



## **Comparison of proficiency testing results on antimicrobial susceptibility testing of Salmonella and Campylobacter obtained by laboratories from the ECDC FWD network (public health) and the EURL-AR network (veterinary/food) 2011.**

**Karlslose, Susanne; Cavaco, Lina; Hendriksen, Rene S.; Aarestrup, Frank Møller**

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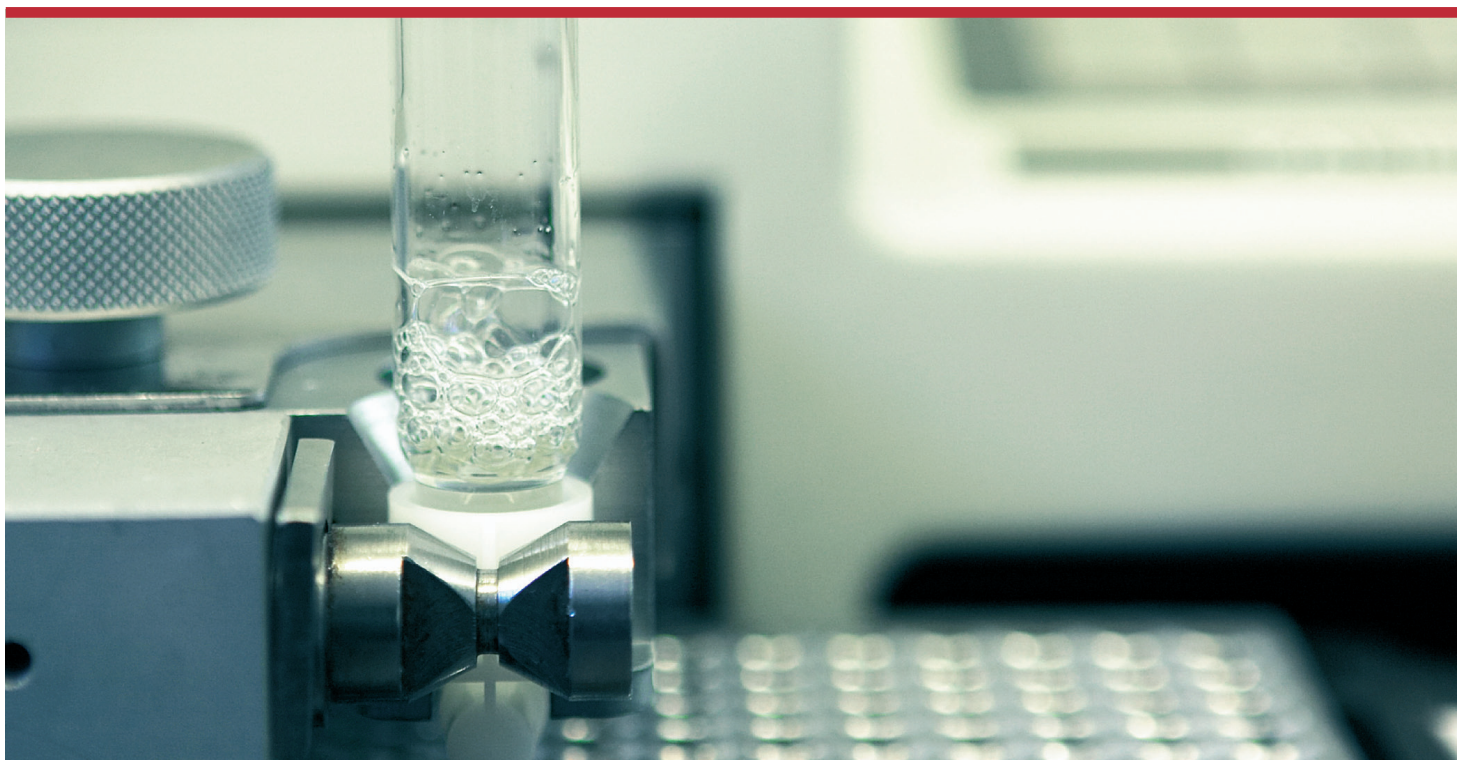
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COMPARISON OF PROFICIENCY TESTING RESULTS ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING OF *SALMONELLA* AND *CAMPYLOBACTER*  
OBTAINED BY LABORATORIES FROM THE ECDC FWD NETWORK  
(PUBLIC HEALTH) AND THE EURL-AR NETWORK (VETERINARY/FOOD) 2011



Susanne Karlsmose  
Lina M. Cavaco  
Rene S. Hendriksen  
Frank M. Aarestrup

# **European Union Reference Laboratory – Antimicrobial Resistance**

**Comparison of proficiency testing results on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* obtained by laboratories from the ECDC FWD network (public health) and the EURL-AR network (veterinary/food) 2011**

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National Food Institute  
Technical University of Denmark  
Kemitorvet, Building 204  
DK-2800 Lyngby



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## Summary

This report summarises and compares results from the proficiency test trial conducted by the National Food Institute (DTU Food) aiming at participants from the public health sector through the Food- and Waterborne Diseases and Zoonoses (FWD) programme of European Centre for Disease Prevention and Control (ECDC) as well as institutes from the veterinary/ food sector (NRL-AR). The objective was to evaluate the quality of the antimicrobial susceptibility data produced by the reference laboratories from the two networks. In addition, it was to identify areas which would require attention to produce reliable and harmonised susceptibility data.

The assessment demonstrated that the AST-results obtained by the FWD-network and the EURL-AR-network are comparable as regards AST of *Salmonella* and *Campylobacter*. The acceptance level for deviations at 5% was met for five (55.6%) of the FWD-network laboratories and for 29 (85.3%) of the NRL-AR's for *Salmonella* AST. For *Campylobacter* AST this was the case for nine (90%) of the FWD-network laboratories and for 22 (84.6%) of the NRL-AR's. For both microorganisms room for improvement is demonstrated, and a repetition of this type of comparative testing therefore appears reasonable.

The interpretation for ciprofloxacin posed a problem for the FWD-network laboratories. Many laboratories in this network perform disc diffusion (DD) for AST of *Salmonella* and as the interpretative criteria (epidemiological cut off value) applied in this proficiency test is much lower ( $R > 0.064 \mu\text{g/mL}$ ) than the clinical breakpoint ( $R \geq 4 \mu\text{g/mL}$ ) and generated differences in interpretation. Also, the FWD-network laboratories to a large extent use diffusion tests for AST of *Campylobacter* and could present good agreement with the expected results using this method. However, for AST of this microorganism, the EURL-AR recommends MIC methods, only.

Detection of ESBL-producing *Enterobacteriaceae* is relevant for both the public health laboratories and the laboratories from the veterinary/food sector as these phenotypes continue to emerge worldwide. Laboratories which have not yet introduced tests to detect ESBL-producing *Enterobacteriaceae* are therefore encouraged to prioritize this area.

## 1. Introduction

In this report, results are summarised and compared from the proficiency test trial conducted by the National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) aiming at two networks as participants, i.e. the *Salmonella* and *Campylobacter* laboratory contact points of the Food- and Waterborne Diseases and Zoonoses Network (FWD-network) under the coordination of European Centre for Disease Prevention and Control, and the EURL-AR network. The FWD-network consists of public health reference level laboratories, and the EURL-AR network consists of institutes from the veterinary/ food sector.

Proficiency testing is considered an important tool for the production of reliable laboratory results of consistently good quality. This proficiency test focuses on *Salmonella* and *Campylobacter* and is the sixth External Quality Assurance System (EQAS) conducted for these microorganisms in the EURL-AR network. In 2011, the EURL-AR through internal



funding was able to offer the public health laboratories participation in the *Salmonella* and *Campylobacter* antimicrobial susceptibility testing (AST) EQAS.

The objective of this EQAS was to assess and compare the quality of the antimicrobial susceptibility data produced by the reference laboratories and to identify areas which would require attention to produce reliable and harmonised susceptibility data.

At the annual EQAS conducted by the EURL-AR, the goal is to have each laboratory performing AST with less than 5% incorrect interpretations (interpretations deviating from the expected results). This performance criterion has also been applied for the present report. Evaluation in detail of the obtained results is presented in separate reports for each of the two networks and is therefore not the objective for the present report. This report will focus on the comparison of the obtained results between the two networks, i.e. between the public health sector and the veterinary-/food sector.

The data in this report are presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential. All conclusions are public.

Participants of an EQAS are expected to evaluate their own results and introduce corrective actions if necessary. The categorization of an uploaded interpretation as incorrect in the EURL-AR EQAS should induce the participant to perform a self-evaluation. This self-evaluation could very well include a comment on the fact that an acceptable deviation for MIC-determination is  $\pm$  one dilution step, which in some cases may affect the interpretation of the result. Therefore, the self-evaluation may lead to arguments which can defend the obtained results internally, yet, incorrect interpretations based on a one step dilution difference is still regarded as a deviation for the overall EQAS reporting, evaluation and in the database.

The EURL-AR is accredited by DANAK (accreditation no. 516) as provider of proficiency test for zoonotic pathogens and indicator organisms in bacterial isolates (serotyping, identification, and antimicrobial susceptibility testing).

## **2. Materials and methods**

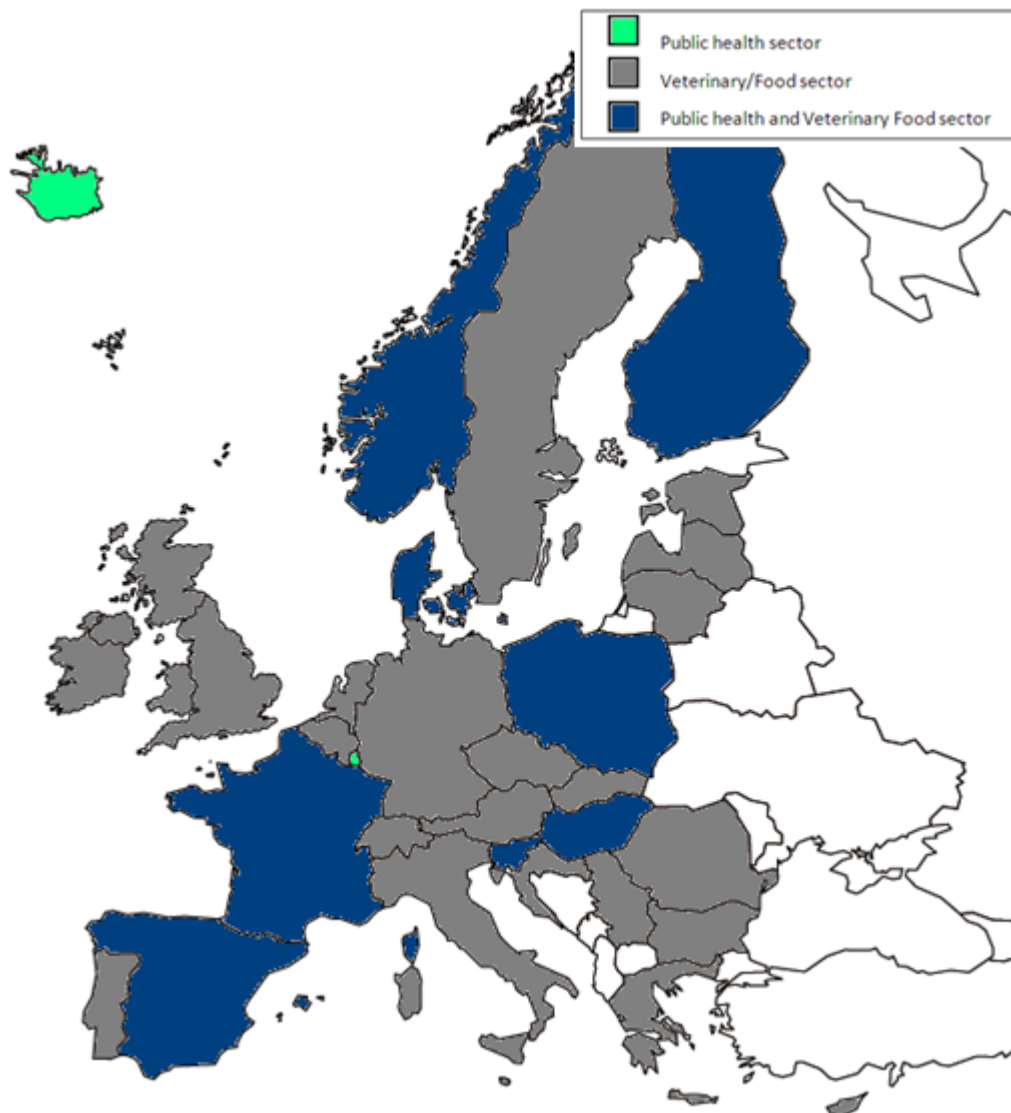
Detailed materials and methods are described in each of the network reports (1, 2).

From the EURL-AR-network, 30 countries delivered 34 sets of *Salmonella* results and 26 sets of *Campylobacter* results, and from the FWD-network, 10 countries delivered 9 sets of *Salmonella* results and 10 sets of *Campylobacter* results (App. 1).

Figure 1 illustrates that from eight countries both laboratories from the public health and from the veterinary/food sector participated, from 22 and two countries, respectively, laboratories from the veterinary/food sector and the public health sector, only, participated.

Eight *Salmonella* strains and eight *Campylobacter* strains were selected for this trial among isolates from the strain collection at DTU Food. Individual sets of the *Salmonella* strains were provided as agar stab cultures and the *Campylobacter* strains as charcoal swabs. The process of preparation, assigning expected values, verification of expected values and shipment handling is described in detail in the EURL-AR network report (1).

The selection of antimicrobials used in the trial for *Salmonella* was: ampicillin, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, ceftiofur, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulfonamides (sulfamethoxazole), tetracycline and trimethoprim. Additionally, cefoxitin was used for detection of ampC, and imipenem, imipenem/EDTA for detection of metallo-beta-lactamases.



**Figure 1:** Countries from each of the networks participating in the antimicrobial susceptibility testing EQAS of *Salmonella* and/or *Campylobacter*.

For *Campylobacter* the following antimicrobials were included: chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline.

In this EQAS, the interpretative criteria which should be used were cut-off values recommended by the European Food Safety Authority (EFSA) and listed in the protocol (1). The participants from the EURL-AR network were instructed to use the method carried out when performing monitoring for EFSA, whereas the participating public health laboratories



could perform the AST using their method routinely employed in their laboratory. In general, participants using DD and E-test were recommended to interpret their results according to their individual routine, categorising the test strains into the terms resistant and susceptible. A categorisation as ‘intermediate’ was not accepted.

In general, agar and broth dilution methods are considered the gold standard as regards antimicrobial susceptibility testing, and the EURL-AR recommends using these methods when performing AST. For *Campylobacter*, the EURL-AR does not recommend the use of either disk diffusion or E-test for AST; i.e. the only type of method recommendable for AST of *Campylobacter* is MIC methods. According to the protocol, the laboratories of the FWD-network could submit results of AST of *Campylobacter* obtained by in-house methods like disk diffusion or E-test, in which cases in-house interpretative criteria should be applied as described in the protocol.

For the EURL-AR network, the detection of ESBL-producing strains was mandatory, whereas it was an optional part of the EQAS for the FWD-network laboratories.

The participants were instructed to enter results from the quality control (QC) reference strains into the database for use as background for the analysis of the obtained results (see each of the network reports (1, 2)). The evaluated results would consist of MIC values or inhibition zone diameters in millimetres for the reference strain *E. coli* (ATCC 25922) and MIC values for *C. jejuni* (ATCC 33560). The results should be in agreement with the quality control ranges according to the relevant guidelines; i.e. the CLSI documents M31-A3 (2008) or M100-S21 (2011); The Sensititre System (Trek Diagnostic Systems Ltd, UK); or E-tests (AB-Biodisk, Sweden).

The database generated evaluation reports assessed the submitted results, describing all deviations from the expected. Deviations in the interpretation as resistant or susceptible were categorised as ‘incorrect’, as was also deviations in confirmation of an isolate as ESBL-producer or ampC.

There are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms ‘susceptible’, ‘intermediate’ and ‘resistant’ should in principle be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data using epidemiological cut-off values, bacteria should be reported as ‘wild-type’ or ‘non-wild-type’ (3). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistant, even in the cases where we are referring to wild-type and non-wild-type strains. The resistance profiles of the included test strains are available in each of the network reports (1, 2).

The database included questions for evaluation of the EQAS as well as questions regarding the individual laboratories’ work in the area of AST. Few laboratories made use of this possibility of sending comments to the EURL-AR; those who did have received direct reply when relevant.



### 3. Results and discussion

The reported results included MIC values or inhibition zone diameters obtained by disk diffusion (DD) together with the categorisation as resistant or susceptible. Only the categorisation was evaluated, whereas the MIC values and disk diffusion inhibition zones were used as supplementary information.

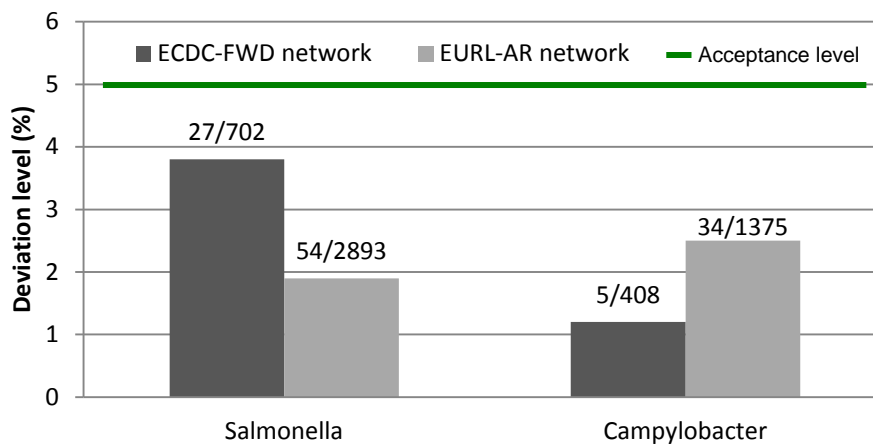
The EURL-AR network has agreed that if less than 75% of the results were correct, based on strain/antimicrobial combination, these results should be further analysed and possibly omitted from evaluation. In the present EQAS this occurred in two cases which both were omitted from evaluation: for the combination of the test strains S-6.2/streptomycin and S-6.6/streptomycin with a level of agreement with the expected results at 55% and 48%, respectively, when assessing the results obtained by the EURL-AR network. Consequently, all results from these two strain/antimicrobial combinations have also been omitted in this analysis.

The methods listed in Table 1 were used for AST by the laboratories of the FWD-network and the EURL-AR-network.

Table 1: Number of laboratories using each method for AST in this proficiency test

	<i>Salmonella</i>			<i>Campylobacter</i>		
	MIC determination	E-test	Disk diffusion	MIC determination	In-house E-test	In-house disk diffusion
ECDC FWD network	1	1	7	2	6	2
EURL-AR network	28	-	6	26	-	(1)*

\* Results disregarded in this report as the method is not accepted for *Campylobacter* in the EURL-AR network

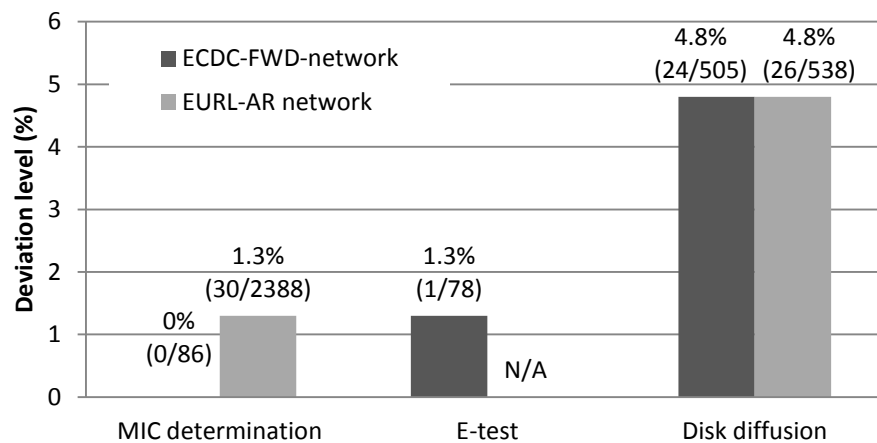


**Figure 2:** A comparison between the results obtained by the FWD-network and the EURL-AR network showing the total percentage of deviations for AST performed by participating laboratories

The percentages of deviations from the expected results of AST performed by laboratories from each of the networks are illustrated in Figure 2. As indicated, both results obtained by each of the networks are well below the 5% acceptance level.

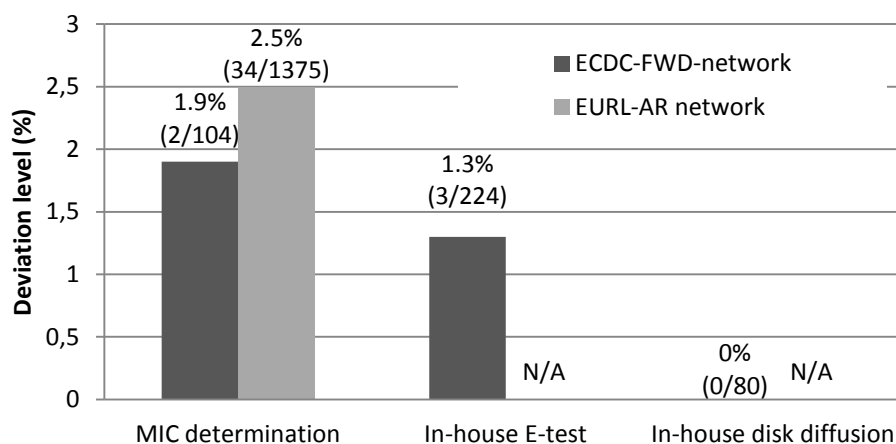
Figures 3 and 4 illustrate the total percentage of deviations from the expected results obtained by the different methods performed, divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively.

Interestingly, the deviation levels for the *Salmonella* AST are exactly the same for MIC determination/EURL-AR-network and E-test/FWD-network, as well as the level of deviations for disk diffusion AST performed by each of the networks. For the EURL-AR network, a significant difference ( $\chi^2$ -test;  $p < 0.01$ ) was obtained when comparing results obtained by the use of disk diffusion and a MIC method. For the FWD-network, the comparison of the MIC-determination and the disk diffusion method also rendered a significant difference ( $\chi^2$ -test;  $p < 0.1$ ). In both cases the MIC-determination exhibiting the better result.



**Figure 3:** The percentage of deviations (number of deviations relative to the total tests performed) for AST's of *Salmonella* test strains performed using each of the available methods.

For the *Campylobacter* AST, the levels of deviation are fairly close as regards the use of MIC determination. Only the FWD-network made use of E-test and disk diffusion for the AST of *Campylobacter*, and a comparison of the results obtained by the MIC method (both networks) shows no significant difference neither to those obtained by E-test ( $p = 0.4$ ) nor to those obtained by disk diffusion ( $p = 0.3$ ).



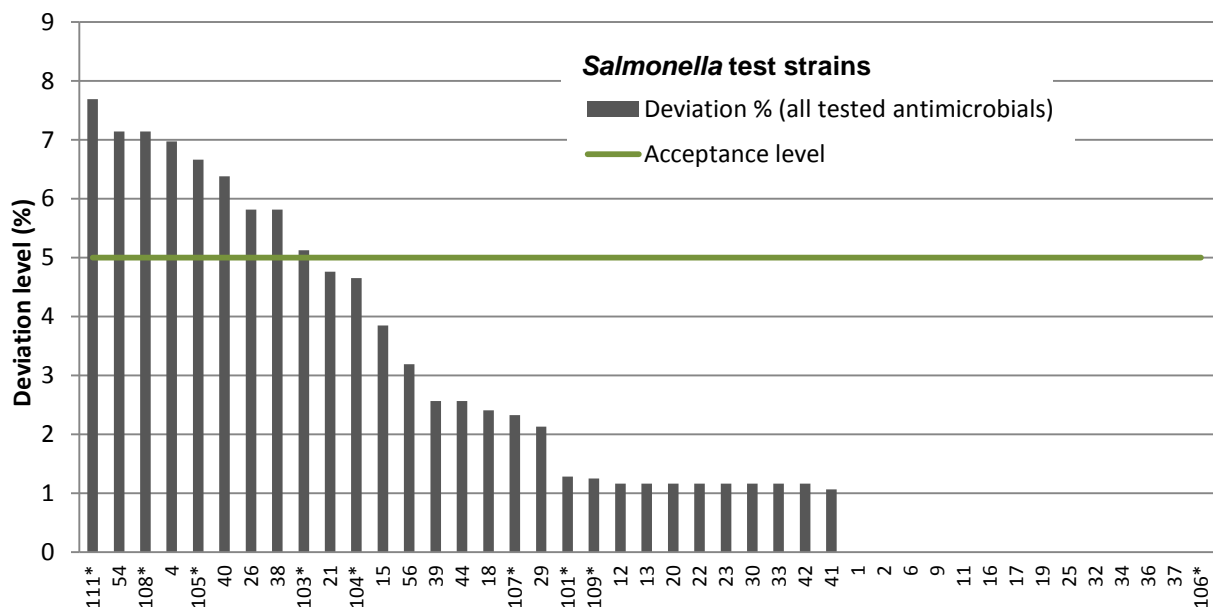
**Figure 4:** The percentage of deviations for AST's of *Campylobacter* test strains performed using each of the available methods.

As for the recommendation by the EURL-AR that the only type of method recommendable for AST of *Campylobacter* is MIC methods, i.e. broth or agar dilution methods, this is based on the fact that internationally recommended interpretative criteria are available for broth and agar

dilution methods, only. These methods have been validated and are recommended by CLSI (Clinical and Laboratory Standards Institute; [www.clsi.org](http://www.clsi.org)) and EUCAST (European Committee on Antimicrobial Susceptibility Testing; [www.eucast.org](http://www.eucast.org)), whereas for the diffusion methods (E-test and disk diffusion) there is for the moment no international references for quality assurance and interpretative criteria.

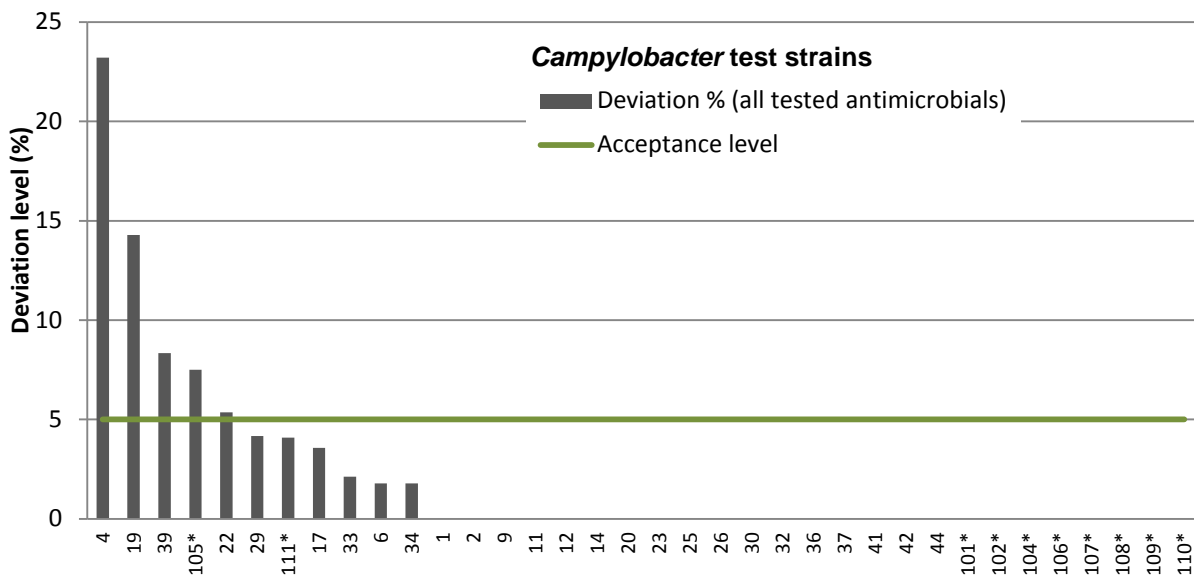
Figures 5 and 6 illustrate the total percentage of deviations from the expected results obtained by each of the laboratories divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively. The laboratories are ranked according to their performance determined by the percentage of deviating results in tests including all antimicrobials but excluding ESBL confirmatory tests.

Assessing results obtained by both networks, the deviation level for the *Salmonella* AST is generally low, with no laboratories exhibiting outlying levels, and with 34 laboratories (79%) performing acceptably according to the acceptance level at 5%.



**Figure 5:** Individual participants' deviations in percent of their total number of *Salmonella* AST's. Laboratory numbers below 100 belong to the EURL-AR-network, whereas laboratory numbers from 101-111 are indicated with an asterisk and belong to the FWD-network

For the *Campylobacter* AST, 31 (86%) laboratories submitted results which meet the acceptance level (<5%), but of the five laboratories with a higher deviation level, one laboratory (#4) counted for 38% of all deviations in the EURL-AR-network. The laboratory has informed the EQAS organizer, that there were some personnel issues which have been handled right away. Laboratory #19 also counted for a large number of deviations, and communicated to the EQAS organizer that they are investigating the reason for these.



**Figure 6:** Individual participants' deviations in percent of their total number of *Campylobacter* AST's. Laboratory numbers below 100 belong to the EURL-AR-network, whereas laboratory numbers from 101-111 are indicated with an asterisk and belong to the FWD-network.

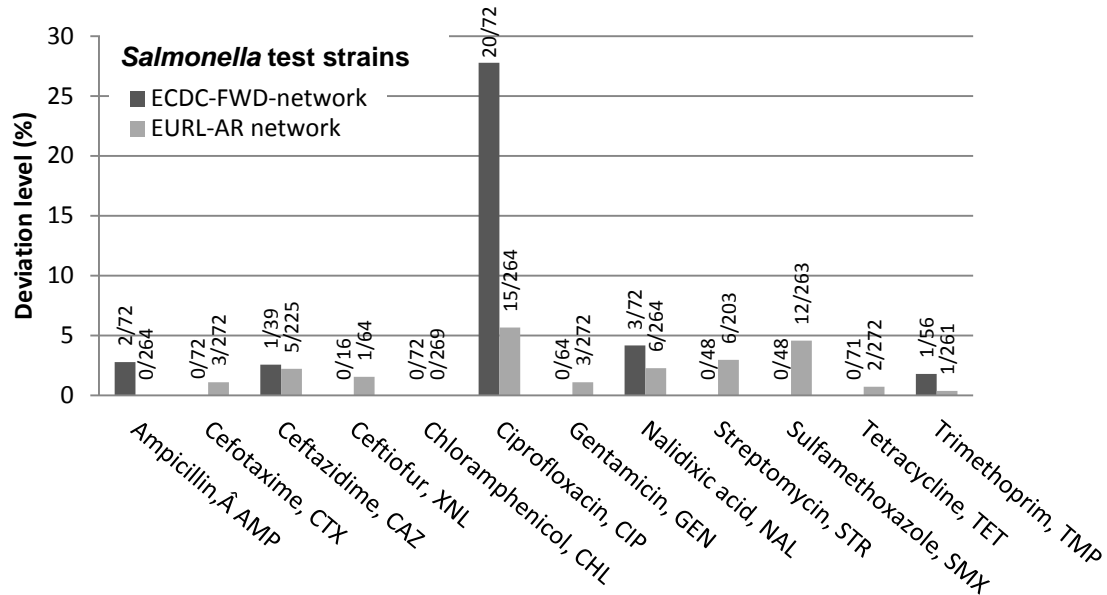
Figures 7 and 8 illustrate the total percentage of deviations from the expected results on each of the antimicrobials divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively.

For *Salmonella*, ciprofloxacin clearly shows the highest deviation level for the FWD-network laboratories (27.8%), and also for the EURL-AR-network (5.7%). The interpretative criteria listed in the protocol refer to EUCAST where the cut off value for this antimicrobial is 0.06 µg/mL. Many of the FWD-network laboratories submitted incorrect interpretations as susceptible, as this epidemiological cut off value is considerably lower than the clinical breakpoint set by CLSI ( $R \geq 4$  µg/mL), and as no corresponding zone diameter is available. Two of the *Salmonella* strains harboured a plasmid-mediated quinolone resistance (PMQR) gene (*S-6.3/qnrB* and *S-6.7/qnrD*) and thus exhibit nalidixic acid susceptibility and low-level ciprofloxacin resistance, the latter not being detectable when applying the routine CLSI methods and guidelines. The consequence of obtaining an incorrect interpretation in this EQAS, i.e. when applying the epidemiological cut off values for interpretation, is however not necessarily an incorrect interpretation in a clinical context. When analysing according to epidemiological cut off values, it is, however, recommended that laboratories performing disk diffusion for AST of *Salmonella* refer to the publication by Cavaco and Aarestrup, 2009 (4), which describes suggestions for disk content and cut off values for AST by disk diffusion of *Salmonella* isolates harbouring a PMQR-gene.

For the *Salmonella* AST, the FWD-network and the EURL-AR-network, respectively, on average tested 9.8 and 10.6 antimicrobials per test strain. A number of the laboratories from the FWD-network did not test ceftiofur (veterinary antimicrobial) and ceftazidime, and the same was the case for the ceftiofur for the EURL-AR-network.

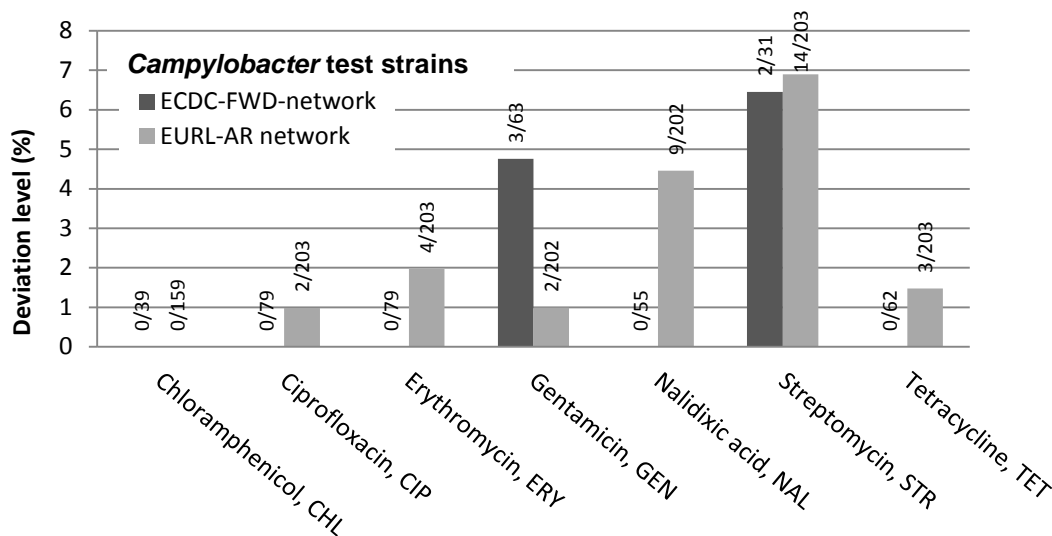
Generally, since only interpretations as resistant or susceptible could be submitted, the laboratories performing disk diffusion for AST should refer to the comment in the protocol that

intermediary results should be interpreted as susceptible. For the FWD-network laboratories, this would have eliminated 4 (15%) of the deviations for the *Salmonella* test strains, as these particular results would be ‘intermediate’ if applying the CLSI guidelines.



**Figure 7:** The percentage of deviations on each of the antimicrobials for AST’s performed on *Salmonella* test strains. Above each bar, the numerator and denominator are given.

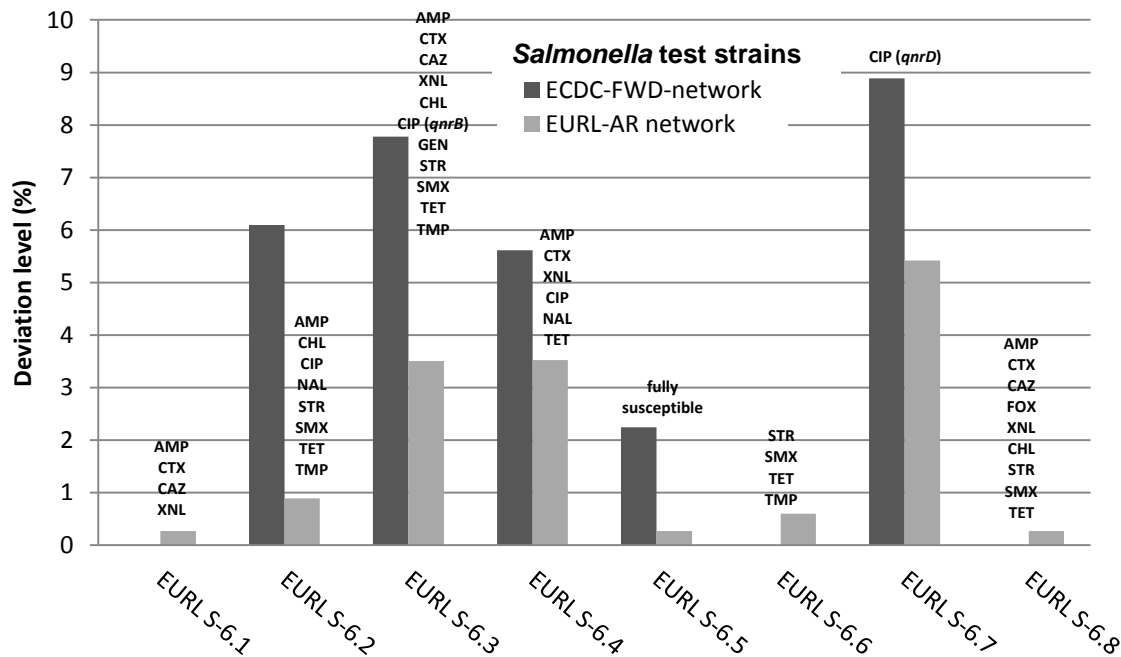
For *Campylobacter*, the FWD-network laboratories demonstrated 5 (1.2%) deviations, all of which were interpretations as resistant of MIC-values that according to the interpretative criteria in the protocol would be susceptible; two laboratories each counted for three and two of these deviations. As for the EURL-AR-network, the deviations (n=34; 2.5%) were to a large extent (n=13; 38% of *Campylobacter* AST deviations) caused by one laboratory due to personnel issues. This laboratory presented deviations for six of the seven antimicrobials.



**Figure 8:** The percentage of deviations on each of the antimicrobials for AST’s performed on *Campylobacter* test strains. Above each bar, the numerator and denominator are given.

Only some laboratories from the FWD-network tested the *Campylobacter* test strains towards streptomycin (n=4) and chloramphenicol (n=5), and in total, the FWD-network and the EURL-AR-network on average tested 5.0 and 6.6 antimicrobials per test strain, respectively.

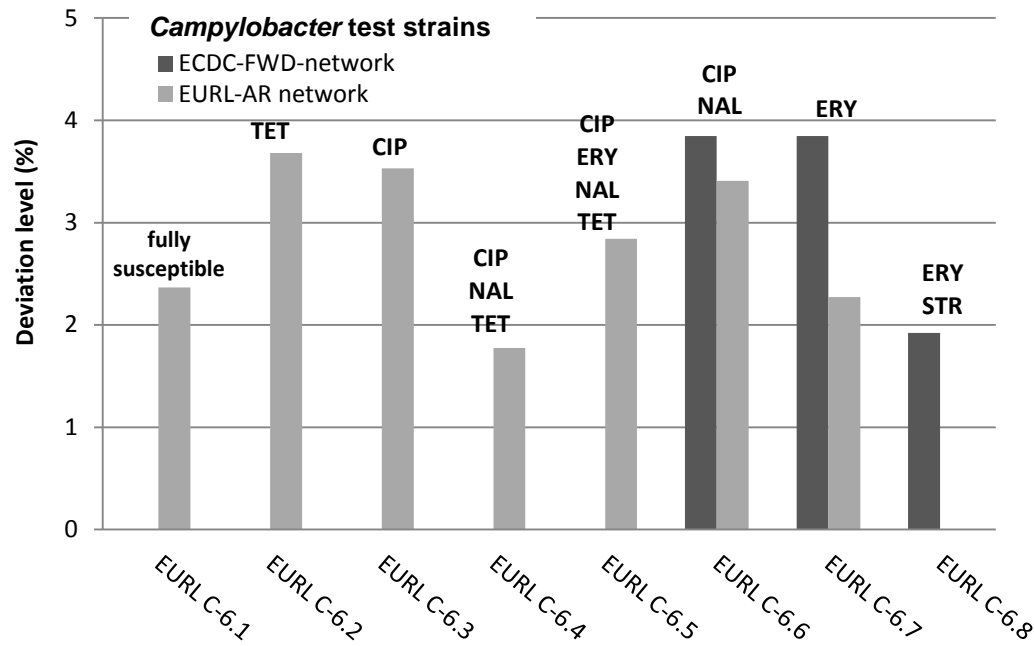
Figures 9 and 10 illustrate the total percentage of deviations from the expected results obtained for each of EQAS test strains divided between each of the networks for AST's performed on *Salmonella* and *Campylobacter* test strains, respectively. The resistance phenotype of each of the strains is indicated.



**Figure 9:** The percentage of deviations on each of the test strains for AST's performed on *Salmonella* test strains. When a strain exhibited resistance to a certain antimicrobial it is indicated by an antimicrobial code; i.e. AMP, ampicillin; CTX, cefotaxime; FOX, cefoxitin; CAZ, ceftazidime; XNL, ceftiofur; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; SMX, sulphonamides; TET, tetracycline; and TMP, trimethoprim.

For *Salmonella*, three strains cause very few deviations or none (S-6.1, S-6.6, S-6.8). Four strains accounted for most deviation for both the networks; i.e. S-6.2, S-6.3, S-6.4 and S-6.7. All of these strains exhibit reduced susceptibility to ciprofloxacin which is the cause of most of the deviating results.

For *Campylobacter*, the five deviations from the FWD-network laboratories belong to three strains (C-6.6, C-6.7, C-6.8), whereas for the EURL-AR-network, the deviations are spread over seven of the eight test strains with one laboratory contributing to the deviations for six of the eight test strains.



**Figure 10:** The percentage of deviations on each of the test strains for AST's performed on *Campylobacter* test strains. For each of the strains, a resistance phenotype is indicated by an antimicrobial code; i.e. CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin; NAL, nalidixic acid; STR, streptomycin; and TET, tetracycline.

### ESBL-producing *Salmonella* test strains

For the EURL-AR network, the detection of ESBL-producing strains was mandatory, whereas it was an optional part of the EQAS for the ECDC FWD-network laboratories. The details of the ESBL-detection and confirmation are addressed in the EURL-AR report (1).

Four test strains; S-6.1, S-6.3, S-6.4 and S-6.8 were ESBL-producers, i.e. three were so-called 'true ESBLs', harbouring *bla*<sub>TEM-52</sub> (S-6.1), *bla*<sub>CTX M-15</sub> and *bla*<sub>SHV-12</sub> (S-6.3) and *bla*<sub>CTX M-15</sub> (S-6.4), whereas one was and ampC-producing strain harbouring *bla*<sub>CMY-2</sub> (S-6.8).

The ESBL-production was confirmed by the majority (n=32, 32, 32 and 31 for the strains S-6.1, S-6.3, S-6.4 and S-6.8) of the 34 laboratories from the EURL-AR network participating in the *Salmonella* EQAS. Of the nine FWD-network laboratories submitting results on the *Salmonella* test strains, six participated in the ESBL component and all of these submitted correct results on the confirmation of ESBL-production except one laboratory, which incorrectly categorised the ampC-producing strain (S-6.8) as ESBL-producer. All nine laboratories tested the *Salmonella* test strains towards at least one cephalosporin, and five included testing of susceptibility towards ceftazidime for selected test strains (n=4) or for all eight *Salmonella* test strains (n=1).

### Deviations by reference strains

In the following section, deviations are defined as results of antimicrobial susceptibility tests on the reference strain that are outside the quality control (QC) acceptance intervals (App. 2). All



laboratories from the FWD-network submitted results for the quality control of the method relevant for both the *Salmonella* (9 laboratories) and the *Campylobacter* (8 laboratories) testing. For the EURL-AR, all 26 laboratories performing MIC for AST of *Campylobacter*, and all 34 laboratories submitting MIC-results for *Salmonella* uploaded QC-strain results. Five of the six NRL-AR's performing disk diffusion for AST of *Salmonella* uploaded QC-results.

The results from the reference strain should be assessed as part of the quality assurance of the values obtained when performing AST on the test strains, and are therefore especially important for laboratories which have deviations listed in their evaluation report. The obtained values for the QC-reference strains are further evaluated in the separate reports for each of the two networks.

The submitted results from testing the *C. jejuni* reference strain could be evaluated for two of the nine FWD-network laboratories, as the remaining uploaded values were E-test MIC-values and inhibition zone diameters where no evaluation criteria is available.

#### 4. Conclusions

The objective of providing the EURL-AR EQAS to the FWD-network this year, was to assess and compare the quality of the antimicrobial susceptibility data produced by the reference laboratories from the two networks. In addition, it was to identify areas which would require attention to produce reliable and harmonised susceptibility data.

This assessment demonstrates that the AST-results obtained by the FWD-network and the EURL-AR-network are comparable as regards AST of *Salmonella* and *Campylobacter*. The goal as to acceptance level for deviations for each laboratory was set at 5%. This goal was met for 5 (55.6%) of the FWD-network laboratories and for 29 (85.3%) of the NRL-AR's for *Salmonella* AST. For *Campylobacter* AST this was the case for 9 (90%) of the FWD-network laboratories and for 22 (84.6%) of the NRL-AR's.

The EURL-AR recommends MIC methods, only, i.e. broth or agar dilution methods, for AST of *Campylobacter* due to the fact that for the diffusion methods there are for the moment no international references for quality assurance and interpretative criteria. In this EQAS, however, no deviations were detected when disc diffusion was applied for the AST of *Campylobacter*.

Especially for the FWD-network laboratories, the interpretation of ciprofloxacin posed a problem. Many laboratories in this network perform DD for AST of *Salmonella* and as this breakpoint is much lower than the clinical breakpoint, this generates a difference in interpretation.

The issue about detection of ESBL-producing *Enterobacteriaceae* is critically relevant for both the public health laboratories and the laboratories from the veterinary/food sector as these phenotypes appear to continue to emerge worldwide. Laboratories which have not yet introduced tests to detect ESBL-producing *Enterobacteriaceae* are therefore encouraged to prioritize this area.





## 5. References

1. **Karlsrose S**, Cavaco LM, Hendriksen RS, Aarestrup FM. The 11th EURL-AR Proficiency Testing, *Salmonella* and *Campylobacter* – 2011 available for download from <http://www.eurl-ar.eu/203-reports.htm>
2. **Aikkinen A** and Takkinen, J. *ECDC-network report (draft)* [title and reference of final report not available at the time of printing the present report]
3. **Schwarz S**, Silley P, Simjee S, Woodford N, van DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother* 65: 601-604
4. **Cavaco LM** and **Aarestrup FM**. (2009). Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac(6')Ib-cr*, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *J Clin Microbiol.* 2009 Sep;47(9):2751-8

## Participant list

Salmonella	Campylobacter	Sector	Institute	Country
X	X	Veterinary/Food	Austrian Agency for Health and Food Safety	Austria
X	X	Veterinary/Food	Institute of Public Health	Belgium
X	-	Veterinary/Food	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	X	Veterinary/Food	Croatian Veterinary Institute	Croatia
X	X	Veterinary/Food	Veterinary Services	Cyprus
X	X	Veterinary/Food	State Veterinary Institute Praha	Czech Republic
X	X	Veterinary/Food	The National Food Institute	Denmark
		Public Health	Statens Serum Institut	Denmark
X	X	Veterinary/Food	Estonian Veterinary and Food Laboratory	Estonia
X	X	Veterinary/Food	Finnish Food Safety Authority EVIRA	Finland
		Public Health	National Institute for Health and Welfare (THL)	Finland
X	-	Veterinary/Food	ANSES Maisons Alfort	France
-	X	Veterinary/Food	ANSES Ploufragan	France
X	-	Veterinary/Food	ANSES Lyon	France
X	-	Veterinary/Food	ANSES Fougères	France
		Public Health	Hôpital Pellegrin	France
X	X	Veterinary/Food	Federal Institute for Risk Assessment	Germany
X	-	Veterinary/Food	Veterinary Laboratory of Chalkis	Greece
X	X	Veterinary/Food	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
		Public Health	National Center for Epidemiology	Hungary
		Public Health	Landspítali University Hospital	Iceland
X	X	Veterinary/Food	Central Veterinary Research Laboratory	Ireland
X	-	Veterinary/Food	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	Veterinary/Food	Institute of Food Safety, Animal Health and Environment „BIOR”	Latvia
X	X	Veterinary/Food	National Food and Veterinary Risk Assessment Institute	Lithuania
		Public Health	Laboratoire National de Santé	Luxembourg
X	X	Veterinary/Food	Public Health Laboratory	Malta
X	X	Veterinary/Food	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	Veterinary/Food	Veterinærinstituttet	Norway
		Public Health	Norwegian Institute of Public Health	Norway
X	X	Veterinary/Food	National Veterinary Research Institute	Poland
		Public Health	Institute of Public Health - National Institute of Hygiene (NIZP-PZH)	Poland
X	X	Veterinary/Food	Laboratório Nacional de Investigação Veterinária	Portugal
X	X	Veterinary/Food	Institute for Hygiene and Veterinary Public Health	Romania
X	X	Veterinary/Food	Institute of Veterinary Medicine of Serbia	Serbia
X	X	Veterinary/Food	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	Veterinary/Food	National Veterinary Institute	Slovenia
		Public Health	ZAVOD ZA ZDRAVSTVENO VARSTVO NOVA GORICA	Slovenia
		Public Health	Institute of Public Health of the Republic of Slovenia	Slovenia
-	-	Veterinary/Food	Laboratorio Central de Sanidad, Animal de Santa Fe (only Staph)	Spain
X	X	Veterinary/Food	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	-	Veterinary/Food	Centro nacional de Alimentacion. Agencia Espanola de Seguridad	Spain
		Public Health	Instituto de Salud Carlos III (ISCIII)	Spain
X	X	Veterinary/Food	National Veterinary Institute, SVA	Sweden
X	X	Veterinary/Food	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	X	Veterinary/Food	The Veterinary Laboratory Agency	United Kingdom
X	X	Veterinary/Food	Centre for Infections Health Protection Agency	United Kingdom

Designated NRL-AR by the competent authority of the member state  
 Not a Member State of the EU

## QC ranges for reference strains

<i>E. coli</i> ATCC 25922			
Antimicrobial	MIC	E-test	DD (disc content)
Ampicillin, AMP	2-8	2-8	16-22 (10µg)
Cefotaxime, CTX	0.03-0.12	0.03-0.12	29-35 (30µg)
Cefoxitin, FOX	2-8	None	23-29 (30µg)
Ceftazidime, CAZ	0.06-0.5	0.06-0.5	25-32 (30µg)
Ceftiofur, XNL	0.25-1	None	26-31 (30µg)
Chloramphenicol, CHL	2-8	None	21-27 (30µg)
Ciprofloxacin, CIP	0.004-0.016	None	30-40 (5µg)
Gentamicin, GEN	0.25-1	None	19-26 (10µg)
Imipenem, IMI	0.06-0.25	0.06-0.25	26-32 (10µg)
Nalidixic acid, NAL	1-4	1-4	22-28 (30µg)
Streptomycin, STR	4-16	2-8	12-20 (10µg)
Sulfisoxazole, FIS	8-32	32-128	15-23 (250/300µg)
Tetracycline, TET	0.5-2	0.5-2	18-25 (30µg)
Trimethoprim, TMP	0.5-2	0.5-2	21-28 (5µg)

MIC ranges and disc diffusion ranges are according to CLSI M100 S21 with the following exceptions: The MIC range for streptomycin is according to Sensititre and the range for ceftiofur is according to M31-A3. Additionally, the range for ciprofloxacin is extended to include 0.016 as well.

E-test ranges are according to AB-Biodisk

<i>Campylobacter jejuni</i> ATCC 33560				
Antimicrobial	Microbroth (36-37°C/48h)	Microbroth (42°C/24h)	Agar dilution (36-37°C/48h)	Agar dilution (42°C/24h)
Chloramphenicol, CHL	1-8	1-4	None	None
Ciprofloxacin, CIP	0.06-0.25	0.03-0.12	0.12-1	0.06-0.5
Erythromycin, ERY	0.5-2	0.25-2	1-8	1-4
Gentamicin, GEN	0.5-2	0.25-2	0.5-2	0.5-4
Nalidixic acid, NAL	4-16	4-16	None	None
Tetracycline, TET	0.25-2	0.25-1	None	None

Ranges are according to CLSI (M31-A3)

National Food Institute  
Technical University of Denmark  
Kemitorvet, 204  
DK - 2800 Kgs Lyngby

Tel. 35 88 70 00  
Fax 35 88 70 01

[www.food.dtu.dk](http://www.food.dtu.dk)

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