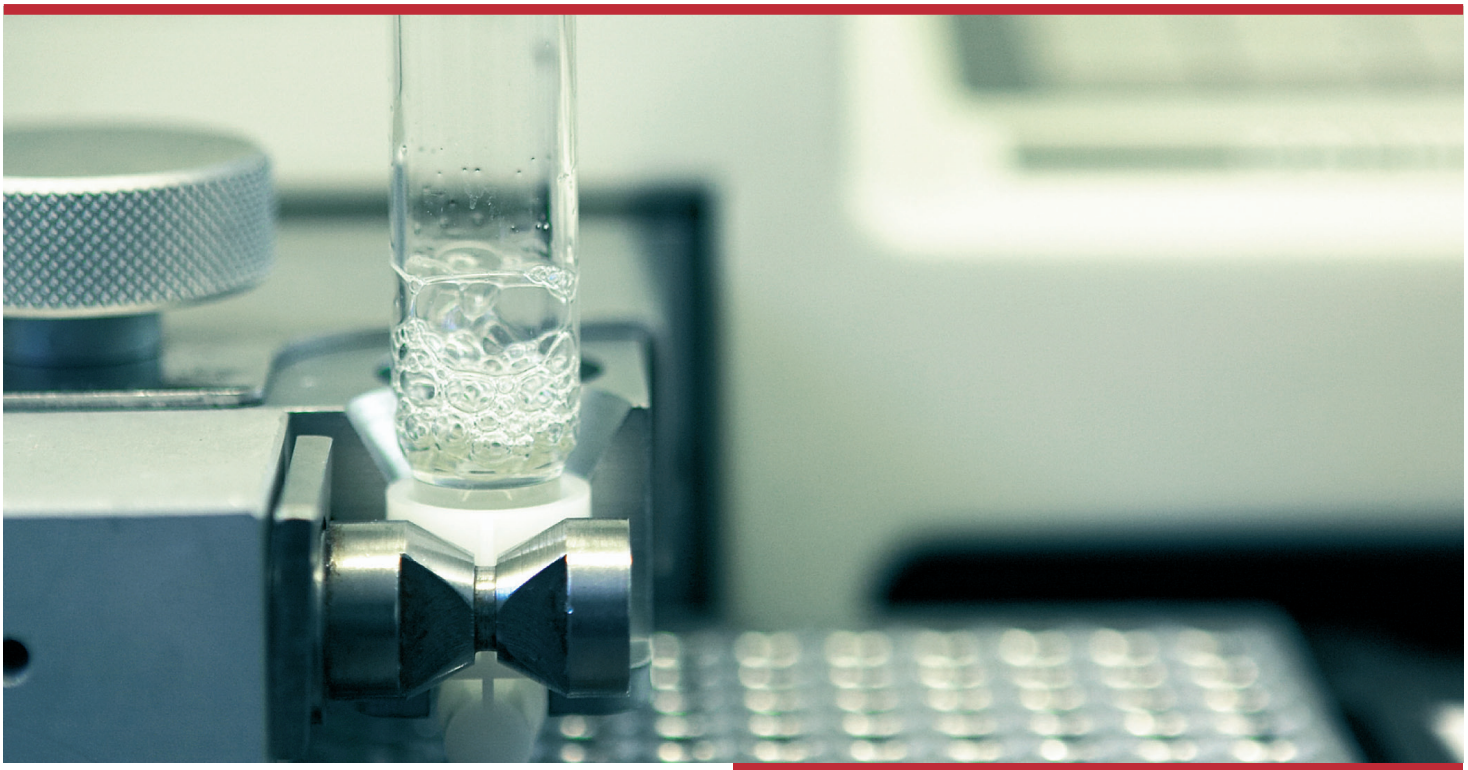
The 13th EURL-AR Proficiency Test *Salmonella*, *Campylobacter* and genotypic characterisation 2012



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1. Introduction

This report summarises results from the thirteenth proficiency test trial conducted by the National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). This proficiency test focuses on *Salmonella* and *Campylobacter* and is the seventh External Quality Assurance System (EQAS) conducted for these microorganisms (the first was EQAS 2006). In addition, the proficiency test for the fourth time includes an optional element consisting of genotypic characterization by PCR/sequencing of antimicrobial resistance genes. This optional component included characterization of genes related to production of extended spectrum beta-lactamases (ESBL) in the ESBL-producing *Salmonella* test strains.

The objective of the EQAS is to monitor the quality of the antimicrobial susceptibility data produced by the NRL-AR and to identify areas or laboratories, for which guidance or assistance would be required as means of producing reliable susceptibility data. The goal until the 2008 iteration was to have all laboratories performing antimicrobial susceptibility testing (AST) with less than 7% incorrect interpretations. This was reconsidered at the EURL-AR workshop 2009, and as of the 2009 iterations, the goal is to have each laboratory performing AST with less than 5% incorrect interpretations (interpretations deviating from the expected results). For the optional genotypic characterisation, no specific acceptance level has been set.

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 and known only to the EURL-AR and the EU Commission. All conclusions are public.

The technical advisory group for the EURL-AR EQAS scheme consists of competent representatives from all National Reference Laboratories for Antimicrobial Resistance (NRLs), who meet annually at the EURL-AR workshop.

The AST data reported to EFSA by the Member States (MS) is based on the interpretation of the AST results. The basis for this EQAS evaluation is the interpretation of the AST result; as is also stated in the protocol, the “main objective of this EQAS is to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by the different NRLs”. In addition, the participants of an EQAS should 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 ± 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 are still regarded as a deviation for the overall EQAS reporting, evaluation and is kept as such 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

2.1 Participants in EQAS 2012

A pre-notification (App. 1) of the EURL-AR EQAS on AST of *Salmonella* and *Campylobacter* was distributed on the 16th July 2012 by e-mail to the 41 NRLs in the EURL-AR-network including all EU countries (except Luxembourg where no NRL has been designated) and including Croatia, Iceland, Norway, Serbia and Switzerland. One laboratory did not participate as they had neither *Salmonella* nor *Campylobacter* AST as their field of responsibility. In addition, Iceland and Serbia did not participate in this iteration. In addition to the AST of *Salmonella* and *Campylobacter*, an optional genotypic characterization by PCR/sequencing of antimicrobial resistance genes of the ESBL-producing *Salmonella* test strains was offered.

Appendix 2 shows that 32 of the 38 participating NRLs were appointed by the individual Member States. Two NRLs were enrolled on equal terms as the designated NRLs, based on their participation in an EU funded concerned action (FAIR5-QLK2-2002-01146), the ARBAO II project (Antibiotic Resistance in Bacteria of Animal Origin). The laboratories in Croatia, Norway, Switzerland and Turkey were charged a fee for their participation in the EQAS, whereas the NRLs from EU Member States participated free of charge.

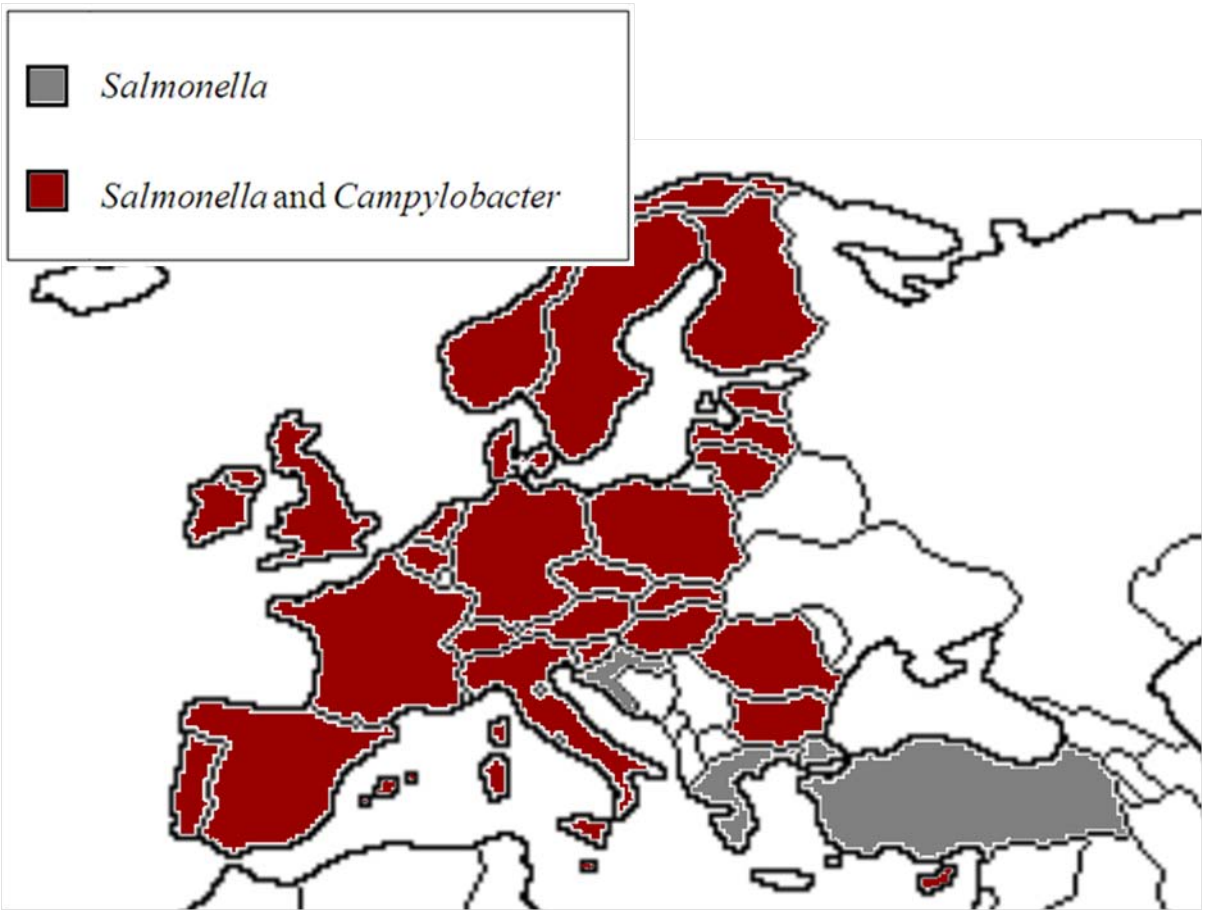

Figure 1: Participating countries that performed antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter.*

Figure 1 illustrates that of the 30 participating countries, 27 tested both *Salmonella* and *Campylobacter*. Three countries, for various reasons, uploaded *Salmonella* results, only, for evaluation (Croatia, Greece and Turkey). Eleven laboratories participated in the optional genotypic characterisation of the ESBL-producing *Salmonella* test strains (not illustrated in Figure 1; see Appendix 2).

The results from the NRLs designated by the MS are presented and evaluated in this report in addition to national reference laboratories in affiliated non-MS; i.e. results from 30 countries consisting of 35 laboratories submitting *Salmonella* results and 29 laboratories submitting *Campylobacter* results. Results from the two laboratories not designated by the MS but enrolled on equal terms as these are not further presented or evaluated in this report.

2.2 Strains

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 in transport media (Stuarts).

The shipment of strains included the lyophilised international reference strains for antimicrobial susceptibility testing; *Escherichia coli* CCM 3954 (ATCC 25922) and *Campylobacter jejuni* CCM 6214 (ATCC 33560) purchased at Czech Collection of Micro-organisms (CCM), the Czech Republic for the NRLs which had not received these reference strains in previous EQAS's conducted by DTU Food.

Prior to distribution of the strains, AST was performed on the *Salmonella* and *Campylobacter* strains at DTU Food and verified by the US Food and Drug Administration (FDA). When MIC-values were not in agreement (+/- one MIC-step), the value obtained by DTU Food was selected as the reference value. The obtained MIC values served as reference for the test strains (App. 3a and 3b). Results from the following antimicrobials were not verified by FDA: cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, imipenem, imipenem/EDTA, and trimethoprim for *Salmonella* and furthermore, chloramphenicol and streptomycin for *Campylobacter*.

2.3 Antimicrobials

The antimicrobials tested in this EQAS are listed in the protocol (App. 4b) and were included mainly according to the recommendations of the European Food Safety Authority (EFSA) monitoring programme [Report of the Task Force of Zoonoses Data Collection including a proposal for a harmonized monitoring scheme of antimicrobial resistance in *Salmonella* in fowl (*Gallus gallus*), turkeys, and pigs and *Campylobacter jejuni* and *C. coli* in broilers, the EFSA Journal (2007), 96,1-46]. A few additional antimicrobials have been added as indicated in the protocol due to the included element on detection of ESBL production.

The selection of antimicrobials used in the trial for *Salmonella* were: ampicillin (AMP), cefotaxime (CTX), cefotaxime/clavulanic acid (CTX/Cl), ceftazidime (CAZ), ceftazidime/clavulanic acid (CAZ/Cl), ceftiofur (XNL), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), nalidixic acid (NAL), sulfonamides (sulfamethoxazole) (SMX), tetracycline (TET) and trimethoprim (TMP). Additionally, cefoxitin (FOX) was used for detection of ampC, and imipenem (IMI), imipenem/EDTA for detection of metallo-beta-lactamases (MBL).

Minimum Inhibitory Concentration (MIC) determination of the *Salmonella* test strains was performed using the Sensititre system from Trek Diagnostic Systems Ltd, UK. For ESBL confirmatory test, the analysis included MIC

determination by microbroth dilution (panel code ESB1F), and in addition, for the antimicrobials cefotaxime/clavulanic acid, cefoxitin, ceftazidime/clavulanic acid, tests were performed using E-test from AB-Biodisk, Sweden. The method guidelines used were according to the Clinical and Laboratory Standards Institute (CLSI) document M7-A9 (2012), "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" (Approved Standard - Ninth Edition), document M100-S22 (2012) "Performance Standards for Antimicrobial Susceptibility Testing" (Twenty-Second Informational Supplement) and document M31-A3 (2008) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals" (Approved Standard – Third Edition).

For *Campylobacter* the following antimicrobials were included: chloramphenicol (CHL), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), nalidixic acid (NAL), streptomycin (STR), and tetracycline (TET). MIC determination was performed using the Sensititre systems from Trek Diagnostic Systems Ltd, UK, according to guidelines from the CLSI document M45-A2 (2010) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria" (Approved Guideline – Second Edition) and M31-A3 (2008) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals" (Approved Standard – Third Edition).

2.4 Distribution

On October 1st, 2012, the cultures and a welcome letter (App. 4a) were dispatched in double pack containers (class UN 6.2) to the participating laboratories as UN3373, biological substance category B, according to the International Air Transport Association (IATA) regulations.

2.5 Procedure

Through the EURL-AR website, http://www.eurl-ar.eu/, the laboratories were provided with protocols and information regarding the handling of the test strains and reference strains (App. 4b, c, d, e). The participants were instructed to subculture the strains according to the description in the protocol prior to performing the AST. Furthermore, participants receiving an ATCC reference strain were requested to save and maintain this for future proficiency tests.

The aim is that only MIC methods are used when performing AST for monitoring conducted by the Commission, and thereby also when performing the EURL-AR EQAS's. Consequently, it was decided in May 2007 by the participants at the EURL-AR workshop that the NRLs should work towards harmonising to MIC methods for these AST analyses. Additionally, it was agreed that all NRLs should work towards covering the antimicrobial panel and epidemiological cut-off values recommended by the EURL-AR. For this EQAS, the participants were instructed to use as many as possible of the antimicrobials listed, using the method carried out when performing monitoring for EFSA.

The cut-off values recommended by EFSA should be used (listed in the protocol). All cut-off values used in the interpretation of the *Campylobacter* MIC results have been developed by EUCAST (www.eucast.org). This is also the case for *Salmonella* with the exception of sulphonamides, where the value from CLSI was used according to the description in the protocol (App. 4b).

Participants using disk diffusion (DD) or 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. The breakpoints used were submitted to the web

based database. Breakpoints applied for disk diffusion for *Salmonella* are listed in Appendix 5.

It should be noted that for AST of *Campylobacter* the EURL-AR does not recommend the use of either disk diffusion or E-test for AST of *Campylobacter*. I.e. only results obtained by broth or agar dilution methods are accepted for this EQAS, as also agreed at the EURL-AR workshop 2009.

The laboratories were instructed to upload obtained MIC values (mg/L) or inhibition zone diameters (mm) and the susceptibility categories (resistant or susceptible) to the database through a secured individual login. Alternatively, the record sheets from the protocol could be sent by fax to DTU Food. The website was open for data entry in the period from the 25th of October 2012 to the 22nd of December 2012.

Detection of ESBL-producing strains should be performed and interpreted according to recommendations by EUCAST described in the protocol. Concerning the cephalosporins used when detecting ESBL-producing strains, MIC values and interpretations of these should be reported as found.

Results from the reference strains should also be entered into the database. The results would consist of MIC values for the reference strains *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560) or, for *E. coli* (ATCC 25922), the inhibition zone diameters in millimetres. 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-S22 (2012); The Sensititre System (Trek Diagnostic Systems Ltd, UK) (App. 7).

For the optional genotypic characterisation of the ESBL-producing *Salmonella* test strains, participating laboratories were requested to report the genes conferring resistance to extended-spectrum beta lactam antimicrobials. The genes listed in the table in the protocol (App. 4b) were included in the test. Identification of additional genes not listed in the protocol was not evaluated by the database. The results were evaluated based on the actual genes identified. Relevant variants of ESBL-genes were additionally evaluated.

The participating laboratories were encouraged to use their own laboratory's method(s) for the genotypic characterisation. The expected results for this component of the EQAS were obtained by whole-genome-sequencing and subsequent analysis using the ResFinder 1.3 platform available at http://cge.cbs.dtu.dk/services/ResFinder/. The positive identifications of genes was not verified elsewhere.

Subsequent to the submission deadline, the laboratories were instructed to login to the secured database once again to retrieve the database-generated, individual evaluation report. The evaluation included assessment of the submitted results and a description of all deviations from the expected. Deviations in the interpretation as resistant or susceptible were categorised as 'incorrect', as was also deviations concerning confirmation of an isolate as ESBL-producer or ampC.

The EURL-AR is aware that there are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should 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' (Schwarz et al., 2010). 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 cases where we are referring

to wild-type and non-wild-type strains.

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 used these features for sending comments to the EURL-AR. When relevant, those who did received direct reply.

3. Results

The participants were asked to report results, including MIC values or inhibition zone diameters obtained by 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.

At the EURL-AR workshop 2008, the network 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 one case: for the combination of the test strain C-7.1/tetracycline with a level of agreement with the expected results at 71% (Appendix 8b presents the total number of correct/incorrect results for this strain/antimicrobial-combination).

The expected MIC (2 mg/L, susceptible) was determined by two different institutions; DTU Food and FDA, and was within one fold dilution difference from the cut-off value (>2 mg/L).

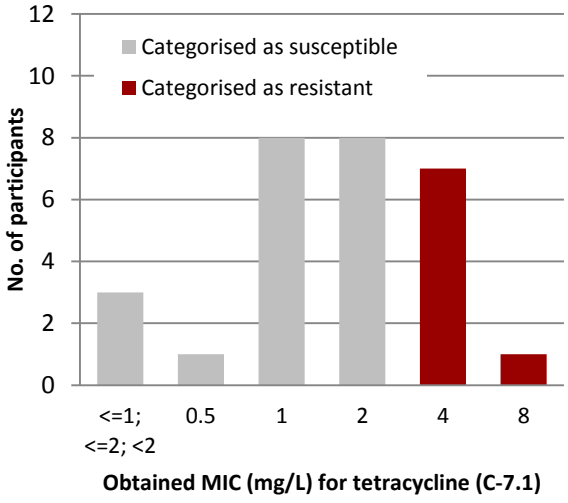

Figure 2: Distribution of the different MIC values obtained by participants for the combination C-7.1/tetracycline.

Figure 2 illustrates the distribution of the MIC values together with the interpretation of these values obtained by participants for the combination of strain C-7.1/tetracycline. The figure shows a distribution of MIC's with the expected value at 2mg/L and a high number of participants obtaining AST results one MIC-dilution higher than the expected result.

Based on the facts that the precision of the method relies on various factors, including the media content the type of microbroth panels, and the fact that an MIC result obtained by the microbroth method or agar dilution can vary +/- one dilution step from the obtained MIC, this strain/antimicrobial combination has been excluded from the evaluation.

3.1 Methods

In the *Salmonella* trial, 30 laboratories used MIC determination (28 used microbroth and two agar dilution), and five laboratories used disk diffusion. For the *Campylobacter* trial, all 29 included laboratories reported the use of MIC determination (microbroth or agar dilution).

3.2 Deviations, overall

The list of deviations is shown in Appendix 9a and 9b. Figure 3 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. The internal control strain mainly followed the trend in deviation level of the different EQAS trials (Figure 3). The deviation level in 2012 is acceptable for both the *Salmonella* and the *Campylobacter* trials.

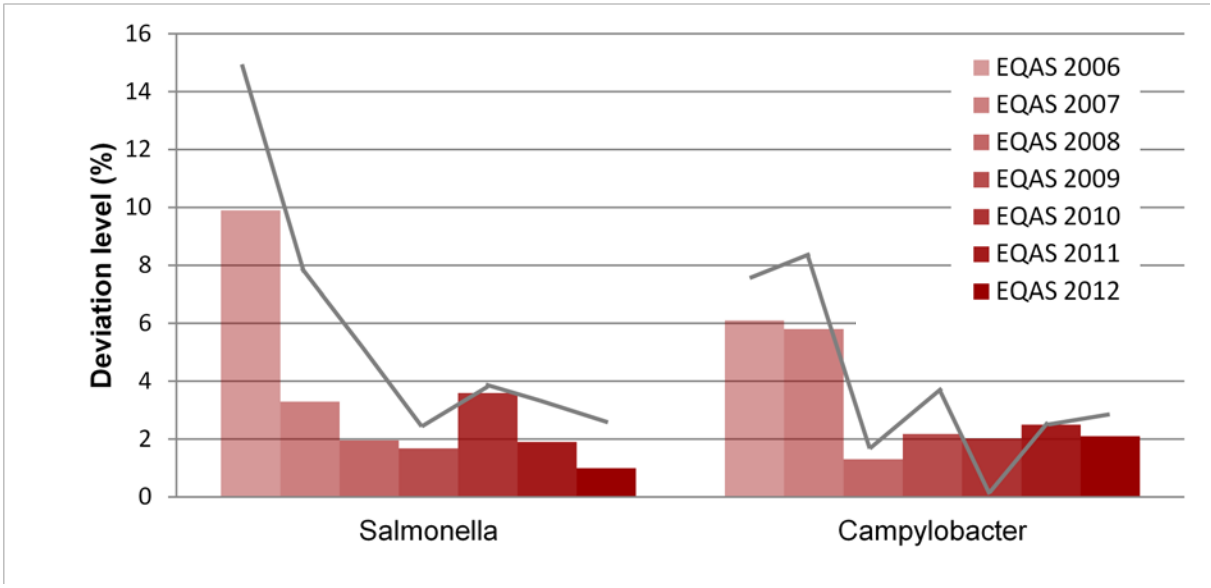

Figure 3: A comparison between the EURL-AR EQAS's since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing performed by participating laboratories.

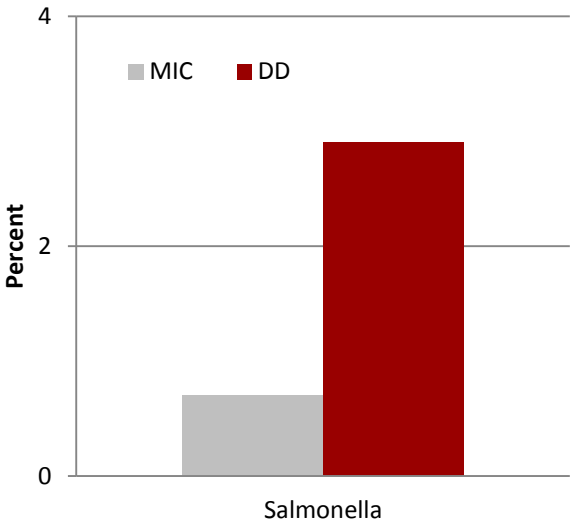

Figure 4: The total percentage of deviations for AST's performed using MIC-methods as opposed to disk diffusion.

3.2.1 *Salmonella* trial

For the *Salmonella* strains, 99.0% of the AST's were interpreted correctly. Figure 4 shows the total percentage of deviations from the expected results of AST performed by MIC-methods as opposed to disk diffusion. The deviation percentage is significantly higher ($p<0.01$) when AST is performed by disk diffusion compared to a MIC-method.

The number of AST's performed and the percentage of correct results for the individual strains in the EQAS, are listed in Table 1. Variations of obtained correct results ranged from 97.4-100% for *Salmonella*. Table 2 illustrates the percentage of correct AST per antimicrobial by bacterial species. The level of correct AST was above 97.4% for the *Salmonella* test strains. Ciprofloxacin exhibited the lowest deviation level.

ESBL-producing *Salmonella* test strains

It was decided on the EURL-AR workshop 2008 that the testing of ESBL production in *Salmonella* should be mandatory. The laboratories were asked to detect the ESBL-producing *Salmonella* strains and to perform confirmatory testing on all relevant strains resistant to cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) according to the protocol (App. 4b).

The four test strains; S-7.1, S-7.2, S-7.3 and S-7.6 were ESBL or ampC-producers, which was confirmed by the majority of the 35 laboratories

Table 1. The number of AST performed and the percentage of correct results for each strain of *Salmonella* and *Campylobacter.*

EQAS 2012 – *Salmonella*			EQAS 2012 – *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-7.1	356	98,6	C-7.1 (*C. coli*)	168	98,2
S-7.2	354	100	C-7.2 (*C. coli*)	196	98,5
S-7.3	352	97,4	C-7.3 (*C. coli*)	197	98,0
S-7.4	352	99,1	C-7.4 (*C. coli*)	197	95,9
S-7.5	354	99,7	C-7.5 (*C. jejuni*)	196	97,4
S-7.6	353	99,4	C-7.6 (*C. jejuni*)	197	99,0
S-7.7	351	99,4	C-7.7 (*C. jejuni*)	197	98,5
S-7.8	351	98,0	C-7.8 (*C. jejuni*)	197	97,5

Table 2: Percentage of correct antimicrobial susceptibility tests per antimicrobial by microorganism. In grey, antimicrobials recommended in the EFSA zoonosis monitoring manual.

Antimicrobial	*Salmonella*	*Campylobacter*
Ampicillin	100.0	-
Cefotaxime	99.3	-
Ceftazidime	99.2	-
Ceftiofur	98.8	-
Chloramphenicol	99.3	100.0
Ciprofloxacin	97.4	97.4
Erythromycin	-	97.0
Gentamicin	99.3	100.0
Nalidixic acid	98.9	96.5
Streptomycin	-	96.6
Sulphonamides	97.9	-
Tetracycline	99.6	98.0
Trimethoprim	98.9	-

participating in the *Salmonella* EQAS. Three of the ESBL-producing strains were so-called 'true ESBLs' (S-7.1, S-7.2 and S-7.3), and one was an ampC-producing strain (Table 3). As the ESBL detection part is mandatory in this EQAS, all results are evaluated below.

There is a difference in the number of cephalosporins used by the laboratories in their routine test for ESBL production; five compounds are included in this proficiency test: cefotaxime, ceftazidime, ceftiofur, cefotaxime/clavulanic acid and ceftazidime/clavulanic acid. The first three are used for initial screening whereas the last two are used for confirmatory test (the combination disk method). The proportion of laboratories using different combinations of cephalosporins for screening for ESBL-production were nine laboratories using the combination CTX, CAZ, XNL, 21 laboratories using CTX, CAZ, one laboratory using CTX, XNL, and four laboratories using CTX.

Strain S-7.1 and S-7.6 exhibited unusual phenotypes and genotypes and will be further discussed below.

Overall, in 49 cases the expected confirmation of the ESBL- or ampC-producing strain was incorrectly detected. Eight of these deviations were due to two laboratories not performing the confirmatory testing (laboratory #44 and #57). Additional 27 deviations were caused by the strain S-7.6 with an unusual phenotype, two deviating results were caused by S-7.4, a non-ampC-strain registed as ampC-producer, five cases represent participants who have detected and confirmed the test strains as expected but incorrectly submitted 'confirmed' as a result in the database. The remaining seven deviations were related to handling of the strains or other procedures in the laboratory and caused an incorrect positive or an incorrect negative answer.

In three cases, resistance to cephalosporins was registered for a non-ESBL-producing strain

Table 3: Overview of ESBL-producing *Salmonella* test strains and proportion of laboratories that obtained the expected result; number and percentages of laboratories which correctly detected and confirmed the ESBL-producing *Salmonella* strains. Fields shaded in grey with numbers in *italics* indicate an unexpected result.

	Strain S-7.1	Strain S-7.2	Strain S-7.3	Strain S-7.6
ESBL-genes harboured in the test strain	***bla*$_{CTX-M-15}$** ***bla*$_{OXA-30}$** ***bla*$_{TEM-1}$**	***bla*$_{CTX-M-15}$** ***bla*$_{OXA-10}$** ***bla*$_{TEM-1}$**	***bla*$_{CTX-M-9}$** ***bla*$_{TEM-1}$**	***bla*$_{ACC-1}$**
ESBL-producing or *ampC*-producing strain	ESBL	ESBL	ESBL	ampC
Confirmed ESBL-producer	30/35 (86%)	32/35 (91%)	31/35 (89%)	*5/35 (14%)*
***ampC* confirmed**	*4/35 (11%)*	*2/35 (6%)*	-	11/35 (31%)
Confirmed MBL	-	-	-	*1/35 (3%)*

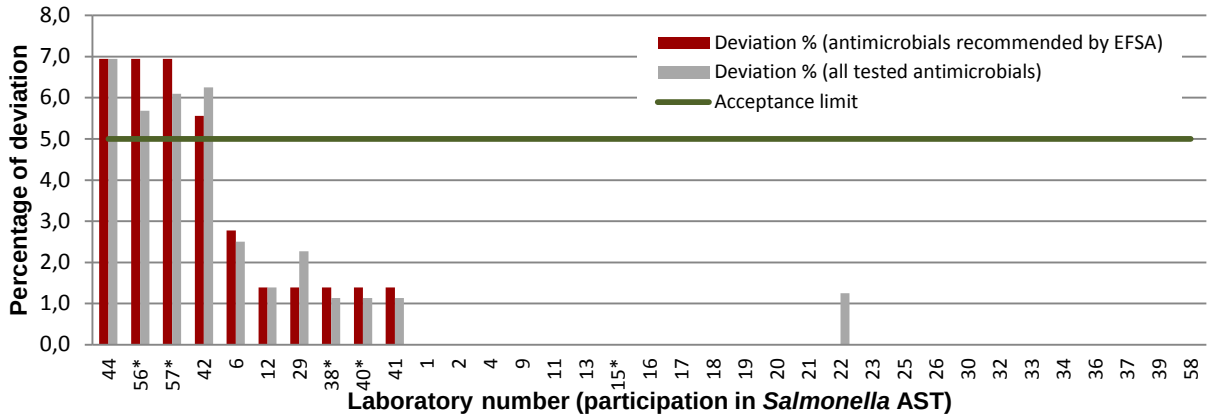

Figure 5: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory performed AST using disk diffusion

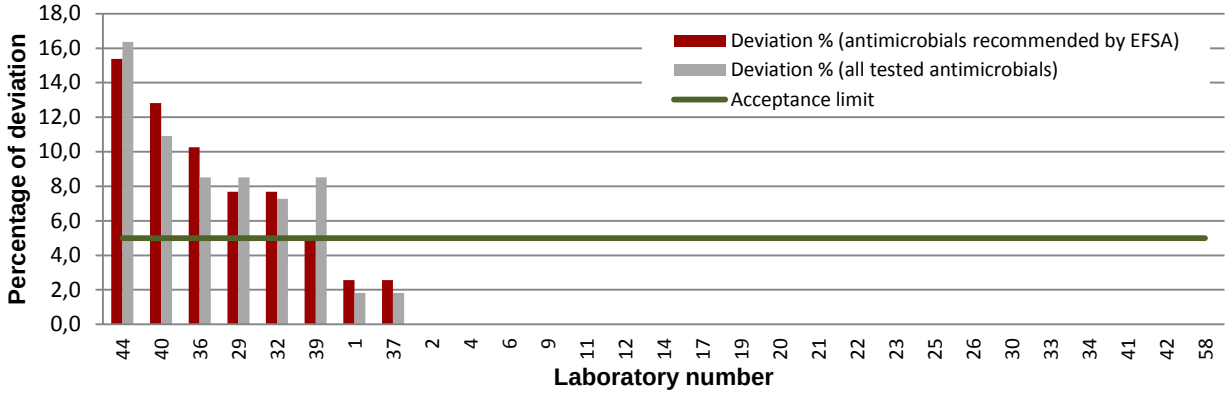

Figure 6: Individual participants' deviations in percent of their total number of *Campylobacter* AST's.

(S-7.4), in one of these cases confirmatory tests were not performed and the strain was not confirmed as an ESBL-producer, in the two other cases the laboratories (#15 and #18) found S-7.4 resistant to cefoxitin, and consequently registered this strain as ampC-producing

3.2.2 *Campylobacter* trial

For the *Campylobacter* strains, 97.9% of AST's were correctly tested. Table 1 presents that the variation in the obtained correct results ranged

from 95.9-99.0% and Table 2 illustrates that the percentage of correct AST per antimicrobial was above 96.5% for the *Campylobacter* test strains with nalidixic acid and streptomycin exhibiting the lowest level.

3.3 Deviations by laboratory

Figure 5 and 6 illustrate the percentage of deviations for each participating laboratory. The laboratories are ranked according to their performance determined by the percentage of deviating results in tests with antimicrobials recommended by EFSA. These results will be the focus of the evaluation in the following sections. Obtained results including all antimicrobials mentioned in the protocol are additionally indicated.

3.3.1 *Salmonella* trial

Thirty-one of the laboratories obtained a result within the acceptance limit at 5% deviations for the *Salmonella* strains. The maximum percentage of deviations was 6.9%. The performance of four (11%) laboratories resulted in a deviation level above the level of performance expected by the EURL-AR (#42, #44, #56, and #57), however, none of the laboratories are regarded as outliers. As illustrated in Figure 5, deviation levels including all antimicrobials mentioned in the protocol generally do not vary much from the deviation levels regarding EFSA-antimicrobials, only.

3.3.2 *Campylobacter* trial

In the *Campylobacter* trial most laboratories performed very well. Applying the 5% acceptance threshold, 23 of 29 participating laboratories performed acceptably, with 21 laboratories having no deviations (Figure 6). Six laboratories present a deviation level above the 5% acceptance level (#29, #32, #36, #39, #40 and #44) and of these, the three with deviation levels at 10.3%, 12.8%, and 15.4% were regarded as outliers (#36, #40, and #44).

Deviation levels including results obtained for all antimicrobials mentioned in the protocol generally do not vary much from the deviation levels including results obtained for antimicrobials recommended by EFSA, only.

Table 4 Obtained values for AST of *E. coli* ATCC 25922 by MIC determination. AMP; ampicillin, CTX; cefotaxime, FOX; cefoxitin, CAZ; ceftazidime, XNL; ceftiofur, CHL; chloramphenicol, CIP; ciprofloxacin, GEN; gentamicin, IMI; imipenem, NAL; nalidixic acid, SMX; sulphonamides, TET; tetracycline, TMP; trimethoprim.

MIC determination *E. coli* ATCC 25922			
Anti-microbial	Proportion outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
AMP	0/29 (0%)	-	-
CTX	0/29 (0%)	-	-
FOX	0/6 (0%)	-	-
CAZ	0/25 (0%)	-	-
XNL	0/5 (0%)	-	-
CHL	0/29 (0%)	-	-
CIP	4/29 (14%)	-	1 step
GEN	1/29 (3%)	-	1 step
IMI	0/5 (0%)	-	-
NAL	0/29 (0%)	-	-
SMX	1/21 (5%)	2 steps	-
TET	0/29 (0%)	-	-
TMP	1/28 (4%)	-	4 steps

Table 5 Obtained values for AST of *C. jejuni* ATCC 33560 by MIC determination. CHL; chloramphenicol, CIP; ciprofloxacin, ERY; erythromycin, GEN; gentamicin, NAL; nalidixic acid, TET; tetracycline.

MIC determination *C. jejuni* ATCC 33560			
Anti-microbial	Proportion outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
CHL	0/20 (0%)	-	-
CIP	1/28 (4%)	-	4 steps
ERY	1/28 (4%)	1 step	-
GEN	1/27 (4%)	1 step	-
NAL	0/26 (0%)	-	-
TET	0/26 (0%)	-	-

3.4 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. 7). Values from the participants' testing of the QC strains are listed in Appendix 6a and 6b, and in Table 4. For the *Salmonella* trial, all laboratories but one (#44) performed QC testing of the reference strain. For the *Campylobacter* trial, all 29 participating laboratories uploaded data from QC-testing on the reference strain.

Appendix 6a indicates that of laboratories performing disk diffusion to test the *E. coli* reference strain (ATCC 25922), all but one (#57 for ceftiofur) of the obtained results were within the QC-range.

The use of MIC determination for AST of the reference strain *E. coli* ATCC 25922 resulted in submission of data from 29 laboratories, six of which produced one value each outside the QC-limit as illustrated in Table 4.

All 29 participating laboratories performed MIC determination for the *C. jejuni* reference strain ATCC 33560. Table 5 presents the proportion of the laboratories with results for the QC strain below or above the QC interval. Three deviations were seen, divided between two laboratories (#21 and #29).

3.5 Genotypic characterisation

For the optional genotypic characterisation of the ESBL-producing *Salmonella* test strains, 11 laboratories participated. In Appendix 10, information is collected on detected genes, genes which were tested but not detected, primers used, and references for the method used. One laboratory performed whole genome sequencing of the ESBL-producing *Salmonella*, two laboratories performed microarray, and eight laboratories performed various types of conventional PCR to identify the relevant genes.

Table 6 indicates that the results on gene level are very good, whereas for the variants some laboratories have submitted results not corresponding to the expected. Also, for each of the four ESBL-producing test strains, additional genes/variants not correlating with the expected have been suggested. One of the additional genes (S-7.6; TEM) was suggested by a laboratory performing microarray whereas the remaining addition genes/variants were identified by laboratories performing conventional PCR.

Table 6: Results from the participation of eleven laboratories in the optional genotypic characterisation component of the EQAS

Test strain	Expected gene	Proportion of correct results (gene level)	Proportion of correct results (variant level)	Additional genes/variants identified
S-7.1	CTXM-15	9/9 (100%)	6/7 (86%)	CTXM-1 OXA-31 SHV
	OXA-30	6/6 (100%)	2/3 (67%)	
	TEM-1	7/7 (100%)	5/5 (100%)	
S-7.2	CTXM-15	9/9 (100%)	6/7 (86%)	CTXM-1 OXA-30 OXA-31
	OXA-10	6/6 (100%)	1/3 (33%)	
	TEM-1	7/7 (100%)	5/5 (100%)	
S-7.3	CTXM-9	9/9 (100%)	6/7 (86%)	CTXM-4
	TEM-1	6/6 (100%)	4/4 (100%)	
S-7.6	ACC-1	9/9 (100%)	4/4 (100%)	TEM

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct antimicrobial susceptibility test results of *Salmonella* was 99.0%. The majority (n=31) of participants obtained satisfactory results according to the level of acceptance (<5% deviation). A significant difference ($p<0.01$) was obtained when comparing results obtained by the use of disk diffusion and a MIC method.

As indicated in Figure 3, the overall quality of the results in the 2012-EQAS would appear to be at the same level or increasing compared to the performance in the former iterations.

Salmonella test strain S-7.3 had the lowest level of compliance between submitted and expected results (97.4%) and the analysis of possible causes for deviations indicate that they are mainly caused by a mix-up of strains in laboratory #42.

The testing of ciprofloxacin towards strains exhibiting reduced susceptibility to this antimicrobial and the correct interpretation of these results when performing disk diffusion caused problems for one laboratory (#57). This laboratory has subsequently been encouraged to apply the recommendations published by Cavaco and Aarestrup (2009). These guidelines describe the use of 1µg or 5µg ciprofloxacin disks together with a lower cut off value for detection of plasmid-mediated resistance phenotypes when performing DD for AST and would detect the resistance phenotype in the *Salmonella* test strain S-7.8 (*qnrS1*) which had an increased ciprofloxacin-MIC but did not exhibit nalidixic acid resistance. The guidelines appear to have been applied by the other four participants of the EURL-AR *Salmonella* EQAS performing DD for AST.

As indicated by Figure 5, deviation levels higher than 5% were exhibited by four laboratories (#42, #44, #56, and #57). In two cases (#42 and #44), these deviation levels appear to be caused by a technical error causing mix-up of strains in the laboratory, whereas one laboratory (#56) appears to have an issue with reading the inhibition zone of sulphonamides (a bacteriostatic antimicrobial for which the inhibition zone must be read at 80% inhibition) and one laboratory (#57) performs AST by DD which requires the introduction of the guidelines recommended in the protocol to detect low-level resistance phenotypes. None of these laboratories were defined as outliers.

For the *E. coli* reference strain, the obtained results were in general in agreement with the CLSI recommendations. The number of laboratories performing AST on *Salmonella* by the use of disk diffusion was five. Four of these uploaded data for the testing of the reference strain with a total of 100% within range. For the laboratories performing AST on *Salmonella* by an MIC-method, two laboratories each submitted a result one MIC-step above the QC-range whereas one laboratory (#4) for sulphonamides and trimethoprim submitted a value two steps below and four steps above the QC-range, respectively. This laboratory should perform trouble shooting to follow up on the reason for the unexpected result. Laboratory #44 did not submit QC-results of the *E. coli* reference strain and is recommended to do so for future EURL-AR EQAS.

Laboratories #4, #26, #38, and #40 which had a deviation level above the acceptance limit in EQAS 2011 with values of 5.1%, 6.4%, 5.1%, and 7.7%, respectively, have increased their performance considerably to a deviation level in the 2012-iteration at 0%, 0%, 1.5%, and 1.4%, respectively. Laboratory #54 (deviation level at 9.3% in 2011) did not participate in the 2012-iteration.

ESBL-producing *Salmonella* test strains

ESBL-producing microorganisms are an

emerging problem worldwide, and it should be of a high priority for the NRLs to be able to detect them. It was therefore decided at the EURL-AR Workshop in June 2008, that the detection of ESBL-producing test strains should be included as a mandatory test in this EQAS.

Four of the *Salmonella* test strains were ESBL-producers (S-7.1, S-7.2, S-7.3 and S-7.6), and the participants were asked to interpret their results according to the description in the protocol. Of the 35 laboratories which tested *Salmonella*, two (#44 and #57) did not submit results for confirmatory testing of ESBL-production and therefore they obtained an evaluation as incorrect.

Two of the included ESBL-producing strains exhibited an unusual phenotype. One was an extremely drug resistant strain (S-7.1) that among resistance to many other antimicrobials also exhibited phenotypic resistance to cefoxitin with an MIC at 16. The strain harboured OXA-30 together with TEM-1 and CTX-M-15. For this particular strain, synergy was seen between both CTX:CTX/Cl and CAZ:CAZ/Cl and a high MIC for cefoxitin. In this case, the high MIC for cefoxitin does not, however, indicate an ampC-genotype. This is based on the fact that a strain with an ampC-genotype together with an ESBL-genotype, would not show phenotypically as synergy between CTX:CTX/Cl or CAZ:CAZ/Cl. The other strain with an unusual pheno-/genotype was strain S-7.6. The strain exhibited resistance to cefotaxime and ceftazidime (and ceftiofur), but when attempting to confirm the ESBL-production by testing for synergy with clavulanic acid, ESBL-production could not be confirmed, nor did the strain show resistance to cefoxitin. Consequently, the results from the phenotypic testing could not confirm ampC- or ESBL-production. In a genotypic analysis, however, the strain was found to harbour bla_{ACC-1}. The organizers concluded that the fact that this strain is phenotypically resistant to cephalosporins should induce the participant to suspect that the strain harboured one type or another of ESBL- or ampC-producing gene and should then demand further investigation, including molecular testing.

If leaving out of account the results from the strain with the particularly unusual phenotype (S-7.6) and the two laboratories which did not perform confirmatory testing, the results submitted from the 33 laboratories performing confirmatory testing were 94% in accordance with the expected. The seven results not in concordance with the expected and relevant for discussion were caused by various problems with confirmatory testing causing an incorrect positive or an incorrect negative answer. These deviations were submitted by five different laboratories and thus do not indicate methodical issues at particular laboratories.

In general, it is recommended that more than one cephalosporin is used for the detection of an ESBL-producing *Salmonella* when initially screening the isolate. The cephalosporins cefotaxime, cefpodoxime, ceftiofur, ceftriaxone, and ceftazidime were all found useful in detecting isolates with ESBL or plasmidic ampC by Aarestrup *et al.* (2010), however, cefotaxime, cefpodoxime, and ceftriaxone were superior to ceftiofur and ceftazidime.

Laboratory #44 and #57 have been contacted directly due to the absent confirmatory tests.

4.2 *Campylobacter* trial

The overall percentage of correct antimicrobial susceptibility test results of *Campylobacter* was 97.9%. The performance varied from no deviations to 15.4% deviations, with 23 laboratories performing satisfactorily according to the established acceptance ranges. Six laboratories (#29, #32, #36, #39, #40, and #44) obtained deviation levels above 5%, three of these were defined as outliers (#36, #40, and #44) with deviation levels at 10.3%, 12.8%, and 15.4%. For one laboratory (#29), the values obtained for the QC-strain indicate that trouble shooting might be necessary to identify if any

methodical issues is the reason for the obtained deviations. For two laboratories (#36 and #44) it appears that the interpretation of the obtained MIC-values has been done with reference to other interpretative criteria than those in the protocol. For the remaining number of laboratories, no obvious type of mistake appears, however it is notable that for each of laboratories #32, #39, and #40, three, three and four of the deviations are on AST's of one of the tested strains; C-7.5, C-7.8 and C-7.4, respectively, indicating an issue with that particular strain.

All participating laboratories uploaded data from tests performed on the *C. jejuni* reference strain and the proportion of results within the QC intervals was 98.1%. The three values outside the QC intervals were obtained by two laboratories (#21 and 29) of which one had no deviation in the test strains and one had a deviation level at 7.7% (laboratory #29).

Laboratories #4, #19, and #39 which were regarded as outliers with deviation levels in EQAS 2011 at 25%, 17.5%, and 10% in 2011, respectively, all increased their performance in the 2012-iteration and obtained deviation levels at 0%, 0% and 5.1%, respectively.

4.3 Genotypic characterisation

The focus on genotypic characterization of microorganisms is increasing in the EU and worldwide. In EU, method proposals are underway for the detection and confirmation of ESBL-producing *Enterobacteriaceae* and in this context the inclusion of sequencing methods for the genotypic characterisation of relevant genes are currently being considered. The optional genotypic characterisation offered as a supplementary part of this EQAS should therefore be seen as an important possibility for the NRL-AR's to introduce this method in the laboratory and thereby be at the forefront when the method proposals are adopted. This year, 11 laboratories participated in this optional EQAS item and all obtained satisfying results.

5. Conclusions

The goal of the EURL-AR EQAS is to have all participating NRLs performing antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* with a deviation level below 5%. This seems within reach for *Salmonella* as well as for *Campylobacter*.

The performance of the NRL's appear to be at the same level for *Salmonella* AST's in this EQAS (99.0%) when compared to the results from the EQAS 2009, 2010 and 2011 (98.4%, 97.8% and 98.1). Regarding *Campylobacter* AST's, the level of deviation also appears to be quite stable with a level at 2.1% in 2012 compared to 2.2%, 2.0% and 1.9% in 2009, 2010 and 2011. Three laboratories were regarded as outliers for the *Campylobacter* AST (#36, #40, and #44) with deviation levels at 10.3%, 12.8%, and 15.4%

The current EQAS iteration included both the phenotypic and the genotypic testing of ESBL-producing *Enterobacteriaceae*. These bacteria are of great concern in the EU and worldwide due to their emergence in various reservoirs and the detection of these resistances should therefore be prioritized by the national reference laboratories performing AST of *Enterobacteriaceae*.

6. References

Aarestrup FM, Hasman H, Veldman K, Mevius D. (2010). Evaluation of eight different cephalosporins for detection of cephalosporin resistance in *Salmonella enterica* and *Escherichia coli*. Microbiol drug res, 16:253-261

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

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

DTU Food
National Food Institute

PRENOTIFICATION:

M00-06-001/01.12.2011

EQAS 2012

FOR *SALMONELLA, CAMPYLOBACTER* AND OPTIONAL GENOTYPIC CHARACTERISATION

The EURL-AR announces the launch of another EQAS, thus providing the opportunity for proficiency testing which is considered an essential tool for the generation of reliable laboratory results of consistently good quality.

This EQAS consists of antimicrobial susceptibility testing of eight *Salmonella* isolates and eight *Campylobacter* isolates. For the optional genotypic characterisation, the ESBL-genes in the relevant strains should be detected. Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214) will be distributed to new participants.

This EQAS is specifically for NRL's on antimicrobial resistance. Therefore, laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. Participation is free of charge for all designated NRL-AR's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is "UN3373, Biological Substance Category B": Eight *Salmonella* strains, eight *Campylobacter* and for new participants also the QC strains mentioned above. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g. specific text that should be written on the proforma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped in October 2012. The protocol for this proficiency test will be available for download from the website (www.eurl-ar.eu).

Submission of results: Results must be submitted to the National Food Institute **no later than December 14th 2012** via the password-protected website.
Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.
EQAS report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

Next EQAS: The next EURL-AR EQAS that we will have is on antimicrobial susceptibility testing of *E. coli*, staphylococci and enterococci which will be carried out in June 2013.

Please contact me if you have comments or questions regarding the EQAS.

Sincerely,

Susanne Karlsmose (suska@food.dtu.dk)
EQAS-Coordinator

Participant list

Salmonella	*Campylobacter*	Genotypic characterisation	Institute	Country
X	X	-	Austrian Agency for Health and Food Safety	Austria
X	X	X	Institute of Public Health	Belgium
X	X	-	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	-	-	Croatian Veterinary Institut	Croatia
X	X	-	Veterinary Services	Cyprus
X	X	X	State Veterinary Institute Praha	Czech Republic
X	X	X	National Food Institute	Denmark
X	X	-	Estonian Veterinary and Food Laboratory	Estonia
X	X	-	Finnish Food Safety Authority EVIRA	Finland
X	-	-	ANSES Maisons Alfort	France
-	X	-	ANSES Ploufragan	France
X	X	-	ANSES Lyon	France
X	-	-	ANSES Fougères	France
X	X	X	Federal Institute for Risk Assessment	Germany
X	-	-	Veterinary Laboratory of Chalkis	Greece
X	X	-	Central Agricultural Office Veterinary Diagnostic Directorate	Hungary
X	X	-	Central Veterinary Research Laboratory	Ireland
X	X	X	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	-	Institute of Food Safety, Animal Health and Enviroment "BIOR"	Latvia
X	X	-	National Food and Veterinary Risk Assessment Institute	Lithuania
X	X	-	Public Health Laboratory	Malta
X	X	-	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	-	Veterinærinstituttet	Norway
X	X	-	National Veterinary Research Institute	Poland
X	X	-	Laboratorio National de Investigacáo Veterinaria	Portugal
X	X	-	Institute for Hygiene and Veterinary Public Health	Romania
X	X	X	Institute for Diagnosis and Animal Health	Romania
X	-	-	Institute of Veterinary Medicine of Serbia	Serbia
X	X	-	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	-	National Veterinary Institute	Slovenia
-	-	-	Laboratorio Central de Sanidad, Animal de Santa Fe (only Staph)	Spain
X	X	X	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	-	VISAVET Health Surveillance Center, Complutense University	Spain
X	-	-	Centro nacional de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricion	Spain
X	X	X	National Veterinary Institute, SVA	Sweden
X	X	X	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	-	-	National Food Reference Laboratory	Turkey
X	X	-	Centre for Infections Health Protection Agency	United Kingdom
X	X	X	The Veterinary Laboratory Agency	United Kingdom

Designated NRL-AR by the compentent authority of the member state
Non-NRL-AR enroled by the EURL
Not a Member State of the EU

Salmonella test strains and reference values (MIC-value and interpretation)

	Ampicillin AMP		Cefotaxime CTX		ESBL CTX:CTX/Cl	Ceftazidime CAZ		ESBL CAZ:CAZ/Cl	Cefoxitin		Ceftiofur XNL		Chloramphenicol CHL		Ciprofloxacin CIP		Gentamicin GEN		Imipenem IMI		Nalidixic acid NAL		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP	
EURL S-7.1	> 32	RESIST	> 64	RESIST	ratio >= 8	= 64	RESIST	ratio >= 8	16	RESIST	> 8	RESIST	> 64	RESIST	> 4	RESIST	> 16	RESIST	<= 0.5	SUSC	> 64	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-7.2	> 32	RESIST	> 64	RESIST	ratio >= 8	= 128	RESIST	ratio >= 8	8	SUSC	> 8	RESIST	> 64	RESIST	> 4	RESIST	> 16	RESIST	<= 0.5	SUSC	> 64	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-7.3	> 32	RESIST	= 16	RESIST	ratio >= 8 (E-test: phantom)	= 1	SUSC	ratio < 8	4	SUSC	> 8	RESIST	= 4	SUSC	= 0.5	RESIST	<= 0.5	SUSC	<= 0.5	SUSC	> 64	RESIST	= 32	SUSC	> 32	RESIST	<= 1	SUSC
EURL S-7.4	> 32	RESIST	= 0.25	SUSC		= 0.5	SUSC				= 1	SUSC	= 16	SUSC	= 0.5	RESIST	<= 0.5	SUSC			> 64	RESIST	> 1024	RESIST	= 4	SUSC	> 32	RESIST
EURL S-7.5	<= 1	SUSC	<= 0.12	SUSC		= 0.25	SUSC				<= 0.5	SUSC	= 8	SUSC	= 0.03	SUSC	= 1	SUSC			= 4	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-7.6	= 32	RESIST	= 16	RESIST	ratio < 8	= 32	RESIST	ratio < 8	4	SUSC	> 8	RESIST	= 8	SUSC	<= 0.015	SUSC	= 1	SUSC	<= 0.5	SUSC	= 4	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-7.7	= 2	SUSC	= 0.25	SUSC		= 0.25	SUSC				= 1	SUSC	= 4	SUSC	= 0.25	RESIST	= 0.5	SUSC			> 64	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-7.8	<= 1	SUSC	<= 0.12	SUSC		= 0.5	SUSC				<= 0.5	SUSC	> 64	RESIST	= 0.5	RESIST	= 0.5	SUSC			= 4	SUSC	= 32	SUSC	<= 2	SUSC	<= 1	SUSC

Resistant

Campylobacter test strains and reference values (MIC-value and interpretation)

Species	Code	Chloramphenicol CHL		Ciprofloxacin CIP		Erythromycin ERY		Gentamicin GEN		Nalidixic acid NAL		Streptomycin STR		Tetracycline TET	
C. coli	EURL C-7.1	= 4	SUSC	= 0.25	SUSC	> 64	RESIST	= 0.5	SUSC	= 8	SUSC	= 2	SUSC	= 2	SUSC
C. coli	EURL C-7.2	= 4	SUSC	= 0.25	SUSC	> 64	RESIST	=1	SUSC	= 8	SUSC	= 4	SUSC	= 64	RESIST
C. coli	EURL C-7.3	= 8	SUSC	= 0.06	SUSC	= 1	SUSC	= 0.5	SUSC	= 4	SUSC	<= 1	SUSC	= 0.5	SUSC
C. coli	EURL C-7.4	= 8	SUSC	= 32	RESIST	= 8	SUSC	= 0.5	SUSC	> 64	RESIST	> 16	RESIST	> 64	RESIST
C. jejuni	EURL C-7.5	= 4	SUSC	= 8	RESIST	= 1	SUSC	= 0.5	SUSC	> 64	RESIST	> 16	RESIST	= 0.12	SUSC
C. jejuni	EURL C-7.6	= 4	SUSC	= 16	RESIST	> 64	RESIST	> 32	RESIST	> 64	RESIST	> 16	RESIST	= 64	RESIST
C. jejuni	EURL C-7.7	<= 2	SUSC	= 0.12	SUSC	= 1	SUSC	= 0.25	SUSC	= 4	SUSC	<= 1	SUSC	= 32	RESIST
C. jejuni	EURL C-7.8	= 4	SUSC	> 4	RESIST	= 2	SUSC	= 0.25	SUSC	> 64	RESIST	<= 1	SUSC	= 32	RESIST

Resistant

DTU Food
National Food Institute

M00-06-001/01.12.2011

Welcomeletter:
EURL-AR External Quality Assurance System 2012
- *Salmonella*, *Campylobacter* and optional genotypic characterisation

Id: «Lab_no_»
«Name»
«Institute__»
«Country»

Kgs. Lyngby, October 2012

Dear «Name»,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2012. Upon arrival to your laboratory, the strains should be stored dark and at 4°C for stabs, and dark and cool for freeze-dried strains. Charcoal swabs must be subcultured straight away.

On the EURL-AR-website (www.eurl-ar.eu) the following documents relevant for the EURL-AR EQAS are available:

- Protocol for *Salmonella* and *Campylobacter* including test forms
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Control Strains

We ask you to examine the eight *Salmonella* and the eight *Campylobacter* strains that we send to you by performing antimicrobial susceptibility testing. The ESBL-producing *Salmonella* strains should be characterised genotypically (optional) according to the description in the protocol. In the protocol you can find detailed description of the procedures to follow. Additionally, you can find a description of the procedure to enter your results into the interactive web database. For accessing the database, you need this username and password.

Your username: «Username»

Your password: «Password»

Please keep this document
Your username and password will not appear in other documents

Results should be returned to us no later than December 14th 2012.

Please acknowledge receipt of this parcel immediately upon arrival (to suska@food.dtu.dk).
Do not hesitate to contact us for further information.

Yours sincerely,

Susanne Karlsmose
EQAS-Coordinator

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2012

DTU Food
National Food Institute

PROTOCOL

For antimicrobial susceptibility testing of *Salmonella, Campylobacter* and optional genotypic characterisation of ESBL-producing strains

1 INTRODUCTION

One of the tasks as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) is to organise and conduct an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *Salmonella* and *Campylobacter*. The *Salmonella* and *Campylobacter* EQAS 2012 includes susceptibility testing of eight *Salmonella* and eight *Campylobacter* strains together with susceptibility testing of the reference strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214). Additionally, optional characterisation of the genes conferring ESBL-production in the *Salmonella* test strains is offered.

For new participants of the EQAS who have not already received the mentioned reference strains, these are included in the parcel. The reference strains will not be included in the years to come. The reference strains are original certified cultures and are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the manual ‘Subculture and Maintenance of QC Strains’. Please use them for future internal quality control for susceptibility testing in your laboratory.

For this EQAS, members of the Food- and Waterborne Diseases and Zoonoses Programme (FWD) based at ECDC are also participating, however, for these participants the EQAS has been slightly adjusted. Description of this can be found in this protocol, i.e. that for antimicrobial susceptibility testing (AST) of *Campylobacter*, results obtained by in-house methods like disk diffusion or E-test are also accepted.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of susceptibility testing of pathogens originating from food and animal sources, especially *Salmonella* and *Campylobacter*. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by different laboratories on *Salmonella* and *Campylobacter* and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2012

3.1 Shipping, receipt and storage of strains

In October 2012, the EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing eight *Salmonella* and eight *Campylobacter* strains. QC reference strains will be included for participants who have not previously received these. Some of the *Salmonella* test strain are ESBL-producing, and are included as test strains in the optional part of the EQAS consisting of characterisation of genes conferring ESBL-production.

The reference strains are shipped lyophilised, the *Campylobacter* test strains are shipped as a charcoal swabs and the *Salmonella* test strains are stab cultures. On arrival, the stab cultures and the charcoal swabs must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please see the document 'Instructions for opening and reviving lyophilised cultures' on the EURL-AR-website (see www.eurl-ar.eu).

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the method used in the laboratory when performing monitoring for EFSA. For

MIC the cut off values listed in Tables 1 and 2 should be used. The epidemiological cut-off values allow two categories of characterisation – resistant or sensitive.

Participants using disk diffusion are recommended to interpret the results according to their individual breakpoints, categorising them into the terms resistant and susceptible. A categorization as intermediary is not accepted; therefore **intermediary results should be interpreted as susceptible**. Interpretations in concordance with the expected value will be categorised as 'correct', whereas interpretations that deviate from the expected interpretation will be categorised as 'incorrect'.

The cut off values used in the interpretation of the MIC results are developed by EUCAST (www.eucast.org).

With regard to MIC range and/or disc content we ask you to fill in these pieces of information in the database. Also, if you ***do not use*** the cut-off values listed in the protocol for interpretation of the susceptibility results, please fill in or update the breakpoints used, in the database.

3.3.1 *Salmonella*

Testing of gentamicin may be of value for monitoring. Please, do not take into account in this study, that the CLSI guidelines state that for aminoglycosides *Salmonella* should not be reported as susceptible.

Antimicrobials for *Salmonella*	MIC (µg/mL) **R is >**
Ampicillin (AMP)	8
Cefotaxime (CTX)	0.5
Ceftazidime (CAZ)**	2
Ceftiofur (XNL)**	2
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.06
Gentamicin (GEN)	2
Nalidixic acid (NAL)	16
Sulphonamides (SMX)*	256
Tetracycline (TET)	8
Trimethoprim (TMP)	2

Table 1: Interpretative guidelines for *Salmonella*
* CLSI
** Not part of the EFSA monitoring programme (used for confirmatory tests for ESBL production)

Also, when following EUCAST epidemiological cut-off values, *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin. When using disc diffusion and CLSI clinical breakpoints this connection between nalidixic acid and ciprofloxacin is not taken into

account. Thus, the result in this situation with regard to ciprofloxacin will deviate from the expected result in this EQAS.

Important notes*: beta-lactam resistance:*

Confirmatory tests for ESBL production is **mandatory** on all strains resistant to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftiofur (XNL).

Confirmatory test for ESBL production requires use of both cefotaxime (CTX) and ceftazidime (CAZ) alone and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/CL or CAZ : CAZ/CL ratio ≥ 8) or ii) a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Confirmatory test for Metallo-beta-lactamase (MBL) production requires use of imipenem (IMI) and IMI/EDTA. Synergy is defined as a ≥ 3 twofold concentration decrease in the MIC for the combination IMI/EDTA vs. MIC for IMI alone (E-test 3 dilution steps difference, MIC IMI : IMI/EDTA ratio ≥ 8; CLSI M100, Table 2A; Enterobacteriaceae). The presence of synergy indicates MBL production.

Detection of AmpC-type beta-lactamases can be performed by testing the bacterium for susceptibility to cefoxitin (FOX). Resistance to FOX could indicate the presence of an AmpC-type beta-lactamase, that should be verified by PCR and sequencing.

The EURL-AR aims to harmonise with EUCAST expert rules. Accordingly, MIC values and relative interpretation of cefotaxime, ceftazidime and/or ceftiofur used for detection of beta-lactamase-producing strains in this EQAS should be reported as found.

3.3.2 *Campylobacter*

Please find information on the test forms showing which test strains are *C. jejuni* and *C. coli*, respectively.

For AST of *Campylobacter* only MIC methods are recommendable, i.e. broth or agar dilution methods. The EURL-AR does not recommend the use of either disk diffusion or E-test for AST of *Campylobacter*. Laboratories in the EURL-AR network should test the sub-cultured *Campylobacter* by the use of microbroth or agar dilution using incubation at 36-37ºC for 48 hours or 42ºC for 24 hours.

Antimicrobials for *Campylobacter*	MIC (µg/mL) **R is >** *C. jejuni*	MIC (µg/mL) **R is >** *C. coli*
Chloramphenicol*	16	16
Ciprofloxacin	0.5	0.5
Erythromycin	4	8
Gentamicin	2	2
Nalicixic acid*	16	16
Streptomycin	4	4
Tetracycline	1	2

Table 2: Interpretative guidelines for *Campylobacter*
*Not part of the EFSA monitoring programme

For the laboratories of the FWD-network, results of AST of *Campylobacter* may be obtained by in-house methods like disk diffusion or E-test. In this case, in-house interpretative criteria must be applied.

3.4 Optional genotypic characterisation

For the optional genotypic characterisation of the ESBL-producing *Salmonella* test strains, the requested results are the genes conferring ESBL-production harboured in the test strains. The genes included in the test are the following: ACC, ACT, CMY, CTX, DHA, FOX, GES, IMP, KPC, MOX, NDM, OXA, PER, SHV, TEM, VEB, and VIM. The database lists the relevant variants of the genes.

When uploading the results in the database, the identified genes will be evaluated against the expected results. The results will be evaluated on the detected gene (ACC-, ACT-, CMY-, etc.) as well as the variant identified.

The method used for the genotypic characterisation should be your laboratory's routine method. The expected results listed in the database are those obtained by the EURL-AR.

EU Reference Laboratory for Antimicrobial Resistance
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4 REPORTING OF RESULTS AND EVALUATION

Test forms are available for recording your results before you enter them into the interactive web database. We kindly ask you to report in the database the tested MIC range and/or antimicrobial disk content. **If you did not use the cut-off values recommended in the protocol for interpretation of AST results, please report the breakpoints used.**

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than 14th December 2012.** After the deadline, the database will be closed and you will be able to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

If you do not have access to the Internet, or if you experience difficulties in entering your results, try a few days later or, alternatively, return the completed test forms by e-mail, fax or mail to the National Food Institute, Denmark.

All results will be summarised in reports available to all participants. The data will be collected in an overall summary report in which anonymous laboratory results will be analyzed. This summary report will focus on comparing the results from the EURL-AR network, and public health laboratories (FWD-laboratories) to assess the level of harmonization need.

In addition, separate reports for the EURL-AR network (by DTU) and for public health laboratories (by ECDC) will be prepared.

The data in the report will be presented with laboratory codes. A laboratory code is only known to the individual laboratory, while the complete list of laboratories and their respective codes is confidential and only known to the EURL-AR (all participants), the ECDC (FWD-laboratories) and the EU Commission (NRL-ARs). All conclusions and all three reports will be publicly available.

If you have any questions, please do not hesitate to contact the EQAS Coordinator:

Susanne Karlsmose
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby
Denmark
Tel: +45 3588 6601
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E-mail: suska@food.dtu.dk

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DTU Food
National Food Institute

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read this passage before entering the web page. Before you go ahead, you need your test form by your side together with your breakpoint values.

You are able to browse back and forth by using the forward and back keys or click on the EURL logo.

You enter the EURL-AR EQAS web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by the National Food Institute. If you have problems with the login please contact us.

Click on either "*Salmonella* test results" or "*Campylobacter* test results" depending on your results. The below description is aimed at *Salmonella* entry but is exactly the same as for *Campylobacter* entry.

Click on "Start of Data Entry - Methods and Breakpoints for Salm."

In the next page you navigate to fields with the Tab-key and mouse.

Fill in what kind of method you have used for the susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays etc.

Fill in the relevant information, either disk content or MIC range. If you use disk diffusion, please upload the breakpoints used.

You will find one more box to fill in on this page when testing *Campylobacter*: Fill in the actual incubation condition used for susceptibility testing of *Campylobacter* – 36°C/48h or 42°C/24h.

Click on "save and go to next page"

In the data entry pages for each *Salmonella* and *Campylobacter* strain, you enter the obtained value and the interpretation as R or S.

For *Salmonella*, you also type in results for the ESBL tests.

If you have not used an antimicrobial, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains please enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc. If you do not use CLSI guidelines for AST on the reference strains, please add a comment on the method used.

Click on "save and go to next page"

This page is a menu, from where you can review the input pages, approve your input and finally see and print the evaluated results:

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Browse through the pages and make corrections if necessary. Remember to save a page if you make any corrections. If you save a page without changes, you will see an error screen, and you just have to click on "back" to get back to the page and "go to next page" to continue.

Please fill in the evaluation form.

Approve your input. Be sure that you have filled in all the results before approval, as YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.

If you have performed the optional genotypic characterisation:

Click on “Gene test” and follow the description in the database for upload of the results of the optional genotypic characterisation. Approve your input. Be sure that you have filled in all the results before approval. The approval blocks your data entry in the interactive database, but allows you to see the evaluated results.

Salmonella and *Campylobacter*, genetic characterisation

TEST FORMS

Name:
Name of laboratory:
Name of institute:
City:
Country:
E-mail:
Fax:

Comments:

TEST FORM

Does your laboratory have an accreditation for *Salmonella* AST? ☐ Yes ☐ No

Does your laboratory have an accreditation for other laboratory methods/tests? ☐ Yes ☐ No

Which method did you use for antimicrobial susceptibility testing of *Salmonella* in this EQAS:

- ☐ Broth Microdilution
- ☐ Agar dilution
- ☐ E-test (strips)
- ☐ Disk diffusion (paper disks)
- ☐ Rosco Neo Sensitabs (tablets)

Brand of microdilution plate, strips or disks:

Method used for detection of ESBL-producing strains, see pictures of the methods on http://www.eurl-ar.eu/201-resources.htm

- ☐ E-test
- ☐ Double disk
- ☐ Combination disk
- ☐ MIC determination (microbroth)
- ☐ Selective media please specify:
- ☐ Other, please specify

Comments or additional information:

TEST FORM

Breakpoints used (zonediameters) and general info regarding disk content and test-range used for MIC:

- Please fill in the disk content or the test-range used for MIC, respectively.
- Please, *only* fill in breakpoints if you did not use a MIC method, that is, *only* if you used other breakpoints/cut-off values than the ones listed in the protocol for interpretation of AST results for *Salmonella*. Otherwise leave breakpoint fields empty.

Antimicrobial	General info The relevant information in the two columns below should be filled in		Zonediameter (mm) Please, *only* fill in breakpoint information if you did not use the cut-off values listed in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Ampicillin, AMP			≤		≥
Cefotaxime, CTX			≤		≥
Ceftazidime, CAZ			≤		≥
Ceftiofur, XNL			≤		≥
Chloramphenicol, CHL			≤		≥
Ciprofloxacin, CIP			≤		≥
Gentamicin, GEN			≤		≥
Nalidixic acid, NAL			≤		≥
Streptomycin, STR			≤		≥
Sulphamethoxazole, SMX			≤		≥
Tetracycline, TET			≤		≥
Trimethoprim, TMP			≤		≥

TEST FORM

Does your laboratory have an accreditation for *Campylobacter* AST? ☐ Yes ☐ No

Does your laboratory have an accreditation for other laboratory methods/tests? ☐ Yes ☐ No

Incubation conditions: ☐ 36-37ºC / 48h ☐ 42ºC / 24h

Method used for antimicrobial susceptibility testing of *Campylobacter* in this EQAS::

- ☐ Microbroth
- ☐ Agardilution
- ☐ In-house (disk diffusion)
- ☐ In-house (E-test)

Brand of broth/agar:

Additional comments:

How many *Campylobacter* isolates does your laboratory annually isolate:

How many *Campylobacter* isolates does your laboratory annually susceptibility test:

If using an in-house method (disk diffusion or E-test),
- Please fill in the disk content or the test-range used for E-test, respectively.
- Please fill in interpretative criteria if you used other breakpoints/cut-off values than the ones listed in the protocol for interpretation of AST results for *Campylobacter*. Otherwise leave breakpoint fields empty.

Antimicrobial	General info The relevant information in the two columns below should be filled in		Zonediameter (mm) Please, *only* fill in breakpoint information if you did not use the cut-off values listed in the protocol		
	Disk content (µg)	Test-range for MIC (µg/mL)	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
Chloramphenicol			≤		≥
Ciprofloxacin			≤		≥
Erythromycin			≤		≥
Gentamicin			≤		≥
Nalidixic Acid			≤		≥
Streptomycin			≤		≥
Tetracycline			≤		≥

TEST FORM

Strain	Antimicrobial	Interpretation		
		☐ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella EURL S-6.X	Ampicillin, AMP			
	Cefotaxime, CTX			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulfonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) should be included for confirmatory tests for ESBL production.

See further description of confirmatory tests above in section '3.3.1 *Salmonella*'.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable	Incr. in zone diam	☐ Incr. ≥ 5 mm (synergy) ☐ Incr.< 5 mm
Cefoxitin, FOX mic value	☐ MIC value > 16 ☐ MIC value ≤ 16	Zone diameter	☐ D ≤ 14 mm ☐ D > 14 mm
Imipenem, IMI mic value	☐ MIC value > 1 ☐ MIC value ≤ 1	☐ Confirmed ESBL ☐ Confirmed AmpC ☐ Confirmed Metallo betalactamase	
IMI/E : IMI mic ratio	☐ MIC ratio ≥ 8 (synergy) ☐ MIC ratio < 8 ☐ Phantom zone (synergy) ☐ Deformation (synergy) ☐ Not determinable		

Comments:

TEST FORM

Susceptibility testing of *E. coli* referencestrain ATCC 25922

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
E. coli ATCC 25922	Ampicillin, AMP	
	Cefotaxime, CTX	
	Cefoxitin, FOX	
	Ceftazidime, CAZ	
	Ceftiofur, XNL	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Gentamicin, GEN	
	Imipenem, IMI	
	Nalidixic acid, NAL	
	Streptomycin, STR	
	Sulfisoxazole, FIS*	
	Tetracycline, TET	
	Trimethoprim, TMP	

*The antimicrobial which is mentioned in the CLSI M100 performance standard as a representative for the sulfonamides as regards acceptable limits for quality control strains (CLSI M100, Table 3)

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter EURL C-6.1 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-6.2 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-6.3 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-6.4 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)	
		36 °C/48 hours	42 °C/24 hours
C. jejuni ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Nalidixic Acid		
	Tetracycline		

For Agar dilution:

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)
C. jejuni ATCC 33560	Ciprofloxacin	
	Doxycycline	
	Erythromycin	
	Gentamicin	
	Meropenem	
	Nalidixic Acid	
	Tetracycline	

TEST FORM – genotypic characterisation

Genotypic characterisation of the test strains

EURL GEN 3.X	PCR-method used
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:

Comments:

INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Manual from Czech Collection of Microorganisms (CCM)
Masaryk University
Tvrdého 14
602 00 BRNO
Czech Republic

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

a. Check the number of the culture on the label inside the ampoule
b. Make a file cut on the ampoule near the middle of the plug
c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
e. Remove the pointed end of the ampoule into disinfectant
f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
g. Incubate the inoculated medium at appropriate conditions for several days
h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

Please note that:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has published a guideline for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test results.

1.2 References

M100-S21, January 2011 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A8, January 2009 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time

1.4 Important Considerations

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC
- CLSI requires that QC be performed either on the same day or weekly (only after 30 day QC validation)
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides

- Periodically perform colony counts to check the inoculum preparation procedure
- Ideally, test values should be in the middle of the acceptable range
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

1.5 Storage of Reference Strains

Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen. (Alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

1.6 Frequency of Testing

Weekly vs. daily testing

Weekly testing is possible if the lab can demonstrate satisfactory performance with daily testing as follows:

- Documentation showing reference strain results from 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more than 3 out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

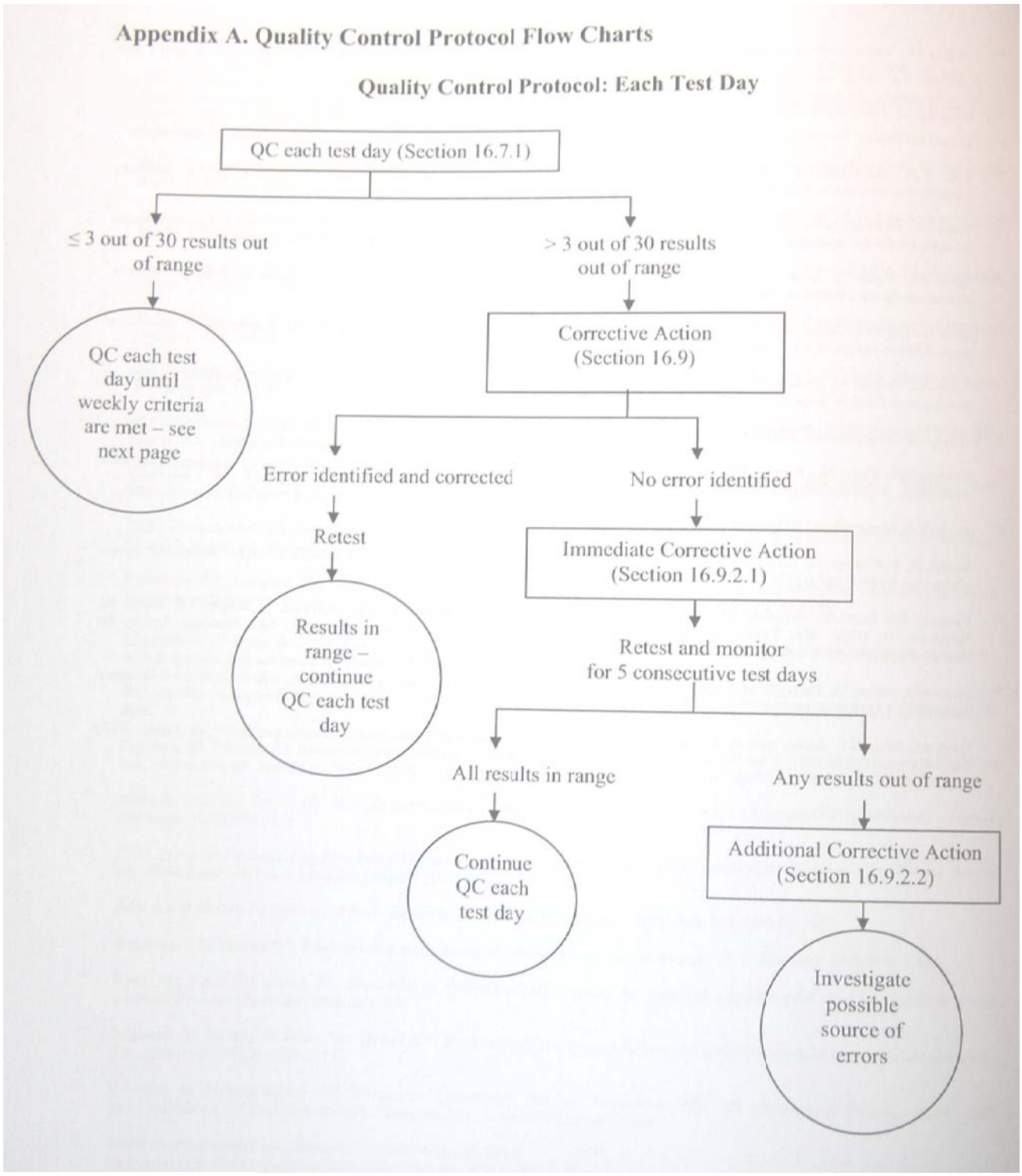
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

The problem is considered resolved only after the reference strain is tested for 5 consecutive days and each drug/organism result is within specification on each day.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

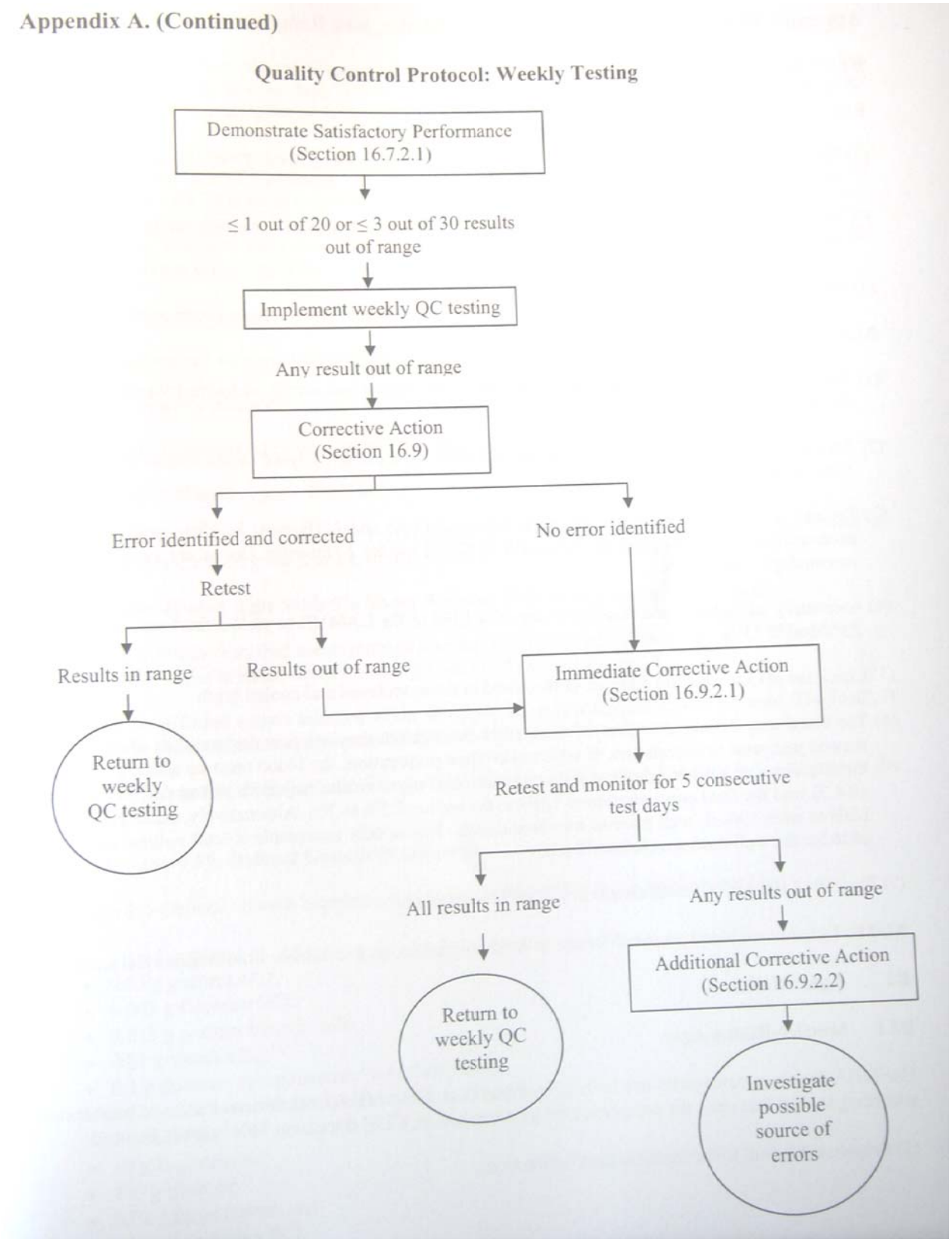
DAILY MIC QC CHART



Reference: CLSI M7-A8, page 44

WEEKLY MIC QC CHART

Appendix A. (Continued)



Reference: CLSI M7-A8, page 45

Disk content and breakpoints used in daily routine (disk diffusion) - *Salmonella*

Antimicrobial	Lab No	Disk content (ug)	R <= (mm)	I = (mm)	S >= (mm)
Ampicillin, AMP	38	10	13	14-16	17
Ampicillin, AMP	40	10	13		
Ampicillin, AMP	57	25	13	14-16	17
Ampicillin, AMP	56	25	13	14-16	17
Cefotaxime, CTX	40	30	14		
Cefotaxime, CTX	57	30	22	23-25	26
Cefotaxime, CTX	56	30	22	23-25	26
Cefotaxime, CTX	15	30	22		26
Cefotaxime, CTX	38	30	22	23-25	26
Ceftazidime, CAZ	40	30	14		
Ceftazidime, CAZ	57	30	17	18-20	21
Ceftazidime, CAZ	56	30	17	18-20	21
Ceftazidime, CAZ	38	30	17	18-20	21
Ceftazidime, CAZ	15	30	20		26
Ceftiofur, XNL	40	30	14		
Ceftiofur, XNL	56	30	17	18-20	21
Ceftiofur, XNL	15	30	17		21
Ceftiofur, XNL	38	30	17	18-20	21
Chloramphenicol, CHL	57	30	12	13-17	18
Chloramphenicol, CHL	56	30	12	13-17	18
Chloramphenicol, CHL	38	30	12	13-17	18
Chloramphenicol, CHL	40	30	12		
Chloramphenicol, CHL	15	30	19		22
Ciprofloxacin, CIP	56	5	15	16-20	21
Ciprofloxacin, CIP	38	5	15	16-20	21
Ciprofloxacin, CIP	40	5	30		
Ciprofloxacin, CIP	57	10	20	21-30	31
Gentamicin, GEN	56	10	12	13-14	15
Gentamicin, GEN	38	10	12	13-14	15
Gentamicin, GEN	40	10	12		
Gentamicin, GEN	15	15	15		18
Gentamicin, GEN	57	30	12	13-14	15
Nalidixic acid, NAL	57	30	13	14-18	19
Nalidixic acid, NAL	56	30	13	14-18	19
Nalidixic acid, NAL	38	30	13	14-18	19
Nalidixic acid, NAL	40	30	13		
Nalidixic acid, NAL	15	30	14		20
Streptomycin, STR	38	10	11	12-14	15
Streptomycin, STR	40	10	11		
Streptomycin, STR	15	10 UI	12		15
Sulfamethoxazole, SMX	15	200	11		17
Sulfamethoxazole, SMX	56	250	12	13-16	17
Sulfamethoxazole, SMX	57	300	12	13-16	17
Sulfamethoxazole, SMX	38	300	12	13-16	17
Sulfamethoxazole, SMX	40	300	12		
Tetracycline,TET	57	30	11	12-14	15
Tetracycline,TET	56	30	11	12-14	15
Tetracycline,TET	38	30	11	12-14	15
Tetracycline,TET	40	30	11		
Tetracycline,TET	15	30 UI	16		19
Trimethoprim, TMP	57	5	10	11-15	16
Trimethoprim, TMP	56	5	10	11-15	16
Trimethoprim, TMP	38	5	10	11-15	16
Trimethoprim, TMP	40	5	10		
Trimethoprim, TMP	15	5	11		16

Test results from the reference strain *E. coli* ATCC 25922

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
1	Ampicillin, AMP	=	4	2	8	1	MIC
1	Cefotaxime, CTX	<=	0.125	0.03	0.125	1	MIC
1	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
1	Chloramphenicol, CHL	=	4	2	8	1	MIC
1	Ciprofloxacin, CIP	<=	0.015	0.004	0.016	1	MIC
1	Gentamicin, GEN	=	2	0.25	1	**0**	MIC
1	Nalidixic acid, NAL	<=	4	1	4	1	MIC
1	Tetracycline, TET	<=	2	0.5	2	1	MIC
1	Trimethoprim, TMP	<=	1	0.5	2	1	MIC
2	Ampicillin, AMP	=	4	2	8	1	MIC
2	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
2	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
2	Chloramphenicol, CHL	=	4	2	8	1	MIC
2	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
2	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
2	Nalidixic acid, NAL	<=	4	1	4	1	MIC
2	Sulfisoxazole, FIS	=	16	8	32	1	MIC
2	Tetracycline, TET	<=	1	0.5	2	1	MIC
2	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
4	Ampicillin, AMP	=	8	2	8	1	MIC
4	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
4	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
4	Chloramphenicol, CHL	=	4	2	8	1	MIC
4	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
4	Gentamicin, GEN	=	1	0.25	1	1	MIC
4	Nalidixic acid, NAL	=	4	1	4	1	MIC
4	Sulfisoxazole, FIS	=	2	8	32	**0**	MIC
4	Tetracycline, TET	=	0.5	0.5	2	1	MIC
4	Trimethoprim, TMP	=	32	0.5	2	**0**	MIC
6	Ampicillin, AMP	=	4	2	8	1	MIC
6	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
6	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
6	Chloramphenicol, CHL	=	4	2	8	1	MIC
6	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
6	Gentamicin, GEN	=	1	0.25	1	1	MIC
6	Nalidixic acid, NAL	<	4	1	4	1	MIC
6	Tetracycline, TET	<	1	0.5	2	1	MIC
6	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
9	Ampicillin, AMP	=	4	2	8	1	MIC
9	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
9	Cefoxitin, FOX	=	4	2	8	1	MIC
9	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
9	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
9	Chloramphenicol, CHL	=	4	2	8	1	MIC
9	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
9	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
9	Imipenem, IMI	=	0.12	0.06	0.25	1	MIC
9	Nalidixic acid, NAL	=	4	1	4	1	MIC
9	Sulfisoxazole, FIS	=	16	8	32	1	MIC
9	Tetracycline, TET	=	1	0.5	2	1	MIC
9	Trimethoprim, TMP	=	1	0.5	2	1	MIC

11	Ampicillin, AMP	=	4	2	8	1	MIC
11	Cefotaxime, CTX	=	0.03	0.03	0.125	1	MIC
11	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
11	Chloramphenicol, CHL	<=	2	2	8	1	MIC
11	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
11	Gentamicin, GEN	=	1	0.25	1	1	MIC
11	Nalidixic acid, NAL	=	2	1	4	1	MIC
11	Sulfisoxazole, FIS	=	16	8	32	1	MIC
11	Tetracycline, TET	<=	1	0.5	2	1	MIC
11	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
12	Ampicillin, AMP	=	4	2	8	1	MIC
12	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
12	Chloramphenicol, CHL	=	4	2	8	1	MIC
12	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	MIC
12	Gentamicin, GEN	=	1	0.25	1	1	MIC
12	Nalidixic acid, NAL	<=	2	1	4	1	MIC
12	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
12	Tetracycline, TET	=	1	0.5	2	1	MIC
12	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
13	Ampicillin, AMP	=	8	2	8	1	MIC
13	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
13	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
13	Chloramphenicol, CHL	=	4	2	8	1	MIC
13	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
13	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
13	Nalidixic acid, NAL	<=	4	1	4	1	MIC
13	Sulfisoxazole, FIS	=	32	8	32	1	MIC
13	Tetracycline, TET	<=	1	0.5	2	1	MIC
13	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
15	Cefotaxime, CTX	=	35	29	35	1	DD
15	Cefoxitin, FOX	=	27	23	29	1	DD
15	Ceftazidime, CAZ	=	32	25	32	1	DD
15	Ceftiofur, XNL	=	29	26	31	1	DD
15	Chloramphenicol, CHL	=	26	21	27	1	DD
15	Gentamicin, GEN	=	26	19	26	1	DD
15	Nalidixic acid, NAL	=	24	22	28	1	DD
15	Tetracycline, TET	=	25	18	25	1	DD
15	Trimethoprim, TMP	=	25	21	28	1	DD
16	Ampicillin, AMP	=	4	2	8	1	MIC
16	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
16	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
16	Chloramphenicol, CHL	=	8	2	8	1	MIC
16	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
16	Gentamicin, GEN	=	1	0.25	1	1	MIC
16	Nalidixic acid, NAL	=	2	1	4	1	MIC
16	Tetracycline, TET	=	2	0.5	2	1	MIC
16	Trimethoprim, TMP	=	2	0.5	2	1	MIC
17	Ampicillin, AMP	=	4	2	8	1	MIC
17	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
17	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
17	Chloramphenicol, CHL	=	4	2	8	1	MIC
17	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
17	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
17	Nalidixic acid, NAL	<=	4	1	4	1	MIC
17	Sulfisoxazole, FIS	=	32	8	32	1	MIC
17	Tetracycline, TET	<=	1	0.5	2	1	MIC
17	Trimethoprim, TMP	=	1	0.5	2	1	MIC

18	Ampicillin, AMP	=	4	2	8	1	MIC
18	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
18	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
18	Chloramphenicol, CHL	=	4	2	8	1	MIC
18	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
18	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
18	Nalidixic acid, NAL	<=	4	1	4	1	MIC
18	Sulfisoxazole, FIS	=	32	8	32	1	MIC
18	Tetracycline, TET	<=	1	0.5	2	1	MIC
18	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
19	Ampicillin, AMP	=	4	2	8	1	MIC
19	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
19	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
19	Chloramphenicol, CHL	=	4	2	8	1	MIC
19	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
19	Gentamicin, GEN	=	1	0.25	1	1	MIC
19	Nalidixic acid, NAL	<=	4	1	4	1	MIC
19	Sulfisoxazole, FIS	=	32	8	32	1	MIC
19	Tetracycline, TET	<=	1	0.5	2	1	MIC
19	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
20	Ampicillin, AMP	=	4	2	8	1	MIC
20	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
20	Cefoxitin, FOX	<=	4	2	8	1	MIC
20	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
20	Chloramphenicol, CHL	=	8	2	8	1	MIC
20	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
20	Gentamicin, GEN	=	1	0.25	1	1	MIC
20	Imipenem, IMI	<=	0.5	0.06	0.25	1	MIC
20	Nalidixic acid, NAL	<=	4	1	4	1	MIC
20	Tetracycline, TET	=	2	0.5	2	1	MIC
20	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
21	Ampicillin, AMP	=	4	2	8	1	MIC
21	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
21	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
21	Chloramphenicol, CHL	=	4	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
21	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	16	8	32	1	MIC
21	Tetracycline, TET	=	2	0.5	2	1	MIC
21	Trimethoprim, TMP	=	1	0.5	2	1	MIC
22	Ampicillin, AMP	=	8	2	8	1	MIC
22	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
22	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
22	Chloramphenicol, CHL	=	4	2	8	1	MIC
22	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
22	Gentamicin, GEN	=	1	0.25	1	1	MIC
22	Nalidixic acid, NAL	<	4	1	4	1	MIC
22	Sulfisoxazole, FIS	=	32	8	32	1	MIC
22	Tetracycline, TET	=	2	0.5	2	1	MIC
22	Trimethoprim, TMP	=	1	0.5	2	1	MIC

23	Ampicillin, AMP	=	2	2	8	1	MIC
23	Cefotaxime, CTX	<	0.25	0.03	0.125	1	MIC
23	Cefoxitin, FOX	<	4	2	8	1	MIC
23	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
23	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
23	Chloramphenicol, CHL	=	4	2	8	1	MIC
23	Ciprofloxacin, CIP	<	0.015	0.004	0.016	1	MIC
23	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
23	Imipenem, IMI	<	0.5	0.06	0.25	1	MIC
23	Nalidixic acid, NAL	=	2	1	4	1	MIC
23	Sulfisoxazole, FIS	=	32	8	32	1	MIC
23	Tetracycline, TET	<	4	0.5	2	1	MIC
25	Ampicillin, AMP	=	4	2	8	1	MIC
25	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
25	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
25	Chloramphenicol, CHL	=	8	2	8	1	MIC
25	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
25	Gentamicin, GEN	=	1	0.25	1	1	MIC
25	Nalidixic acid, NAL	<=	4	1	4	1	MIC
25	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
25	Tetracycline, TET	=	2	0.5	2	1	MIC
25	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
26	Ampicillin, AMP	=	4	2	8	1	MIC
26	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
26	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
26	Chloramphenicol, CHL	=	4	2	8	1	MIC
26	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
26	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
26	Nalidixic acid, NAL	<=	4	1	4	1	MIC
26	Tetracycline, TET	<=	1	0.5	2	1	MIC
26	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
29	Ampicillin, AMP	=	2	2	8	1	MIC
29	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
29	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
29	Chloramphenicol, CHL	=	4	2	8	1	MIC
29	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
29	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
29	Nalidixic acid, NAL	=	2	1	4	1	MIC
29	Tetracycline, TET	=	1	0.5	2	1	MIC
29	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
30	Ampicillin, AMP	=	4	2	8	1	MIC
30	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
30	Cefoxitin, FOX	<=	4	2	8	1	MIC
30	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
30	Chloramphenicol, CHL	=	4	2	8	1	MIC
30	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	MIC
30	Gentamicin, GEN	=	1	0.25	1	1	MIC
30	Imipenem, IMI	<=	0.5	0.06	0.25	1	MIC
30	Nalidixic acid, NAL	<=	4	1	4	1	MIC
30	Sulfisoxazole, FIS	=	16	8	32	1	MIC
30	Tetracycline, TET	=	2	0.5	2	1	MIC
30	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC

32	Ampicillin, AMP	=	4	2	8	1	MIC
32	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
32	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
32	Chloramphenicol, CHL	=	4	2	8	1	MIC
32	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	MIC
32	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
32	Nalidixic acid, NAL	<=	4	1	4	1	MIC
32	Sulfisoxazole, FIS	=	16	8	32	1	MIC
32	Tetracycline, TET	<=	1	0.5	2	1	MIC
32	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
33	Ampicillin, AMP	=	4	2	8	1	MIC
33	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
33	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
33	Gentamicin, GEN	=	1	0.25	1	1	MIC
33	Nalidixic acid, NAL	=	2	1	4	1	MIC
33	Sulfisoxazole, FIS	=	32	8	32	1	MIC
33	Tetracycline, TET	=	1	0.5	2	1	MIC
33	Trimethoprim, TMP	=	1	0.5	2	1	MIC
34	Ampicillin, AMP	=	4	2	8	1	MIC
34	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
34	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
34	Chloramphenicol, CHL	=	4	2	8	1	MIC
34	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
34	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
34	Nalidixic acid, NAL	<=	4	1	4	1	MIC
34	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
34	Tetracycline, TET	<=	1	0.5	2	1	MIC
34	Trimethoprim, TMP	=	1	0.5	2	1	MIC
36	Ampicillin, AMP	=	2	2	8	1	MIC
36	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
36	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
36	Chloramphenicol, CHL	=	8	2	8	1	MIC
36	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	MIC
36	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
36	Nalidixic acid, NAL	=	2	1	4	1	MIC
36	Sulfisoxazole, FIS	=	32	8	32	1	MIC
36	Tetracycline, TET	<=	1	0.5	2	1	MIC
36	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
37	Ampicillin, AMP	=	8	2	8	1	AGA
37	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	AGA
37	Chloramphenicol, CHL	=	4	2	8	1	AGA
37	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	AGA
37	Gentamicin, GEN	=	0.5	0.25	1	1	AGA
37	Nalidixic acid, NAL	<=	2	1	4	1	AGA
37	Tetracycline, TET	=	1	0.5	2	1	AGA
37	Trimethoprim, TMP	=	0.5	0.5	2	1	AGA

38	Ampicillin, AMP	=	16	16	22	1	DD
38	Cefotaxime, CTX	=	34	29	35	1	DD
38	Cefoxitin, FOX	=	27	23	29	1	DD
38	Ceftazidime, CAZ	=	30.7	25	32	1	DD
38	Ceftiofur, XNL	=	27.5	26	31	1	DD
38	Chloramphenicol, CHL	=	27.0	21	27	1	DD
38	Ciprofloxacin, CIP	=	38.7	30	40	1	DD
38	Gentamicin, GEN	=	26.0	19	26	1	DD
38	Imipenem, IMI	=	29.7	26	32	1	DD
38	Nalidixic acid, NAL	=	22.0	22	28	1	DD
38	Tetracycline, TET	=	20.6	18	25	1	DD
38	Trimethoprim, TMP	=	21.1	21	28	1	DD
39	Ampicillin, AMP	=	2	2	8	1	MIC
39	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
39	Chloramphenicol, CHL	=	4	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	MIC
39	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
39	Nalidixic acid, NAL	=	4	1	4	1	MIC
39	Sulfisoxazole, FIS	=	32	8	32	1	MIC
39	Tetracycline, TET	=	1	0.5	2	1	MIC
39	Trimethoprim, TMP	=	1	0.5	2	1	MIC
40	Ampicillin, AMP	=	18	16	22	1	DD
40	Cefotaxime, CTX	=	31	29	35	1	DD
40	Cefoxitin, FOX	=	27	23	29	1	DD
40	Ceftazidime, CAZ	=	27	25	32	1	DD
40	Chloramphenicol, CHL	=	24	21	27	1	DD
40	Ciprofloxacin, CIP	=	35	30	40	1	DD
40	Gentamicin, GEN	=	25	19	26	1	DD
40	Imipenem, IMI	=	30	26	32	1	DD
40	Nalidixic acid, NAL	=	24	22	28	1	DD
40	Tetracycline, TET	=	23	18	25	1	DD
40	Trimethoprim, TMP	=	27	21	28	1	DD
41	Ampicillin, AMP	=	4	2	8	1	MIC
41	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
41	Cefoxitin, FOX	=	4	2	8	1	MIC
41	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
41	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
41	Chloramphenicol, CHL	=	8	2	8	1	MIC
41	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
41	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
41	Nalidixic acid, NAL	=	2	1	4	1	MIC
41	Tetracycline, TET	=	1	0.5	2	1	MIC
41	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
42	Ampicillin, AMP	=	4	2	8	1	MIC
42	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
42	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
42	Chloramphenicol, CHL	=	4	2	8	1	MIC
42	Ciprofloxacin, CIP	=	0.05	0.004	0.016	**0**	MIC
42	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
42	Nalidixic acid, NAL	<=	4	1	4	1	MIC
42	Sulfisoxazole, FIS	=	32	8	32	1	MIC
42	Tetracycline, TET	<=	1	0.5	2	1	MIC
42	Trimethoprim, TMP	=	1	0.5	2	1	MIC

56	Ampicillin, AMP	=	22	16	22	1	DD
56	Cefotaxime, CTX	=	33	29	35	1	DD
56	Cefoxitin, FOX	=	25	23	29	1	DD
56	Ceftazidime, CAZ	=	29	25	32	1	DD
56	Ceftiofur, XNL	=	26	26	31	1	DD
56	Chloramphenicol, CHL	=	25	21	27	1	DD
56	Ciprofloxacin, CIP	=	32	30	40	1	DD
56	Gentamicin, GEN	=	21	19	26	1	DD
56	Nalidixic acid, NAL	=	26	22	28	1	DD
56	Sulfisoxazole, FIS	=	16	15	23	1	DD
56	Tetracycline, TET	=	24	18	25	1	DD
56	Trimethoprim, TMP	=	21	21	28	1	DD
57	Ampicillin, AMP	=	20	16	22	1	DD
57	Cefotaxime, CTX	=	30	29	35	1	DD
57	Cefoxitin, FOX	=	28	23	29	1	DD
57	Ceftazidime, CAZ	=	26	25	32	1	DD
57	Ceftiofur, XNL	=	24	26	31	**0**	DD
57	Chloramphenicol, CHL	=	25	21	27	1	DD
57	Ciprofloxacin, CIP	=	35	30	40	1	DD
57	Gentamicin, GEN	=	25	19	26	1	DD
57	Nalidixic acid, NAL	=	26	22	28	1	DD
57	Sulfisoxazole, FIS	=	21	15	23	1	DD
57	Tetracycline, TET	=	23	18	25	1	DD
57	Trimethoprim, TMP	=	24	21	28	1	DD
58	Ampicillin, AMP	=	8	2	8	1	MIC
58	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
58	Cefoxitin, FOX	<=	4	2	8	1	MIC
58	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
58	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
58	Chloramphenicol, CHL	=	8	2	8	1	MIC
58	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
58	Gentamicin, GEN	=	1	0.25	1	1	MIC
58	Imipenem, IMI	<=	0.5	0.06	0.25	1	MIC
58	Nalidixic acid, NAL	<=	4	1	4	1	MIC
58	Sulfisoxazole, FIS	=	32	8	32	1	MIC
58	Tetracycline, TET	<=	1	0.5	2	1	MIC
58	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC

Test results from the reference strain *C. jejuni* ATCC 33560

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
1	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
1	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
1	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
1	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
1	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
1	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
2	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
2	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
2	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
2	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
2	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
2	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
4	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
4	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
4	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
4	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
4	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
4	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
6	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
6	Ciprofloxacin, CIP	<	0.06	0.03	0.125	1	MIC		X
6	Erythromycin, ERY	<	0.5	0.25	2	1	MIC		X
6	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
6	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
6	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
9	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
9	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
9	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
9	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
9	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
9	Tetracycline, TET	=	0.5	0.25	2	1	MIC	X	
11	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
11	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
11	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
11	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
11	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
12	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
12	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
12	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
12	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
12	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
14	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
14	Ciprofloxacin, CIP	=	0.125	0.03	0.125	1	MIC		X
14	Erythromycin, ERY	<=	0.5	0.25	2	1	MIC		X
14	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
14	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
14	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
17	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
17	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
17	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
17	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
17	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
17	Tetracycline, TET	=	1	0.25	2	1	MIC	X	

19	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
19	Ciprofloxacin, CIP	=	0.12	0.03	0.125	1	MIC		X
19	Erythromycin, ERY	<=	0.5	0.25	2	1	MIC		X
19	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
19	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
19	Tetracycline, TET	=	1	0.25	1	1	MIC		X
20	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
20	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
20	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
20	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
20	Nalidixic acid, NAL	=	4	4	16	1	MIC	X	
20	Tetracycline, TET	=	0.5	0.25	2	1	MIC	X	
21	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
21	Ciprofloxacin, CIP	=	0.12	0.03	0.125	1	MIC		X
21	Erythromycin, ERY	<=	0.5	0.25	2	1	MIC		X
21	Gentamicin, GEN	=	0.12	0.25	2	**0**	MIC		X
21	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
21	Tetracycline, TET	=	0.25	0.25	1	1	MIC		X
22	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
22	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
22	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
22	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
22	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
23	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
23	Ciprofloxacin, CIP	=	0.12	0.03	0.125	1	MIC		X
23	Erythromycin, ERY	<	0.5	0.25	2	1	MIC		X
23	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
23	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
23	Tetracycline, TET	=	1	0.25	1	1	MIC		X
25	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
25	Ciprofloxacin, CIP	<=	0.12	0.06	0.25	1	MIC	X	
25	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
25	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
25	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
25	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
26	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
26	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
26	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
26	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
26	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
26	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
29	Ciprofloxacin, CIP	=	4	0.06	0.25	**0**	MIC	X	
29	Erythromycin, ERY	=	0.25	0.5	2	**0**	MIC	X	
29	Gentamicin, GEN	=	2	0.5	2	1	MIC	X	
29	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
29	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
30	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
30	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
30	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
30	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
30	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
30	Tetracycline, TET	=	2	0.25	2	1	MIC	X	

32	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
32	Ciprofloxacin, CIP	=	0.125	0.06	0.25	1	MIC	X	
32	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
32	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
32	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
32	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
33	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
33	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
33	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
33	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
33	Tetracycline, TET	=	0.5	0.25	2	1	MIC	X	
34	Chloramphenicol, CHL	=	2	1	8	1	MIC	X	
34	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
34	Erythromycin, ERY	=	0.5	0.5	2	1	MIC	X	
34	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
34	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
34	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
36	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
36	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
36	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
36	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
36	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
37	Ciprofloxacin, CIP	=	0.25	0.12	1	1	AGA	X	
37	Erythromycin, ERY	=	2	1	8	1	AGA	X	
37	Gentamicin, GEN	=	1	0.5	2	1	AGA	X	
39	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
39	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
39	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
39	Nalidixic acid, NAL	=	4	4	16	1	MIC	X	
39	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
40	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
40	Ciprofloxacin, CIP	=	0.12	0.03	0.125	1	MIC		X
40	Erythromycin, ERY	=	2	0.25	2	1	MIC		X
40	Gentamicin, GEN	=	0.5	0.25	2	1	MIC		X
40	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
40	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
41	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
41	Ciprofloxacin, CIP	=	0.06	0.03	0.125	1	MIC		X
41	Erythromycin, ERY	=	0.5	0.25	2	1	MIC		X
41	Gentamicin, GEN	=	1	0.25	2	1	MIC		X
41	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
41	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
42	Chloramphenicol, CHL	<=	2				MIC	37°C 24 h	
42	Ciprofloxacin, CIP	=	0.12				MIC	37°C 24 h	
42	Erythromycin, ERY	<=	0.5				MIC	37°C 24 h	
42	Gentamicin, GEN	<=	0.12				MIC	37°C 24 h	
42	Nalidixic acid, NAL	=	8				MIC	37°C 24 h	
42	Tetracycline, TET	=	4				MIC	37°C 24 h	
44	Ciprofloxacin, CIP	<	1	0.12	1	1	AGA	X	
44	Erythromycin, ERY	<	4	1	8	1	AGA	X	
44	Gentamicin, GEN	<	2	0.5	2	1	AGA	X	
58	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
58	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
58	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
58	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
58	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
58	Tetracycline, TET	=	1	0.25	2	1	MIC	X	

QC ranges for reference strains

E. coli ATCC 25922		
Antimicrobial	**MIC**	**DD (disc content)**
Ampicillin, AMP	2-8	16-22 (10µg)
Cefotaxime, CTX	0.03-0.12	29-35 (30µg)
Cefoxitin, FOX	2-8	23-29 (30µg)
Ceftazidime, CAZ	0.06-0.5	25-32 (30µg)
Ceftiofur, XNL	0.25-1	26-31 (30µg)
Chloramphenicol, CHL	2-8	21-27 (30µg)
Ciprofloxacin, CIP	0.004-0.016	30-40 (5µg)
Gentamicin, GEN	0.25-1	19-26 (10µg)
Imipenem, IMI	0.06-0.25	26-32 (10µg)
Nalidixic acid, NAL	1-4	22-28 (30µg)
Sulfisoxazole, FIS	8-32	15-23 (250/300µg)
Tetracycline, TET	0.5-2	18-25 (30µg)
Trimethoprim, TMP	0.5-2	21-28 (5µg)

MIC ranges and disc diffusion ranges are according to CLSI M100 S22 with the following exceptions: The range for ceftiofur is according to M31-A3 and the range for ciprofloxacin is extended to include 0.016 as well.

E-test ranges are according to AB-Biodisk

Campylobacter jejuni 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)

Salmonella - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Ampicillin, AMP	EURL S-7.1	R	100%	0%	34	0
	EURL S-7.2	R	100%	0%	34	0
	EURL S-7.3	R	100%	0%	34	0
	EURL S-7.4	R	100%	0%	34	0
	EURL S-7.5	S	0%	100%	34	0
	EURL S-7.6	R	100%	0%	34	0
	EURL S-7.7	S	0%	100%	34	0
	EURL S-7.8	S	0%	100%	34	0
Cefotaxime, CTX	EURL S-7.1	R	97%	3%	34	1
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	R	100%	0%	35	0
	EURL S-7.4	S	3%	97%	34	1
	EURL S-7.5	S	0%	100%	35	0
	EURL S-7.6	R	100%	0%	35	0
	EURL S-7.7	S	0%	100%	35	0
	EURL S-7.8	S	0%	100%	35	0
Ceftazidime, CAZ	EURL S-7.1	R	100%	0%	31	0
	EURL S-7.2	R	100%	0%	31	0
	EURL S-7.3	S	6%	94%	29	2
	EURL S-7.4	S	0%	100%	30	0
	EURL S-7.5	S	0%	100%	30	0
	EURL S-7.6	R	100%	0%	31	0
	EURL S-7.7	S	0%	100%	30	0
	EURL S-7.8	S	0%	100%	30	0
Ceftiofur, XNL	EURL S-7.1	R	100%	0%	13	0
	EURL S-7.2	R	100%	0%	11	0
	EURL S-7.3	R	89%	11%	8	1
	EURL S-7.4	S	0%	100%	10	0
	EURL S-7.5	S	0%	100%	12	0
	EURL S-7.6	R	100%	0%	10	0
	EURL S-7.7	S	0%	100%	10	0
	EURL S-7.8	S	0%	100%	10	0
Chloramphenicol, CHL	EURL S-7.1	R	97%	3%	34	1
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	S	3%	97%	34	1
	EURL S-7.4	S	0%	100%	35	0
	EURL S-7.5	S	0%	100%	35	0
	EURL S-7.6	S	0%	100%	35	0
	EURL S-7.7	S	0%	100%	35	0
	EURL S-7.8	R	100%	0%	35	0
Ciprofloxacin, CIP	EURL S-7.1	R	100%	0%	34	0
	EURL S-7.2	R	100%	0%	34	0
	EURL S-7.3	R	97%	3%	33	1
	EURL S-7.4	R	97%	3%	33	1
	EURL S-7.5	S	0%	100%	34	0
	EURL S-7.6	S	3%	97%	33	1
	EURL S-7.7	R	97%	3%	33	1
	EURL S-7.8	R	91%	9%	30	3

Gentamicin, GEN	EURL S-7.1	R	97%	3%	34	1
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	S	3%	97%	34	1
	EURL S-7.4	S	0%	100%	35	0
	EURL S-7.5	S	0%	100%	35	0
	EURL S-7.6	S	0%	100%	35	0
	EURL S-7.7	S	0%	100%	35	0
	EURL S-7.8	S	0%	100%	35	0
Nalidixic acid, NAL	EURL S-7.1	R	97%	3%	34	1
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	R	100%	0%	35	0
	EURL S-7.4	R	100%	0%	35	0
	EURL S-7.5	S	0%	100%	35	0
	EURL S-7.6	S	0%	100%	35	0
	EURL S-7.7	R	100%	0%	35	0
	EURL S-7.8	S	6%	94%	33	2
Sulphonamides, SMX	EURL S-7.1	R	100%	0%	35	0
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	S	6%	94%	33	2
	EURL S-7.4	R	97%	3%	34	1
	EURL S-7.5	S	3%	97%	34	1
	EURL S-7.6	S	3%	97%	34	1
	EURL S-7.7	R	100%	0%	35	0
	EURL S-7.8	S	3%	97%	34	1
Tetracycline, TET	EURL S-7.1	R	100%	0%	35	0
	EURL S-7.2	R	100%	0%	35	0
	EURL S-7.3	R	100%	0%	35	0
	EURL S-7.4	S	0%	100%	35	0
	EURL S-7.5	S	0%	100%	35	0
	EURL S-7.6	S	0%	100%	35	0
	EURL S-7.7	R	100%	0%	35	0
	EURL S-7.8	S	3%	97%	34	1
Trimethoprim, TMP	EURL S-7.1	R	97%	3%	33	1
	EURL S-7.2	R	100%	0%	34	0
	EURL S-7.3	S	3%	97%	33	1
	EURL S-7.4	R	100%	0%	34	0
	EURL S-7.5	S	0%	100%	34	0
	EURL S-7.6	S	0%	100%	34	0
	EURL S-7.7	R	97%	3%	32	1
	EURL S-7.8	S	0%	100%	34	0

Campylobacter - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Chloramphenicol, CHL	EURL C-7.1	S	0%	100%	23	0
	EURL C-7.2	S	0%	100%	23	0
	EURL C-7.3	S	0%	100%	23	0
	EURL C-7.4	S	0%	100%	23	0
	EURL C-7.5	S	0%	100%	23	0
	EURL C-7.6	S	0%	100%	23	0
	EURL C-7.7	S	0%	100%	23	0
	EURL C-7.8	S	0%	100%	23	0
Ciprofloxacin, CIP	EURL C-7.1	S	0%	100%	29	0
	EURL C-7.2	S	0%	100%	29	0
	EURL C-7.3	S	0%	100%	29	0
	EURL C-7.4	R	97%	3%	28	1
	EURL C-7.5	R	97%	3%	28	1
	EURL C-7.6	R	97%	3%	28	1
	EURL C-7.7	S	3%	97%	28	1
	EURL C-7.8	R	93%	7%	27	2
Erythromycin, ERY	EURL C-7.1	R	100%	0%	29	0
	EURL C-7.2	R	100%	0%	29	0
	EURL C-7.3	S	3%	97%	28	1
	EURL C-7.4	S	14%	86%	25	4
	EURL C-7.5	S	3%	97%	28	1
	EURL C-7.6	R	100%	0%	29	0
	EURL C-7.7	S	3%	97%	28	1
	EURL C-7.8	S	0%	100%	29	0
Gentamicin, GEN	EURL C-7.1	S	0%	100%	29	0
	EURL C-7.2	S	0%	100%	29	0
	EURL C-7.3	S	0%	100%	29	0
	EURL C-7.4	S	0%	100%	29	0
	EURL C-7.5	S	0%	100%	29	0
	EURL C-7.6	R	100%	0%	29	0
	EURL C-7.7	S	0%	100%	29	0
	EURL C-7.8	S	0%	100%	29	0
Nalidixic acid, NAL	EURL C-7.1	S	3%	97%	28	1
	EURL C-7.2	S	4%	96%	27	1
	EURL C-7.3	S	3%	97%	28	1
	EURL C-7.4	R	97%	3%	28	1
	EURL C-7.5	R	97%	3%	28	1
	EURL C-7.6	R	97%	3%	28	1
	EURL C-7.7	S	0%	100%	29	0
	EURL C-7.8	R	93%	7%	27	2
Streptomycin, STR	EURL C-7.1	S	7%	93%	27	2
	EURL C-7.2	S	7%	93%	27	2
	EURL C-7.3	S	3%	97%	28	1
	EURL C-7.4	R	93%	7%	27	2
	EURL C-7.5	R	97%	3%	28	1
	EURL C-7.6	R	100%	0%	29	0
	EURL C-7.7	S	0%	100%	29	0
	EURL C-7.8	S	0%	100%	29	0
Tetracycline, TET	*EURL C-7.1**	*S*	*29%*	*71%*	*20*	*8*
	EURL C-7.2	R	100%	0%	29	0
	EURL C-7.3	S	3%	97%	28	1
	EURL C-7.4	R	100%	0%	29	0
	EURL C-7.5	S	4%	96%	27	1
	EURL C-7.6	R	100%	0%	29	0
	EURL C-7.7	R	97%	3%	28	1
	EURL C-7.8	R	97%	3%	28	1

*Strain/antimicrobial-combination excluded from the evaluation

Deviations - *Salmonella*

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
4	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
6	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
6	EURL S-7.8	Nalidixic acid, NAL	R	>64	S	= 4	MIC
6	EURL S-7.8	Tetracycline, TET	R	<=32	S	<= 2	MIC
9	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
11	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
12	EURL S-7.2	Confirmed AmpC	Yes		No		MIC
12	EURL S-7.2	Confirmed ESBL	No		Yes		MIC
12	EURL S-7.3	Confirmed ESBL	No		Yes		MIC
12	EURL S-7.4	Sulfamethoxazole, SMX	S	256	R	> 1024	MIC
15	EURL S-7.4	Confirmed AmpC	Yes		No		DD
15	EURL S-7.6	Confirmed AmpC	No		Yes		DD
15	EURL S-7.6	Confirmed ESBL	Yes		No		DD
16	EURL S-7.1	Confirmed AmpC	Yes		No		MIC
16	EURL S-7.1	Confirmed ESBL	No		Yes		MIC
18	EURL S-7.4	Confirmed AmpC	Yes		No		MIC
19	EURL S-7.1	Confirmed AmpC	Yes		No		MIC
19	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
19	EURL S-7.6	Confirmed ESBL	Yes		No		MIC
20	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
22	EURL S-7.3	Ceftazidime, CAZ	R	1	S	= 1	MIC
22	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
22	EURL S-7.6	Confirmed ESBL	Yes		No		MIC
23	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
25	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
26	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
29	EURL S-7.3	Ceftiofur, XNL	S	19mm	R	> 8	MIC
29	EURL S-7.3	Confirmed ESBL	No		Yes		MIC
29	EURL S-7.8	Ciprofloxacin, CIP	S	0.5	R	= 0.5	MIC
30	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
32	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
32	EURL S-7.6	Confirmed MBL	Yes		No		MIC
34	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
37	EURL S-7.6	Confirmed AmpC	No		Yes		AGA
38	EURL S-7.1	Confirmed AmpC	Yes		No		DD
38	EURL S-7.2	Confirmed AmpC	Yes		No		DD
38	EURL S-7.6	Confirmed AmpC	No		Yes		DD
38	EURL S-7.6	Confirmed ESBL	Yes		No		DD
38	EURL S-7.8	Nalidixic acid, NAL	R	10.8	S	= 4	DD
39	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
40	EURL S-7.1	Gentamicin, GEN	S	14	R	> 16	DD
41	EURL S-7.1	Confirmed ESBL	No		Yes		MIC
41	EURL S-7.6	Ciprofloxacin, CIP	R	0.25	S	<= 0.015	MIC
41	EURL S-7.6	Confirmed AmpC	No		Yes		MIC
41	EURL S-7.6	Confirmed ESBL	Yes		No		MIC

42	EURL S-7.3	Ceftazidime, CAZ	R	>16	S	= 1	MIC
42	EURL S-7.3	Chloramphenicol, CHL	R	>64	S	= 4	MIC
42	EURL S-7.3	Gentamicin, GEN	R	>32	S	<= 0.5	MIC
42	EURL S-7.3	Sulfamethoxazole, SMX	R	>1024	S	= 32	MIC
42	EURL S-7.3	Trimethoprim, TMP	R	>32	S	<= 1	MIC
44	EURL S-7.1	Cefotaxime, CTX	S	<1	R	> 64	AGA
44	EURL S-7.1	Chloramphenicol, CHL	S	<8	R	> 64	AGA
44	EURL S-7.1	Confirmed ESBL	No		Yes		AGA
44	EURL S-7.1	Nalidixic acid, NAL	S	<16	R	> 64	AGA
44	EURL S-7.1	Trimethoprim, TMP	S	<2	R	> 32	AGA
44	EURL S-7.2	Confirmed ESBL	No		Yes		AGA
44	EURL S-7.3	Confirmed ESBL	No		Yes		AGA
44	EURL S-7.4	Cefotaxime, CTX	R	>1	S	= 0.25	AGA
44	EURL S-7.6	Confirmed AmpC	No		Yes		AGA
56	EURL S-7.1	Confirmed AmpC	Yes		No		DD
56	EURL S-7.1	Confirmed ESBL	No		Yes		DD
56	EURL S-7.3	Sulfamethoxazole, SMX	R	6	S	= 32	DD
56	EURL S-7.5	Sulfamethoxazole, SMX	R	6	S	= 64	DD
56	EURL S-7.6	Confirmed AmpC	No		Yes		DD
56	EURL S-7.6	Sulfamethoxazole, SMX	R	6	S	= 64	DD
56	EURL S-7.8	Ciprofloxacin, CIP	S	28	R	= 0.5	DD
56	EURL S-7.8	Sulfamethoxazole, SMX	R	6	S	= 32	DD
57	EURL S-7.1	Confirmed ESBL	No		Yes		DD
57	EURL S-7.2	Confirmed ESBL	No		Yes		DD
57	EURL S-7.3	Ciprofloxacin, CIP	S	28	R	= 0.5	DD
57	EURL S-7.3	Confirmed ESBL	No		Yes		DD
57	EURL S-7.4	Ciprofloxacin, CIP	S	27	R	= 0.5	DD
57	EURL S-7.6	Confirmed AmpC	No		Yes		DD
57	EURL S-7.7	Ciprofloxacin, CIP	S	30	R	= 0.25	DD
57	EURL S-7.7	Trimethoprim, TMP	S	27	R	> 32	DD
57	EURL S-7.8	Ciprofloxacin, CIP	S	31	R	= 0.5	DD
58	EURL S-7.6	Confirmed AmpC	No		Yes		MIC

AGA	Agar dilution
DD	Disk diffusion
ET	E-test
MIC	Microbroth dilution

Deviations - *Campylobacter*

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	EURL C-7.4	Erythromycin, ERY	R	16.0	S	= 8	MIC
29	EURL C-7.3	Erythromycin, ERY	R	64	S	= 1	MIC
29	EURL C-7.3	Nalidixic acid, NAL	R	64	S	= 4	MIC
29	EURL C-7.3	Tetracycline, TET	R	4	S	= 0.5	MIC
29	EURL C-7.7	Tetracycline, TET	S	<0.12	R	= 32	MIC
32	EURL C-7.5	Ciprofloxacin, CIP	S	<=0.125	R	= 8	MIC
32	EURL C-7.5	Nalidixic acid, NAL	S	<=8	R	> 64	MIC
32	EURL C-7.5	Streptomycin, STR	S	<=2	R	> 16	MIC
32	EURL C-7.7	Ciprofloxacin, CIP	R	<=4	S	= 0.12	MIC
36	EURL C-7.1	Streptomycin, STR	R	8	S	= 2	MIC
36	EURL C-7.2	Streptomycin, STR	R	4	S	= 4	MIC
36	EURL C-7.3	Streptomycin, STR	R	4	S	<= 1	MIC
36	EURL C-7.4	Erythromycin, ERY	R	8	S	= 8	MIC
37	EURL C-7.4	Erythromycin, ERY	R	16	S	= 8	AGA
39	EURL C-7.6	Nalidixic acid, NAL	S	1	R	> 64	MIC
39	EURL C-7.8	Ciprofloxacin, CIP	S	<=0.06	R	> 4	MIC
39	EURL C-7.8	Nalidixic acid, NAL	S	2	R	> 64	MIC
39	EURL C-7.8	Tetracycline, TET	S	<=0.12	R	= 32	MIC
40	EURL C-7.1	Streptomycin, STR	R	16	S	= 2	MIC
40	EURL C-7.2	Streptomycin, STR	R	16	S	= 4	MIC
40	EURL C-7.4	Ciprofloxacin, CIP	S	0.06	R	= 32	MIC
40	EURL C-7.4	Erythromycin, ERY	R	32	S	= 8	MIC
40	EURL C-7.4	Nalidixic acid, NAL	S	2	R	> 64	MIC
40	EURL C-7.4	Streptomycin, STR	S	2	R	> 16	MIC
44	EURL C-7.1	Nalidixic acid, NAL	R	>16,<32	S	= 8	AGA
44	EURL C-7.2	Nalidixic acid, NAL	R	>16, <32	S	= 8	AGA
44	EURL C-7.4	Streptomycin, STR	S	>4	R	> 16	AGA
44	EURL C-7.5	Erythromycin, ERY	R	>16	S	= 1	AGA
44	EURL C-7.5	Tetracycline, TET	R	>128	S	= 0.12	AGA
44	EURL C-7.6	Ciprofloxacin, CIP	S	>0.5	R	= 16	AGA
44	EURL C-7.7	Erythromycin, ERY	R	>16	S	= 1	AGA
44	EURL C-7.8	Ciprofloxacin, CIP	S	<0.5	R	> 4	AGA
44	EURL C-7.8	Nalidixic acid, NAL	S	<16	R	> 64	AGA

AGA Agar dilution
MIC Microbroth dilution

Optional genotypic characterisation

Lab no.	Strain	Gene tested		Not detected	Primer used 5'→3'	Primer used 3'→5'	PCR-method	Reference
I	EURL S-7.1	CTX	M-15					Whole genome sequencing
I	EURL S-7.1	OXA	-30					Whole genome sequencing
I	EURL S-7.1	TEM	-1					Whole genome sequencing
I	EURL S-7.2	CTX	M-15					Whole genome sequencing
I	EURL S-7.2	OXA	-10					Whole genome sequencing
I	EURL S-7.2	TEM	-1					Whole genome sequencing
I	EURL S-7.3	CTX	M-9					Whole genome sequencing
I	EURL S-7.3	TEM	-1					Whole genome sequencing
I	EURL S-7.6	ACC	-1					Whole genome sequencing
III	EURL S-7.1	CTX	M-15				Published	Carattoli et al. JCM 2008
III	EURL S-7.1	OXA	-31				Published	Guerra et al. AAC 2000
III	EURL S-7.1	TEM	-1				Published	Guerra et al. AAC 2001
III	EURL S-7.1	ACC		X			Published	Pérez-Perez JCM 2002
III	EURL S-7.1	ACT		X				Provided by EURL-AR (Hasman)
III	EURL S-7.1	CMY-2		X			Published	Zhao 2001
III	EURL S-7.1	DHA		X			Published	Pérez-Perez JCM 2002
III	EURL S-7.1	FOX		X			Published	Pérez-Perez JCM 2002
III	EURL S-7.1	GES		X				Provided by EURL-AR (Hasman)
III	EURL S-7.1	IMP		X			Published	Dallene JAC 2010
III	EURL S-7.1	KPC		X			Published	Dallene JAC 2010
III	EURL S-7.1	MOX		X			Published	Pérez-Perez JCM 2002
III	EURL S-7.1	NDM		X			Published	Poirel DMID 2011
III	EURL S-7.1	PER		X				Provided by EURL-AR (Hasman)
III	EURL S-7.1	SHV		X			Published	Weill JCM 2004
III	EURL S-7.1	VEB		X				Provided by EURL-AR (Hasman)
III	EURL S-7.1	VIM		X			Published	Dallene JAC 2010
III	EURL S-7.2	CTX	M-15					
III	EURL S-7.2	OXA	-31					
III	EURL S-7.2	TEM	-1					
III	EURL S-7.2	ACC		X				
III	EURL S-7.2	ACT		X				
III	EURL S-7.2	CMY-2		X				
III	EURL S-7.2	DHA		X				
III	EURL S-7.2	FOX		X				
III	EURL S-7.2	GES		X				
III	EURL S-7.2	IMP		X				
III	EURL S-7.2	KPC		X				
III	EURL S-7.2	MOX		X				
III	EURL S-7.2	NDM		X				
III	EURL S-7.2	PER		X				
III	EURL S-7.2	SHV		X				
III	EURL S-7.2	VEB		X				
III	EURL S-7.2	VIM		X				
III	EURL S-7.3	CTX	M-9					
III	EURL S-7.3	TEM	-1					

Lab no.	Strain	Gene tested		Not detected	Primer used 5'→3'	Primer used 3'→5'	PCR-method	Reference
III	EURL S-7.3	ACC		X				
III	EURL S-7.3	ACT		X				
III	EURL S-7.3	CMY		X				
III	EURL S-7.3	DHA		X				
III	EURL S-7.3	FOX		X				
III	EURL S-7.3	GES		X				
III	EURL S-7.3	IMP		X				
III	EURL S-7.3	KPC		X				
III	EURL S-7.3	MOX		X				
III	EURL S-7.3	NDM		X				
III	EURL S-7.3	OXA		X				
III	EURL S-7.3	PER		X				
III	EURL S-7.3	SHV		X				
III	EURL S-7.3	VEB		X				
III	EURL S-7.3	VIM		X				
III	EURL S-7.6	ACC	-1					
III	EURL S-7.6	ACT		X				
III	EURL S-7.6	CMY		X				
III	EURL S-7.6	CTX		X				
III	EURL S-7.6	DHA		X				
III	EURL S-7.6	FOX		X				
III	EURL S-7.6	GES		X				
III	EURL S-7.6	IMP		X				
III	EURL S-7.6	KPC		X				
III	EURL S-7.6	MOX		X				
III	EURL S-7.6	NDM		X				
III	EURL S-7.6	OXA		X				
III	EURL S-7.6	PER		X				
III	EURL S-7.6	SHV		X				
III	EURL S-7.6	TEM		X				
III	EURL S-7.6	VEB		X				
III	EURL S-7.6	VIM		X				
IV	EURL S-7.1	CTX	M-15				PCR (published)	J Clin Microbiol 2008;46:103-8
IV	EURL S-7.2	CTX	M-15				PCR (published)	J Clin Microbiol 2008;46:103-8
IV	EURL S-7.3	CTX	M-9					Emerg Infect Dis 2006;12:807-12
IV	EURL S-7.6	ACC	-1				PCR (published)	J Clin Microbiol. 2002 Jun;40(6):2153-62
VI	EURL S-7.1	CTX	M-15		CCATGGTTAAAAAATCACTGCG	CCGCTSRTTCTGGTSACYTAYTTYACCCA	PCR (published)	Pediatr Infect Dis J. 28:814-818.2009
VI	EURL S-7.1	OXA	-30		ATGAAAAACACAATACATATCAACTTCG	AATGCGATCACCCATTCTAAAGACAC	PCR (published)	Journal of Antimicrobial Chemotherapy (2009) 64, 1
VI	EURL S-7.1	TEM	-1		GCGGAACCCCTATTTG	CTCACTGATTAAGCATTGGT	PCR (published)	Antimicrobial Agents and Chemotherapy. 2009. 53:17
VI	EURL S-7.1	CMY		X	GCACTTAGCCACCTATACGGCAG	CCTGGCGCATTCTTGAAAAGC	PCR (published)	J. Antimicrob. Chemother. 2006 56:115-121
VI	EURL S-7.1	SHV		X	TTATCTCCCTGTTAGCCACC	CCGAGCGAAATCAGCAAATC	PCR (published)	FEMS Microbiol Lett. 1997. 152:163-7.
VI	EURL S-7.2	CTX	M-15		5'-CCATGGTTAAAAAATCACTGCG-3'	CCGCTSRTTCTGGTSACYTAYTTYACCCA	PCR (published)	Pediatr Infect Dis J. 28:814-818.2009
VI	EURL S-7.2	OXA	**-30**		5'-ATGAAAAACACAATACATATCAACTTCG-3'	AATGCGATCACCCATTCTAAAGACAC	PCR (published)	Journal of Antimicrobial Chemotherapy (2009) 64, 1
VI	EURL S-7.2	TEM	-1		5'-GCGGAACCCCTATTTG-3'	CTCACTGATTAAGCATTGGT	PCR (published)	Antimicrobial Agents and Chemotherapy. 2009. 53:17
VI	EURL S-7.2	CMY		X	5'-GCACTTAGCCACCTATACGGCAG-3'	CCTGGCGCATTCTTGAAAAGC	PCR (published)	J. Antimicrob. Chemother. 2006 56:115-121
VI	EURL S-7.2	SHV		X	5'-TTATCTCCCTGTTAGCCACC-3'	CCGAGCGAAATCAGCAAATC	PCR (published)	FEMS Microbiol Lett. 1997. 152:163-7.
VI	EURL S-7.3	CTX	M-9		GTGACAAAGAGAGTGCAACGG	GGCTTCAGCGGCGAGAATCAT	PCR (published)	J Antimicrob Chemother. 2005. 56:1107-10.
VI	EURL S-7.3	TEM	-1		GCGGAACCCCTATTTG	CTCACTGATTAAGCATTGGT	PCR (published)	Antimicrobial Agents and Chemotherapy. 2009. 53:17
VI	EURL S-7.3	CMY		X	GCACTTAGCCACCTATACGGCAG	CCTGGCGCATTCTTGAAAAGC	PCR (published)	.J. Antimicrob. Chemother.2006 56:115-121
VI	EURL S-7.3	OXA		X	ATGAAAAACACAATACATATCAACTTCG	AATGCGATCACCCATTCTAAAGACAC	PCR (published)	Journal of Antimicrobial Chemotherapy (2009) 64,
VI	EURL S-7.3	SHV		X	TTATCTCCCTGTTAGCCACC	CCGAGCGAAATCAGCAAATC	PCR (published)	FEMS Microbiol Lett. 1997. 152:163-7.
VII	EURL S-7.1	CTX			AAA AAT CAC TGC GYC AGT TC	AGC TTA TTC ATC GCC ACG TT	PCR (published)	Woodford et al.2006
VII	EURL S-7.1	OXA			ACA CAA TAC ATA TCA ACT TCG C	AGT GTG TTT AGA ATG GTG ATC	PCR (published)	Fang et al. 2006
VII	EURL S-7.1	TEM			CGC CGC ATA CAC TAT TCT CAG AAT GA	ACG CTC ACC GGC TCC AGA TTT AT	PCR (published)	Fang et al. 2006
VII	EURL S-7.2	CTX			AAA AAT CAC TGC GYC AGT TC	AGC TTA TTC ATC GCC ACG TT	PCR (published)	Woodford et al.2006
VII	EURL S-7.2	OXA			ACA CAA TAC ATA TCA ACT TCG C	AGT GTG TTT AGA ATG GTG ATC	PCR (published)	Fang et al. 2006
VII	EURL S-7.2	TEM			CGC CGC ATA CAC TAT TCT CAG AAT GA	ACG CTA ACC GGC TCC AGA TTT AT	PCR (published)	Fang et al. 2006
VII	EURL S-7.3	CTX			CAA AGA GAR TGC AAC GGA TG	ATT GGA AAG CGT TCA TCA CC	PCR (published)	Woodford et al.2006
VII	EURL S-7.3	TEM			CGC CGC ATA CAC TAT TCT CAG AAT GA	ACG CTC ACC GGC TCC AGA TTT AT	PCR (published)	Fang et al. 2006
VII	EURL S-7.6	ACC			AAC AGC CTC AGC AGC CGG TTA	TTC GCC GCA ATC ATC CCT AGC	PCR (published)	Pérez-Perez and Hanson,2002

Lab no.	Strain	Gene tested		Not detected	Primer used 5'→3'	Primer used 3'→5'	PCR-method	Reference
VIII	EURL S-7.1	CTX						
VIII	EURL S-7.1	OXA						
VIII	EURL S-7.1	TEM						
VIII	EURL S-7.1	ACC		X				
VIII	EURL S-7.1	ACT		X				
VIII	EURL S-7.1	CMY		X				
VIII	EURL S-7.1	DHA		X				
VIII	EURL S-7.1	FOX		X				
VIII	EURL S-7.1	MOX		X				
VIII	EURL S-7.1	PER		X				
VIII	EURL S-7.1	SHV		X				
VIII	EURL S-7.2	CTX						
VIII	EURL S-7.2	OXA						
VIII	EURL S-7.2	TEM						
VIII	EURL S-7.2	ACC		X				
VIII	EURL S-7.2	ACT		X				
VIII	EURL S-7.2	CMY		X				
VIII	EURL S-7.2	DHA		X				
VIII	EURL S-7.2	FOX		X				
VIII	EURL S-7.2	MOX		X				
VIII	EURL S-7.2	PER		X				
VIII	EURL S-7.2	SHV		X				
VIII	EURL S-7.3	CTX						
VIII	EURL S-7.3	TEM						
VIII	EURL S-7.3	ACC		X				
VIII	EURL S-7.3	ACT		X				
VIII	EURL S-7.3	DHA		X				
VIII	EURL S-7.3	FOX		X				
VIII	EURL S-7.3	OXA		X				
VIII	EURL S-7.3	PER		X				
VIII	EURL S-7.3	SHV		X				
VIII	EURL S-7.6	ACC						
VIII	EURL S-7.6	**TEM**						
VIII	EURL S-7.6	ACT		X				
VIII	EURL S-7.6	CTX		X				
VIII	EURL S-7.6	DHA		X				
VIII	EURL S-7.6	FOX		X				
VIII	EURL S-7.6	OXA		X				
VIII	EURL S-7.6	PER		X				
VIII	EURL S-7.6	SHV		X				
IX	EURL S-7.1	CTX	M-15				PCR (published)	
IX	EURL S-7.1	**SHV**						
IX	EURL S-7.1	TEM	-1				PCR (published)	
IX	EURL S-7.2	CTX	M-15					
IX	EURL S-7.2	TEM	-1					
IX	EURL S-7.2	SHV		X				
IX	EURL S-7.3	CTX	M-9					
IX	EURL S-7.3	TEM	-1					
IX	EURL S-7.3	SHV		X				
IX	EURL S-7.6	CMY		X				
IX	EURL S-7.6	CTX		X				
IX	EURL S-7.6	SHV		X				
IX	EURL S-7.6	TEM		X				
X	EURL S-7.1	CTX	M-15		TTAGGAARTGTGCCGCTGYA	CGATATCGTTGGTGGTRCCAT	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.1	OXA			GGCACCAGATTCAACTTTCAAG	GACCCCAAGTTTCCTGTAAGTG	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.1	TEM	-1		CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.2	CTX	M-15		TTAGGAARTGTGCCGCTGYA	CGATATCGTTGGTGGTRCCAT	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.2	OXA			GGCACCAGATTCAACTTTCAAG	GACCCCAAGTTTCCTGTAAGTG	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.2	TEM	-1		CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.3	CTX	M-9		TCAAGCCTGCCGATCTGGT	TGATTCTCGCCGCTGAAG	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498
X	EURL S-7.6	ACC	-1		CACCTCCAGCGACTTGTTAC	GTTAGCCAGCATCACGATCC	PCR (published)	JAC 2010; 65: 490–495;doi:10.1093/jac/dkp498

Lab no.	Strain	Gene tested		Not detected	Primer used 5'→3'	Primer used 3'→5'	PCR-method	Reference
XI	EURL S-7.1	CTX	M-1				PCR (published)	
XI	EURL S-7.2	CTX	M-1					
XI	EURL S-7.3	CTX	M-4				PCR (published)	
XI	EURL S-7.6	ACC						
XII	EURL S-7.1	ACC		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.1	CMY		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.1	DHA		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.1	FOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.1	MOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.2	ACC		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.2	CMY		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.2	DHA		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.2	FOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.2	MOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.3	ACC		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.3	CMY		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.3	DHA		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.3	FOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.3	MOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.6	ACC					PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.6	CMY		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.6	DHA		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.6	FOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XII	EURL S-7.6	MOX		X			PCR (published)	Journal of Clin. Microbiology Jun 2002 p2153-2162
XIII	EURL S-7.1	ACC		X	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.1	DHA		X	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.1	FOX		X	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.1	MOX		X	GCTGCTCAA GGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.2	ACC		X	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.2	DHA		X	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.2	FOX		X	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.2	MOX		X	GCTGCTCAA GGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.3	ACC		X	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.3	DHA		X	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.3	FOX		X	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.3	MOX		X	GCTGCTCAA GGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.6	ACC			AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.6	DHA		X	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.6	FOX		X	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol
XIII	EURL S-7.6	MOX		X	GCTGCTCAA GGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	PCR (published)	F. J. Perez-Perez et al., 2002, J.Clin.Microbiol

Legend:
Fields shaded grey indicate that the gene was expected
Genes in bold were detected but not expected

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