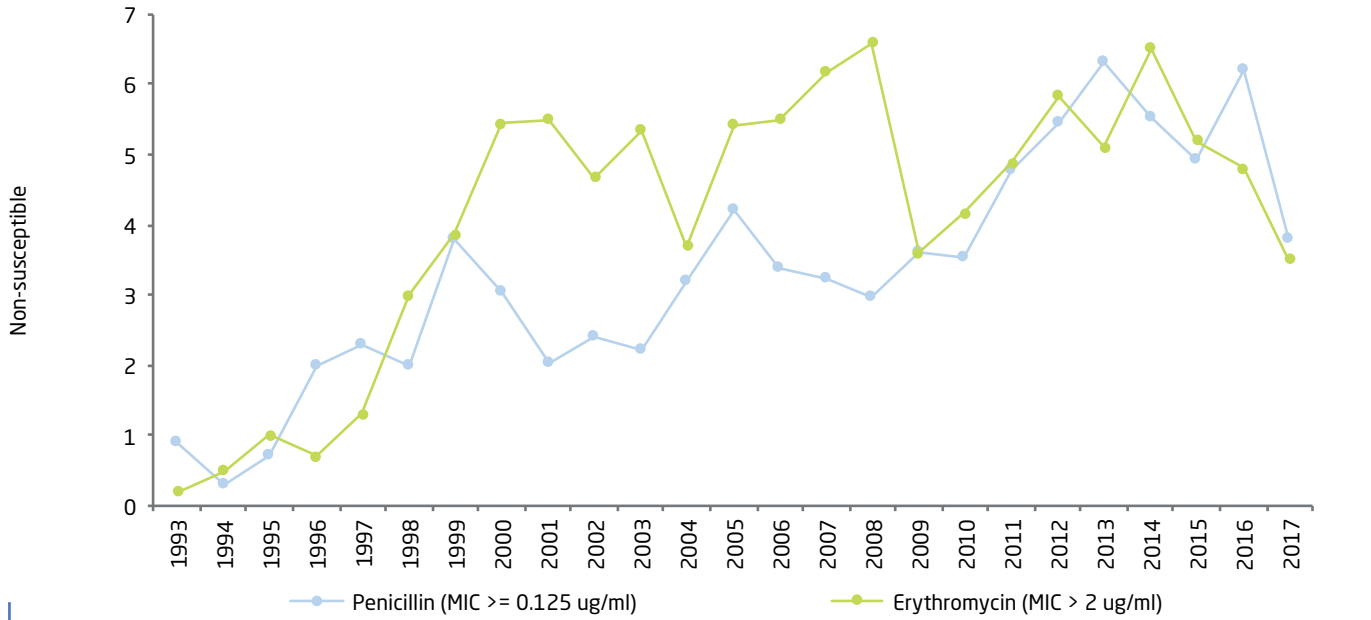

Table 8.6.1 Number of isolates and distribution of resistance in the most common sero-types of pneumococci from blood and spinal fluid, Denmark

DANMAP 2017

Serotype	Number of isolates	PEN-S, ERY-S	PEN-S, ERY-R	PEN-I, ERY-S	PEN-I, ERY-R
8	192	191	1		
12F	69	68	1		
22F	58	58			
3	57	57			
9N	56	55			1
20	26	26			
16F	21	20		1	
35B	20	18		2	
11A	19	17	1	1	
24F	19	15	4		
23A	18	18			
15A	16	10		1	5
15B	14	13	1		
6C	13	12	1		
19F	13	9	2		2
33F	13	12	1		
35F	13	13			
23B	11	3		7	1
17F	10	8		2	

Streptococcus pyogenes (group A streptococci; GAS) causes pharyngitis, tonsillitis, otitis media, wound infections, and superficial skin infections, but also more severe infections such as bacteraemia, necrotising myofasciitis, and rarely meningitis. 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 and may cause meningitis and septicaemia in the newborn due to transmission during labor. In addition, GBS infections have increasingly been observed in elderly and immuno-compromised patients in recent years.

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

This section presents data on antimicrobial resistance in non-duplicate invasive isolates (i.e. from blood or cerebrospinal fluid) of BHS submitted in 2017 to the Neisseria and Streptococci Reference laboratory (NSR) at Statens Serum Institut. Isolates are received from all DCMs in Denmark. Submission is voluntary and not all DCMs submit BHS of all groups.

Infections with BHS are usually treated with penicillins or macrolides, hence all submitted isolates of BHS group A, B, C and G are tested for susceptibility to penicillin, erythromycin and clindamycin as well as inducible clindamycin resistance. Susceptibility testing is performed with disk diffusion methods. If resistance is detected, MIC is determined with Etest. All results are interpreted according to MIC breakpoints issued by EUCAST (http://www.eucast.org/).

Figure 8.6.3 shows the resistance findings for the years 2013 through 2017. The numbers of submitted isolates of GAS, GBS, GCS and GGS changed in 2017 compared to 2016: GAS; 204 isolates (+17%), GBS; 150 isolates (-18%), GCS; 121 isolates (+13%), and GGS; 223 (-5%). Although the trend is less clear, also for the BHS there seems to be an increasing trend in the number of invasive isolates throughout the surveillance years that follows the trends for most bacteraemias described in the introduction of this chapter. All isolates were fully susceptible to penicillin. The erythromycin resistance rate was virtually unchanged for GBS and GGS, but increased from 5.2% to 7.4% for GAS and decreased from 8.4% to 3.3% for GCS. The clindamycin resistance rate increased for all groups except GCS. The percentage of strains with inducible clindamycin resistance was virtually unchanged for GAS and GGS, but showed a marked decrease for GBS and GCS. The percentage of fully susceptible isolates was unchanged for GBS and GGS, but continued to decrease for GAS (from 95% to 92%) and increased for GCS (from 91% to 97%).

Conclusions

The number of submitted isolates in 2017 compared to 2016 increased for GAS and GCS and decreased for GBS and GGS. As for most other bacteraemias the total number of BHS has been increasing over the years. The erythromycin resistance rate increased for GAS but remained unchanged or declining for GBS, GCS and GGS.

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Figure 8.6.2 Penicillin and erythromycin susceptibility among *Streptococcus pneumoniae* serotypes

DANMAP 2017

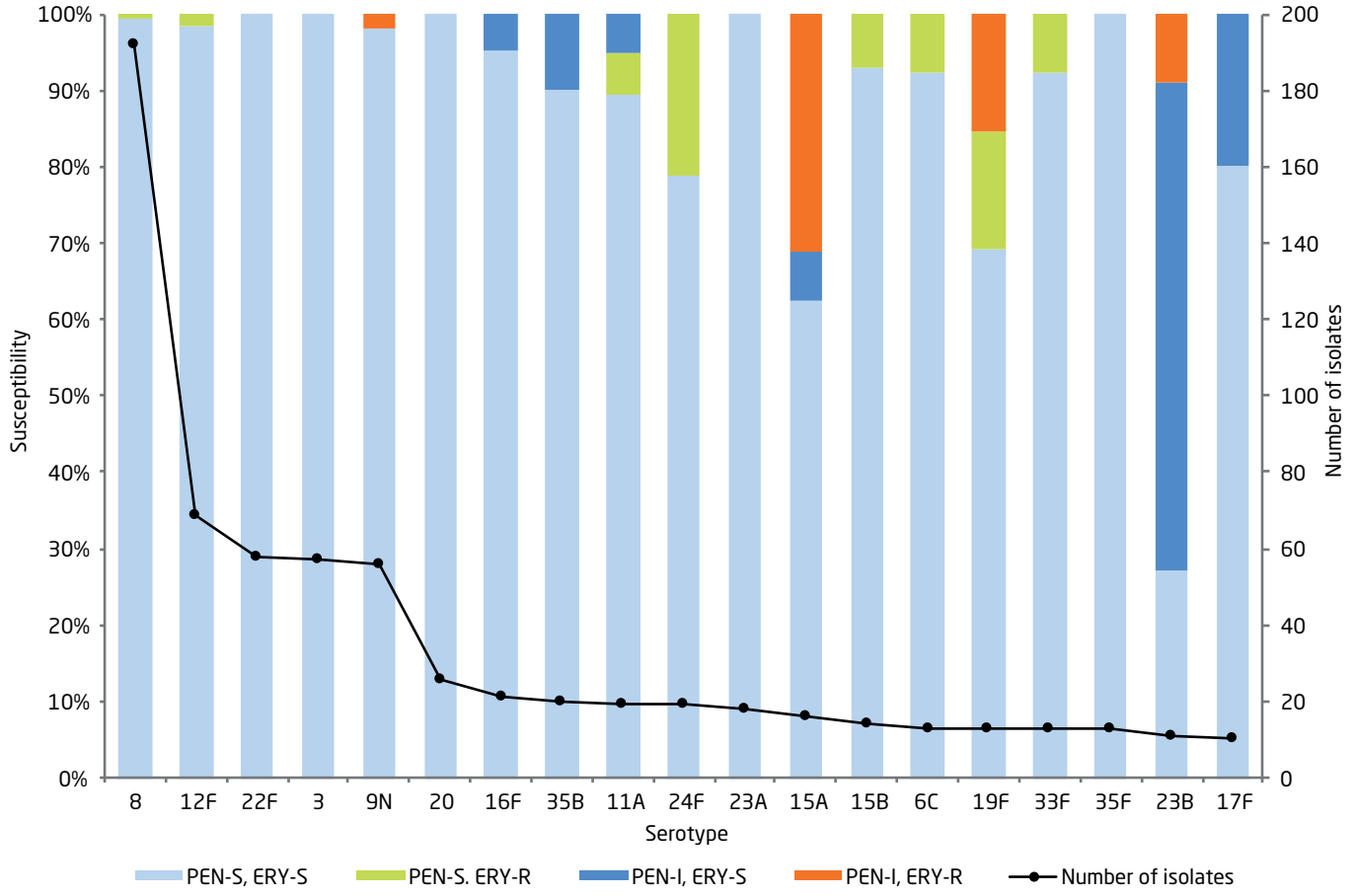
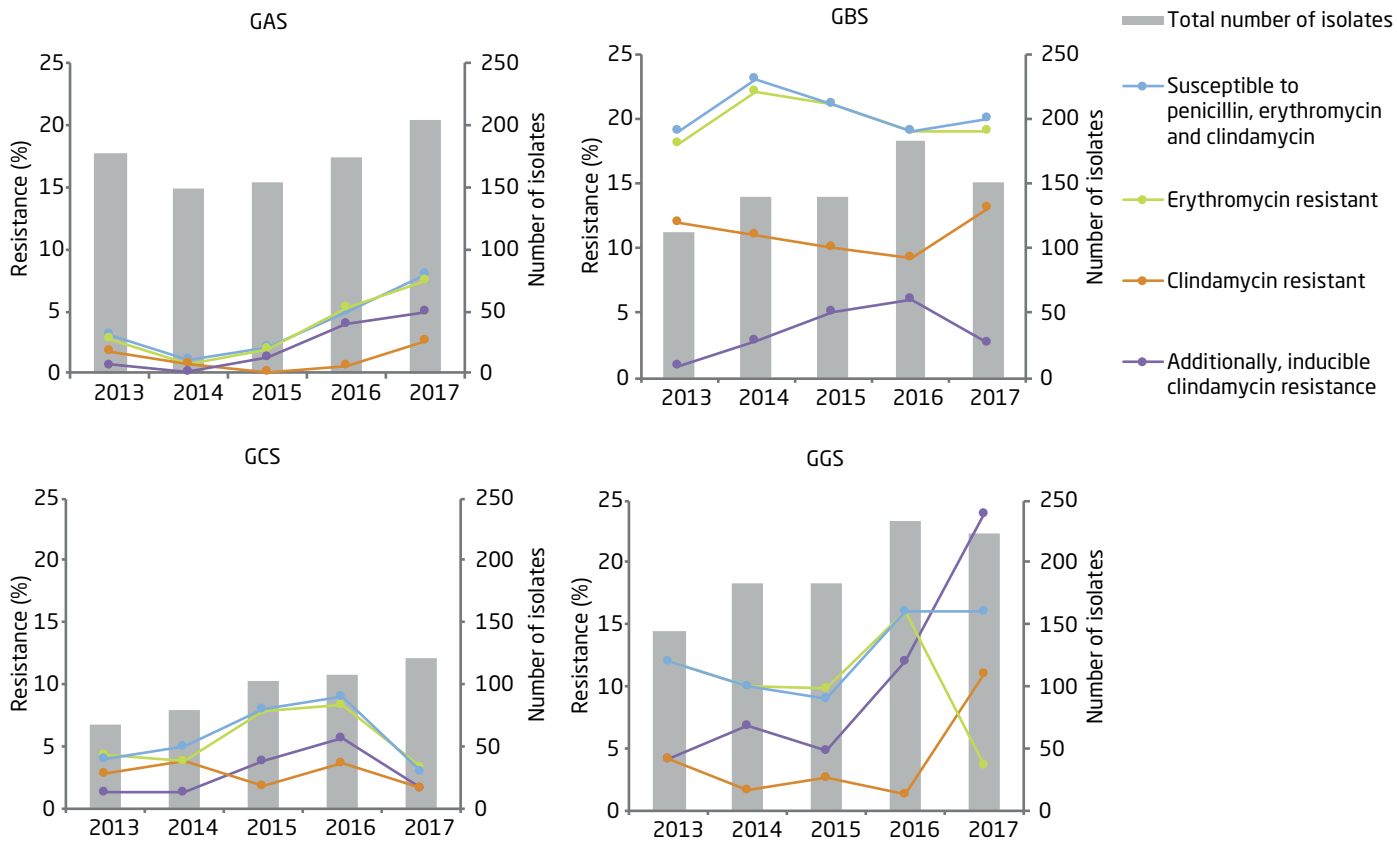


Figure 8.6.3 Beta-haemolytic streptococci, 2013-2017, Denmark: Results of susceptibility testing

DANMAP 2017



Textbox 8.4

Surveillance of invasive isolates of *Haemophilus influenzae*

This is the first report of invasive *Haemophilus influenzae* cases for DANMAP.

Haemophilus influenzae is part of the normal upper respiratory tract flora, where colonisation varies with age. *H. influenzae* can also be the cause of infections, with otitis media and bacterial sinusitis being the most common clinical manifestations. Invasive infections with *H. influenzae* happen rarely and occur predominantly in the very young and the elderly but may also afflict individuals with underlying conditions, such as chronic obstructive pulmonary disease or cancer. *H. influenzae* can be divided into six capsular serotypes (a, b, c, d, e and f), as well as non-capsular (non-typeable, NTHi). Introduction of the polysaccharide type B vaccine in 1993 significantly reduced the number of cases with systemic infection caused by *H. influenzae* type b (Hib) isolates. The dominating serotype is now NTHi for which no vaccine exists.

Invasive *Haemophilus influenzae*:
Surveillance of invasive Hib is mandatory by submission of the isolate to Statens Serum Institut (SSI). By tradition, non-invasive Hib and non-typable NTHi isolates are often voluntarily submitted as well. The received isolates are serotyped by the reference laboratory at SSI. Adherence to surveillance is not complete, but the remaining cases can be identified through data extraction from the Danish Microbiological Database (MiBa). Thus for all invasive infections, data results of antibiotic susceptibility testing is available, while data on serotype is only available for isolates submitted to SSI.

The present report includes all episodes of invasive *H. influenzae* identified in MiBa through January 2014 to December 2017. A total of 469 cases of invasive infections with *H. influenzae* were identified through MiBa, covering data from all 10 Departments of Clinical Microbiology (DCM) in Denmark. In 72.1% (338/469) of the identified episodes isolates were submitted to SSI, while information on the remaining 131 cases was only available through MiBa. Only the data for two antimicrobial agents, ampicillin and cefuroxime, was selected for the present analysis as these are the most frequently tested (410 tested for ampicillin and 367 for cefuroxime, 353 tested for both).

The overall prevalence of invasive *H. influenzae* cases was stable throughout the four-year period. The highest number of cases was observed in 2014 (n = 128) and the lowest number in 2016 (n = 106). The number of cases in 2017 was 115.

The distribution of collection sites was as follows: Blood (n = 428; 91.6%), cerebrospinal fluid (n = 21; 4.5%), pleura effusion (n = 15; 3.2%) and synovial fluid (n = 4; 0.9%). Additionally, one single isolate of *H. influenzae* was extracted from brain tissue. The highest proportion of invasive *H. influenzae* cases was observed in the groups of infants and children between zero and two years of age and in adults above 55 years of age, (Figure 1).

For all invasive *H. influenzae* isolates, a mean susceptibility of 82% was found for ampicillin and a mean susceptibility of 84% was found for cefuroxime in the four-year period. Rates of non-susceptibility for ampicillin and cefuroxime for either one (Table 1 and 2) as well as in combination (Figure 2 and Table 3) were relatively stable during all four years.

Out of all the *H. influenzae* isolates submitted to the National Reference Laboratory at SSI during the four-year period, the prevalence of *H. influenzae* type b isolates was 7.1% (24/338). During the period, a statistically significant increase (p-value: 0.0174) of Hib-positive isolates was observed in 2017 (11/115; 9.5%) compared to 2016 (1/106; 0.9%), 2015 (8/120; 6.6%) and 2014 (4/128; 3.1%). Four of the 24 Hib-positive cases were associated with meningitis disease, two of these appeared in the group of neonates (2/4; 2016 and 2017). The majority of overall Hib-positive isolates was recognised in young children in the age between 0 and 4 years (5/24; 20.8%) and adults above 65 years of age (11/24; 45.8 %).

The prevalence of non-susceptibility to ampicillin and cefuroxime in Hib-positive isolates remained low during all four years. For ampicillin, 18% were found non-susceptible (4/22; two isolates with unknown susceptibility) and all of the nine tested isolates from 2017 were susceptible to ampicillin. For cefuroxime, only one single isolate (5%) was found non-susceptible (1/20; four isolates with unknown susceptibility), and all of the eleven tested isolates from 2017 were susceptible to cefuroxime. One Hib isolate found in 2016 was non-susceptible to both antimicrobials.

Textbox 8.4 continued...

No resistant isolates of type a (n = 3) or type e (n = 7) were detected. Non-susceptibility for type f (n = 43) was 2% for ampicillin (1/41, excluding two isolates with unknown susceptibility) and 3% for cefuroxime (1/38, five isolates with unknown susceptibility). Non-susceptibility for NTHi (n = 261) was 20% (50/244; 17 isolates with unknown susceptibility) on average for ampicillin and 14% (32/221; 40 isolates with unknown susceptibility) for cefuroxime.

Conclusion:
In conclusion, antimicrobial non-susceptibility in Danish isolates of invasive *H. influenzae* is low but highly dependent on serotype, with the non-typeable isolates showing the highest degree of non-susceptibility. Since the non-typeable isolates account for the highest prevalence in invasive *H. influenza* infections, it should be considered to include these strains in the program on mandatory submissions.

Acknowledgements: We wish to thank the 10 Departments of Clinical Microbiology in Denmark for the voluntary submission of invasive *H. influenzae* of all types to the reference laboratory.

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Figure 1 Proportion of different serotypes in invasive *H. influenzae* cases according to age (n = 469), 2014-2017, Denmark

DANMAP 2017

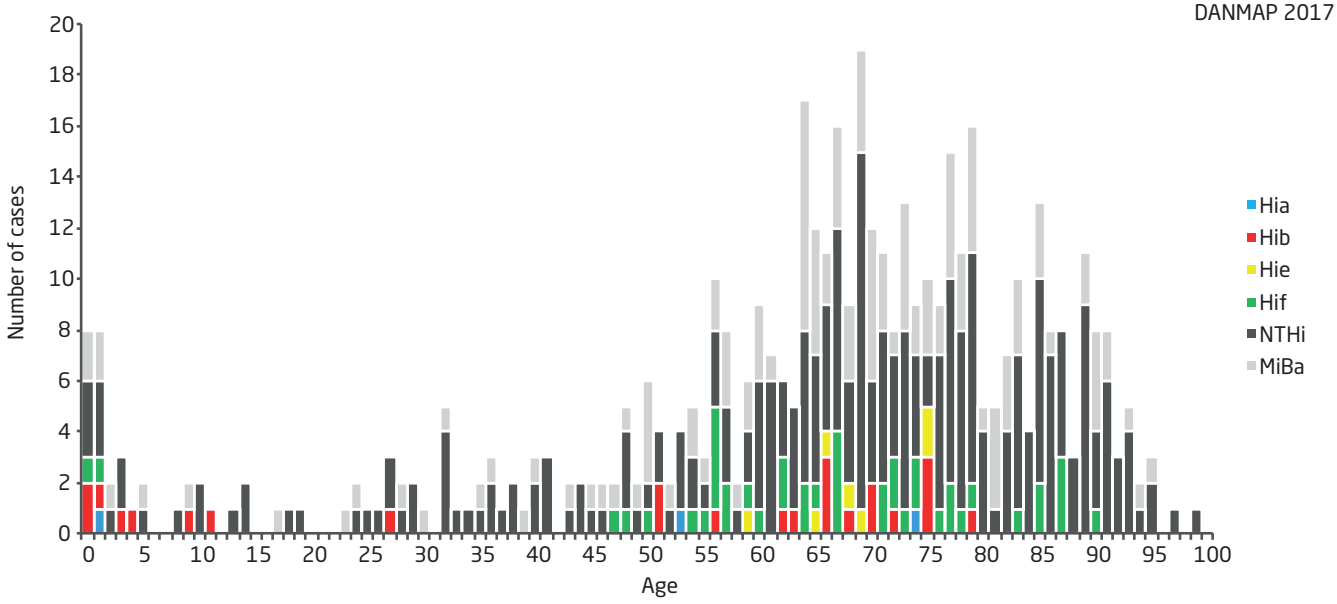
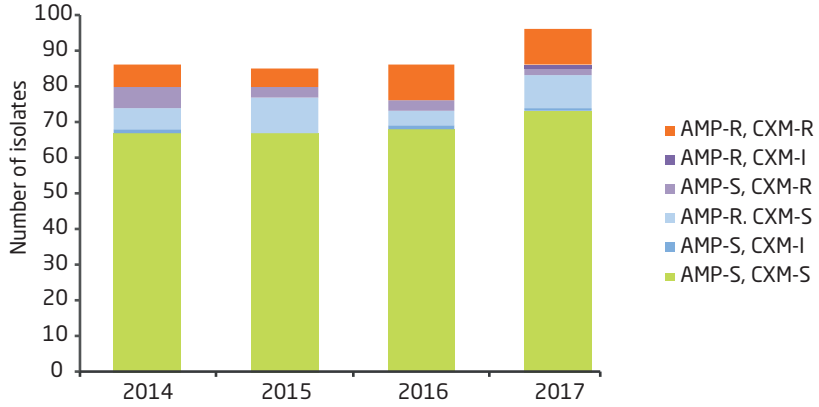


Figure 2 Combined results of susceptibility to ampicillin and cefuroxime in invasive *H. influenzae* cases (n = 353), 2014-2017, Denmark

DANMAP 2017



Note: AMP = ampicillin and CXM = cefuroxime

Tabel 1 Susceptibility (abs. and %) to ampicillin in invasive *H. influenzae* cases (n = 469), 2014-2017, Denmark DANMAP 2017

Year	Total number of isolates	ND[1]	Ampicillin-S	Ampicillin-R	% S[2]	% R[3]
2014	128	22	87	19	82	18
2015	120	16	87	17	84	16
2016	106	10	81	15	84	16
2017	115	11	81	23	78	22
Overall	469	59	337	74	82	18

ND[1] - Results for susceptibility testing for Ampicillin are unknown
% S[2] - Percent of susceptible isolates
% R[3] - Percent of resistant isolates

Tabel 2 Susceptibility (abs. and %) to cefuroxime in invasive *H. influenzae* cases (n = 469), 2014-2017, Denmark DANMAP 2017

Year	Total number of isolates	ND[1]	Cefuroxime-S	Cefuroxime-I	Cefuroxime-R	% S[2]	% non-S[3]
2014	128	39	75	1	13	84	16
2015	120	32	77	0	11	88	13
2016	106	17	72	1	16	81	19
2017	115	14	86	2	13	85	15
Overall	469	102	310	4	53	84	16

ND[1] - Results for susceptibility testing for Ampicillin are unknown
% S[2] - Percent of susceptible isolates
% non-S[3] - Percent of non-susceptible isolates

Table 3 Combined results of susceptibility testing to ampicillin and cefuroxime in invasive *H. influezae* cases (n = 353), 2014-2017, Denmark DANMAP 2017

Year	Total number of isolates tested	AMP-S	CXM-S	AMP-S	CXM-I	AMP-R	CXM-S
2014	86	67	1	6	6	0	6
2015	85	67	0	10	3	0	5
2016	86	68	1	4	3	0	10
2017	96	73	1	9	2	1	10
Overall	353	275	3	29	14	1	31
Percent	100%	78%	1%	8%	4%	0%	9%

AMP = ampicillin and CXM = cefuroxime

8.7 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora of the skin and mucosa in approximately 50% of humans. Some people only carry *S. aureus* intermittently, whereas others carry *S. aureus* for longer time. *S. aureus* causes infections ranging from superficial skin infections i.e. impetigo and boils to more invasive infections such as post-operative wound infections, infections related to intravenous catheters and prosthetic devices, septic arthritis, osteomyelitis, endocarditis and bacteraemia. Some of these may have a fulminant progress and are associated with high mortality.

In Denmark, a voluntary surveillance program of all *S. aureus* bacteraemia (SAB) cases was established in 1957. The adherence to surveillance is high. A comparison to the number of bacteraemia cases registered in the Danish Microbiology Database (MiBa) since 2010 proofed the number of cases reported to SSI to be almost complete (94-97%).

Laboratory and clinical notification of Methicillin-resistant *S. aureus* (MRSA) has existed since November 2006.

At SSI, all isolates are initially tested using a multiplex PCR detecting: the *spa*, *mecA*, *hsd, scn* and *pvl* gene (*lukF-PV*). *spa* is used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing, *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to CC398, respectively. PVL is rarely found in methicillin-susceptible *S. aureus* (MSSA) causing bacteraemia but has been closely associated with certain community acquired (CA) MRSA strains. PVL has also been closely linked to skin abscesses and the very rare condition of severe necrotising pneumonia. Isolates positive for *mecA* and the CC398 specific *hsd* fragment but negative for *scn* (human adaptive factor) and *pvl* genes are considered typical Livestock MRSA (LA-MRSA) and are not *spa* typed. All others including human adapted CC398 isolates are *spa* typed. All bacteraemia cases and *mecA* negative presumptive MRSA are tested for presence of the *mecC* gene.

A representative selection of bacteraemia isolates are tested for antimicrobial susceptibility against 17 antimicrobials. In 2017 testing was performed in approximately every fourth strain (n = 551). For MRSA cases, demographic and epidemiological information is registered. Based on the epidemiological information each case is classified with respect to possible place of acquisition: hospital (HA), community (CA), healthcare-associated with a community onset (HACO), import (IMP) and livestock-associated (LA) MRSA. For CA and HACO, classification is separated into known and not known exposure.

Table 8.7.1 Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark DANMAP 2017

Antimicrobial agent	2008 %	2009 %	2010 %	2011 %	2012 %	2013 %	2014 %	2015 %	2016 %	2017 %
Methicillin	1.3	1.6	1.4	1.4	1.2	1.7	2.9	1.5	2.1	2.2
Penicillin	77	77	75	77	74	76	77	71	71	72
Erythromycin	5	7	5	7	6	7	8	7	7	6
Clindamycin	4	6	4	6	6	6	8	7	6	5
Tetracycline	3	2	3	2	2	3	5	4	3	3
Fusidic acid	9	9	13	13	14	15	15	16	12	14
Rifampicin	<1	<1	<1	<1	<1	0	<1	<1	<1	<1
Norfloxacin	2	2	3	4	4	5	6	6	4	4
Kanamycin	1	1	1	<1	1	2	2	3	1	1
Linezolid	0	0	0	0	0	0	0	0	0	0
Mupirocin	<1	<1	<1	<1	<1	<1	<1	<1	0	<1
Trimethoprim-sulfamethoxazole	nt	nt	nt	<1	1	1	1	<1	<1	<1

Note: nt = not tested
n web annex table A8.1 the distribution of MICs and resistance for all tested antimicrobial agents are shown.

Surveillance of bacteraemia

In 2017, a total of 2,104 *S. aureus* bacteraemia cases corresponding to 36.6 cases per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. This is the first increase in three years, after a plateau of approximately 1,900 annual cases from 2014 to 2016. A decade ago, in 2008, 1,344 cases were reported, corresponding to 24.5 cases per 100,000 inhabitants. Forty-six (2.2%) of the bacteraemia cases in 2017 were caused by MRSA. The percentage of methicillin-resistant bacteremias has increased slightly but steadily within the last decade, measured at 1.3% in 2008. Still, Denmark remains at a notably lower level than the majority of the other countries participating in EARS-Net, where the EU/EEA population-weighted mean MRSA percentage was 13.7% in 2016 [EARS-Net 2016]. Four of the 46 MRSA cases were caused by LA-MRSA CC398 (7 LA-MRSA CC398 in 2016). The 30 days mortality was 23% (486 patients), while the mortality for the MRSA bacteraemia cases was 20% (n = 9).

Antimicrobial resistance in *S. aureus* bacteraemia isolates from 2008–2017 is presented in Table 8.7.1. For most antimicrobial agents, the susceptibility remained, in 2017, at the same level as the previous years, the highest frequency of resistance to other antimicrobials than penicillin being observed for fusidic acid (14%), erythromycin (6%), clindamycin (5%) and norfloxacin (4%). Together with resistance to methicillin, the resistance to fusidic acid is the only one with slight but continuous increases for the past decade.

Typing revealed 623 different *spa* types distributed in 28 different CC groups (the ten most prevalent *spa* types representing 33% of the total amount are presented in Table 8.7.2). The PVL toxin was present in 26 (1.2%) cases of which seven were MRSA. The 26 PVL presenting isolates were distributed among 20 different *spa* types and 11 different CC groups.

Surveillance of methicillin-resistant *S. aureus*

In 2017, a total of 3,579 MRSA cases were detected (62.3 per 100,000 inhabitants). This was close to the number observed in Denmark in 2016 (3,550; Figure 8.7.1). A case was defined when a person for the first time tested positive for a specific MRSA strain without regard to infection or colonisation. MRSA isolates were confirmed by detection of either the *mecA* or more uncommonly, the *mecC* gene.

Although the number of cases leveled in 2017 compared to 2016, an increasing trend has been observed since 2009. CC398 cases constituted 35% (n = 1,251) of new MRSA cases, of which 1,212 belonged to the LA-MRSA CC398 and the 39 other to a human adapted variant harboring the PVL encod-

Figure 8.7.1 Number of new methicillin-resistant *Staphylococcus aureus* cases, with a three years moving average, Denmark, 1994-2017

DANMAP 2017

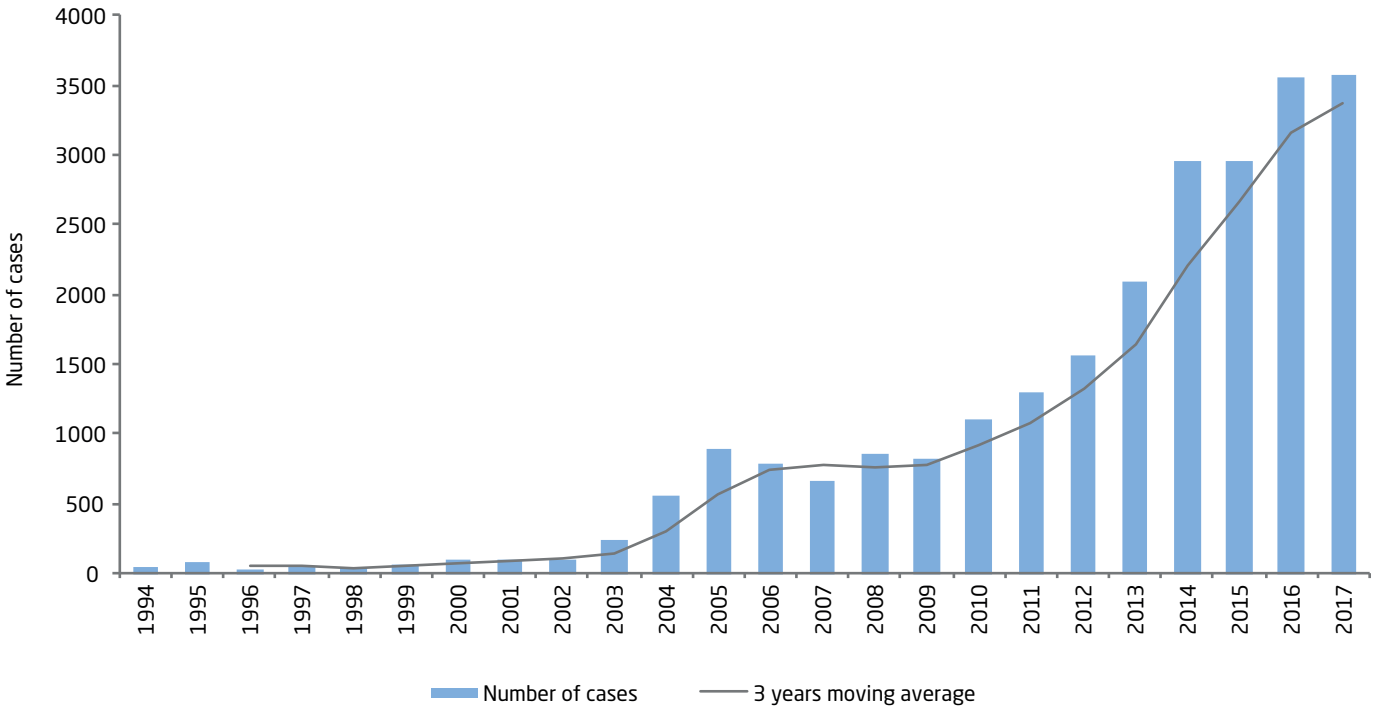


Table 8.7.2 The ten most prevalent spa types demonstrated in SAB and in non LA-CC398 MRSA cases, Denmark 2017 DANMAP 2017

SAB			MRSA			
spa type	CC group[a]	No. of cases	spa type	CC group[a]	No. of cases	No. causing infections (%)
t127	CC1	128	t304	CC8	249	90 (36)
t091	CC7	85	t223	CC22	199	66 (33)
t002	CC5	81	t002	CC5	162	91 (56)
t084	CC15	75	t127	CC1	141	63 (45)
t230	CC45	70	t019	CC30	103	75 (73)
t012	CC30	65	t008	CC8	99	59 (60)
t021	CC30	52	t005	CC22	93	41 (44)
t701	CC8	51	t044	CC80	49	30 (61)
t008	CC8	44	t437	CC59	38	29 (76)
t015	CC45	43	t690	CC88	34	7 (21)

[a] CC = Clonal complex

ing genes. The number of LA-MRSA CC398 was slightly lower compared to 2016. Screening for the carriage of LA-MRSA upon contact with the healthcare system (being admitted to hospital, undergoing planned surgery or when being pregnant) was included in the Danish MRSA prevention program in 2012. Many farmers were voluntarily screened in 2014 and 2015, following screening of the animals at their farms or when household screening was performed due to a positive sample from a family member. The leveling in number of cases with LA-MRSA may be influenced by the fact that only new cases are registered in the surveillance and many people in contact with livestock have already been tested positive.

Figure 8.7.2 Number of MRSA infections according to epidemiological classification, Denmark, 2007 - 2017 DANMAP 2017

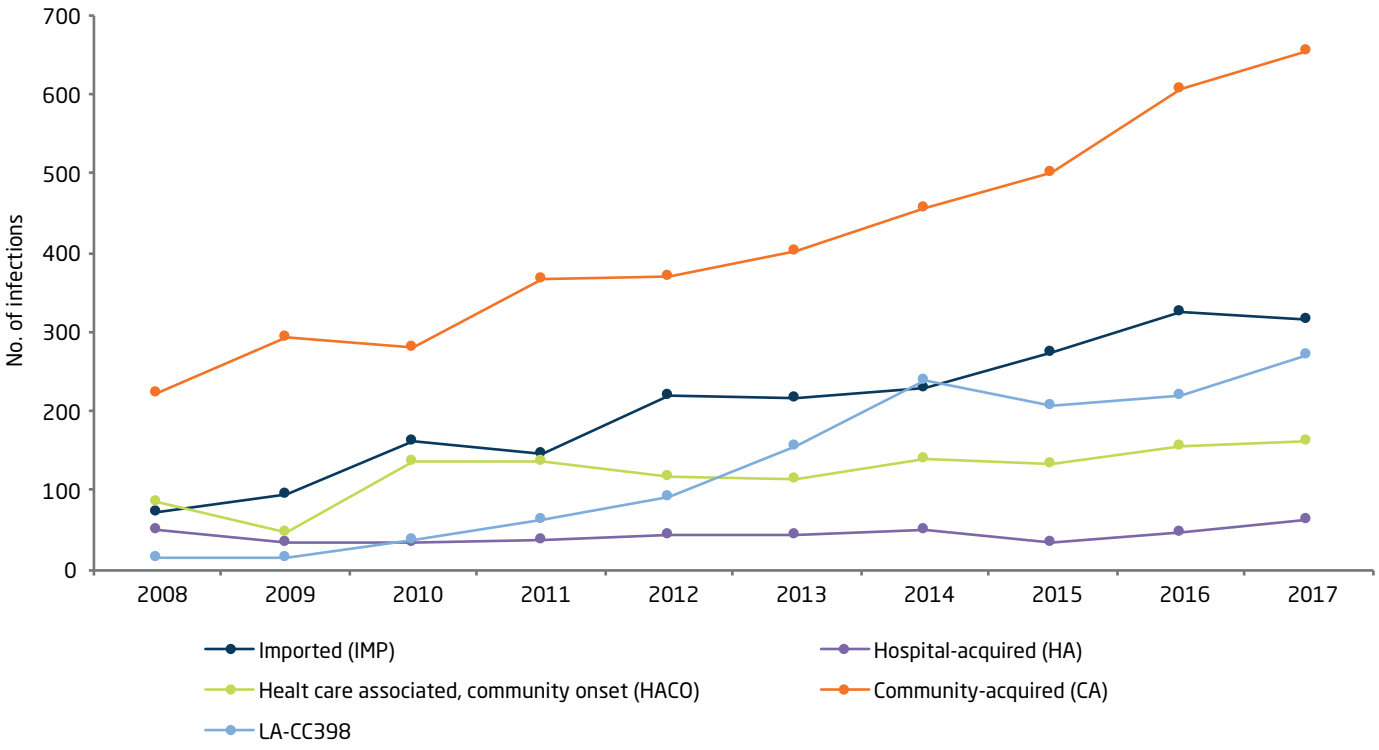


Table 8.7.3 Epidemiological classification of new MRSA cases, Denmark, 2017

DANMAP 2017

Epidemiologic classification	Exposure	No. of cases (% of total)	No. (%) of cases with infections
Imported (IMP)		**581 (16)**	316 (54)
Hospital-acquired (HA)		**100 (3)**	44 (44)
Health-care associated, community onset (HACO)		**218 (6)**	
	with known exposure	39	18 (46)
	without known exposure	179	141 (79)
Health care worker		**54 (2)**	21 (39)
Community-acquired (CA)		**1402 (39)**	
	with known exposure	744	144 (19)
	without known exposure	658	510 (78)
LA-MRSA CC398		**1212 (34)**	
	with known exposure	1019	174 (17)
	without known exposure	193	98 (51)
Unknown/ missing		**12**	5

Note: Numbers shown in bold are totals

MRSA isolates carrying *mec*C were detected in 35 cases (1.0%) in 2017 (9 in 2009, 21 in 2010, 37 in 2011, 24 in 2012, 41 in 2013, 53 in 2014, 61 in 2015 and 45 in 2016). Twenty-two of the cases (63%) had infections at the time of diagnosis. One patient had contact to cattle, another to sheep, both known reservoirs for *mec*C MRSA, while the remaining patients reported no known contact to any livestock.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.7.3. Most of the cases (84%) were acquired in Denmark. At the time of diagnosis, 41% (n = 1,471) of cases had infection, which was higher compared to 2015 and 2016 but similar to previous years.

The epidemiological classification of MRSA infections 2007-2017 is shown in Figure 8.7.2. The number of hospital acquired infections (n = 63) has increased compared to previous years but is still at a low level. The increase may partly be explained by several outbreaks in neonatal wards. In 2017, the number of CA infections continued the increasing trend and were by far the largest group (n = 654), while infections caused by LA-MRSA CC398 increased to 272 (Figure 8.7.2). The number of health care workers with MRSA (infections or colonisations) increased from 40 cases in 2016 to 54 cases in 2017.

Molecular typing of the MRSA strains.

In total, *spa* typing revealed 340 different strain types, not including isolates belonging to LA-CC398, of which 260 types were associated with clinical infections. The 10 dominating non LA-CC398 *spa* types isolated in 2017 are listed in Table 8.7.2. They constituted 50% of the total number of non LA-CC398 MRSA isolates. Ten *spa* types constituted 48% of the 1,175 clinical infections with non LA-CC398 MRSA. The *pvl* gene was detected in 30% of the infections and in 11% of the asymptomatic carriers and most often in relation to isolates with *spa* types t019 (n = 99), t008 (n = 69), t005 (n = 53), t044 (n = 47) and t002 (n = 44).

Resistance among MRSA isolates

Resistance data for non-LA-CC398 isolates is presented in Table 8.7.4. Every other non-LA-CC398 isolate received in 2017 was tested (n = 1,193). Resistance prevalences were similar to previous years.

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Table 8.7.4 Resistance (%) in non LA-CC398 MRSA isolates, Denmark 2017

DANMAP 2017

	% non-CC398
Erythromycin	34
Clindamycin	27
Tetracycline	24
Fusidic acid	16
Rifampicin	1
Norfloxacin	20
Kanamycin	26
Linezolid	0
Mupirocin	<1
Trimethoprim-sulfamethoxazole	3
Number of tested isolates	1193

Textbox 8.5

Incidence of multiresistant bacteria in Greenland

Background: Greenland has a population of 55,860 inhabitants (January 2017) and Nuuk is the capital with around 16,000 inhabitants. 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 that cannot be treated at Dronning Ingrids Hospital are transferred to Denmark or Iceland for further treatment e.g. haemodialysis, cancer treatment, brain surgery etc.

Resistant bacteria: From 2000 to 2017, 46 patients have been diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA), 87 patients with extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, three patients with vancomycin-resistant enterococci (VRE), and 147 patients with *Clostridium difficile* infection, among whom 49 had the 027 type.

MRSA: Up to 2015 there were only altogether 14 patients with MRSA, but since a nearly 4-fold increase in incidence of MRSA has been observed (see Figure 1). During 2017, the number of patients with MRSA increased due to an outbreak in Tasiilaq. This outbreak, consisting of 12 persons, is to date the biggest MRSA-outbreak ever described in Greenland and at the first report of MRSA from the East coast.

The first person in the outbreak was a new-born. This child was born by caesarean section at the hospital in Tasiilaq. Few days after discharge, the child was readmitted due to conjunctivitis in both eyes. Unexpectedly, this infection was due to MRSA and therefore screening procedures for MRSA were established in the child's family (a large household consisting of 17 persons) as well as at the hospital including all hospital staff (38 persons). Furthermore, a mother and new-born child, who had shared room with the MRSA-positive child during the hospital stay (including their household of 13 persons), were screened.

In the family of the index case, one more child and four adults were tested MRSA-positive (a half-brother to the new-born, the mother, the father, the grandmother, and the uncle).The remaining six MRSA-positive persons were all hospital staff. It is unclear where the spread originated but all 12 persons carried the same MRSA-strain (t304 CC6) which is a common strain in Denmark but never seen in Greenland before.

After treatment for MRSA-carrier state according to the MRSA guideline - in some cases treatment was performed twice - all were MRSA-negative except the new-born child, who was positive in samples from the perineum. In families with children below the age of two years, it can be very difficult to treat the MRSA-carrier state and because most children spontaneously "loose" MRSA before the age of two years, treatment of these families is usually not done. However, because the child was living in a big household, a treatment attempt was made.

No further spread of MRSA was seen in the family/household or at the hospital.

This outbreak illustrates the fact that transmission of MRSA in hospital is mainly seen at wards with new-born/premature children due to close contact between the children and healthcare workers, and to close contact in families.

VRE: In spite of ongoing VRE outbreaks in Denmark, so far only three patients have been diagnosed with VRE in Greenland. Two patients were colonised with VRE in the rectum and one patient had pleurisy – in all three cases VRE occurred after hospitalisation in Denmark. No transmission was seen within 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 *Enterobacteriaceae*, 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 at the hospitals, and transmission within the country occurred. But due to a great effort on infection prevention and control from the hospital staff, these outbreaks were quickly controlled. Of the 11 new *C. difficile* cases in 2017, two belonged to the 027 type.

Consumption of antimicrobial agents: All antimicrobial agents in Greenland are purchased and distributed from the National Pharmacy. Due to a new IT-system at the National Pharmacy, it is not possible to show data for antimicrobial consumption/purchase at the present time.

Conclusion: Through many years, the incidence of MRSA in Greenland has been very low but since 2015 an almost 4-fold increase has been recorded due to several outbreaks. The increase in incidence might be explained by factors such as import of the strains from Denmark and abroad from persons hospitalised or traveling, and possible transmission within Greenland. In some of the outbreaks the epidemiology was unknown.

The continuing of surveillance, compliance to screening procedures (especially of patients admitted to hospitals abroad), a persistent focus on the use of broad-spectrum antimicrobial agents, and compliance to guidelines for infection prevention and control are necessary in order to combat MRSA and other multiresistant bacteria in Greenland also in the future.

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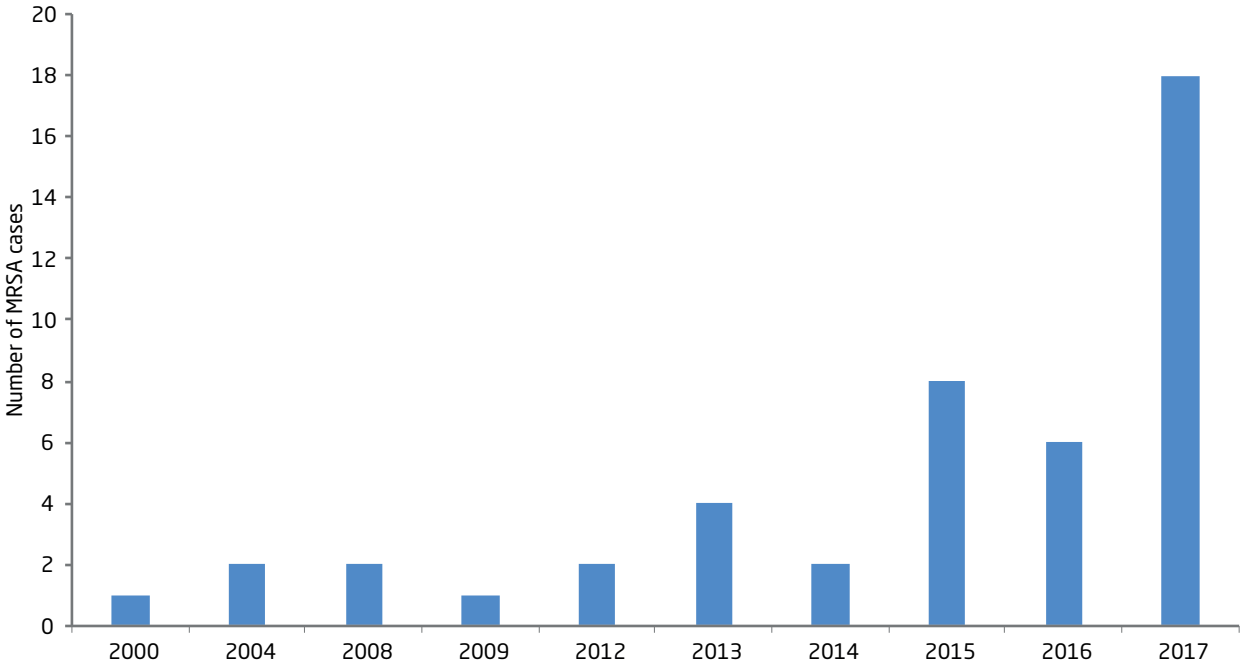
Figure 1 Increasing numbers of MRSA in Greenland

DANMAP 2017



8.8 *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 sometimes be detected 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. In both males and females, 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 inoculation.

Surveillance

Since 1962, the Departments of Clinical Microbiology in Denmark have submitted isolates of gonococci to the Neisseria and Streptococcus Reference (NSR) laboratory at Statens Serum Institut for national surveillance of antimicrobial resistance. Most of the received isolates are from urethra or cervix, while clinicians only rarely obtain specimens from rectum and pharynx. Occasionally, the NSR laboratory receives strains isolated from other anatomical sites, such as conjunctivae, joint fluid, blood, Bartholin's abscess, etc.

At the NSR laboratory, ceftriaxone, ciprofloxacin and azithromycin MICs are determined using the Etest® on chocolate agar incubated at 35 °C in 5% CO_2. The breakpoints used are those defined by EUCAST. Both resistant and intermediary susceptible isolates are categorised as resistant in this report. The Nitrocephin assay is used to test for penicillinase production.

As part of NSR's participation in ECDC's surveillance of sexually transmitted infections since 2009, 110-120 gonococcus isolates are consecutively collected every year and investigated for susceptibility to an expanded panel of antimicrobial agents. This panel includes cefixime and in selected years also spectinomycin and sometimes gentamicin.

Submitted isolates and resistance results

In 2017, isolates from 1,473 unique cases of gonorrhoea were retrieved. The annual number has increased markedly from 2011 through 2016 (Figure 8.8.1), partly because the now widespread use of combined nucleic acid amplifications tests for *Chlamydia trachomatis* and *N. gonorrhoeae* has identified unexpected cases of gonorrhoea (followed by culture), and partly due to an increasing incidence of gonorrhoea, especially among young heterosexual persons, among whom an increasing proportion are women.

The ciprofloxacin resistance rate was 28% in 2017 (18% in 2016, 29% in 2015 and 46% in 2014), thus still markedly lower than the peak of 75% in 2009 (Figure 8.8.1). The percentage of strains producing penicillinase was 9%. It fluctuated between 22% in 2005 and 7% in 2016. Azithromycin resistance was detected in 10% of the cases.

Ceftriaxone resistant gonococci (MIC > 0.125 mg/L) as well as ceftriaxone treatment failure in patients with gonorrhoea has occurred in several countries in recent years. During 2003 through 2009, the proportion of isolates with ceftriaxone MIC ≥ 0.008 mg/L gradually increased from 40% to nearly 75% (Figure 8.8.2), but during recent years this shift has nearly reversed (44% in 2014 and 17% in 2015). In 2017, there was a single case among the submitted isolates with ceftriaxone MIC of 0.25 mg/L and azithromycin MIC of 0.25 mg/L.

The National Board of Health issued guidelines in 2015 for the diagnosis and treatment of sexually transmitted infections. A combination of high dose ceftriaxone (500 mg i.m.) and azithromycin (2 g) is recommended for the treatment of gonorrhoea. Ciprofloxacin 500 mg p.o. may be used for treatment if the strain is fully susceptible.

In a subset of 118 isolates, resistance against cefixime (MIC > 0.125 mg/L) was 0.8% in 2017 (0% in 2016 and 2015). Cefixime is an orally administered cephalosporin that has never been used in Denmark. Resistance against spectinomycin was 0% in 2017 like in 2016 and 2015. MIC values for gentamicin were 1 to 4 mg/L, but no breakpoints are defined for this agent against gonococci. However, the distribution of gentamicin MICs were a little lower than during preceding years.

Conclusions

The incidence of gonorrhoea remained at the high level it had reached in 2016 following substantial increases. The ciprofloxacin resistance rate increased in 2017 for the first time after almost a decade of decreasing. Although resistance problems are still not present in Denmark, the emerging ceftriaxone treatment failures in other countries underlines the importance of maintaining the centralised national surveillance of antimicrobial resistance in gonococci.

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Figure 8.8.1 Number of submitted gonococcus isolates from males and females, and the percentage of isolates containing ciprofloxacin resistance, azithromycin resistance and penicillinase production, Denmark, 2003-2017

DANMAP 2017

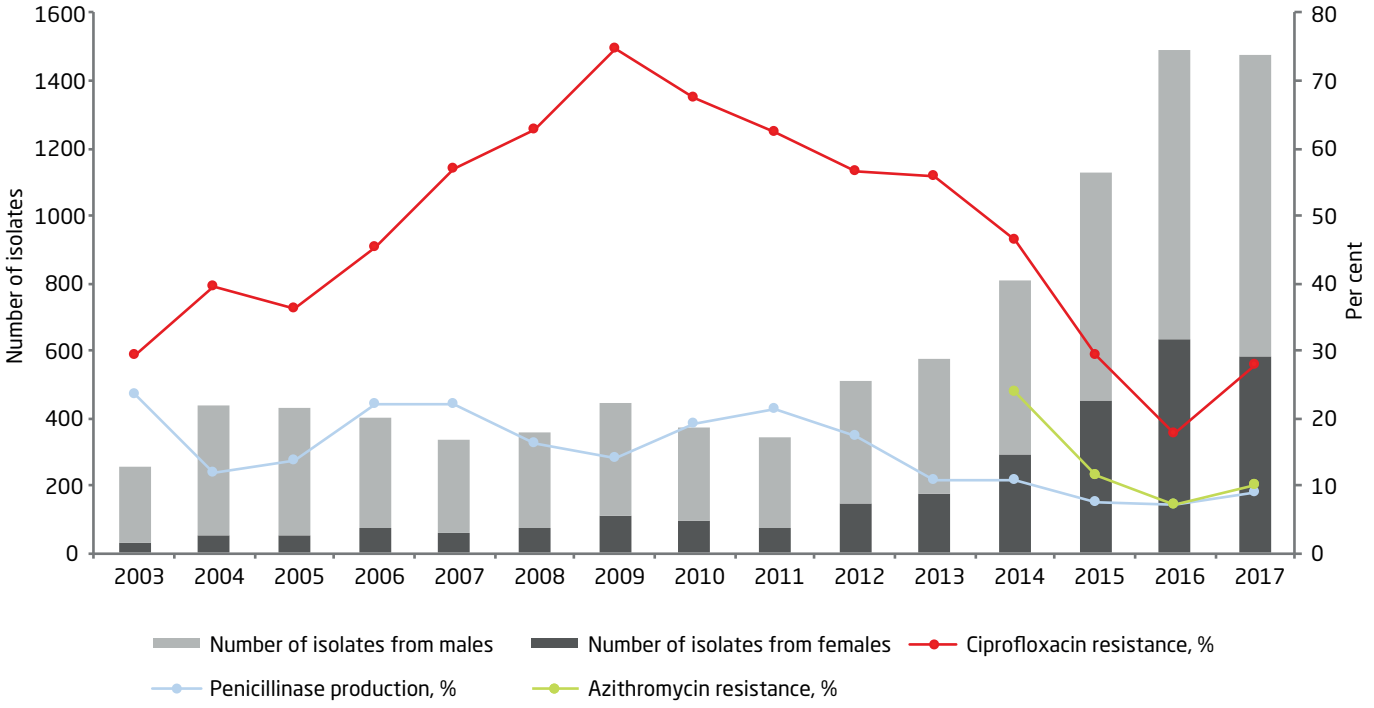
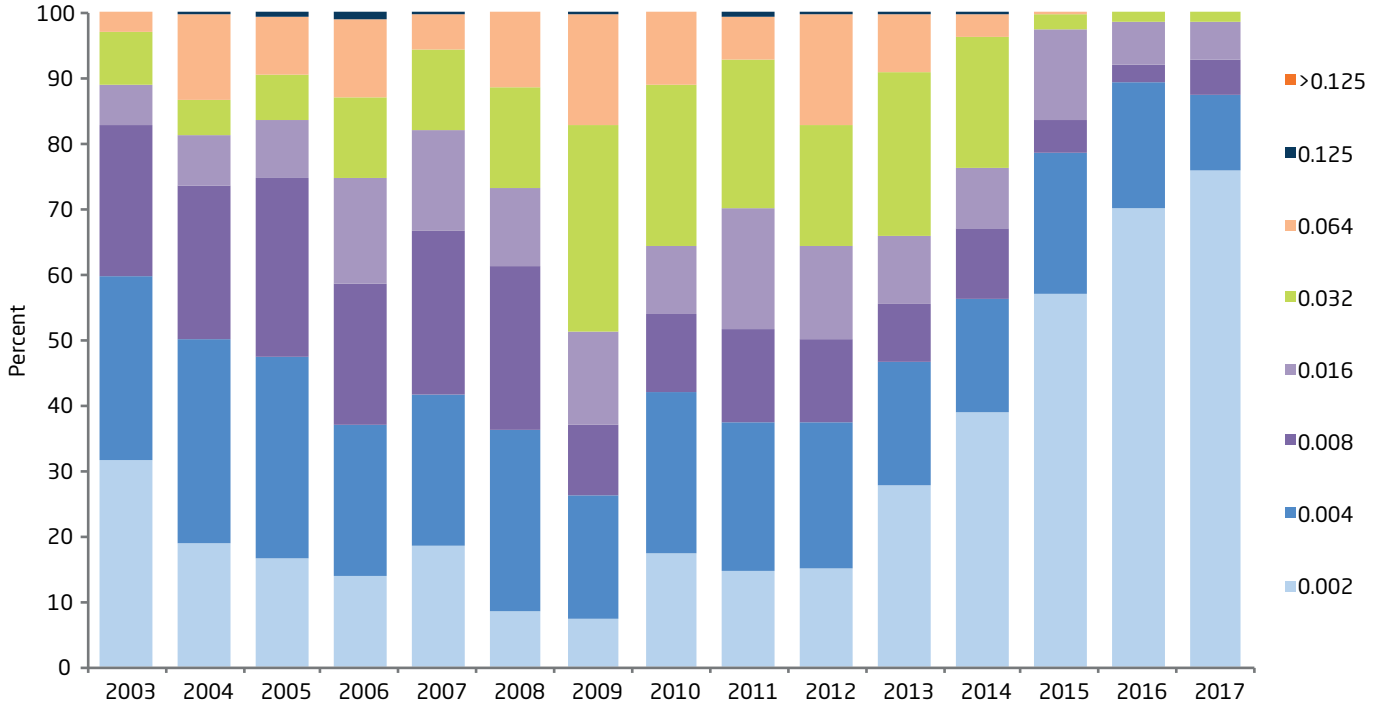


Figure 8.8.2 Distribution of ceftriaxone MIC (mg/L) values in gonococci, 2003-2017, Denmark

DANMAP 2017



Textbox 8.6

Mycoplasma genitalium

Background: *Mycoplasma genitalium* is a relatively newly discovered sexually transmitted bacterium with the first report of isolation appearing in 1981. It is extremely fastidious and slow to grow in culture, and its role as a pathogen could not be determined until the development of PCR-based methods in the 1990s. *M. genitalium* causes non-gonococcal urethritis (NGU) in men and women and cervicitis and pelvic inflammatory disease (PID) in women. It has been associated with infertility by serology, but causality is not proven. The association between *M. genitalium* and adverse pregnancy outcome is not completely established either; a meta-analysis showed a significant association, but in Denmark, the prevalence in pregnant women is low, and thus, it is not considered to be a major problem in pregnancy.

Epidemiology: In population-based studies of individuals not seeking health care, *M. genitalium* is found with the highest prevalence in the age group 15-35 years of age, but unlike chlamydia, older age groups are also relatively commonly represented. The overall prevalence in the population is slightly lower than that of *C. trachomatis* (1-3% *M. genitalium* positive in most studies) but much higher than that of *Neisseria gonorrhoeae*.

In symptomatic patients, *M. genitalium* is detected in 15-25% of NGU and 20-30% of those with *C. trachomatis* negative NGU. Due to the difficulty in treatment of the infection, 35-50% of men with persistent NGU are *M. genitalium* positive. It is estimated that 5-15% of PID is caused by *M. genitalium*.

Diagnosis: The only relevant diagnostic method is nucleic acid amplification tests (NAATs) on first-void urine from men and vaginal swab material from women. All positive results should be followed by testing for macrolide resistance mediating mutations and treatment should await the result of this test if possible.

Treatment: *M. genitalium* is susceptible to tetracyclines in vitro, but treatment with doxycycline eradicates only around 30% of the infections.

Azithromycin has traditionally been the first line antimicrobial with an eradication rate of approximately 85% in studies performed before 2009, but in recent years, this rate has declined dramatically due to a rapid development of high-level macrolide resistance, though. The resistance rate varies significantly between countries (see Figure 1 for a compilation of resistance studies in Europe) from <20% in Sweden to >50% in Denmark and Norway. The reason for this difference in resistance rates between the Nordic countries is most likely the widespread use of azithromycin 1 g single-dose for treatment of chlamydia in Denmark and Norway compared to doxycycline for first-line treatment of chlamydia and urethritis/cervicitis of unknown aetiology in Sweden. A dramatic example of selection of macrolide resistance is provided from Greenland where nearly 100% of the infections are caused by strains with macrolide resistance. This is most likely due to the very high prevalence of *C. trachomatis* infection and the resulting frequent use of 1 g single-dose therapy with azithromycin.

It is estimated that each round of azithromycin treatment of a susceptible infection leads to selection of resistance in 10% of the treated patients. This is the background for the recommendation of a test of cure after more than three weeks after initiation of treatment.

Second-line treatment for patients failing azithromycin treatment or with documented macrolide resistance at the time of diagnosis is moxifloxacin (400 mg once daily for 7 days). However, resistance to fluoroquinolones is also increasing worldwide. At present, approx. 5% of Danish *M. genitalium* patients carry strains with mutations associated with fluoroquinolone resistance and most of these infections are also macrolide resistant, leading to a situation where no registered treatment alternatives are available in Denmark. Some success in treating these multidrug-resistant (MDR) strains has been achieved with pristinamycin, a streptogramin class antimicrobial only registered in France. However, pristinamycin eradicates only 75% of the infections and is available only on a special permit (the clinic for sexually transmitted diseases at Bispebjerg Hospital has pristinamycin available).

Discussion: *M. genitalium* is rapidly developing resistance to all available antimicrobials and treatment should always be directed with regard to macrolide resistance testing. The use of azithromycin 1 g single-dose for chlamydia and urethritis/cervicitis of unknown aetiology should be minimised and first-line treatment should be changed to doxycycline 100 mg twice daily for 7 days. This is in accordance with the newest European guidelines, since it also has a better effect on rectal chlamydia, which is more common than previously recognised and a suspected cause of re-infection.

Screening for *M. genitalium* in asymptomatic patients is not recommended, since there is no evidence of benefit for the patient or on a population level.

Systematic surveillance of antimicrobial resistance is being established in Denmark and is needed to guide treatment recommendations. New antimicrobials and combinations of the already available ones are subject to a number of in vitro studies in Denmark and Internationally.

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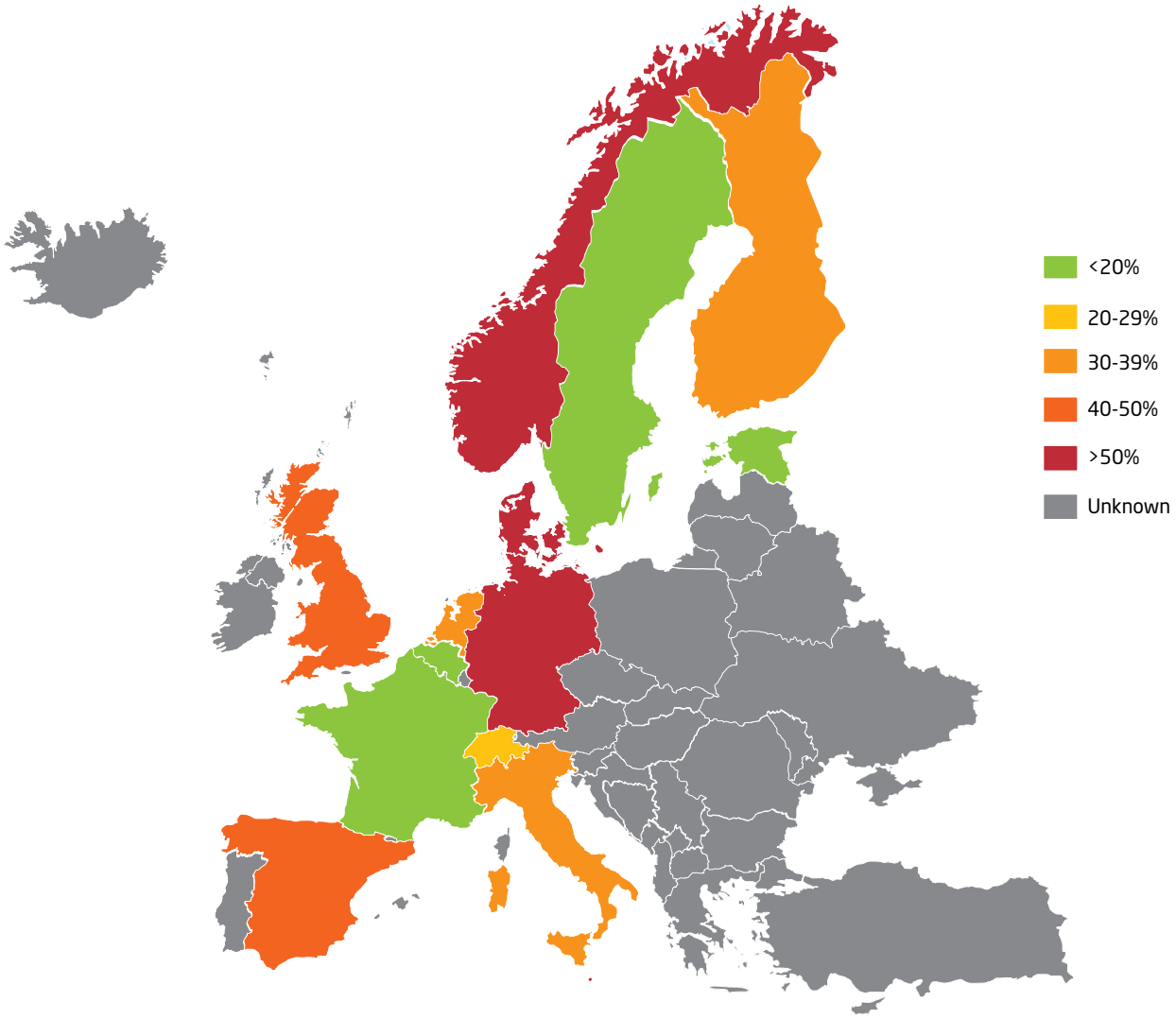
Figure 1 Prevalence of macrolide resistance mediating mutations in *M. genitalium* in Europe. Data compiled from published data sources

DANMAP 2017



9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2017 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on 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.

An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 3.2.

9.2 Data on antimicrobial consumption in animals

9.2.1 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. However, since April 2007, the monopoly was suspended and private companies (four in 2017) were given license to sell prescribed veterinary medicinal products for animals, when following strict guidelines, identical to those applied to pharmacies. Furthermore, in 2007 price setting of antibiotic was liberalised, which allowed for discounts to veterinarians, when buying larger quantities.

A pharmacy or company either sells the medicine to veterinarians for use in their practice or for re-sale to farmers, or sells the medicine directly to the animal holder on presentation of a prescription. By law, veterinarians are allowed only very small profits on their sale of medicine (5%), to limit the economic incentive to overprescribe.

In 2017, 99% of antimicrobial agents were purchased through pharmacies and the drug trading companies, while 1% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 82% of antimicrobial agents used in 2017 were purchased from pharmacies, whereas 10 years ago more than 80% of the antimicrobial agents used in cattle was purchased through the veterinarian. In aquaculture, approximately 60% is purchased through the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database called VetStat, which is hosted by the Danish Veterinary and Food Administration. Prior to 2001, all data on antimicrobial sales were derived from pharmaceutical companies. 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 the writing of invoices. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and type of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at SSI and to VetStat. However, VetStat does not have any validation on data entry and slight typing errors from vets may occur.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) have also been collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark for the past twenty years. In very rare instances, medicines are prescribed on special license and will not be included in VetStat (i.e. medicines not approved for marketing in Denmark).

The VetStat database contains detailed information about source 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 field [www. whocc.no]. The data presented in DANMAP 2017 were extracted from VetStat on 6th August 2018.

9.2.2 Methods

In DANMAP, we report use of antimicrobials dispersed in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound, to enable an overall crude comparison of consumption in different animal species and in the veterinary and human sectors.

Thereafter, a more detailed comparison of the quantity of antimicrobials used is performed, taking into account their

potency, formulation, route of administration and the age of the animals (where relevant), by generating Defined animal daily doses (DADDs).

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 for each antimicrobial agent, administration route and animal species and when appropriate, also age group. 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).

A DADD group is defined for each antimicrobial agent by administration route, pharmaceutical form and animal species; and when appropriate also by age group;

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products are given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group are 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 group/indication or formulation.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - biomass

Trends in antimicrobial consumption in pigs are presented in DADD per 1,000 animals per day (DAPD). 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.

Pig production: The estimation was based on the number of pigs produced, including exports at different ages [Statistics Denmark; Danish Agriculture and Food Council], productivity data for each year [Danish Agriculture and Food Council, 2016] and census data for breeding animals [Statistics Denmark, 2016]. The average weight and life span for the growing animals (piglets, weaners and finishers) were estimated from the annual productivity numbers. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this could not be 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 and gender categories.

Broiler (Gallus gallus): The live biomass was estimated based on number of broilers produced [Statistics Denmark; Danish Agriculture and Food Council], an average live weight at slaughter of 1.97 kg after an estimated average life span of 30 days. The mean live biomass per broiler is assumed to be half of the weight at slaughter.

Turkey production: The live biomass is estimated based on the number of turkeys produced [Statistics Denmark; Danish Agriculture and Food Council] and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Fur animals: The live biomass of mink is estimated from production data [Kopenhagen Fur] and carried out as described by Jensen et al., 2016 [Prev. Vet. Med., 26: 170].

Pet animals: Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The population is based on census data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture: The estimation is based on data from the Danish AgriFish Agency (Ministry of Environment and Food) on produced amounts in each subtype of production, and information on the typical lifespan and entrance and exit body weights, and were calculated in cooperation with Danish Aquaculture [N.H. Henriksen, Danish Aquaculture].

Treatment proportion - DAPD - DADD per 1,000 animals per day

The treatment proportion is calculated as the number of DADDs administered to an animal species during a year (in thousands) divided by the number of standard animals at risk per day. The number of standard animals at risk per day takes

into account species differences in average body-mass and lifespan. When relevant, the numbers of DADDs and standard animals at risk are estimated for specific age groups, or simply as number of doses (DADDs) used to treat one kg of animal divided with the total estimated biomass (in tonnes).

DAPD is a statistical measure, which provides a rough estimate of the proportion of animals treated daily with an average maintenance dose of 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. The DAPD is also referred to as the treatment proportion or treatment intensity. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, Defined daily dose per 1000 inhabitants per day (DID), see Section 9.9.2. In 2017, DAPD calculations were carried out for pigs only.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of consumption per pig was calculated. The adjustment is based on the assumption that pigs exported at 30 kg, on average, received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg. Antimicrobial use per pig produced (adjusted) is calculated as:

[DADDs + DADDw + (1+Q)*DADDf] / (biomass-days-total + Nw*5,800(kg*days)), where

DADDs= the amount of antimicrobial agents used in sows

DADDw = the amount of antimicrobial agents used in weaners

DADDf = the amount of antimicrobial agents used in finishers

Q is the proportion of weaning pigs exported around 30 kg

Nw= the number of pigs exported at 30 kg bodyweight

Nw*5,800= the the number of biomass days the exported pigs would have contributed to the live biomass if not exported

9.3 Collection of bacterial isolates - animals and meat

9.3.1 Animals

Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. The legislation requires, in addition to sampling for the national *Salmonella* control programmes in poultry farms, sampling of broilers and fattening turkeys at slaughter in even years (2014-2020) and sampling of fattening pigs and cattle < 1 year at slaughter in odd years (2015-2019). In 2017, samples from pigs were examined for ESBL/AmpC and carbapenemase producing *E. coli,* indicator *E. coli, Salmonella and Enterococcus faecalis.* Samples from cattle were examined for ESBL/AmpC and carbapenemase producing *E. coli,* indicator *E. coli and Campylobacter jejuni.* Additionally, sampling of *Campylobacter jejuni* and indicator *E. coli* from broilers was carried out (Table 9.1).

Meat inspection staff or abattoir personnel at the slaughter houses collected caecal samples from healthy pigs, cattle (< 1 year) and broilers. For pigs and cattle, the samples were collected throughout 2017, and the sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse. For broilers, the sampling took place in the two major Danish slaughterhouses during August and September; in order to collect isolates during the expected high-prevalence period of *Campylobacter*. Four intact caeca from each broiler flock were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal.

All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted, including antimicrobial susceptibility testing and *Salmonella* serotyping.

Salmonella from layers, broilers, turkeys and cattle are not included in DANMAP 2017 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2017].

9.3.2 Meat

The EU harmonised monitoring requires, in addition to sampling for the national *Salmonella* control programmes at slaughter, sampling at retail of broiler meat in even years (2014-2020) and sampling of pork and beef in odd years (2015-2019) [Decision 2013/652/EU].

In 2017, ESBL/AmpC producing *E. coli* were isolated from packages of fresh, chilled pork and beef collected in Danish wholesale and retail outlets throughout the year by the regional DVFA officers (Table 9.1). Products with added saltwater or other types of marinade were excluded and the packages were selected without pre-selecting based on the country of origin. 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 product (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory.

The *Salmonella* isolates from pork originate from the national control programme at the slaughterhouses (Table 9.1), where the carcasses are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). The numbers of swabs collected depend on the slaughterhouse capacity. All samples were processed at Industry laboratories. Isolates from all *Salmonella* positive samples were send to the DVFA laboratory, where one isolate per sample was serotyped and susceptibility tested.

Salmonella from broiler meat and beef are not included in DANMAP 2017 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2017]. *Campylobacter* from broiler meat for DANMAP

originate from the national control program: Intensified Control of Salmonella and Campylobacter in fresh meat. However, in 2017 very few *Campylobacter* isolates from domestically produced broiler meat found and therefore not included in DANMAP.

9.4 Microbiological methods – isolates from animals and meat

9.4.1 *Salmonella*

Salmonella was isolated in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] or Annex D, ISO 6579 [ISO6579:2002/Amd 1:2007]. Serotyping of isolates was performed 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. Only one isolate per serotype was selected from each herd, flock or slaughter batch.

9.4.2 *Campylobacter*

Campylobacter from broilers and cattle was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] followed by species-determination by BAX® rtPCR assay. Pre-enrichment in Bolton broth was used for cattle samples, whereas direct spread of caecal sample on to selective agar was used for broiler samples. Only one *C. jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

9.4.3 Indicator *E. coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread of caecal sample material 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.

For specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* from caecal samples, the present EURL-AR laboratory protocol describing the selctive enrichment procedures was applied in accordance with the EU harmonized monitoring. Only one ESBL/AmpC producing *E. coli* isolate per pig herd, cattle herd and meat sample was selected (no isolates of carbapenemase-producing *E. coli* was detected).

9.4.5 *Enterococcus*

Enterococcus from pigs were isolate from an adequate amount of caecal material suspended in 2 ml BPW. The suspension was streaked onto Slanetz Bartley agar and incubated 48h at 41.5°C. Two colonies resembling typical *E. faecalis* were sub-cultivated on blood agar. Species identification was done by rtPCR. Only one isolate of *E. faecalis* was selected per herd.

All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted including *Salmonella* serotyping, except for isolation of *Salmonella* from pig carcasses, which was carried out at Industry laboratories.

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2017

DANMAP 2017

Bacteria	Origin of isolates	Legislative sampling frequency (2013/652/EU)	Number of samples in 2017
Salmonella spp.	On-farm samples from laying hens, broilers and fattening turkeys (breeder and production flocks)	Annually	4993 flocks
	Caecal samples from fattening pigs	Odd years	295 pigs
	Carcasses of broilers	Annually	259 flocks
	Carcasses of fattening pigs and cattle <1 year	Annually	4466 cattle carcasses, 11155 pig carcasses
Campylobacter jejuni	Caecal samples from broilers	Even years	163 flocks
	Caecal samples from cattle <1 yr		297 cattle
Indicator *E. coli*	Caecal samples from broilers	Even years	135 flocks
	Caecal samples from fattening pigs and cattle <1 yr	Odd years	190 cattle, 193 pigs
Specific monitoring of ESBL/AmpC – producing *E. coli*	Caecal samples from fattening pigs and cattle <1 yr	Odd years	297 cattle, 295 pigs
	Fresh pork and beef at retail	Odd years	290 beef samples, 277 pork samples
	WGS data for collected ESBL/AmpC isolates		17 isolates
Enterococcus spp.	Caecal samples from fattening pigs		295 pigs

Note: All animal samples originate from different flocks/herds. All food samples originate from different slaughter batches, batches of Danish or imported meat ready for retail or packages of fresh meat at retail

9.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella, Campylobacter, E. coli* and *Enterococcus* was performed as Minimum Inhibitory Concentration (MIC) determination using broth microdilution by Sensititre (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. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonized monitoring of antimicrobial resistance.

The relevant quality control strains were used at the laboratories: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.'
Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted that is accredited by DANAK (the national body for accreditation).

9.6 Whole genome sequencing –isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (Section 9.4.1), whole genome sequencing (WGS) and in silico bioinformatic 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 followed by bioinformatics analysis at DTU National Food Institute from Centre for Genomic Epidemiology (www.genomicepidemiology.org; https://cge.cbs.dtu.dk//services/all.php) including:

1. De novo assembly using SPAdes [Bankevich et al. 2012. J Comput Biol. 19(5): 455];
2. MLST using MLST Finder 2.0 [Larsen et al. 2012. J Clin Micobiol. 50(4): 1355];
3. Detection of antimicrobial resistance genes using ResFinder 3.0 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];
4. Detection of virulence genes using VirulenceFinder 1.5 [Joensen et al. 2014. J Clin Micobiol. 52(5): 1501], and;
5. Detection of plasmid replicons using PlasmidFinder 1.3 [Carattoli et al. 2014. Antimicrob Agents Chemother. 58(7): 3895].

9.7 Data handling – isolates from animals and meat

For the samples processed at the DFVA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were electronically transferred to DTU National Food Institute. At the DTU National Food Institute, data were harmonised and one isolate per epidemiological unit was selected for reporting. All data are stored in an Oracle database at isolate level (9i Enterprise Edition®). The database contains all antimicrobial data reported in DANMAP or to EFSA since 2007 (partial dataset from 2001-2006).

Variables include: bacterial species (subtype where applicable), date of sampling, animal species or food type, herd identifier and country of origin whenever possible. MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant cut-off from 2017 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at www.danmap.org. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals calculated as 95% binomial proportions presenting Wilson intervals. Classification of CPE, ESBL and AmpC phenotypes was done according to the scheme provided by EFSA [EFSA 2018. EFSA Journal 16(2):5182]:

1. CPE phenotype if meropenem MIC >0.12;
2. ESBL phenotype if cefotaxime/ceftazidime MIC >1 and meropenem MIC <=0.12 and cefoxitin MIC <=8 and synergy (clavulanic acid and cefotaxime/ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 and meropenem MIC <=0.12 and cefoxitin MIC >8 and synergy (clavulanic acid and cefotaxime/ceftazidime);
4. AmpC phenotype if cefotaxime/ceftazidime MIC >1 and meropenem MIC<=0.12 and cefoxitin MIC>8 and no-synergy (clavulanic acid and cefotaxime/ceftazidime);
5. Other phenotype if not in 1-4.

Synergy is defined by MIC value of clavulanic acid combined with cefotaxime or ceftazidime at least 8-times higher than the MIC of cefotaxime or ceftazidime alone, respectively.
All handling, validation and analysis of results were carried out using Microsoft Excel, Oracle and SAS®Software, SAS Enterprise Guide 6.1.

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 6.1 using univariable Chi-square, or Fisher's Exact Tests as appropriate. All changes and differences yielding $p<0.05$ were commented on in the text, whereas the remaining data was visualised in figures or tables only.

Some types of resistances were looked for, but not found by the DANMAP monitoring system, yielding a prevalence of zero. It is not possible for surveys to prove freedom from diseases or resistances in populations, but with a defined confidence, surveys can identify the maximum possible prevalence given that the survey failed to find any positives [Textbox 6.2, DANMAP 2016]. This maximum prevalence was calculated for the report using 95% confidence and assuming a perfect test by a probability formula to substantiate freedom from disease [Cameron and Baldock 1998, Prev. Vet. Med].

Link to calculation example: http://epitools.ausvet.com.au/content.php?page=HerdSens5&Sens=1&SampleSize=100&Population=&TargetSeH=0.95.

Table 9.2 Interpretation criteria for MIC-testing by EUCAST epidemiological cut-off values and the corresponding EUCAST clinical breakpoints

DANMAP 2017

Antimicrobial agent	*Salmonella*		*E. coli*		*E. faecalis*		*C. jejuni*		*S. aureus*
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml
Ampicillin	>8	>8	>8	>8	>4	>8			
Azithromycin	>16 [a]	>0	>16 [a]	>0					
Cefepime	>0.125 [a]	>4	>0.125	>4					
Cefotaxime	>0.5	>2	>0.25	>2					
Cefotaxime/ clavulansyre	>0.5 [a]	>0	>0.25 [a]	>0					
Cefoxitin	>8	>0	>8	>0					>4
Ceftaroline									>1
Ceftazidime	>2	>4	>0.5	>4					
Ceftazidime/ clavulansyre	>2 [a]	>0	>0.5 [a]	>0					
Ceftobiprole									>1
Chloramphenicol	>16	>8	>16	>8	>32	>0			
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>4	>0	>0.5	>0.5	>1
Clindamycin									>0.25 [c]
Colistin	>2 [b]	>2	>2	>2					
Daptomycin					>4	>0			>1
Ertapenem	>0.064	>1	>0.064	>1					
Erythromycin					>4	>0	>4	>4	>1
Fusidic acid									>0.5
Gentamicin	>2	>4	>2	>4	>32	>0	>2	>0	>2
Imipenem	>1	>8	>0.5	>8					
Kanamycin									>8
Linezolid					>4	>4			>4
Meropenem	>0.125	>8	>0.125	>8					
Mupirocin									>1
Nalidixic acid	>16	>0	>16	>0			>16	>0	
Norfloxacin									>4
Penicillin									>0.125
Quinupristin/ dalfopristin						>0			
Rifampicin									>0.03
Streptomycin							>4	>0	
Sulfamethoxazole/ Trimethoprim									>0.5
Sulfonamide	>256 [a]	>0	>64	>0					
Teicoplanin					>2	>2			
Temocillin	>32 [a]	>0	>32 [a]	>0					
Tetracycline	>8	>0	>8	>0	>4	>0.5	>1	>2	>1
Tigecycline	>1	>2	>1	>2	>0.25	>0.25			
Trimethoprim	>2	>4	>2	>4					
Vancomycin					>4	>4			>2

Note: EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted

a) The EUCAST ECOFF (>2) for colistin was applied for *S.* Typhimurium and other serotypes, except for *S.* Enteritidis and *S.* Dublin where ECOFF >8 was applied according to investigations presented in DANMAP 2011

b) No current EUCAST ECOFF is available, apply complementary interpretative thresholds as suggested by EFSA [EFSA Supporting publication 2017:EN-1176]

c) Inducible clindamycin resistance is included

9.8 Data on antimicrobial consumption in humans

9.8.1 Data registration

Antimicrobial consumption at hospitals is reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Before 2012, data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. From 2013 onwards, all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at SSI.

Reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between the hospital and different medical suppliers. Detailed information is given on the different drugs delivered on ATC5 level. The reporting corresponds relatively well to the overall consumption at department level, but for many clinical departments it is difficult to establish the use of antimicrobials dispersed on the different patient categories, since several clinical specialties are brought together within the same clinical entity. Also, some clinical departments have close collaboration with other specialties, which also may include helping each other out in situations of shortage of certain drugs. Finally, a clinical entity may be geographically spread across several hospital buildings and dispersing of deliverances of medicine will not always be announced to the pharmacy.

In case of production failures and shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency. These special deliverances are reported separately to DANMAP through the Danish Health Authority. In 2017, there were shortages in deliverance of piperacillin with tazobactam and pivemecillinam as well as mecillinam. 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.

Data on treatment at patient level is available at very few of the hospitals and has so far been used in local quality assurance only but has not been available to the national surveillance system. Improvement of patient monitoring has been included in the newest electronical patient journal systems but in several of the regions, this is not fully implemented yet, thus a national account of the prudence of use of antimicrobials at hospitals has so far not been possible.

Earlier reporting on human antimicrobial consumption in Denmark exist. These were performed through 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 the pharmacies. This reporting became less reliable over time, since there was an increasing amount of parallel imported drugs from the late 1980´s, which were not covered by this registration.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported through the pharmacies by a code relating to the defined package. The information from the code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The sale also report the age, gender and regional residence of the patient. In 2017, clinical information on the indication to prescribe the drug, was available for 90% of prescriptions, (an increase from 86% in 2016). Yet some indication codes, like the term "infection", suffer from being unspecific. Specific indications account for only 69%. Better and more precise indication codes will give the opportunity to register and evaluate on a more prudent use of antimicrobials in the future.

9.8.2 Method

Only 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 (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2017 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary health care and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitant-days).

The consumption in hospitals is expressed as DBDs, the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days). 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). Finally, the consumption of antibacterial agents at hospitals has also been calculated in DIDs, primarily for comparison with primary health care. These can be found in web annex. All consumption is provided with two decimal digits, if possible.

9.8.3 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the date of discharge minus the date of admission and rounded up to the nearest whole day. Every new admission to a new hospital department counts as a new bed-day. These were extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

9.8.4 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific ward (i.e. one patient can be registered as admitted multiple times if transferred between wards during the same hospital stay). The admissions were extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

9.9 *Salmonella* and *Campylobacter* in humans

9.9.1 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. Exact figures of the proportion tested and the sampling strategy for the different species can be found in Sections 6.1 and 6.2. 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 within the week prior to the onset of disease.

9.9.2 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.

9.9.3 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.

9.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) 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.

9.10 *Staphylococcus aureus* including MRSA in humans

9.10.1 Data source

Blood isolates are 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 are sent to the reference laboratory.

9.10.2 Microbiological methods

At SSI, all isolates are 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 is 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 are tested for presence of the mecC gene.

spa-negative isolates are confirmed as *S. aureus* by MALDI-TOF. Based on the spa type and known association with MLST typing, each isolate is assigned to a clonal complex (CC).

9.10.3 Susceptibility testing

Antimicrobial susceptibility testing of *Staphylococcus aureus* was performed by Minimum Inhibitory Concentration (MIC) determination using a custom-made panel (DKSSP2, 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. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonized monitoring of antimicrobial resistance.

Staphylococcus aureus ATCC 29213 was included as quality control for each batch of resistance determination.

9.10.4 Data handling

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 colonization), 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 hospitalizations, 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

9.11 Invasive *Streptococcus pneumoniae* in humans

9.11.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all invasive isolates nationwide are sent to SSI for identification or confirmation as well as serotyping and susceptibility testing.

9.11.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* is based on: visual evaluation of colonies, positive optochin test and test with either latex omni serum or Neufeld based Omni serum. If challenging results occur, are MALDI-TOF and bile solubility test perform to further confirm the correct species identification.

Serotype identification of invasive *S. pneumoniae* are performed by using latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Hillerød, Denmark) and serotype specific antisera by the Quellung test (SSI Diagnostica, Hillerød, Denmark).

9.11.3 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 Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST. Both fully and intermediary resistant isolates are counted as non-susceptible.

9.11.4 Data handling

Data on susceptibility testing of isolates were stored as MICs in a Microsoft® Access database linked to a SQL server at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

9.12 Invasive Beta-haemolytic streptococci (Group A, B, C and G Streptococci) in humans

9.12.1 Data source

Isolates are submitted to SSI on a voluntary basis. Only isolates from blood and spinal fluid are included in the DANMAP report

9.12.2 Microbiological methods

Identification of streptococcal group is performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Roskilde, Denmark).

9.12.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin resistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Roskilde, Denmark) on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Hillerød, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with E-test (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Müller-Hinton agar. The breakpoints used were as defined by the EUCAST. Both fully and intermediary resistant isolates were categorized as resistant.

9.12.3 Data handling

Data on susceptibility testing of isolates were stored as inhibition zone diameters and if indicated also MICs in a Microsoft® Access database linked to a SQL server at SSI.

9.13 *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter* spp., *E. faecium* and *E. faecalis* in humans

9.13.1 Data source

Susceptibility data on all isolates are provided from all DCMs, this includes blood samples (*E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter spp., E. faecium* and *E. faecalis*) and urines samples (*E. coli* and *K. pneumonia*)

No samples were collected from healthy humans

9.13.2 Microbiological methods

All microbiological analysis, including susceptibility testing and interpretation of test results is being performed by the DCMs. Test results from susceptibility testing are reported to SSI based on S-I-R interpretation. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST breakpoints and the EUCAST disk diffusion method for most species. Interpretation mainly follows EUCAST principles but for some DCMs local interpretation rules are applied on the susceptibility in specific species, primarily from invasive infections (e.g. susceptibility to mecillinam in *E. coli* obtained from blood samples).

9.13.3 Data handling

All DCMs in Denmark provided data on antimicrobial susceptibility test results from all *E. coli, K. pneumoniae*, invasive *P. aeruginosa*, invasive *Acinetobacter spp.*, invasive *E. faecium* and invasive *E. faecalis* isolates. Data were extracted from the following laboratory information systems:

ADBakt (Autonik AB, Skoldinge, Sweden) for the DCMs at Hvidovre, Herlev and Aalborg Hospitals.

MADS (DCM Skejby Hospital, Aarhus, Denmark) for the DCMs at Rigshospitalet, Slagelse/Region Zealand, Odense University, Sønderborg, Esbjerg, Vejle and Aarhus University (Skejby) Hospitals.

Resistance data on the first isolate per patient per sample material per year were included. Generally, resistance data on a specific antimicrobial agent were excluded if the DCM had only performed susceptibility tests on that agent on a limited number of isolates.

9.14 ESBL-producing bacteria in human patients

9.14.1 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 Statens Serum Institut. Since January 1st 2018, 3rd generation cephalosporin resistant *Klebsiella pneumoniae* from bloodstream infections isolates have been included as well (data not available at this point).

9.14.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and *in silico* bioinformatics analysis have been applied for characterization of the genetic background of the ESBL and AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.14.3 Data handling

The Bacterial Analysis Pipeline – Batch upload version 1.0 from Center of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/cgs/) 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/services/myDbFinder-1.2/).

9.15 CPO in human patients

9.15.1 Data source

The Danish DCMs have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at Statens Serum Institut.

9.15.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were subjected to WGS. More than one isolate from the same patient were included, only if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases.

9.15.3 Data handling

The Bacterial Analysis Pipeline – Batch upload version 1.0 from Center of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/cgs/) 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*).

9.16 VRE in human patients

9.16.1 Data source

The Danish DCMs have on a voluntary basis, submitted VRE for species identification, genotyping and surveillance to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

9.16.2 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.

9.16.3 Data handling

The Bacterial Analysis Pipeline – Batch upload version 1.0 from Center of Genomic Epidemiology (https://cge.cbs.dtu.dk/services/cgs/) 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.

9.17 Statistical tests for human data

Significance tests for trends in rates of resistance in human bacteria were performed by applying the Cochran-Armitage test. The significance levels were calculated using the DescTools v0.99.19 package in R version 3.5.0. A p-value of < 0.05 is generally considered significant. Presented in this report are results from trend analysis for five years- and ten years trend, respectively.

The test was applied to several bacteria's resistance to a broad range of antimicrobials. One-sided tests were chosen because of preliminary expected trend directions. Cochran-Armitage test calculates probability in binomial proportions across one single, levelled variable. In this report, the test has been performed on susceptibility data from the past 10 years containing numbers of resistant/intermediate and susceptible cases, respectively. The resulting p-values are reported in chapter eight, supplied by an arrow indicating trend direction. Note that the significance levels serve to support the graphs and thus should be interpreted with caution.

10

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
CA	Community-acquired
CC	Clonal complex
CDI	Clostridium difficile infections
CHR	Central Husbandry Register
CPE	Carbapenemase producing Enterobactereales
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
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/1000 inhabitant days)
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
ESBL	Extended spectrum beta-lactamase
GP	General Practitioner
HAI	Hospital-acquired infections
HCAI	Health care associated infections
HACO	Health care associated community onset
HAIBA	Hospital-acquired infections database
HLGR	High-level gentamicin resistance
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
OIE	World Organisation for Animal Health
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
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 (www.whocc.no/ atcddd/ index database).

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 cover all patients attended to at somatic hospitals only. Admission-days are 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. The current report is the first to update data 10 years back.

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). In contrast to the ADD previously used in DANMAP, the DADD has not been defined for each antimicrobial agent, administration route and animal species but at product level. In DANMAP 2012, the DADD replaces the ADD (as defined in VetStat), which had been used since DANMAP 2003. For more details, see Chapter 9, Materials and Methods. The DADDs used in DANMAP 2015 are presented 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 life-span. 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 (Section 4.3 and Chapter 9, 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. The current report is the first to update data retrospectively for 10 years. Every patient admitted to hospital accounts for one bed-day, independent of the actual length of stay within every 24 hours. This corresponds to the actual hours at hospital divided by 24 hours and rounded up to the next whole number. For patients transferred between wards each transfer will count as a new bed day. Non-ended hospital stays are not included.

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 inhabitant-days.

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.

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 depend 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 one 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.

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