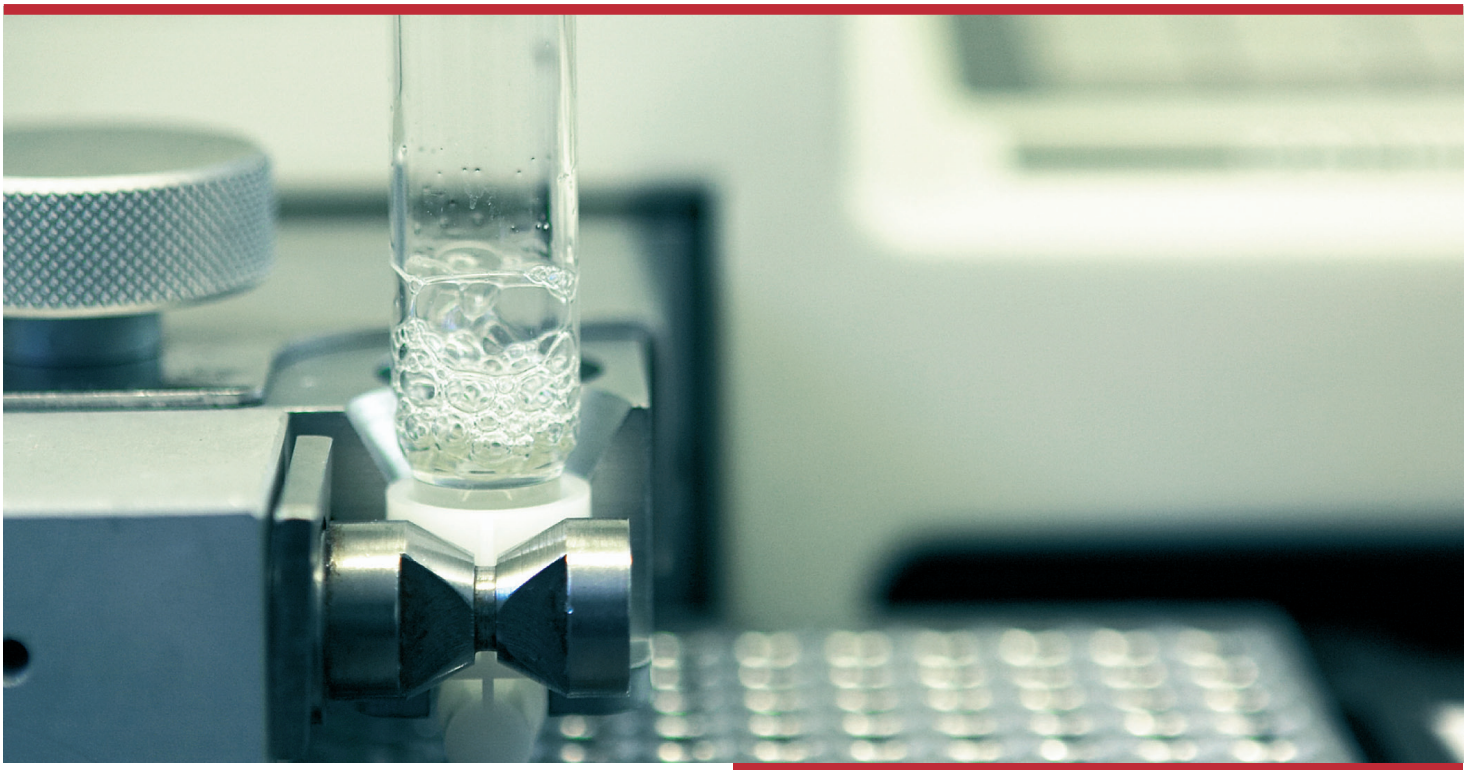
The 9th EURL-AR Proficiency Testing *Salmonella* and *Campylobacter* 2010



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The 9th EURL-AR Proficiency Testing

Salmonella and *Campylobacter* 2010

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1. Introduction

In this report, results are summarised from the ninth 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 fifth External Quality Assurance System (EQAS) conducted for these microorganisms (the first was EQAS 2006).

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).

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 (NRLs), who meet once a year 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. This is the basis for this EQAS evaluating the interpretation; 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 the MIC value for strain frequently varies by one dilution step either way, which in some cases affect the interpretation of the result. Therefore, the self-evaluation may lead to arguments which can defend the obtained results internally, yet, incorrect interpretations based on a one step dilution difference is still regarded as a deviation for the overall EQAS reporting, evaluation and in the database.

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

2. Materials and methods

2.1 Participants

A pre-notification (App. 1) of the EURL-AR EQAS on AST of *Salmonella* and *Campylobacter* was distributed on the 20th August 2010 by e-mail to the 40 NRLs in the EURL-AR-network (including Iceland, Norway, Serbia and Switzerland). In addition, to the AST of *Salmonella* and *Campylobacter*, an optional genotypic characterization by PCR/sequencing of antimicrobial resistance genes of a selected *Enterococcus faecium* and *Shigella* spp. isolate was offered. The pre-notification was sent to NRLs in all EU countries except Luxemburg, where no NRL has been designated. All 37 laboratories responded. One laboratory declined to participate as they had neither *Salmonella* nor *Campylobacter* as their field of responsibility. In addition, Iceland and Serbia did not participate in this iteration.

Appendix 2 shows that 33 of the 37 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 Iceland, Norway, Serbia and Switzerland were charged a fee for their participation in the EQAS, whereas the NRLs from EU Member States participated free of charge.

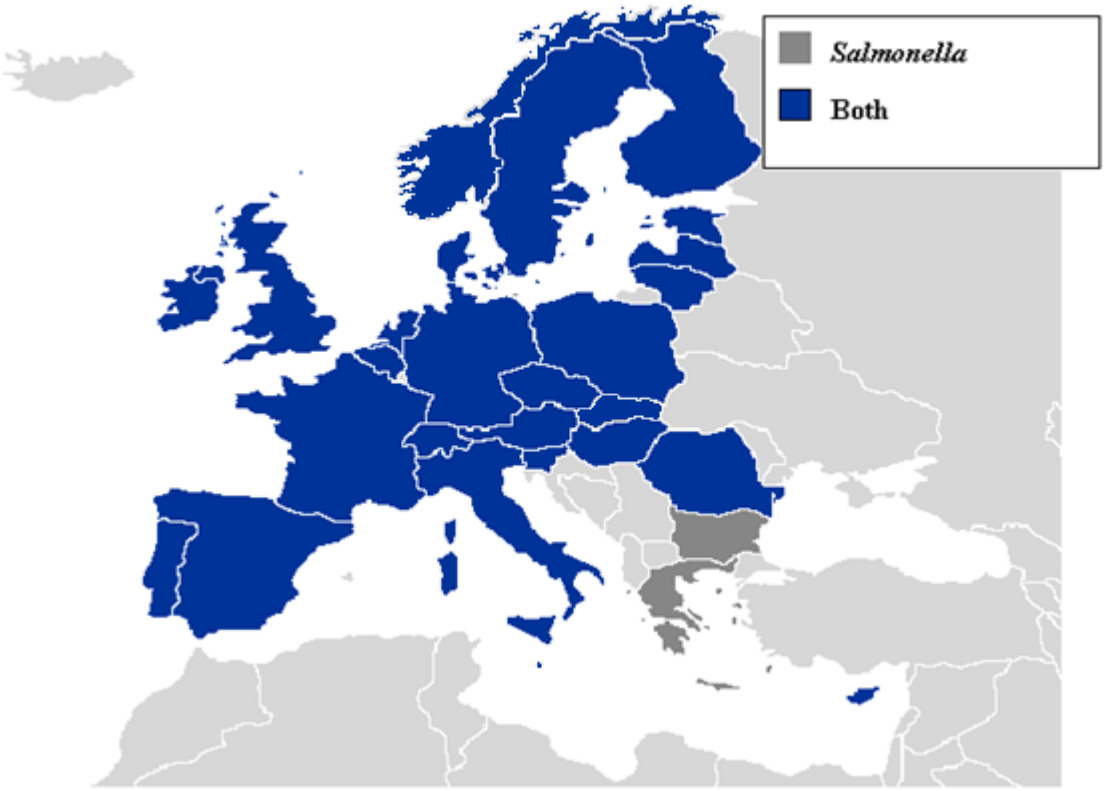

Figure 1: Participating countries that performed antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter*.

Figure 1 shows that out of 28 participating countries, two uploaded only the *Salmonella* results (Bulgaria and Greece), whereas 26 tested both *Salmonella* and *Campylobacter*. The results from the designated NRLs are presented and evaluated in this report; i.e. results from 26

countries consisting of 31 sets of *Salmonella* results and 27 sets of *Campylobacter* results. Four laboratories participated in the optional genotypic characterisation of the *E. faecium* and/or the *Shigella* spp. isolate (not illustrated in Figure 1).

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.

The shipment of strains also 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. This was relevant only for the NRLs which had not been provided with these reference strains in previous EQAS's conducted by DTU Food.

Prior to distribution of the strains, antimicrobial susceptibility testing (AST) on the *Salmonella* and *Campylobacter* strains was performed at DTU Food and verified by the US Food and Drug Administration (FDA). The obtained MIC values served as reference for the test strains (App. 3a and 3b). However, 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*. Furthermore, chloramphenicol and streptomycin for *Campylobacter*.

The test strains offered for optional genotypic characterisation were an *Enterococcus faecium* (EURL GEN 2.1) exhibiting resistance to ampicillin, erythromycin, kanamycin, moxifloxacin, penicillin, streptomycin, tetracycline, and vancomycin, and a *Shigella* spp. (EURL GEN 2.2) exhibiting resistance to ampicillin, cephalothin, cefpodoxime, ceftiofur, chloramphenicol, ciprofloxacin, nalidixic acid, spectinomycin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim (selection of antimicrobials was different from those used for the AST in this EQAS).

2.3 Antimicrobials

The antimicrobials used in the 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 included element on detection of ESBL production.

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

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 microbroth dilution MIC determination (including imipenem), and for the antimicrobials cefotaxime/clavulanic acid, cefoxitin, ceftazidime/clavulanic acid additional tests using E-test from AB-Biodisk, Sweden. The method guidelines used were according to the Clinical and Laboratory Standards Institute (CLSI) document M7-A7 (2006), "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" (Approved Standard - Seventh Edition), document M100-S20 (2010) "Performance Standards for Antimicrobial Susceptibility Testing" (Twentieth 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, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline. MIC determination was performed using the Sensititre systems from Trek Diagnostic Systems Ltd, UK, according to guidelines from the CLSI document M45-A (2006) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria" (Approved Guideline) 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 25th, 2010, 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, they were requested to save and maintain the ATCC reference strain(s) 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) and E-test were recommended to interpret the results according to their individual routine, categorising the test strains into the terms resistant and susceptible. A categorisation as 'intermediate' was not accepted. In these cases, the breakpoints used were submitted to the web based database, from which the relevant breakpoints (disk diffusion for *Salmonella*) are listed in Appendix 5.

It should be noted that 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*. In addition, when reporting monitoring data to EFSA these have to be submitted as MIC-results. It was agreed at the EURL-AR workshop 2009 that only MIC results for *Campylobacter* ASTs are accepted.

The laboratories were instructed to upload the obtained MIC values (mg/L) or inhibition zone diameters (mm) and the susceptibility categories (resistant or susceptible) to an electronic record sheet in the EURL-AR web based 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 4th of November 2010 to the 17th of January 2011.

Detection of ESBL-producing strains should be performed and interpreted according to recommendations by EUCAST described in the protocol. Concerning cefotaxime, ceftazidime and/or ceftiofur used when detecting ESBL-producing strains in this EQAS, MIC values and interpretations for these antimicrobials 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; the CLSI documents M31-A3 (2008) / M100-S20 (2010) / M45-A (2006); The Sensititre System (Trek Diagnostic Systems Ltd, UK); or E-tests (AB-Biodisk, Sweden) (App. 7).

For the optional PCR-testing of the selected Gram-positive and Gram-negative isolate, participating laboratories were requested to report the genes harboured in the test strain. 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. The results were evaluated based on the actual genes identified. The variants of TEM-, CTX-, SHV-, CMY-, OXA-genes as well as the gyrA-mutations and parC-mutations were additionally evaluated. For gyrA and parC, the point of mutation at a specific codon was evaluated in the same way as the genes.

The participating laboratories were encouraged to use their own laboratory's method(s) for the PCR-testing. The expected results for the Gram-positive strain were obtained by the EURL-AR's routine PCR-methods, whereas the expected results for the Gram-negative strain were obtained at the EURL-AR by using miniaturized microarrays (Identibac Amr-ve array tubes; New Haw, Addlestone, Surrey, United Kingdom) containing probes for most relevant Gram-negative antimicrobial resistance gene groups such as quinolone, sulfonamide, tetracycline,

aminoglycoside, carbenicillinase, chloramphenicol exporter/acetyltransferase, florfenicol, trimethoprim, plasmidic AmpC, beta-lactam antimicrobials as well as class 1/2 integrase. Analysis was performed as recommended by the manufacturer. PCR was conducted for confirmation of weak array results. The results for both strains were verified by the US FDA.

After submitting the data, the laboratories were instructed to retrieve the instantly generated, individual evaluation report from the secured web site. The evaluation reports assessed the submitted results, describing all deviations from the expected. Deviations in the interpretation as resistant or susceptible were categorised as 'incorrect', as was also deviations in confirmation of an isolate as ESBL-producer or AmpC.

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 the 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, those who did have received direct reply when relevant. Test ranges for concentrations used when performing MIC for AST were collected in Appendix 8.

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 two cases: for the combination of the test strains S-5.2/streptomycin and S-5.3/streptomycin with a level of agreement with the expected results at 47% and 27%, respectively (Appendix 9a and 9b present the total percentage of correct/incorrect results for each strain/antimicrobial-combinations).

In both cases, the expected MIC (32 mg/L, resistant) and the cut-off value (>16 mg/L) were within one fold dilution difference. The expected values were determined by two different institutions; DTU Food and FDA and were consistent with MIC results of 32mg/L or ≤32mg/L. For both test strains, S-5.2 and S-5.3, the presence of *aadA* was confirmed by PCR by the EURL-AR, whereas the genes *strA* and *strB* were not detected in either of the two test strains.

Figure 2 illustrates the distribution of the different MIC values together with the interpretation of these values obtained by participants performing MIC for the combination of strain S-5.2/streptomycin and S-5.3/streptomycin. The figure shows a distribution of MIC's with the

expected value at 32mg/L and the majority of participants obtaining AST results one MIC-dilution below the expected result. Results from four participants performing disk diffusion have been excluded from these particular analyses.

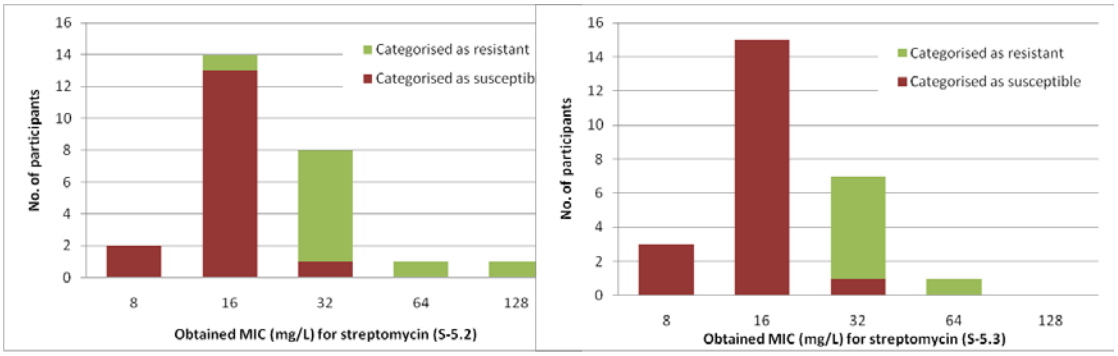

Figure 2: Distribution of the different MIC values obtained by participants performing MIC for the combination S-5.2/streptomycin and S-5.3/streptomycin.

This data was presented and discussed at the EURL-AR workshop, and it was concluded to exclude these two strain/antimicrobial combinations of the evaluation. This conclusion was based on the fact that the precision of the method relies on various factors, including the media content, the type of microbroth panels as well as a number of others, and the fact that an MIC result obtained by the microbroth method or agar dilution can vary +/- one dilution step from the obtained MIC.

3.1 Methods used by EQAS-participants

In the *Salmonella* trial, 27 laboratories used MIC determination, and four laboratories used disk diffusion. For the *Campylobacter* trial, all 27 laboratories reported the use of MIC determination (microbroth or agar dilution).

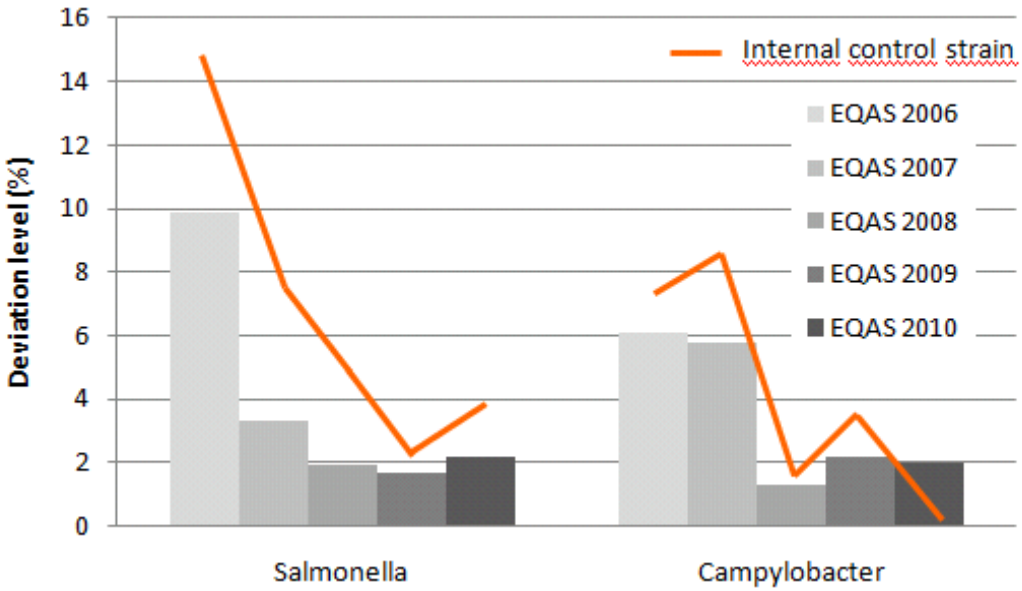

Figure 3: A comparison between the EURL-AR EQAS's since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing performed by participating laboratories

3.2 Deviations by strain and antimicrobial

The list of deviations is shown in Appendix 10a and 10b. Figure 3 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. For the *Salmonella* strains, 97.8% of the AST's were interpreted correctly. For the *Campylobacter* strains, 98.0% of AST's were correctly tested. The internal control strains have mainly followed the trend in deviation level of the different EQAS trials (Figure 3). However, for the *Campylobacter* trial, the internal control strain caused no deviations. The deviation level in 2010 is acceptable for both the *Salmonella* and the *Campylobacter* trials.

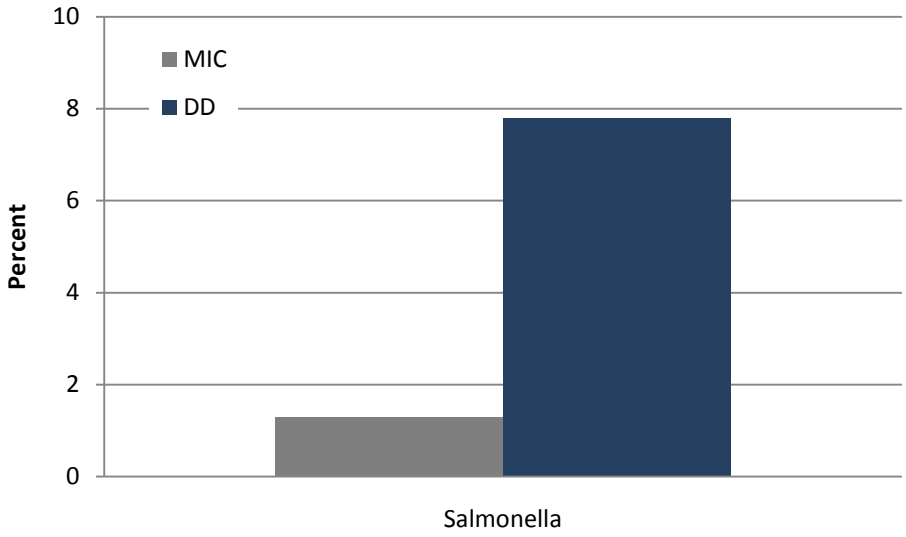

Figure 4: The total percentage of deviations for AST's performed using MIC-methods as opposed to disk diffusion.

Figure 4 shows the total percentage of deviations from the expected results of AST performed by MIC-methods as opposed to disk diffusion. This is relevant for the *Salmonella* trial for which the deviation percentage is significantly higher ($p<0.01$) when AST is performed by disk diffusion compared to a MIC-method.

EQAS 2010 – *Salmonella*			EQAS 2010 – *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-5.1	341	98.8	C-5.1 (*C. jejuni*)	182	100.0
S-5.2	311	97.4	C-5.2 (*C. coli*)	182	97.3
S-5.3	310	97.4	C-5.3 (*C. coli*)	181	97.2
S-5.4	341	99.4	C-5.5 (*C. coli*)	181	95.6
S-5.5	341	96.5	C-5.5 (*C. coli*)	182	97.3
S-5.6	341	97.7	C-5.6 (*C. coli*)	182	98.4
S-5.7	340	96.2	C-5.7 (*C. jejuni*)	174	98.3
S-5.8	342	99.1	C-5.8 (*C. jejuni*)	174	100.0

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

The number of AST's performed and the percentage of correct results for the individual *Salmonella* and *Campylobacter* strains in the EQAS, are listed in Table 1. Variations of

obtained correct results ranged from 96.2-99.4% for *Salmonella* and from 95.6-100% for *Campylobacter*.

For *Salmonella*, the test strain S-5.7 was also included in former EQAS's as internal reference strain. Figure 3 indicates the variation in deviation level over the years. This strain is resistant to ampicillin, cefotaxime, ceftiofur, ciprofloxacin, nalidixic acid, and tetracycline. In former EQASs, this strain was also regarded as resistant towards ceftazidime, but due to a change in the EUCAST expert rules, MIC's for all cephalosporins should be evaluated and reported as found.

Table 2 illustrates the percentage of correct AST per antimicrobial by bacterial species. When testing *Salmonella*, it appeared that the antimicrobial with the lowest percentage of correct AST was ciprofloxacin (90.8%) which could be attributed to some of to the six test strains exhibiting reduced susceptibility towards this antimicrobial.

EQAS 2010 Antimicrobial	% correct	
	Salmonella	*Campylobacter*
Ampicillin, AMP	99.6	-
Cefotaxime, CTX	99.6	-
Ceftazidime, CAZ	97.9	-
Ceftiofur, XNL	100.0	-
Chloramphenicol, CHL	99.2	100.0
Ciprofloxacin, CIP	91.1	99.1
Erythromycin, ERY	-	99.1
Gentamicin, GEN	98.8	100.0
Nalidixic acid, NAL	96.0	97.7
Streptomycin, STR	96.3	95.6
Sulphonamides, SMX	98.4	-
Tetracycline, TET	98.4	94.8
Trimethoprim, TMP	100.0	-

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

For *Campylobacter*, none of the antimicrobials had a notably outlying 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 two test strains S-5.7 and S-5.8 were ESBL-producers, and this was confirmed by the majority of the 31 laboratories participating in the *Salmonella* EQAS. As the ESBL detection part is mandatory in this EQAS, all results are evaluated below.

Both ESBL-producing strains were so-called 'true ESBLs, harbouring $bla_{\text{CTX M-15-like}}$ (S-5.7) and $bla_{\text{CTX M-15}}$ (S-5.8) (Table 3).

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).

		Strain S-5.7 (CTX M-15 like)	**Strain S-5.8 (CTX M-15)**
Proportion of laboratories succesfully using different cephalosporins for screening (correct confirmation of ESBL production)	**CTX, CAZ, XNL**	6/6 (100%)	6/6 (100%)
	CTX, CAZ	18/18 (100%)	17/18 (94%)
	CTX, XNL	1/1 (100%)	1/1 (100%)
	CTX	4/6 (67%)	4/6 (67%)
Confirmed ESBL-producer		29/31 (93%)	28/31 (90%)
FOX^{S}		31/31 (100%)	31/31 (100%)
AmpC not confirmed		31/31 (100%)	31/31 (100%)

Table 3: Proportion of laboratories that obtained the expected result. Number and percentages of laboratories which correctly detected and confirmed the ESBL-producing *Salmonella* strains.

In five occasions, the ESBL-producing strain was not detected. Four of these deviations were due to two laboratories which did not perform the confirmatory testing (laboratory #38 and #39) which was also the case in EURL-EQAS 2009. The remaining case appears to be a lapse

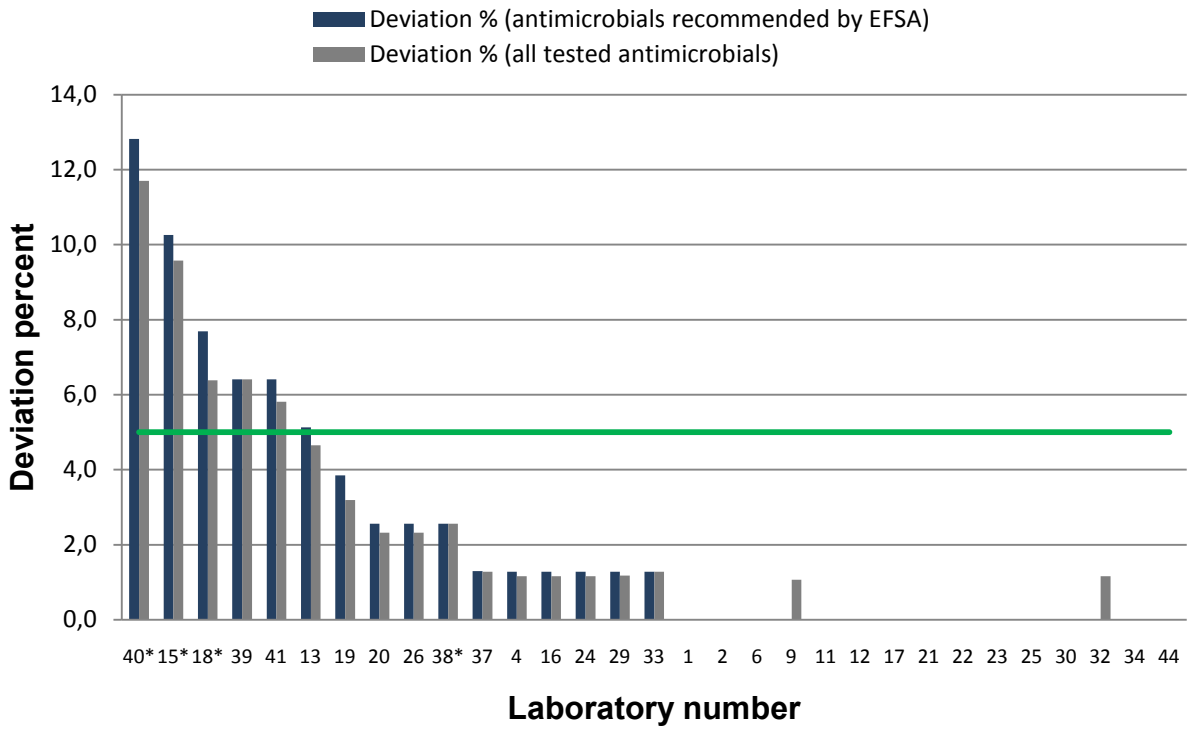

Figure 5: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory performed AST using disk diffusion

of registration in the database, as the uploaded screening and confirmatory results were all in agreement with the expected.

Thirteen laboratories uploaded an MIC-ratio as a result, and 14 uploaded the increase of inhibition zone diameter, additionally, two laboratories uploaded both an MIC and an inhibition zone diameter result. All results uploaded on confirmatory tests were in accordance with the expected, and led to the correct confirmation of ESBL production in all cases with the exception mentioned above.

According to the expected results, none of the laboratories reported resistance to cephalosporins for any of the non-ESBL-producing strains.

3.3 Deviations by laboratory

Figure 5 and 7 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. In Figure 6 and 8, the total amount of deviations in percentages is illustrated by number of laboratories.

3.3.1 *Salmonella* trial

Twenty-five of the laboratories obtained a result within the acceptance limit at 5% deviations for the *Salmonella* strains. The maximum percentage of deviations was 12.8%.

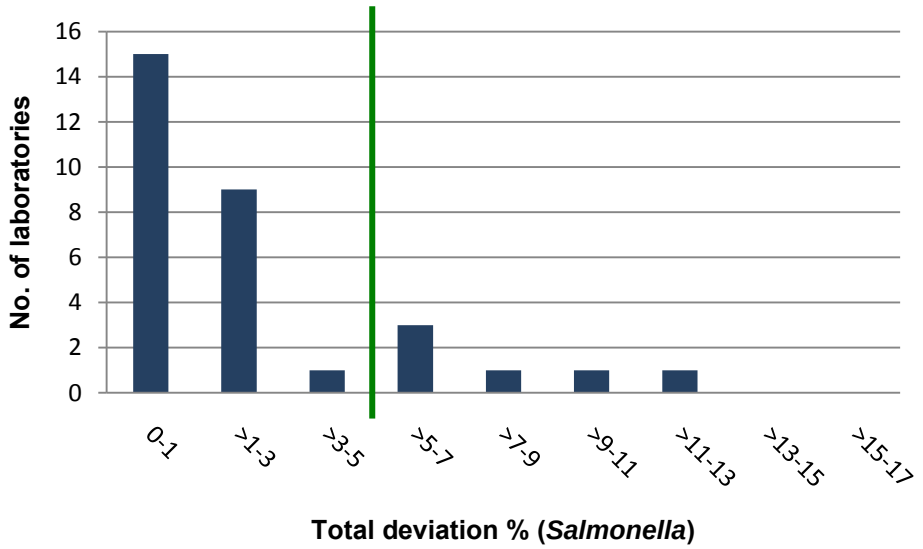

Figure 6: The number of laboratories listed in intervals of percent of total deviations. The green line marks the 5% acceptance limit set by the EURL-AR

Figure 6 illustrates the performance of six (19%) laboratories which resulted in a deviation level above the level of performance expected by the EURL-AR (#13, #15, #18, #39, #40, and #41), however, none of the laboratories are regarded as outliers. As illustrated in Figure 5,

deviation levels including all antimicrobials mentioned in the protocol 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, 22 of 27 participating laboratories performed acceptably, with 17 laboratories having no deviations (Figure 7 and 8). Five laboratories present a deviation level above the 5% acceptance level (#19, #21, #22, #30, and #44). No laboratories are regarded as outliers.

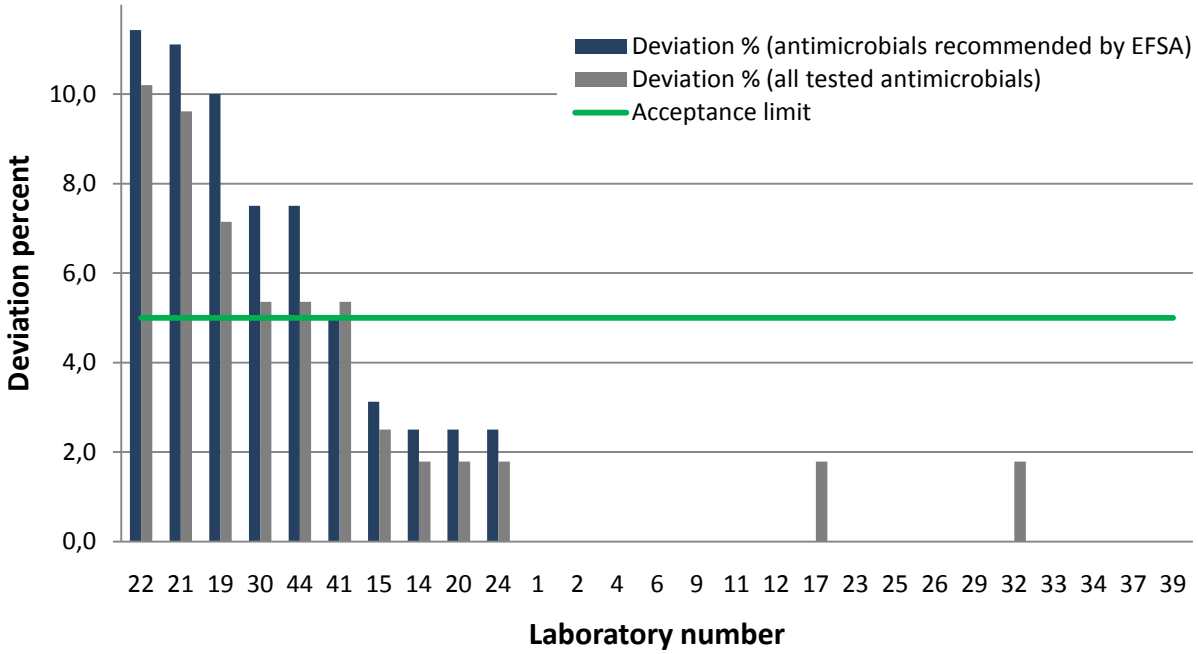

Figure 7: Individual participants' deviations in percent of their total number of *Campylobacter* AST's.

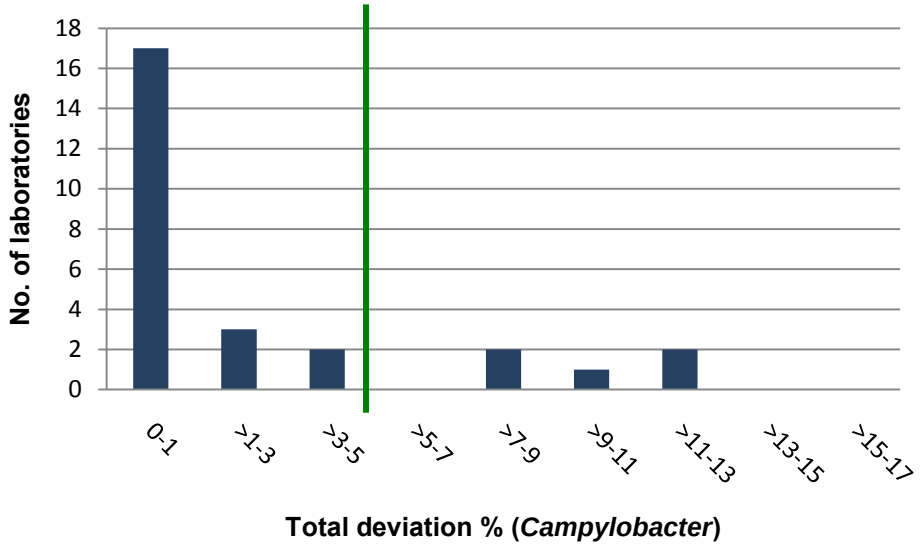

Figure 8: The number of laboratories listed in intervals of percent of total deviations.

Deviation levels including results obtained for all antimicrobials mentioned in the protocol vary to a relatively high extent from the deviation levels including results obtained for antimicrobials recommended by EFSA, only. The higher deviation levels generally showing for the latter group of antimicrobials.

3.4 Deviations by reference strains

In this 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 Tables

EQAS 2010 Antimicrobial	**Disk diffusion *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in mm inhibition zones (min/max)	
		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	1/4 (25%)	-	2
Cefotaxime, CTX	1/4 (25%)	-	1
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/3 (0%)	-	-
Ceftiofur, XNL	1/3 (33%)	2	-
Chloramphenicol, CHL	0/4 (0%)	-	-
Ciprofloxacin, CIP	0/4 (0%)	-	-
Gentamicin, GEN	0/4 (0%)	-	-
Imipenem, IMI	1/3 (33%)	-	3
Nalidixic acid, NAL	0/4 (0%)	-	-
Streptomycin, STR	0/4 (0%)	-	-
Sulphonamides, SMX	0/3 (0%)	-	-
Tetracycline, TET	0/4 (0%)	-	-
Trimethoprim, TMP	0/4 (0%)	-	-

Table 4: Obtained values for AST of *E. coli* ATCC 25922 by disk diffusion.

EQAS 2010 Antimicrobial	**MIC determination *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/27 (0%)	-	-
Cefotaxime, CTX	0/26 (0%)	-	-
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/19 (0%)	-	-
Ceftiofur, XNL	0/2 (0%)	-	-
Chloramphenicol, CHL	0/27 (0%)	-	-
Ciprofloxacin, CIP	2/27 (8%)	-	1 step
Gentamicin, GEN	0/27 (0%)	-	-
Nalidixic acid, NAL	0/26 (0%)	-	-
Streptomycin, STR	1/25 (4%)	1 step	-
Sulphonamides, SMX	0/17 (0%)	-	-
Tetracycline, TET	0/27 (0%)	-	-
Trimethoprim, TMP	0/25 (0%)	-	-

Table 5: Obtained values for AST of *E. coli* ATCC 25922 by MIC determination

4, 5 and 6 which summarize results from the laboratories' quality control. For the *Salmonella* trial, all laboratories performed QC testing of the reference strain. For the *Campylobacter* trial, 25 of the 27 participating laboratories uploaded data from QC-testing on the reference strain.

Table 4 presents the proportion of laboratories that obtained values out of range for the *E. coli* reference strain (ATCC 25922), when performing disk diffusion. For four out of 14 antimicrobials, a value outside the QC-range was obtained. Three of these values were uploaded by one laboratory (#15).

The use of MIC determination for AST of the reference strain *E. coli* ATCC 25922 resulted in submission of data from twenty-six laboratories, three of which produced one value each outside the QC-limit as illustrated in Table 5.

EQAS 2010	**MIC determination *C. jejuni* ATCC 33560**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	0/16 (0%)	-	-
Ciprofloxacin, CIP	1/25 (4%)	-	3 steps
Erythromycin, ERY	2/25 (8%)	1 step	1 step
Gentamicin, GEN	4/18 (22%)	1 step	-
Nalidixic acid, NAL	0/22 (0%)	-	-
Tetracycline, TET	2/22 (9%)	-	1 step

Table 6: Obtained values for AST of *C. jejuni* ATCC 33560 using MIC determination

Twenty-five laboratories performed MIC determination for the *C. jejuni* reference strain ATCC 33560. Table 6 presents the proportion of the laboratories with results for the QC strain below or above the QC interval. Deviations were seen for four antimicrobials with ciprofloxacin exhibiting the largest deviation (3 steps above the upper QC-limit).

3.5 Genotypic characterisation

For the optional PCR-testing of selected isolates, one and four laboratories performed the genotypic characterization on the Gram positive test strain GEN 2.1 and the Gram negative test strain GEN 2.2, respectively. In Appendix 11, there is information on detected genes, on genes which were tested but not detected, on primers used, and references for the method used. Table 7 shows that for all the uploaded results there is good correlation with the expected genes.

For GEN 2.1, the participating laboratory reported the presence of the tet(K) gene in addition to the genes expected by the EURL. The primers used at the EURL-AR for amplifying the tet(K)-gene are known to also amplify the tet(L)-gene and thereby render a false positive result for tet(K) when tet(L) is present and tet(K) is not. Therefore, both PCR products were sequenced to detect a possible false positive result; both the presumptive tet(K) and the tet(L) were verified as amplicons of tet(L).

For the GEN 2.2, two laboratories recorded the expected result, CTX-M-14, whereas one recorded the CTX-M-9-group (which also includes the CTX-M-14 variant). In addition, due to the fact that the EQAS-organisers during the preparations were not able to attribute the detected OXA-gene to either OXA-1 or OXA-30, it was decided to add both of these to the list of expected genes for the Gram-negative isolate.

Two of the four participants recorded that the majority or all of the utilized PCR-methods were published, whereas one made use of in-house PCR-methods and one did not upload information as to the methods used. One laboratory informed of the use of a microarray (Clondiag, VLA, UK; Identibac).

			Lab I		Lab III		Lab IV		Lab V	
EURL GEN 2.1	Aminoglycosides	aadE	1	in	NT		NT		NT	
	Aminoglycosides	aph(3')-III	1	in	NT		NT		NT	
	Glycopeptide	vanB	1	in	NT		NT		NT	
	Macrolides	erm(B)	1	in	NT		NT		NT	
	Penicillin	pbp5	1	in	NT		NT		NT	
	Tetracycline	tet(L)	1	in	NT		NT		NT	
	Tetracycline	tet(M)	1	in	NT		NT		NT	
	Additional genes detected		tet(K)	in	NT		NT		NT	
EURL GEN 2.2	Betalactams	CTX-M-14	1/NT	in	1/1	P	1/NT		1/1	P
	Betalactams	OXA-1	1/NT	in	1/1	P	1/NT		1/NT	P
	Betalactams	OXA-30	1/NT	in	1/NT	P	1/NT		1/1	P
	Chloramphenicol	catA1	1	in	1	P	1		1	P
	Quinolones	gyrA-83	NT		1/1	P	NT		1/1	P
	Quinolones	parC-80	NT		1/1	P	NT		NT	
	Streptomycin	strA	1	in	1	P	1		1	P
	Streptomycin	strB	1	in	1	P	1		1	in
	Streptomycin	aadA	1	in	1	P	1		1	P
	Sulfamethoxazole	sul2	1	in	1	P	1		1	in
	Tetracycline	tetB	1	in	1	P	1		1	P
	Groups detected		CTX-M-9	in						

Table 7: Results from genotypic characterisation.
Legend:
1 indicates identification in accordance with the expected
- indicates identification not in accordance with the expected
1/1 indicates 'correctly identified gene or gene group'/'specific gene or mutation correctly identified'
1/- indicates that the PCR-product was not sequenced to obtain a specific gene- or codon mutation
NT indicates 'Not tested'
P indicates that a published PCR-method was used
in indicates that an in-house protocol was used
Laboratory numbers are not consistent with the numbers otherwise used in this report, but they are consistent with the number used for the genotypic characterisation in the 2009-iteration.

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct antimicrobial susceptibility test results of *Salmonella* was 97.8%. The majority (n=25) 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 2010-EQAS would appear to be at the same level compared to the performance in the former four iterations.

Three (#15, #18, and #40) of the six laboratories exhibiting a deviation level higher than 5% performed disk diffusion for AST and obtained deviation levels at 10.3%, 7.7%, and 12.8% , respectively. The additional three laboratories (#13, #39 and #41) performed MIC for AST and obtained deviation levels at 5.1%, 6.4% and 6.4%, respectively. None of them was defined as outlier.

Ciprofloxacin appeared to cause the majority of the deviations for these six laboratories; for laboratory #41, the use of the low cut-off value for ciprofloxacin presented in the protocol would have eliminated five deviations and thereby resulted in one deviation only; laboratory #18 and #40 failed to record all five *Salmonella* test strains exhibiting reduced susceptibility to ciprofloxacin as resistant. These deviations indicate that the recommendations published by Cavaco and Aarestrup (2009) regarding interpretation of ciprofloxacin results could improve the quality of the results when performing disk diffusion of AST. In addition, laboratory #40 did not detect the *qnr*-positive *Salmonella* test strain (S-5.6), which was, on the contrary, correctly categorised as resistant to ciprofloxacin by laboratory #18.

For laboratory #15, the deviations neither on the test strain nor on the reference strain allow to speculate that a methodical reason should have caused the deviations. Indeed, the ten deviations are caused by combinations of seven test strains and six antimicrobials, and the results obtained for the *E. coli* QC reference strain show no deviations on the six antimicrobials in question. Also, for laboratory #13, the four deviations could not be attributed to a specific reason.

Laboratory #39 obtained seven deviations on the test strains but exhibit no deviations for the *E. coli* QC-reference strain. Two of the deviations were due to correct MIC-value being interpreted according to other interpretative criteria than those listed in the protocol. The additional five deviations are results from testing four different antimicrobials towards the S-5.2 and S-5.5, and unexpectedly obtaining a conclusion that the strain was resistant.

The relatively low performance regarding ciprofloxacin presented in Table 2 (90.8% correct results), was mainly caused by the three laboratories mentioned above (#18, #40, and #41). One additional laboratory (#13) also failed to interpret the obtained MIC value according to the cut-off value. In addition, when performing disk diffusion, the issue regarding the low cut-off value for ciprofloxacin is addressed in the protocol '*Salmonella* strains resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin'. These guidelines appear to have been followed by only two of the four laboratories performing disk diffusion.

The test strain S-5.6 was a *Salmonella* strain harbouring a plasmid-mediated quinolone resistance gene; *qnrS*. This *qnr*-gene confers low-level resistance to ciprofloxacin (MIC=0.5mg/L), but not to nalidixic acid (MIC=16mg/L). The participants generally found this isolate susceptible to nalidixic acid (97%), whereas only 83% found the isolate resistant to ciprofloxacin. The reduced susceptibility towards ciprofloxacin resistance caused by a *qnr*-gene is difficult to detect when performing disk diffusion as the usual association between ciprofloxacin and nalidixic acid is not seen.

For the *E. coli* reference strain, the results obtained were in general in agreement with the CLSI recommendations. The number of laboratories performing AST on *Salmonella* by the use of disk diffusion was four. All of these laboratories uploaded data for the testing of the reference strain, and a total of 92.3% were within range. For the laboratories performing AST on

Salmonella by an MIC-method, all laboratories uploaded QC-results to the database. The proportion of values within the expected range was 98.9%.

Laboratories #15 and #40 which had a deviation level above the acceptance limit in EQAS 2009 showed values of 5.6% and 7.5% in 2009, respectively, which appear to follow the overall trend and have decreased in performance to 10.2% and 12.2% deviations, respectively, in the 2010-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.

Two of the *Salmonella* test strains were ESBL-producers (S-5.7 and S-5.8), and the participants were asked to interpret their results according to the description in the protocol. Of the 30 laboratories which tested *Salmonella*, two did not upload results for confirmatory testing of ESBL-production which resulted in an evaluation as incorrect. The 28 laboratories which uploaded results appear to be confident in detecting and confirming the two ESBL-producers (S-5.7 and S-5.8) with the overall proportion of laboratories confirming S-5.7 and S-5.8 as ESBL-producers being 93% and 90%, respectively.

Comparison of obtained results when performing confirmatory tests by either of the two methods: measurement of inhibition zone diameters (disk diffusion) or by obtaining a MIC-ratio (E-test) does not show indication of differences for the confirmation on ESBL-production.

In this EQAS, it appeared that laboratories performing the initial screening with cefotaxime only, had problems in the detection of the ESBL production. The two laboratories which are registered with incorrect ESBL results for both ESBL-positive strains (#39 and #40) only utilized cefotaxime for screening for ESBL production whereas 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 the other two.

Interestingly, both laboratories obtained results that the test strains S-5.7 and S-5.8 were resistant towards cefotaxime. This result, however, did not lead to confirmatory testing. Laboratory #39 has requested more information and advice on ESBL- detection and confirmation which will be part of the follow-up subsequent to this EQAS cycle. In addition, laboratory #38 will be contacted for clarification of the absent results.

4.2 *Campylobacter* trial

The overall percentage of correct antimicrobial susceptibility test results of *Campylobacter* was 98.0%. The performance varied from no deviations to 11.4% deviations, with 22 laboratories performing satisfactorily according to the established acceptance ranges. Of the five

laboratories (#19, #21, #22, #30 and #44) with deviation levels above 5%, none were defined as outliers.

The deviation levels above 5% appear to be caused by different reasons: Laboratory #19 incorrectly detected streptomycin resistance in three of the *Campylobacter* test strains. No reference values are available for streptomycin for the *C. jejuni* reference strain; for laboratory #21, four different test strains combined with four different antimicrobials resulted in five deviations. This laboratory did not upload values for the *C. jejuni* QC reference strain; for laboratory #22, the test strain C-5.5 apparently posed a problem, as tests against four of the antimicrobials rendered incorrect results. This could be caused by a contamination of the test isolate; Laboratories #30 and #44 had deviation levels close to 5% when including results from all antimicrobials mentioned in the protocol. Both laboratories had two deviations for tetracycline. The result uploaded by laboratory #30 on the reference strain was within range, however, on the top limit, whereas laboratory #44 did not upload a value for the reference strain on this antimicrobial. In addition, laboratory #44 commented that the two isolates incorrectly categorised as resistant to tetracycline, would have been recorded as susceptible, if the daily routine method was followed.

The proportion of results for the *C. jejuni* reference strain within the QC intervals was 93.2% which is the same level as in EQAS 2009. In this year's trial, 25 of 27 participating laboratories uploaded data from tests performed on the reference strain. The eight values outside the QC intervals were obtained by six laboratories, five of which performed well under the 5% acceptance level. The remaining one had a deviation level at 10% (laboratory #19).

A follow-up on the laboratory which was an outlier in the *Campylobacter* trial in EQAS 2009 (#39) shows a tremendous improvement from a deviation level in 2009 at 25% to 0% in 2010.

4.3 Optional genotypic characterisation of selected *Salmonella* test strain

As the focus on molecular aspects appear to be increasing, it is likely that genotypic characterisation of relevant bacterial isolates in the future will gain further interest. The genotypic characterisation offered as an optional supplementary part of this EQAS was performed by four laboratories. All participating laboratories 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 (97.8%) when compared to the results from the EQAS 2008 and 2009 (98.0% and 98.4%). Regarding *Campylobacter* AST's, the level of deviation also appears to be stable with a level at 2.0% in 2010 compared to 1.3% and 2.2% in 2008 and 2009.

Laboratories which have not yet introduced tests to detect ESBL-producing *Enterobacteriaceae*, should prioritize this area, as these antimicrobial resistance mechanisms appear to continue to emerge worldwide. In addition, the genotypic characterisation which was

offered as an optional supplementary part of this EQAS appeared to be of interested to the EURL-AR network, and is likely to be repeated.

6. References

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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

European Union Reference Laboratory Antimicrobial Resistance

EURL-AR EQAS pre-notification

DFVF- M00-06-001/21.05.2010

EQAS 2010 for *Salmonella*, *Campylobacter* and optional genotypic characterisation

The EURL-AR are pleased to announce the launch of another EQAS. The EQAS provides the opportunity for proficiency testing, which is considered an important tool for the production of reliable laboratory results of consistently good quality.

This EQAS offers antimicrobial susceptibility testing of eight *Salmonella* isolates, eight *Campylobacter* isolates and two strains for genotypic characterisation (one Shigella and one *Enterococcus*). Additionally, new participants will be offered the following QC strains: *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214).

This EQAS is specifically for NRL's on antimicrobial resistance. Therefore, you do not need to sign up to be a participant. All who receive this pre-notification are automatically regarded as participants. Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
Please remember to provide the EQAS coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the content of the parcel is "Biological Substance Category B". The strains are expected to arrive at your laboratory in October 2010.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped in October 2010. The protocol will be available on the website (www.eurl-ar.eu).

Returning of results: Results must be returned to the National Food Institute, by December 31st 2010. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

EQAS report: When the EQAS is concluded, the data will be collected in an overall report in which it is possible to see all participants' results in comparison. In the report the laboratories will be coded, which ensures full anonymity; only the National Food Institute and the EU Commission will be given access to un-coded results.

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 2011.

Any comments regarding the EQAS, please contact me by e-mail (suska@food.dtu.dk) or by fax (+45 3588 6341).

Sincerely,

Susanne Karlsmose
EQAS-Coordinator

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Ph: + 45 3588 6601, Fax: + 45 3588 6001, e-mail: suska@food.dtu.dk

Participant list

Salmonella	*Campylobacter*	Genotypic characterisation	Institute	Country
X	X	-	Austrian Agency for Health and Food Safety	Austria
X	X	-	Institute of Public Health	Belgium
X	-	-	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	X	-	Veterinary Services	Cyprus
X	X	-	State Veterinary Institute Praha	Czech Republic
X	X	X	The National Food Institute	Denmark
X	-	-	The National Veterinary 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 Diagnostical Directorate	Hungary
-	-	-	University of Iceland	Iceland
X	X	-	Central Veterinary Research Laboratory	Ireland
X	X	-	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	-	Institute of Food Safety, Animal Health and Environment „BIOR"	Latvia
X	X	-	National Veterinary Laboratory	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	-	National Institute of Research-Development for Microbiology and Immunology "Cantacuzino"	Romania
X	X	-	Institute for Hygiene and Veterinary Public Health	Romania
-	-	-	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	-	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	-	Complutense University of Madrid	Spain
X	-	-	Centro nacional de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricio	Spain
X	X	-	National Veterinary Institute, SVA	Sweden
X	X	-	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	X	-	The Veterinary Laboratory Agency	United Kingdom
X	X	X	Centre for Infections Health Protection 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-confirmation CTX:CTX/Cl		Ceftazidime CAZ		ESBL-confirmation CAZ:CAZ/Cl		Cefoxitin		Ceftiofur XNL		Chloramphenicol CHL		Ciprofloxacin CIP		Gentamicin GEN		Imipenem IMI		Nalidixic acid NAL		Streptomycin STR		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP	
EURL S-5.1	> 32	RESIST	= 0.25	SUSC			= 0.5	SUSC					= 2	SUSC	> 64	RESIST	= 1	RESIST	<= 0.25	SUSC			> 64	RESIST	= 64	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-5.2	> 32	RESIST	= 0.25	SUSC			= 1	SUSC					= 2	SUSC	= 8	SUSC	= 1	RESIST	<= 0.25	SUSC			> 64	RESIST	= 32	RESIST	> 1024	RESIST	= 4	SUSC	> 32	RESIST
EURL S-5.3	= 2	SUSC	= 0.25	SUSC			= 0.5	SUSC					= 2	SUSC	= 64	RESIST	= 0.5	RESIST	= 8	RESIST			> 64	RESIST	= 32	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-5.4	<= 1	SUSC	<= 0.12	SUSC			= 0.5	SUSC					<= 0.5	SUSC	= 4	SUSC	= 0.03	SUSC	= 0.5	SUSC			= 4	SUSC	= 16	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-5.5	> 32	RESIST	<= 0.12	SUSC			= 0.5	SUSC					= 1	SUSC	= 4	SUSC	= 1	RESIST	<= 0.25	SUSC			= 16	SUSC	> 128	RESIST	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-5.6	> 32	RESIST	<= 0.12	SUSC			= 0.25	SUSC					= 0.5	SUSC	= 4	SUSC	= 0.5	RESIST	= 1	SUSC			= 16	SUSC	= 16	SUSC	= 32	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-5.7	> 32	RESIST	> 4	RESIST	>8		= 1	SUSC	<8		= 4	SUSC	> 8	RESIST	<= 4	SUSC	= 0.25	RESIST	= 1	SUSC	<= 0.5	SUSC	> 64	RESIST	= 16	SUSC	= 64	SUSC	= 32	RESIST	<= 1	SUSC
EURL S-5.8	> 32	RESIST	> 4	RESIST	>8		= 128	RESIST	>8		= 4	SUSC	> 8	RESIST	<= 4	SUSC	= 0.03	SUSC	= 1	SUSC	<= 0.5	SUSC	= 4	SUSC	= 16	SUSC	= 64	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. jejuni	EURL C-5.1	= 4	SUSC	= 0.06	SUSC	= 1	SUSC	= 0.25	SUSC	= 4	SUSC	<= 1	SUSC	= 0.12	SUSC
C. coli	EURL C-5.2	= 4	SUSC	= 4	RESIST	> 64	RESIST	= 0.25	SUSC	= 64	RESIST	> 16	RESIST	= 16	RESIST
C. coli	EURL C-5.3	<= 2	SUSC	= 0.25	SUSC	> 64	RESIST	= 0.25	SUSC	= 8	SUSC	<= 1	SUSC	= 1	SUSC
C. coli	EURL C-5.4	= 4	SUSC	= 0.25	SUSC	> 64	RESIST	= 0.5	SUSC	= 8	SUSC	<= 1	SUSC	= 2	SUSC
C. coli	EURL C-5.5	= 4	SUSC	= 4	RESIST	= 0.5	SUSC	= 0.25	SUSC	= 64	RESIST	> 16	RESIST	= 0.25	SUSC
C. coli	EURL C-5.6	= 4	SUSC	= 0.25	SUSC	> 64	RESIST	= 0.25	SUSC	= 8	SUSC	<= 1	SUSC	> 64	RESIST
C. jejuni	EURL C-5.7	<= 2	SUSC	= 0.12	SUSC	= 1	SUSC	= 0.25	SUSC	= 4	SUSC	<= 1	SUSC	= 64	RESIST
C. jejuni	EURL C-5.8	<= 2	SUSC	= 16	RESIST	= 2	SUSC	= 0.25	SUSC	> 64	RESIST	<= 1	SUSC	> 64	RESIST

Resistant

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DFVF- M00-06-001/21.05.2010

European Union Reference Laboratory Antimicrobial Resistance

EURL-AR External Quality Assurance System 2010

- *Salmonella*, *Campylobacter* and optional genotypic characterisation

Id: >>Id<<
>>Institute<<
>>Country<<

Kgs. Lyngby, October 2010

Dear >>name<<,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2010.

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 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 additional strains (EURL GEN 2.1 and EURL GEN 2.2) are included for optional genotypic characterisation. In the protocol you will find detailed description of how to test the strains. Additionally, you will find a description of how to enter your results into the interactive web database. For entering data 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

After receipt, 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.

The results should be returned to us no later than **December 31st, 2010**.

Please acknowledge receipt of parcel immediately on arrival (by email to suska@food.dtu.dk). For further information, please do not hesitate to contact us.

Yours sincerely,

Susanne Karlsmose
EQAS-Coordinator

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2010

DTU Food
National Food Institute

PROTOCOL

For susceptibility testing of *Salmonella*, *Campylobacter* and optional genotypic characterisation of two test 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 susceptibility testing of *Salmonella* and *Campylobacter*. The *Salmonella* and *Campylobacter* EQAS 2010 will include 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 PCR-testing of a selected Gram-negative isolate and a selected Gram-positive isolate 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.

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 2010

3.2 3.1 Shipping, receipt and storage of strains

In October 2010, the EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing eight *Salmonella*, eight *Campylobacter* strains and 2 additional strain(s) for optional PCR (one *Shigella* and one *Entercoccus*). Reference strains will be included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material.

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.

3.3 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).

3.4 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 3.3.1 and 3.3.2 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 sensitive. 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.

3.4.1 *Salmonella*

Testing of gentamicin and streptomycin 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.

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.

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
Streptomycin (STR)	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)

ESBL production

The following tests regarding ESBL production are mandatory: All strains resistant against cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) should be confirmed by confirmatory tests for ESBL production.

The confirmatory tests for ESBL production require testing with a pure antimicrobial (CTX and CAZ) vs. a test with the same antimicrobial combined with a β-lactamase inhibitor (clavulanic acid). Synergy is defined as a 3 dilution steps difference between the two compounds in at least one of the two cases (MIC ratio ≥ 8, E-test 3 dilution steps) or an increase in zone diameter ≥ 5 mm (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Confirmatory tests for Metallo beta lactamase require comparison between imipenem (IMI) and IMI/EDTA, synergy is in this test defined as a MIC ratio ≥ 8 or E-test 3 dilution steps difference (CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

Additionally, AmpC detection can be performed by testing the microorganism to cefoxitin (FOX), resistance to FOX could indicate AmpC. Verification of AmpC requires PCR or sequencing.

The EURL-AR aim to harmonize with EUCAST expert rules. Concerning **cefotaxime, ceftazidime and/or ceftiofur** used when detecting ESBL-producing strains in this EQAS, MIC values and interpretations for these antimicrobials should be reported as found.

3.4.2 *Campylobacter*

Antimicrobials for *Campylobacter*	MIC (µg/mL) **R is >** *C. jejuni*	MIC (µg/mL) **R is >** *C. coli*
Chloramphenicol*	16	16
Ciprofloxacin	1	1
Erythromycin	4	16
Gentamicin	1	2
Nalicixic acid*	16	32
Streptomycin	2	4
Tetracycline	2	2

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

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

The sub-cultured *Campylobacter* should be used for the MIC-testing after incubation at 36-37ºC for 48 hours or 42ºC for 24 hours.

3.5 Optional genotypic characterisation

An optional PCR-testing of a selected *E. faecium* (EURL GEN 2.1) as well as a *Shigella* (EURL GEN 2.2) isolate is offered. If performing the genotypic characterisation of these test strains, the results requested are the genes harboured in the test strain. The genes listed in Tables 3 and 4 below are those included in the test. The test strains may harbour resistance genes not present on these lists; these will not be evaluated by the database, but may be mentioned in the comments-field. When uploading the results in the database, the identified genes will be evaluated against the expected results. The results will be evaluated on the actual gene identified. The groups of TEM-, CTX-, SHV-, CMY-, OXA-genes as well as the gyrA-mutations and parC-mutations will additionally be evaluated on the group selected. For gyrA and parC the codon-no of the site of mutation will be evaluated in the same way as the genes.

The method used for the PCR-testing should be the one(s) used in your laboratory. The expected results listed in the database are those obtained by the CRL (as this is a pilot study the results have not been verified elsewhere).

Antimicrobial	**Gene**
Aminoglycosides	aadE
	aac(6')-aph(2")
	aph(3')-III
Chloramphenicol	catpIP
Glycopeptide	vanA
	vanB
Macrolides	erm(A)
	erm(B)
Oligosaccharides	emtA
Penicillin	pbp5
Streptogramin A	vat(D)
	vat(E)
Streptogramin B	vgbA
Tetracycline	tet(K)
	tet(L)
	tet(M)
	tet(O)
	tet(S)

Table 3: Genes included in the test of the *E. faecium*-strain
Reference: Simjee, S. *et al.* Enterococcus (2006). In: Frank M. Aarestrup (Ed.) Antimicrobial Resistance in Bacteria of Animal Origin, ASM Press, Washington, D.C., pp. 315-328.

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Antimicrobial	Group	Gene/Codon no.
Betalactams	TEM	List of gene numbers in the database
	CTX	List of gene numbers in the database
	SHV	List of gene numbers in the database
	CMY	List of gene numbers in the database
	OXA	List of gene numbers in the database
Chloramphenicol	-	cmlA
	-	catA1
Florphenicol	-	floR
Gentamicin	-	aac(3)-IV
	-	ant(2")-I
	-	aac(3)-II
Neomycin	-	aph(3)-III
	-	aph(3)-II
	-	aph(3)-I
Quinolones	gyrA	Codon 83
	gyrA	Codon 87
	parC	Codon 57
	parC	Codon 78
	parC	Codon 80
	parC	Codon 84
	-	qnrA
	-	qnrB
	-	qnrC
	-	qnrD
	-	qnrS
Streptomycin	-	strA
	-	strB
	-	aadA
Sulfamethoxazole	-	sul1
	-	sul2
	-	sul3
Tetracycline	-	tetA
	-	tetB
	-	tetC
	-	tetD
	-	tetE
	-	tetF
	-	tetG

Table 4: Genes included in the test of the *Shigella*-strain

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4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the test forms, and enter your results into the interactive web database. Please read the detailed description below before entering your results. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest December 31st, 2010.

If you do not have access to the Internet, or if you experience difficulties entering the data, please return results by e-mail, fax or mail to the National Food Institute.

All results will be summarized in a report which will be made available to all participants. The data in the report will be 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 and the EU Commission. All conclusions are public.

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

Fax: +45 3588 6341

E-mail: suska@food.dtu.dk

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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 2010 start 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 optional PCR results. 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

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

- ☐ MIC – Microtitre
- ☐ MIC – Agar dilution
- ☐ Strips – E-test
- ☐ Discs, tablets
- ☐ Rosco, Neo Sensitabs

Brand**:**

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

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

Comments or additional information:

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

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

- ☐ MIC – Microtitre
- ☐ MIC – Agar dilution
- ☐ Strips – E-test
- ☐ Discs, tablets
- ☐ Rosco, Neo Sensitabs

Brand**:**

Incubation conditions: °C/ h

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

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

Comments or additional information:

Antimicrobial	General info The relevant information should be filled in below
	Test-range for MIC (µg/mL)
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-5.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-5.1 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.2 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.3 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.4 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter EURL C-5.5 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.6 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.7 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter EURL C-5.8 *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 2.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’:
Gene: ☐ Found ☐ Tested, not found	☐ Published method , reference:
	☐ In-house method
	Primer used 5’→3’:
	Primer used 3’→5’:

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-S18, January 2008 (Performance Standards for Antimicrobial Susceptibility Testing)

M7-A7, January 2006 (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% fecal 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:

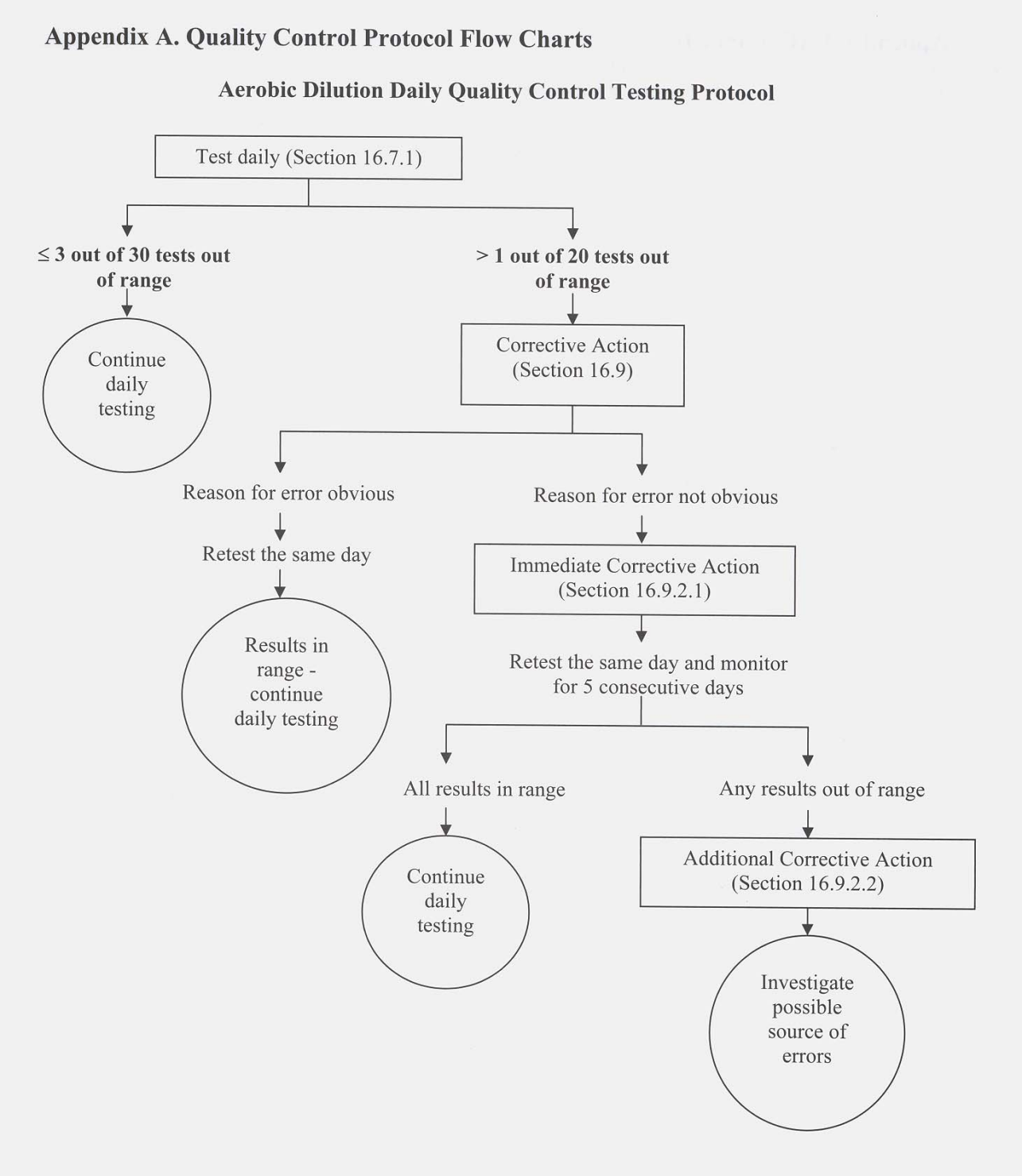
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

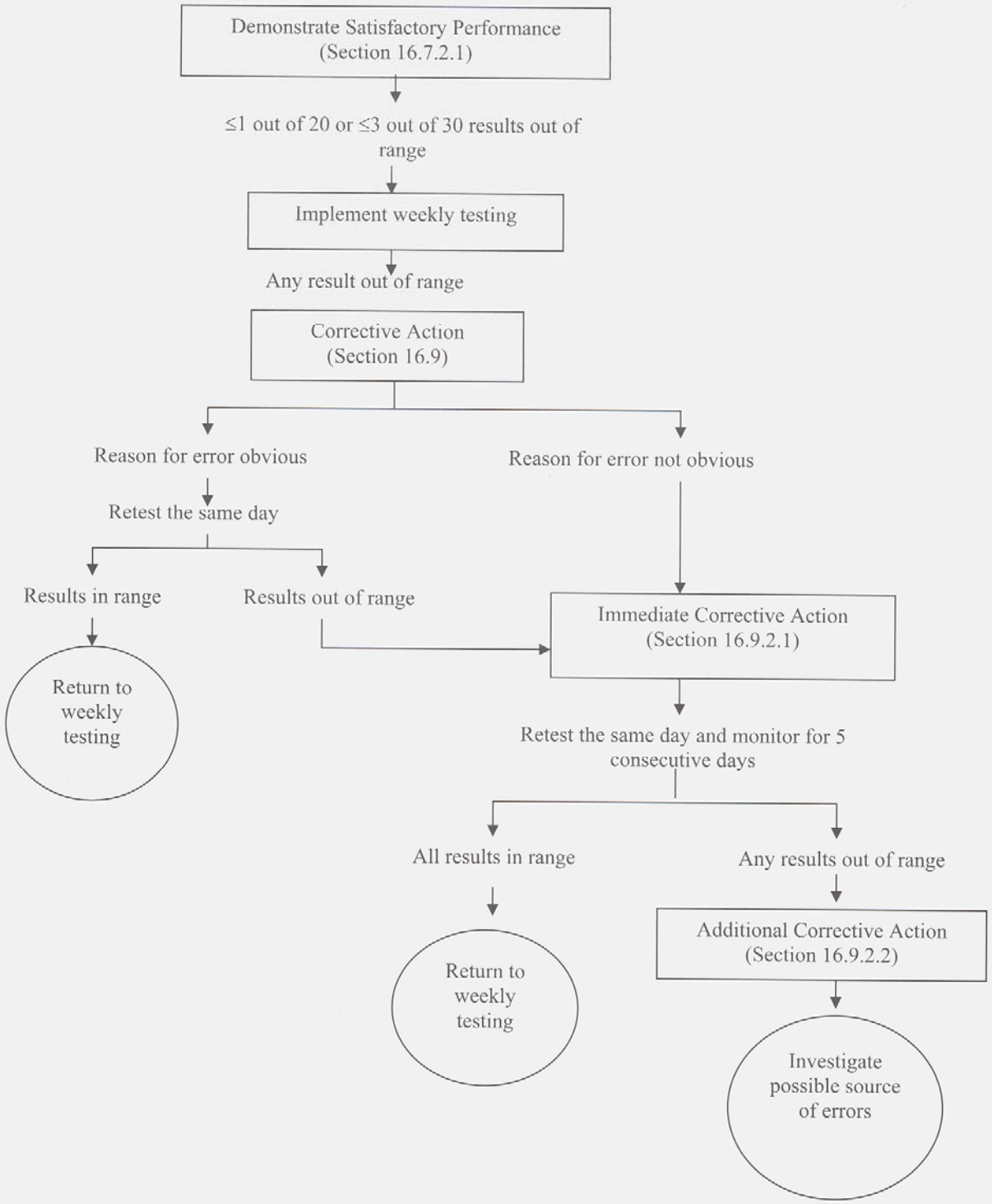


Reference: CLSI M7-A7, page 39

WEEKLY MIC QC CHART

Appendix A. (Continued)

Aerobic Dilution Weekly Quality Control Testing Protocol



Reference: CLSI M7-A7, page 40

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	15	25	13	14-20	21
Ampicillin, AMP	18	10	13	14-16	17
Ampicillin, AMP	38	10	13	14-16	17
Ampicillin, AMP	40	10	13	14-16	17
Cefotaxime, CTX	15	30	22	23-25	26
Cefotaxime, CTX	18	30	22	23-25	26
Cefotaxime, CTX	38	30	22	23-25	26
Cefotaxime, CTX	40	30	14	15-22	23
Ceftazidime, CAZ	15	30	18	19-25	26
Ceftazidime, CAZ	18	30	17	18-20	21
Ceftazidime, CAZ	40	30	14	15-17	18
Ceftiofur, XNL	15	30	17	18-20	21
Ceftiofur, XNL	18	30			
Ceftiofur, XNL	40	30	14		
Chloramphenicol, CHL	15	30	18	19-21	22
Chloramphenicol, CHL	18	30	12	13-17	18
Chloramphenicol, CHL	38	30	12	13-17	18
Chloramphenicol, CHL	40	30	12	13-17	18
Ciprofloxacin, CIP	15		16	17-21	22
Ciprofloxacin, CIP	18	5	15	16-20	21
Ciprofloxacin, CIP	38	5	15	16-20	21
Ciprofloxacin, CIP	40	5	15	16-20	21
Gentamicin, GEN	15	15	15	16-17	18
Gentamicin, GEN	18	10	12	13-14	15
Gentamicin, GEN	38	10	12	13-14	15
Gentamicin, GEN	40	10	12	13-14	15
Nalidixic acid, NAL	15	30	14	15-19	20
Nalidixic acid, NAL	18	30	13	14-18	19
Nalidixic acid, NAL	38	30	13	14-18	19
Nalidixic acid, NAL	40	30	13	14-18	19
Streptomycin, STR	15	10 UI	12	13-14	15
Streptomycin, STR	18	10	11	12-14	15
Streptomycin, STR	38	10	11	12-14	15
Streptomycin, STR	40	10	11	12-14	15
Sulfamethoxazole, SMX	15	200	11	12-16	17
Sulfamethoxazole, SMX	18	300	12	13-16	17
Sulfamethoxazole, SMX	40	300	12	13-16	17
Tetracycline,TET	15	30 UI	16	17-18	19
Tetracycline,TET	18	30	11	12-14	15
Tetracycline,TET	38	30	11	12-14	15
Tetracycline,TET	40	30	11	12-14	15
Trimethoprim, TMP	15	5	11	12-15	16
Trimethoprim, TMP	18	5	10	11-15	16
Trimethoprim, TMP	38	5	10	11-15	16
Trimethoprim, TMP	40	5	10	11-15	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	=	1	0,25	1	1	MIC
1	Nalidixic acid, NAL	<=	4	1	4	1	MIC
1	Streptomycin, STR	<=	8	4	16	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	<=	0.06	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	Streptomycin, STR	=	8	4	16	1	MIC
2	Sulfisoxazole, FIS	=	32	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	=	4	2	8	1	MIC
4	Cefotaxime, CTX	=	0.06	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	Streptomycin, STR	=	8	4	16	1	MIC
4	Sulfisoxazole, FIS	=	32	8	32	1	MIC
4	Tetracycline, TET	=	1	0,5	2	1	MIC
4	Trimethoprim, TMP	=	1	0,5	2	1	MIC
6	Ampicillin, AMP	=	4	2	8	1	MIC
6	Cefotaxime, CTX	<=	0.06	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	=	0.5	0,25	1	1	MIC
6	Nalidixic acid, NAL	<=	4	1	4	1	MIC
6	Streptomycin, STR	=	8	4	16	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	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	Streptomycin, STR	=	8	4	16	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	<=	0.06	0,03	0,125	1	MIC
11	Chloramphenicol, CHL	=	4	2	8	1	MIC
11	Ciprofloxacin, CIP	=	0.016	0,004	0,016	1	MIC
11	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
11	Nalidixic acid, NAL	=	4	1	4	1	MIC
11	Streptomycin, STR	=	16	4	16	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	=	2	2	8	1	MIC
12	Cefotaxime, CTX	=	0.06	0,03	0,125	1	MIC
12	Ceftazidime, CAZ	<=	0.25	0,06	0,5	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	Streptomycin, STR	=	16	4	16	1	MIC
12	Tetracycline, TET	<=	1	0,5	2	1	MIC
12	Trimethoprim, TMP	=	1	0,5	2	1	MIC
13	Ampicillin, AMP	=	4	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	Streptomycin, STR	=	4	4	16	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	Ampicillin, AMP	=	24	16	22	0	DD
15	Cefotaxime, CTX	=	36	29	35	0	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	Ciprofloxacin, CIP	=	35	30	40	1	DD
15	Gentamicin, GEN	=	25	19	26	1	DD
15	Imipenem, IMI	=	35	26	32	0	DD
15	Nalidixic acid, NAL	=	24	22	28	1	DD
15	Streptomycin, STR	=	20	12	20	1	DD
15	Sulfisoxazole, FIS	=	16	15	23	1	DD
15	Tetracycline, TET	=	24	18	25	1	DD
15	Trimethoprim, TMP	=	22	21	28	1	DD
16	Ampicillin, AMP	=	4	2	8	1	MIC
16	Cefotaxime, CTX	=	0.06	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	=	0.5	0,25	1	1	MIC
16	Nalidixic acid, NAL	=	2	1	4	1	MIC
16	Streptomycin, STR	=	8	4	16	1	MIC
16	Sulfisoxazole, FIS	=	32	8	32	1	MIC
16	Tetracycline, TET	<=	1	0,5	2	1	MIC
16	Trimethoprim, TMP	=	1	0,5	2	1	MIC

17	Ampicillin, AMP	=	4	2	8	1	MIC
17	Cefotaxime, CTX	<=	0.06	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	<=	0.008	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	Streptomycin, STR	=	4	4	16	1	MIC
17	Sulfisoxazole, FIS	=	16	8	32	1	MIC
17	Tetracycline, TET	<=	1	0,5	2	1	MIC
17	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
18	Ampicillin, AMP	=	16	16	22	1	DD
18	Cefotaxime, CTX	=	30	29	35	1	DD
18	Cefoxitin, FOX	=	26	23	29	1	DD
18	Ceftiofur, XNL	=	27	26	31	1	DD
18	Chloramphenicol, CHL	=	26	21	27	1	DD
18	Ciprofloxacin, CIP	=	35	30	40	1	DD
18	Gentamicin, GEN	=	21	19	26	1	DD
18	Nalidixic acid, NAL	=	26	22	28	1	DD
18	Streptomycin, STR	=	16	12	20	1	DD
18	Sulfisoxazole, FIS	=	23	15	23	1	DD
18	Tetracycline, TET	=	24	18	25	1	DD
18	Trimethoprim, TMP	=	25	21	28	1	DD
19	Ampicillin, AMP	=	2	2	8	1	MIC
19	Cefotaxime, CTX	=	0.06	0,03	0,125	1	MIC
19	Ceftazidime, CAZ	=	0.5	0,06	0,5	1	MIC
19	Chloramphenicol, CHL	=	8	2	8	1	MIC
19	Ciprofloxacin, CIP	=	0.008	0,004	0,016	1	MIC
19	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
19	Nalidixic acid, NAL	=	4	1	4	1	MIC
19	Streptomycin, STR	=	16	4	16	1	MIC
19	Sulfisoxazole, FIS	=	32	8	32	1	MIC
19	Tetracycline, TET	=	2	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	<=	0.06	0,03	0,125	1	MIC
20	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
20	Chloramphenicol, CHL	=	4	2	8	1	MIC
20	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
20	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
20	Nalidixic acid, NAL	<=	4	1	4	1	MIC
20	Streptomycin, STR	=	8	4	16	1	MIC
20	Sulfisoxazole, FIS	=	16	8	32	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	=	0.12	0,03	0,125	1	MIC
21	Ceftazidime, CAZ	=	0.5	0,06	0,5	1	MIC
21	Chloramphenicol, CHL	=	8	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.008	0,004	0,016	1	MIC
21	Gentamicin, GEN	=	0.25	0,25	1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	32	8	32	1	MIC
21	Tetracycline, TET	=	2	0,5	2	1	MIC
21	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC

22	Ampicillin, AMP	=	2	2	8	1	MIC
22	Chloramphenicol, CHL	=	4	2	8	1	MIC
22	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
22	Sulfisoxazole, FIS	=	16	8	32	1	MIC
22	Tetracycline, TET	<	1	0,5	2	1	MIC
23	Ampicillin, AMP	=	2	2	8	1	MIC
23	Cefotaxime, CTX	=	0.06	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	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	Nalidixic acid, NAL	=	4	1	4	1	MIC
23	Streptomycin, STR	=	4	4	16	1	MIC
23	Sulfisoxazole, FIS	=	32	8	32	1	MIC
23	Tetracycline, TET	=	1	0,5	2	1	MIC
23	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
24	Ampicillin, AMP	=	4	2	8	1	MIC
24	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
24	Cefoxitin, FOX	<=	4	2	8	1	MIC
24	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
24	Chloramphenicol, CHL	=	8	2	8	1	MIC
24	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
24	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
24	Imipenem, IMI	<=	0.5	0,06	0,25	1	MIC
24	Nalidixic acid, NAL	<=	4	1	4	1	MIC
24	Streptomycin, STR	=	4	4	16	1	MIC
24	Tetracycline, TET	<=	1	0,5	2	1	MIC
24	Trimethoprim, TMP	<=	0.5	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	<=	0.008	0,004	0,016	1	MIC
25	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
25	Imipenem, IMI	<=	0.5	0,06	0,25	1	MIC
25	Nalidixic acid, NAL	<=	4	1	4	1	MIC
25	Streptomycin, STR	=	8	4	16	1	MIC
25	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
25	Tetracycline, TET	<=	1	0,5	2	1	MIC
25	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
26	Ampicillin, AMP	=	2	2	8	1	MIC
26	Cefotaxime, CTX	<=	0.06	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	=	1	0,25	1	1	MIC
26	Nalidixic acid, NAL	<=	4	1	4	1	MIC
26	Streptomycin, STR	=	8	4	16	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	=	0.12	0,03	0,125	1	MIC
29	Ceftazidime, CAZ	=	30	25	32	1	DD
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	Streptomycin, STR	=	4	4	16	1	MIC
29	Tetracycline, TET	=	1	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	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	=	0.5	0,25	1	1	MIC
30	Nalidixic acid, NAL	<=	4	1	4	1	MIC
30	Streptomycin, STR	=	4	4	16	1	MIC
30	Sulfisoxazole, FIS	=	16	8	32	1	MIC
30	Tetracycline, TET	<=	1	0,5	2	1	MIC
30	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
32	Ampicillin, AMP	=	2	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	<	0.25	0,25	1	1	MIC
32	Nalidixic acid, NAL	<	4	1	4	1	MIC
32	Streptomycin, STR	=	4	4	16	1	MIC
32	Tetracycline, TET	<	1	0,5	2	1	MIC
32	Trimethoprim, TMP	=	1	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	Cefoxitin, FOX	=	4	2	8	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.03	0,004	0,016	0	MIC
33	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
33	Nalidixic acid, NAL	=	4	1	4	1	MIC
33	Streptomycin, STR	=	4	4	16	1	MIC
33	Sulfisoxazole, FIS	=	32	8	32	1	MIC
33	Tetracycline, TET	<=	1	0,5	2	1	MIC
33	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
34	Ampicillin, AMP	=	8	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	=	8	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	Streptomycin, STR	<=	2	4	16	0	MIC
34	Sulfisoxazole, FIS	=	32	8	32	1	MIC
34	Tetracycline, TET	=	2	0,5	2	1	MIC
34	Trimethoprim, TMP	=	2	0,5	2	1	MIC
37	Ampicillin, AMP	=	4	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	<=	0.008	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	Streptomycin, STR	=	8	4	16	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	=	34.3	29	35	1	DD
38	Cefoxitin, FOX	=	27.9	23	29	1	DD
38	Chloramphenicol, CHL	=	26.2	21	27	1	DD
38	Ciprofloxacin, CIP	=	37.1	30	40	1	DD
38	Gentamicin, GEN	=	23.6	19	26	1	DD
38	Imipenem, IMI	=	29.7	26	32	1	DD
38	Nalidixic acid, NAL	=	23	22	28	1	DD
38	Streptomycin, STR	=	15.1	12	20	1	DD
38	Tetracycline, TET	=	24.2	18	25	1	DD
38	Trimethoprim, TMP	=	24	21	28	1	DD
39	Ampicillin, AMP	=	4	2	8	1	MIC
39	Cefotaxime, CTX	=	0.12	0,03	0,125	1	MIC
39	Ceftiofur, XNL	=	0.5	0,25	1	1	MIC
39	Chloramphenicol, CHL	=	4	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.016	0,004	0,016	1	MIC
39	Gentamicin, GEN	=	1	0,25	1	1	MIC
39	Nalidixic acid, NAL	<	2	1	4	1	MIC
39	Streptomycin, STR	=	8	4	16	1	MIC
39	Tetracycline, TET	=	1	0,5	2	1	MIC
39	Trimethoprim, TMP	=	1	0,5	2	1	MIC
40	Ampicillin, AMP	=	20	16	22	1	DD
40	Cefotaxime, CTX	=	29	29	35	1	DD
40	Cefoxitin, FOX	=	24	23	29	1	DD
40	Ceftazidime, CAZ	=	26	25	32	1	DD
40	Ceftiofur, XNL	=	24	26	31	0	DD
40	Chloramphenicol, CHL	=	24	21	27	1	DD
40	Ciprofloxacin, CIP	=	36	30	40	1	DD
40	Gentamicin, GEN	=	21	19	26	1	DD
40	Imipenem, IMI	=	30	26	32	1	DD
40	Nalidixic acid, NAL	=	24	22	28	1	DD
40	Streptomycin, STR	=	16	12	20	1	DD
40	Sulfisoxazole, FIS	=	20	15	23	1	DD
40	Tetracycline, TET	=	22	18	25	1	DD
40	Trimethoprim, TMP	=	27	21	28	1	DD
41	Ampicillin, AMP	=	8	2	8	1	MIC
41	Cefotaxime, CTX	=	0.12	0,03	0,125	1	MIC
41	Ceftazidime, CAZ	<=	0.25	0,06	0,5	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	<=	4	1	4	1	MIC
41	Streptomycin, STR	=	8	4	16	1	MIC
41	Tetracycline, TET	=	2	0,5	2	1	MIC
41	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
44	Ampicillin, AMP	<=	8	2	8	1	AGA
44	Cefotaxime, CTX	<=	1	0,03	0,125	1	AGA
44	Chloramphenicol, CHL	<=	8	2	8	1	AGA
44	Ciprofloxacin, CIP	<=	0.125	0,004	0,016	1	AGA
44	Gentamicin, GEN	<=	4	0,25	1	1	AGA
44	Nalidixic acid, NAL	<=	16	1	4	1	AGA
44	Streptomycin, STR	<=	16	4	16	1	AGA
44	Sulfisoxazole, FIS	<=	64	8	32	1	AGA
44	Tetracycline, TET	<=	8	0,5	2	1	AGA
44	Trimethoprim, TMP	<=	2	0,5	2	1	AGA

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	=	8	1	8	1	MIC	X	
2	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
2	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
2	Gentamicin, GEN	=	0.25	0,5	2	**0**	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	=	0.12	0,06	0,25	1	MIC	X	
4	Erythromycin, ERY	=	0.5	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	=	0.5	0,25	2	1	MIC	X	
6	Chloramphenicol, CHL	=	4	1	4	1	MIC		X
6	Ciprofloxacin, CIP	=	1	0,03	0,125	**0**	MIC		X
6	Erythromycin, ERY	=	4	0,25	2	**0**	MIC		X
6	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
6	Tetracycline, TET	=	2	0,25	1	**0**	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	=	0.12	0,06	0,25	1	MIC	X	
11	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
11	Gentamicin, GEN	=	1	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	=	8	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
15	Ciprofloxacin, CIP	=	0.064	0,06	0,5	1	AGA		X
15	Erythromycin, ERY	=	0.5	1	4	**0**	AGA		X
15	Gentamicin, GEN	=	0.38	0,5	4	**0**	AGA		X
15	Nalidixic acid, NAL	=	3	0	256	**0**	AGA		X
15	Tetracycline, TET	=	0.38	0	256	**0**	AGA		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	=	1	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	=	0.5	0,25	2	1	MIC	X	
19	Chloramphenicol, CHL	=	4	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	=	2	0,25	1	**0**	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	=	8	4	16	1	MIC	X	
20	Tetracycline, TET	=	1	0,25	2	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	
24	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
24	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
24	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
24	Gentamicin, GEN	<=	0.25	0,5	2	**0**	MIC	X	
24	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
24	Tetracycline, TET	=	2	0,25	2	1	MIC	X	
25	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
25	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
25	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
25	Gentamicin, GEN	<=	0.25	0,5	2	**0**	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	=	0.12	0,06	0,25	1	MIC	X	
29	Erythromycin, ERY	=	0.5	0,5	2	1	MIC	X	
29	Gentamicin, GEN	=	1	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	<=	2	1	8	1	MIC	X	
30	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
30	Erythromycin, ERY	<=	0.5	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	=	1	0,25	2	1	MIC	X	
32	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
32	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
32	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
32	Gentamicin, GEN	=	2	0,5	2	1	MIC	X	

32	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
32	Tetracycline, TET	=	1	0,25	2	1	MIC	X	
33	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
33	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
33	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
33	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
33	Tetracycline, TET	=	1	0,25	2	1	MIC	X	
34	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
34	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
34	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
34	Gentamicin, GEN	=	0.5	0,5	2	1	MIC	X	
34	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
34	Tetracycline, TET	=	2	0,25	2	1	MIC	X	
37	Chloramphenicol, CHL	=	4	0	256	**0**	AGA	X	
37	Ciprofloxacin, CIP	=	0.25	0,12	1	1	AGA	X	
37	Erythromycin, ERY	=	1	1	8	1	AGA	X	
37	Gentamicin, GEN	=	1	0,5	2	1	AGA	X	
37	Nalidixic acid, NAL	=	8	0	256	**0**	AGA	X	
37	Tetracycline, TET	=	1	0	256	**0**	AGA	X	
39	Ciprofloxacin, CIP	=	0.12	0,06	0,25	1	MIC	X	
39	Erythromycin, ERY	=	0.5	0,5	2	1	MIC	X	
39	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
39	Tetracycline, TET	=	1	0,25	2	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	=	0.5	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
44	Chloramphenicol, CHL	=	4	0	256	**0**	AGA	X	
44	Ciprofloxacin, CIP	=	1	0,12	1	1	AGA	X	
44	Erythromycin, ERY	=	4	1	8	1	AGA	X	
44	Gentamicin, GEN	=	0.5	0,5	2	1	AGA	X	
44	Nalidixic acid, NAL	=	8	0	256	**0**	AGA	X	
44	Tetracycline, TET	=	8	0	256	**0**	AGA	X	

QC ranges for reference strains

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

MIC ranges and disc diffusion ranges are according to CLSI M100 S20 with the following exceptions: The MIC range for streptomycin is according to Sensititre and the range for ceftiofur is according to M31-A3. Additionally, the range for ciprofloxacin is extended to include 0.016 as well.

E-test ranges are according to AB-Biodisk

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)

Test range for MIC (µg/mL) - *Salmonella*

Lab no	Method	Test range for MIC (ug/mL)
Ampicillin, AMP		
1	MIC	1-32
2	MIC	0.5-32
4	MIC	0.5-32
6	MIC	0.5-32
9	MIC	0.5-32
11	MIC	0.5-64
12	MIC	1-128
13	MIC	0.5-32
16	MIC	1-128
17	MIC	0.5-32
20	MIC	0.5-32
22	MIC	0.5-32
23	MIC	0.5-32
24	MIC	0.5-32
25	MIC	0.5-32
26	MIC	0.5-32
29	MIC	>4
30	MIC	0.5-32
32	MIC	0,5-32
33	MIC	0.5-64
37	AGA	0.5-64
39	MIC	0.5-64
41	MIC	0.5-32
44	AGA	8 and 128
Cefotaxime, CTX		
1	MIC	0.125-4
2	MIC	0.06 - 4
4	MIC	0.06 - 4
6	MIC	0.06-4
9	MIC	0.06-4
11	MIC	0.06-8
12	MIC	0.016-2
13	MIC	0.06-4
16	MIC	0.015-2
17	MIC	0.06-4
20	MIC	0.06-4
22	MIC	0.06-4
23	MIC	0.06-4
24	MIC	0.06-4
25	MIC	0.06-4
26	MIC	0.06-4
29	MIC	>0.5
30	MIC	0.06-4
32	MIC	0,06-4
33	MIC	0.06-8
37	AGA	0.06-8
39	MIC	0.06-8
41	MIC	0.06-4
44	AGA	1
Ceftazidime, CAZ		
2	MIC	0.25 - 16
4	MIC	0.25 - 16
6	MIC	0.25-16
9	MIC	0.25-16
12	MIC	0.25-16
13	MIC	0.25-16
16	MIC	0.06-8
17	MIC	0.25-16
20	MIC	0.25-16
22	MIC	0.25-16
23	MIC	0.25-16
24	MIC	0.25-16
25	MIC	0.25 - 16
26	MIC	0.25-16
29	MIC	>2
30	MIC	0.25-16
32	MIC	0,25-16
39	MIC	N/A
41	MIC	0.25 - 16
Ceftiofur, XNL		
1	MIC	0.5-8
12	MIC	0.12-16
23	MIC	0,12-8
29	MIC	>2
39	MIC	N/A

Lab no	Method	Test range for MIC (ug/mL)
Chloramphenicol, CHL		
1	MIC	2-64
2	MIC	2-64
4	MIC	2-64
6	MIC	2-64
9	MIC	2-64
11	MIC	2-256
12	MIC	2-64
13	MIC	2-64
16	MIC	2-256
17	MIC	2-64
20	MIC	2-64
22	MIC	2-64
23	MIC	2-64
24	MIC	2-64
25	MIC	2-64
26	MIC	2-64
29	MIC	>16
30	MIC	2-64
32	MIC	2-64
33	MIC	2-256
37	AGA	2-256
39	MIC	2-256
41	MIC	2 - 64
44	AGA	8
Ciprofloxacin, CIP		
1	MIC	0.015-4
2	MIC	0.008-8
4	MIC	0.008-8
6	MIC	0.008-8
9	MIC	0.008-8
11	MIC	0.008-1
12	MIC	0.008-1
13	MIC	0.008-8
16	MIC	0.008-4
17	MIC	0.008-8
20	MIC	0.008-8
22	MIC	0.008-8
23	MIC	0.008-8
24	MIC	0.008-8
25	MIC	0.008-8
26	MIC	0.008-8
29	MIC	>0.06
30	MIC	0.008-8
32	MIC	0.008-8
33	MIC	0.008-8
37	AGA	0.008-8
39	MIC	0.008-1
41	MIC	0.008-8
44	AGA	0.125 and 1
Gentamicin, GEN		
1	MIC	0.5-16
2	MIC	0.25-32
4	MIC	0.25-32
6	MIC	0.25-32
9	MIC	0.25-32
11	MIC	0.25-32
12	MIC	0.12-16
13	MIC	0.25-32
16	MIC	0.12-16
17	MIC	0.25-32
20	MIC	0.25-32
22	MIC	0.25-32
23	MIC	0.25-32
24	MIC	0.25-32
25	MIC	0.25-32
26	MIC	0.25-32
29	MIC	>2
30	MIC	0.25-32
32	MIC	0.25-32
33	MIC	0.25-32
37	AGA	0.25-32
39	MIC	0.25-32
41	MIC	0.25-32
44	AGA	4

Lab no	Method	Test range for MIC (ug/mL)
Nalidixic acid, NAL		
1	MIC	4-64
2	MIC	4-64
4	MIC	4-64
6	MIC	4-64
9	MIC	4-64
11	MIC	2-256
12	MIC	1-128
13	MIC	8-64
16	MIC	1-128
17	MIC	4-64
20	MIC	4-64
22	MIC	4-64
23	MIC	4-64
24	MIC	4-64
25	MIC	4-64
26	MIC	4-64
29	MIC	>16
30	MIC	4-64
32	MIC	4-64
33	MIC	2-256
37	AGA	2-512
39	MIC	2-256
41	MIC	4 - 64
44	AGA	16
Streptomycin, STR		
1	MIC	8-128
2	MIC	2-128
4	MIC	2-128
6	MIC	2-128
9	MIC	2-128
11	MIC	2-256
12	MIC	2-256
13	MIC	2-128
16	MIC	2-256
17	MIC	2-128
20	MIC	2-128
22	MIC	2-128
23	MIC	2-128
24	MIC	2-128
25	MIC	2-128
26	MIC	2-128
29	MIC	>32
30	MIC	2-128
32	MIC	2-128
33	MIC	2-256
37	AGA	2-512
39	MIC	2-256
41	MIC	2 - 128
44	AGA	16 and 128
Sulfamethoxazole, SMX		
1	MIC	64-1024
2	MIC	8-1024
4	MIC	8-1024
6	MIC	8-1024
9	MIC	8-1024
11	MIC	8-1024
12	MIC	16-2048
13	MIC	8-1024
16	MIC	8-1024
17	MIC	8-1024
20	MIC	8-1024
22	MIC	8-1024
23	MIC	8-1024
24	MIC	8-1024
25	MIC	8-1024
26	MIC	8-1024
29	MIC	>256
30	MIC	8-1024
32	MIC	8-1024
33	MIC	8-1024
37	AGA	8-1024
39	MIC	8-1024
41	MIC	8-1024
44	AGA	64

Lab no	Method	Test range for MIC (ug/mL)
Tetracycline, TET		
1	MIC	2-32
2	MIC	1-64
4	MIC	1-64
6	MIC	1-64
9	MIC	1-64
11	MIC	0.5-64
12	MIC	1-128
13	MIC	1-64
16	MIC	1-128
17	MIC	1-64
20	MIC	1-64
22	MIC	1-64
23	MIC	1-64
24	MIC	1-64
25	MIC	1-64
26	MIC	1-64
29	MIC	>8
30	MIC	1-64
32	MIC	1-64
33	MIC	0.5-64
37	AGA	0.5 - 64
39	MIC	0.5-64
Trimethoprim, TMP		
41	MIC	1 - 64
44	AGA	8 and 128
1	MIC	1-32
2	MIC	0.5-32
4	MIC	0.5-32
6	MIC	0.5-32
9	MIC	0.5-32
11	MIC	0.25-32
12	MIC	0.12-16
13	MIC	0.5-32
16	MIC	0.12-16
17	MIC	0.5-32
20	MIC	0.5-32
22	MIC	0.5-32
23	MIC	0.5-32
24	MIC	0.5-32
25	MIC	0.5-32
26	MIC	0.5-32
29	MIC	>2
30	MIC	0.5-32
32	MIC	0,5-32
33	MIC	0.25-32
37	AGA	0.25-32
39	MIC	0.25-32
41	MIC	0.5-32
44	AGA	2

Antimicrobials recommended by EFSA are marked in grey

Participants' ranges covering the EFSA range are marked in grey

MIC: Microbroth dilution
AGA: Agar dilution

Test range for MIC (µg/mL) - *Campylobacter*

Lab no	Method	Test range for MIC (ug/mL)
Chloramphenicol, CHL		
1	MIC	2-32
2	MIC	2 - 64
4	MIC	2 - 32
6	MIC	2-32
9	MIC	2-32
14	MIC	2-32
17	MIC	2-32
19	MIC	2-32
20	MIC	2-32
21	MIC	1-32
22	MIC	2-32
23	MIC	2-32
24	MIC	2-128
25	MIC	2 - 128
26	MIC	2-32
30	MIC	2-32
32	MIC	2-32
34	MIC	1 to 32
37	AGA	2 - 256
39	MIC	N/A
41	MIC	2 - 32
44	AGA	8
Ciprofloxacin, CIP		
1	MIC	0.06-4
2	MIC	0.06-32
4	MIC	0.06-4
6	MIC	0.06-4
9	MIC	0,06-4
11	MIC	0.06-8
12	MIC	0.06-8
14	MIC	0.06-4
15	AGA	0.002-32
17	MIC	0.06-4
19	MIC	0.06-4
20	MIC	0.06-4
21	MIC	0.06-128
22	MIC	0.06-4
23	MIC	0.06-4
24	MIC	0.12-16
25	MIC	0.12-16
26	MIC	0.06-4
29	MIC	0.06-8
30	MIC	0.06-4
32	MIC	0.06-4
33	MIC	0.06-8
34	MIC	0.032 to 32
37	AGA	0.06-8
39	MIC	0.06-8
41	MIC	0.06-4
44	AGA	1
Erythromycin, ERY		
1	MIC	0.5-32
2	MIC	0.25-128
4	MIC	0.5-32
6	MIC	0.5-32
9	MIC	0,5-32
11	MIC	0.5-64
12	MIC	0.5-64
14	MIC	0.5-32
15	AGA	0.016-256
17	MIC	0.5-32
19	MIC	0.5-32
20	MIC	0.5-32
21	MIC	0.12-128
22	MIC	0.5-32
23	MIC	0.5-32
24	MIC	0.5-64
25	MIC	0.5-64
26	MIC	0.5-32
29	MIC	0.5-64
30	MIC	0.5-32
32	MIC	0.5-32
33	MIC	0.5-64
34	MIC	0.125 to 128
37	AGA	0.5-64
39	MIC	0.5-64
41	MIC	0.5-32
44	AGA	4

Lab no	Method	Test range for MIC (ug/mL)
Gentamicin, GEN		
1	MIC	0.125-16
2	MIC	0.12-16
4	MIC	0.12-16
6	MIC	0.12-16
9	MIC	0.12-16
11	MIC	0.12-16
12	MIC	0.12-16
14	MIC	0.125-16
15	AGA	0.016-256
17	MIC	0.12-16
19	MIC	0.12-16
20	MIC	0.12-16
21	MIC	0.12-128
22	MIC	0.12-16
23	MIC	0.12-16
24	MIC	0.25-32
25	MIC	0.25-32
26	MIC	0.12-16
29	MIC	0.12-16
30	MIC	0.12-16
32	MIC	0.125-16
33	MIC	0.12-16
34	MIC	0.125 to 32
37	AGA	0.125-16
39	MIC	0.12-16
41	MIC	0.12-16
44	AGA	4
Nalidixic acid, NAL		
1	MIC	2-64
2	MIC	2 - 256
4	MIC	2 - 64
6	MIC	2-64
9	MIC	2-64
11	MIC	1-64
12	MIC	1-64
14	MIC	2-64
15	AGA	0.016-256
17	MIC	2-64
19	MIC	2-64
20	MIC	2-64
21	MIC	0.12-128
22	MIC	2-64
23	MIC	2-64
24	MIC	1-128
25	MIC	1 - 128
26	MIC	2-64
29	MIC	1-64
30	MIC	2-64
32	MIC	2-64
33	MIC	1-64
34	MIC	0.5 to 64
37	AGA	2 - 256
39	MIC	1-64
41	MIC	2 - 64
44	AGA	16

Lab no	Method	Test range for MIC (ug/mL)
Streptomycin, STR		
1	MIC	1-16
2	MIC	0.5-32
4	MIC	1-16
6	MIC	1-16
9	MIC	1-16
11	MIC	0.5-64
12	MIC	0.5-64
14	MIC	1-16
17	MIC	1-16
19	MIC	1-16
20	MIC	1-16
21	MIC	0.12-128
22	MIC	1-16
23	MIC	1-16
24	MIC	1-128
25	MIC	1-128
26	MIC	1-16
29	MIC	0.5-64
30	MIC	1-16
32	MIC	1-16
33	MIC	0.5-64
34	MIC	0.25 to 64
37	AGA	0.5-32
39	MIC	0.5-64
41	MIC	1 - 16
44	AGA	not tested
Tetracycline,TET		
1	MIC	0.25-16
2	MIC	0.12-64
4	MIC	0.25-16
6	MIC	0.25-16
9	MIC	0,25-16
11	MIC	0.12-16
12	MIC	0.12-16
14	MIC	0.25-16
15	AGA	0.016-256
17	MIC	0.25-16
19	MIC	0.25-16
20	MIC	0.25-16
21	MIC	0.12-128
22	MIC	0.25-16
23	MIC	0.25-16
24	MIC	0.5-64
25	MIC	0.5-64
26	MIC	0.25-16
29	MIC	0.12-16
30	MIC	0.25-16
32	MIC	0.25-16
33	MIC	0.12-16
34	MIC	0.125 to 256
37	AGA	0.125 - 16
39	MIC	0.12-16
41	MIC	0.25 - 16
44	AGA	8 & 128

Antimicrobials recommended by EFSA are marked in grey
Participants' ranges covering the EFSA range are marked in grey

MIC: Microbroth dilution
AGA: Agar dilution

Salmonella - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Ampicillin, AMP	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	R	100	0	31	0
	EURL S-5.3	S	3	97	30	1
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	R	100	0	31	0
	EURL S-5.6	R	100	0	31	0
	EURL S-5.7	R	100	0	31	0
	EURL S-5.8	R	100	0	31	0
Cefotaxime, CTX	EURL S-5.1	S	0	100	31	0
	EURL S-5.2	S	0	100	31	0
	EURL S-5.3	S	0	100	31	0
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	S	3	97	30	1
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	R	100	0	31	0
	EURL S-5.8	R	100	0	31	0
Ceftazidime, CAZ	EURL S-5.1	S	0	100	24	0
	EURL S-5.2	S	0	100	24	0
	EURL S-5.3	S	0	100	24	0
	EURL S-5.4	S	0	100	25	0
	EURL S-5.5	S	0	100	24	0
	EURL S-5.6	S	0	100	24	0
	EURL S-5.7	S	17	83	19	4
	EURL S-5.8	R	100	0	25	0
Ceftiofur, XNL	EURL S-5.1	S	0	100	7	0
	EURL S-5.2	S	0	100	7	0
	EURL S-5.3	S	0	100	7	0
	EURL S-5.4	S	0	100	7	0
	EURL S-5.5	S	0	100	7	0
	EURL S-5.6	S	0	100	7	0
	EURL S-5.7	R	100	0	7	0
	EURL S-5.8	R	100	0	7	0
Chloramphenicol, CHL	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	S	6	94	29	2
	EURL S-5.3	R	100	0	31	0
	EURL S-5.4	S	0	100	30	0
	EURL S-5.5	S	0	100	31	0
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	S	0	100	31	0
	EURL S-5.8	S	0	100	31	0
Ciprofloxacin, CIP	EURL S-5.1	R	94	6	29	2
	EURL S-5.2	R	90	10	28	3
	EURL S-5.3	R	90	10	28	3
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	R	84	16	26	5
	EURL S-5.6	R	84	16	26	5
	EURL S-5.7	R	87	13	27	4
	EURL S-5.8	S	0	100	31	0

Gentamicin, GEN	EURL S-5.1	S	0	100	31	0
	EURL S-5.2	S	0	100	31	0
	EURL S-5.3	R	90	10	27	3
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	S	0	100	31	0
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	S	0	100	31	0
	EURL S-5.8	S	0	100	31	0
Nalidixic acid, NAL	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	R	97	3	30	1
	EURL S-5.3	R	100	0	31	0
	EURL S-5.4	S	3	97	30	1
	EURL S-5.5	S	16	84	26	5
	EURL S-5.6	S	3	97	30	1
	EURL S-5.7	R	97	3	30	1
	EURL S-5.8	S	3	97	30	1
Streptomycin, STR	EURL S-5.1	R	94	6	29	2
	EURL S-5.2*	R	48*	52	15	16
	EURL S-5.3*	R	26*	74	8	23
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	R	100	0	31	0
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	S	6	94	29	2
	EURL S-5.8	S	6	94	29	2
Sulphonamides, SMX	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	R	100	0	31	0
	EURL S-5.3	R	100	0	31	0
	EURL S-5.4	S	3	97	30	1
	EURL S-5.5	S	0	100	31	0
	EURL S-5.6	S	6	94	29	2
	EURL S-5.7	S	3	97	30	1
	EURL S-5.8	S	0	100	31	0
Tetracycline, TET	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	S	6	94	29	2
	EURL S-5.3	R	100	0	31	0
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	S	3	97	30	1
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	R	97	3	30	1
	EURL S-5.8	S	0	100	31	0
Trimethoprim, TMP	EURL S-5.1	R	100	0	31	0
	EURL S-5.2	R	100	0	31	0
	EURL S-5.3	R	100	0	31	0
	EURL S-5.4	S	0	100	31	0
	EURL S-5.5	S	0	100	31	0
	EURL S-5.6	S	0	100	31	0
	EURL S-5.7	S	0	100	31	0
	EURL S-5.8	S	0	100	31	0

*Strain/antimicrobial-combination excluded from the evaluation

Campylobacter - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Chloramphenicol, CHL	EURL C-5.1	S	0	100	21	0
	EURL C-5.2	S	0	100	21	0
	EURL C-5.3	S	0	100	21	0
	EURL C-5.4	S	0	100	21	0
	EURL C-5.5	S	0	100	21	0
	EURL C-5.6	S	0	100	21	0
	EURL C-5.7	S	0	100	20	0
	EURL C-5.8	S	0	100	20	0
Ciprofloxacin, CIP	EURL C-5.1	S	0	100	27	0
	EURL C-5.2	R	100	0	27	0
	EURL C-5.3	S	0	100	27	0
	EURL C-5.4	S	0	100	27	0
	EURL C-5.5	R	96	4	26	1
	EURL C-5.6	S	0	100	27	0
	EURL C-5.7	S	4	96	25	1
	EURL C-5.8	R	100	0	26	0
Erythromycin, ERY	EURL C-5.1	S	0	100	27	0
	EURL C-5.2	R	100	0	27	0
	EURL C-5.3	R	100	0	27	0
	EURL C-5.4	R	100	0	27	0
	EURL C-5.5	S	4	96	26	1
	EURL C-5.6	R	100	0	27	0
	EURL C-5.7	S	4	96	25	1
	EURL C-5.8	S	0	100	26	0
Gentamicin, GEN	EURL C-5.1	S	0	100	27	0
	EURL C-5.2	S	0	100	27	0
	EURL C-5.3	S	0	100	27	0
	EURL C-5.4	S	0	100	27	0
	EURL C-5.5	S	0	100	27	0
	EURL C-5.6	S	0	100	27	0
	EURL C-5.7	S	0	100	26	0
	EURL C-5.8	S	0	100	26	0
Nalidixic acid, NAL	EURL C-5.1	S	0	100	27	0
	EURL C-5.2	R	93	7	25	2
	EURL C-5.3	S	0	100	27	0
	EURL C-5.4	S	4	96	26	1
	EURL C-5.5	R	96	4	26	1
	EURL C-5.6	S	0	100	27	0
	EURL C-5.7	S	4	96	25	1
	EURL C-5.8	R	100	0	26	0
Streptomycin, STR	EURL C-5.1	S	0	100	26	0
	EURL C-5.2	R	100	0	26	0
	EURL C-5.3	S	4	96	24	1
	EURL C-5.4	S	12	88	23	3
	EURL C-5.5	R	92	8	24	2
	EURL C-5.6	S	12	88	23	3
	EURL C-5.7	S	0	100	24	0
	EURL C-5.8	S	0	100	24	0
Tetracycline, TET	EURL C-5.1	S	0	100	27	0
	EURL C-5.2	R	89	11	24	3
	EURL C-5.3	S	15	85	23	4
	EURL C-5.4	S	15	85	22	4
	EURL C-5.5	S	0	100	27	0
	EURL C-5.6	R	100	0	27	0
	EURL C-5.7	R	100	0	26	0
	EURL C-5.8	R	100	0	26	0

Deviations - *Salmonella*

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
4	EURL S-5.5	Nalidixic acid, NAL	R	16	S	=16	MIC
9	EURL S-5.7	Ceftazidime, CAZ	R	0.5	S	=1	MIC
13	EURL S-5.3	Ampicillin,Â AMP	R	>32	S	=2	MIC
13	EURL S-5.3	Gentamicin, GEN	S	4	R	=8	MIC
13	EURL S-5.5	Ciprofloxacin, CIP	S	0.5	R	=1	MIC
13	EURL S-5.6	Ciprofloxacin, CIP	S	0.25	R	=0.50	MIC
15	EURL S-5.2	Chloramphenicol, CHL	R	21	S	=8	DD
15	EURL S-5.2	Tetracycline, TET	R	18	S	=4	DD
15	EURL S-5.4	Nalidixic acid, NAL	R	20	S	=4	DD
15	EURL S-5.4	Sulfamethoxazole, SMX	R	15	S	=64	DD
15	EURL S-5.5	Nalidixic acid, NAL	R	12	S	=16	DD
15	EURL S-5.6	Nalidixic acid, NAL	R	15	S	=16	DD
15	EURL S-5.6	Sulfamethoxazole, SMX	R	15	S	=32	DD
15	EURL S-5.7	Ceftazidime, CAZ	R	29	S	=1	DD
15	EURL S-5.8	Nalidixic acid, NAL	R	19	S	=4	DD
16	EURL S-5.7	Streptomycin, STR	R	32	S	=16	MIC
18	EURL S-5.1	Ciprofloxacin, CIP	S	24	R	=1	DD
18	EURL S-5.2	Ciprofloxacin, CIP	S	26	R	=1	DD
18	EURL S-5.3	Ciprofloxacin, CIP	S	27	R	=0.5	DD
18	EURL S-5.3	Gentamicin, GEN	S	12	R	=8	DD
18	EURL S-5.5	Ciprofloxacin, CIP	S	25	R	=1	DD
18	EURL S-5.7	Ciprofloxacin, CIP	S	28	R	=0.25	DD
19	EURL S-5.5	Ciprofloxacin, CIP	S	0.06	R	=1	MIC
19	EURL S-5.6	Ciprofloxacin, CIP	S	0.06	R	=0.50	MIC
19	EURL S-5.7	Ciprofloxacin, CIP	S	0.06	R	=0.25	MIC
20	EURL S-5.2	Nalidixic acid, NAL	S	<=4	R	>64	MIC
20	EURL S-5.7	Nalidixic acid, NAL	S	<=4	R	>64	MIC
24	EURL S-5.8	Confirmed ESBL	No		Yes		MIC
24	EURL S-5.8	Streptomycin, STR	R	32	S	=16	MIC
26	EURL S-5.7	Streptomycin, STR	R	32	S	=16	MIC
26	EURL S-5.8	Streptomycin, STR	R	32	S	=16	MIC
29	EURL S-5.1	Streptomycin, STR	S	32	R	=64	MIC
32	EURL S-5.7	Ceftazidime, CAZ	R	<=1	S	=1	MIC
33	EURL S-5.5	Nalidixic acid, NAL	R	32	S	=16	MIC
37	EURL S-5.1	Streptomycin, STR	S	32	R	=64	AGA
38	EURL S-5.5	Nalidixic acid, NAL	R	6	S	=16	DD
38	EURL S-5.6	Ciprofloxacin, CIP	S	29.5	R	=0.50	DD
38	EURL S-5.7	Confirmed ESBL	No		Yes		DD
38	EURL S-5.8	Confirmed ESBL	No		Yes		DD
39	EURL S-5.2	Chloramphenicol, CHL	R	256	S	=8	MIC
39	EURL S-5.2	Tetracycline, TET	R	64	S	=4	MIC
39	EURL S-5.5	Cefotaxime, CTX	R	4	S	<=0.12	MIC
39	EURL S-5.5	Nalidixic acid, NAL	R	256	S	=16	MIC
39	EURL S-5.5	Tetracycline, TET	R	16	S	<=2	MIC
39	EURL S-5.7	Confirmed ESBL	No		Yes		MIC
39	EURL S-5.8	Confirmed ESBL	No		Yes		MIC
40	EURL S-5.1	Ciprofloxacin, CIP	S	22	R	=1	DD
40	EURL S-5.2	Ciprofloxacin, CIP	S	21	R	=1	DD

40	EURL S-5.3	Ciprofloxacin, CIP	S	27	R	=0.5	DD
40	EURL S-5.3	Gentamicin, GEN	S	14	R	=8	DD
40	EURL S-5.5	Ciprofloxacin, CIP	S	26	R	=1	DD
40	EURL S-5.6	Ciprofloxacin, CIP	S	26	R	=0.50	DD
40	EURL S-5.6	Sulfamethoxazole, SMX	R	6	S	=32	DD
40	EURL S-5.7	Ceftazidime, CAZ	R	13	S	=1	DD
40	EURL S-5.7	Ciprofloxacin, CIP	S	27	R	=0.25	DD
40	EURL S-5.7	Sulfamethoxazole, SMX	R	6	S	=64	DD
40	EURL S-5.7	Tetracycline, TET	S	12	R	=32	DD
41	EURL S-5.2	Ciprofloxacin, CIP	S	0,5	R	=1	MIC
41	EURL S-5.3	Ciprofloxacin, CIP	S	0,25	R	=0.5	MIC
41	EURL S-5.5	Ciprofloxacin, CIP	S	0,5	R	=1	MIC
41	EURL S-5.6	Ciprofloxacin, CIP	S	0,5	R	=0.50	MIC
41	EURL S-5.7	Ciprofloxacin, CIP	S	0,5	R	=0.25	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
14	EURL C-5.2	Tetracycline, TET	S	1	R	=16	MIC
15	EURL C-5.2	Tetracycline, TET	S	0.38	R	=16	AGA
17	EURL C-5.2	Nalidixic acid, NAL	S	32	R	=64	MIC
19	EURL C-5.3	Streptomycin, STR	R	8	S	<=1	MIC
19	EURL C-5.4	Streptomycin, STR	R	8	S	<=1	MIC
19	EURL C-5.4	Tetracycline, TET	R	4	S	=2	MIC
19	EURL C-5.6	Streptomycin, STR	R	8	S	<=1	MIC
20	EURL C-5.2	Tetracycline, TET	S	2	R	=16	MIC
21	EURL C-5.3	Tetracycline, TET	R	4	S	=1	MIC
21	EURL C-5.4	Streptomycin, STR	R	8	S	<=1	MIC
21	EURL C-5.6	Streptomycin, STR	R	8	S	<=1	MIC
21	EURL C-5.7	Ciprofloxacin, CIP	R	1	S	=0.12	MIC
21	EURL C-5.7	Nalidixic acid, NAL	R	64	S	=4	MIC
22	EURL C-5.4	Tetracycline, TET	R	8	S	=2	MIC
22	EURL C-5.5	Erythromycin, ERY	R	>32	S	=0.5	MIC
22	EURL C-5.5	Ciprofloxacin, CIP	S	0.25	R	=4	MIC
22	EURL C-5.5	Nalidixic acid, NAL	S	8	R	=64	MIC
22	EURL C-5.5	Streptomycin, STR	S	4	R	>16	MIC
24	EURL C-5.3	Tetracycline, TET	R	4	S	=1	MIC
30	EURL C-5.3	Tetracycline, TET	R	4	S	=1	MIC
30	EURL C-5.4	Tetracycline, TET	R	4	S	=2	MIC
30	EURL C-5.5	Streptomycin, STR	S	<= 1	R	>16	MIC
32	EURL C-5.2	Nalidixic acid, NAL	S	<=32	R	=64	MIC
41	EURL C-5.4	Nalidixic acid, NAL	R	64	S	=8	MIC
41	EURL C-5.4	Streptomycin, STR	R	>16	S	<=1	MIC
41	EURL C-5.7	Erythromycin, ERY	R	>32	S	=1	MIC
44	EURL C-5.3	Tetracycline, TET	R	8	S	=1	AGA
44	EURL C-5.4	Tetracycline, TET	R	8	S	=2	AGA
44	EURL C-5.6	Streptomycin, STR	R	8	S	<=1	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
l	EURL GEN-2.1	catpIP		X	5'-GGATATGAAATTTATCCCTC-3'	5'-CAATCATCTACCCTATGAAT-3'	In-house	
l	EURL GEN-2.1	vanA		X	5'-AAAGTGCGAAAAACCTTGC-3'	5'-AACAACTTACGCGGCACT-3'	In-house	
l	EURL GEN-2.1	erm(A)		X	5'-AAGCGGTAAAACCCCTCTGAG-3'	5'-TCAAAGCCTGTCGGAATTGG-3'	In-house	
l	EURL GEN-2.1	emtA		X	5'-GGTCAGCAGATCACTTGTTT-3'	5'-TGAACAATTCTAAGTCCTCG-3'	In-house	
l	EURL GEN-2.1	vat(D)		X	5'-GCTCAATAGGACCAGGTGTA-3'	5'-TCCAGCTAACATGTATGGCG-3'	In-house	
l	EURL GEN-2.1	vat(E)		X	5'-ACTATACCTGACGCAAATGC-3'	5'-GGTTCAAATCTTGGTCCG-3'	In-house	
l	EURL GEN-2.1	vgbA		X	5'-TACAGAGTACCCACTACCGA-3'	5'-TCAATTCCTGCTCCAGCAGT-3'	In-house	
l	EURL GEN-2.1	tet(O)		X	5'-GATGGCATACAGGCACAGAC-3'	5'-CAATATCACCAGAGCAGGCT-3'	In-house	
l	EURL GEN-2.1	tet(S)		X	5'-TGGAACGCCAGAGAGGTATT-3'	5'-ACATAGACAAGCCGTTGACC-3'	In-house	
l	EURL GEN-2.1	aadE			5'-TCAAAACCCCTATTAAAGCC-3'	5'-ATGGAATTATTCCCACCTGA-3'	In-house	
l	EURL GEN-2.1	aph(3´)-III			5'-GCCGATGTGGATTGCGAAAA-3'	5'-GCTTGATCCCCAGTAAGTCA-3'	In-house	
l	EURL GEN-2.1	vanB			5'-GATATTCAAAGCTCCGCAGC-3'	5'-TGATGGATGCGGAAGATACC-3'	In-house	
l	EURL GEN-2.1	erm(B)			5'-GGAACATCTGTGGTATGGCG-3'	5'-CATTTAACGACGAAACTGGC-3'	In-house	
l	EURL GEN-2.1	pbp5			5-'TGAGCAATT TGTCCAAGC-3'	5'-TGATCCAGCTTTTCCTCC-3'	In-house	
l	EURL GEN-2.1	**tet(K)**			5'-TTAGGTGAAGGGTTAGGTCC-3'	5'-GCAAACTCATTCCAGAAGCA-3'	In-house	
l	EURL GEN-2.1	tet(L)			5'-CATTTGGTCTTATTGGTACG-3'	5'-ATTACACTTCCGATTTCGG-3'	In-house	
l	EURL GEN-2.1	tet(M)			5'-GTTAAATAGTGTTCTTGGAG-3'	5'-CTAAGATATGGCTCTAACAA-3'	In-house	
l	EURL GEN-2.2	CMY		X			In-house	
l	EURL GEN-2.2	SHV		X			In-house	
l	EURL GEN-2.2	TEM		X			In-house	
l	EURL GEN-2.2	cmlA		X			In-house	
l	EURL GEN-2.2	floR		X			In-house	
l	EURL GEN-2.2	aac(3)-II		X	5'-TGAAACGCTGACGGAGCCTC-3'	5'-GTCGAACAGGTAGCACTGAG-3'	In-house	
l	EURL GEN-2.2	aac(3)-IV		X			In-house	
l	EURL GEN-2.2	ant(2'')-I		X			In-house	
l	EURL GEN-2.2	aph(3')-I		X	5'-AACGTCTTGCTCGAGGCCGCG-3'	5'-GGCAAGATCCTGGTATCGGTCTGCG-3'	In-house	
l	EURL GEN-2.2	aph(3')-III		X	5'-GCCGATGTGGATTGCGAAAA-3'	5'-GCTTGATCCCCAGTAAGTCA-3'	In-house	
l	EURL GEN-2.2	qnrA		X	5'-GGATGCCAGTTTCGAGGA-3'	5'-TGCCAGGCACAGATCTTG-3'	In-house	
l	EURL GEN-2.2	qnrB		X			In-house	
l	EURL GEN-2.2	qnrC		X	5'-GGGTTGTACATTTATTGAATC-3'	5'-TCCACTTTACGAGGTTCT-3'	In-house	
l	EURL GEN-2.2	qnrD		X	5'-CGAGATCAATTTACGGGGAATA-3'	5'-AACAAGCTGAAGCGCCTG-3'	In-house	
l	EURL GEN-2.2	qnrS		X			In-house	
l	EURL GEN-2.2	sul1		X			In-house	
l	EURL GEN-2.2	sul3		X			In-house	
l	EURL GEN-2.2	tetA		X			In-house	
l	EURL GEN-2.2	tetC		X			In-house	
l	EURL GEN-2.2	tetD		X			In-house	
l	EURL GEN-2.2	tetE		X			In-house	
l	EURL GEN-2.2	tetG		X			In-house	
l	EURL GEN-2.2	CTX	-M-9*		5'-GTGTGTTTAGAATGGTGATCGCATT-3'	5'-ATGATTCTCGCCGCTGAAGCC-3'	In-house	
l	EURL GEN-2.2	OXA					In-house	
l	EURL GEN-2.2	catA1					In-house	
l	EURL GEN-2.2	aadA					In-house	
l	EURL GEN-2.2	strA					In-house	
l	EURL GEN-2.2	strB					In-house	
l	EURL GEN-2.2	sul2					In-house	
l	EURL GEN-2.2	tetB					In-house	

III	EURL GEN-2.2	CMY		X			Published	Zhao et al. 2003 JCM
III	EURL GEN-2.2	SHV none		X			Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	TEM none		X			Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	cmlA		X			Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	floR		X			Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	aac(3)-II		X	ATTCGAAAACTCGGAGTC	CGGAGTGGCTCCGAAGTG	In-house	
III	EURL GEN-2.2	aac(3)-IV		X			Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	ant(2'')-I		X			Published	Frana et al. 2001 AEM 67:445-8
III	EURL GEN-2.2	aph(3')-I		X			Published	Frana et al. 2001 AEM 67:445-8
III	EURL GEN-2.2	aph(3')-II		X			Published	Frana et al. 2001 AEM 67:445-8
III	EURL GEN-2.2	aph(3')-III		X			Published	Gibreel et al. 2004; AAC
III	EURL GEN-2.2	gyrA-87		X			Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	parC-57		X			Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	parC-78		X			Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	parC-84		X			Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	qnrA		X			Published	Wang et al., 2003
III	EURL GEN-2.2	qnrB		X			Published	Jacoby et al., 2006
III	EURL GEN-2.2	qnrC		X			Published	Wang et al., 2009
III	EURL GEN-2.2	qnrD		X			Published	Cavaco et al., 2009
III	EURL GEN-2.2	qnrS		X			Published	Gay et al., 2006
III	EURL GEN-2.2	sul1		X			Published	Sadvang et al., 1997 FEMS Mic Let
III	EURL GEN-2.2	sul3		X			Published	Perreten-Boerlin 2003
III	EURL GEN-2.2	tetA		X			Published	Ng, Lai King 1999 AAC
III	EURL GEN-2.2	tetC		X			Published	Ng, Lai King 1999 AAC
III	EURL GEN-2.2	tetD		X			Published	Ng, Lai King 1999 AAC
III	EURL GEN-2.2	tetE		X			Published	Ng, Lai King 1999 AAC
III	EURL GEN-2.2	tetG		X			Published	Ng, Lai King 1999 AAC
III	EURL GEN-2.2	CTX	-M-14				Published	Batchelor JCM2005; Carattoli et al.,
III	EURL GEN-2.2	OXA	-1				Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	catA1					Published	Guerra et al 2004 MDR 10:83-91
III	EURL GEN-2.2	gyrA	-83				Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	parC	-80				Published	Malorny et al., Vet Rec. 2003
III	EURL GEN-2.2	aadA					Published	Sadvang et al., 1997 FEMS Mic Let
III	EURL GEN-2.2	strA					Published	Madsen et al. 2000 VetMic 75:73-82
III	EURL GEN-2.2	strB					Published	Madsen et al. 2000 VetMic 75:73-82
III	EURL GEN-2.2	sul2					Published	Chu et al., 2001 AAC
III	EURL GEN-2.2	tetB					Published	Ng, Lai King 1999 AAC
IV	EURL GEN-2.2	CTX						
IV	EURL GEN-2.2	OXA						
IV	EURL GEN-2.2	catA1						
IV	EURL GEN-2.2	aadA						
IV	EURL GEN-2.2	strA						
IV	EURL GEN-2.2	strB						
IV	EURL GEN-2.2	sul2						
IV	EURL GEN-2.2	tetB						

V	EURL GEN-2.2	SHV none		X	AGGATTGACTGCCTTTTTG	ATTTGCTGATTTCGCTCG	Published	Colom et al FEMS Micro Lett (2003) 223(2):147-5
V	EURL GEN-2.2	TEM none		X	ATCAGCAATAAACCAGC	CCCCGAAGAACGTTTTC	Published	Colom et al FEMS Micro Lett (2003) 223(2):147-5
V	EURL GEN-2.2	cmlA		X	TGTCATTTACGGCATACTCG	ATCAGGCATCCCATTCCCAT	Published	Guerra et al JAC (2003) 52:489-492
V	EURL GEN-2.2	qnrA		X	ATTTCTCACGCCAGGATTTG	GATCGGCAAAGGTTAGGTCA	Published	Robiscek et al AAC (2006) 50(8):2872-4.
V	EURL GEN-2.2	qnrB		X	GATCGTGAAAGCCAGAAAGG	ACGATGCCTGGTAGTTGTCC	Published	Robiscek et al AAC (2006) 50(8):2872-4.
V	EURL GEN-2.2	qnrS		X	ACGACATTCGTCAACTGCAA	TAAATTGGCACCCTGTAGGC	Published	Robiscek et al AAC (2006) 50(8):2872-4.
V	EURL GEN-2.2	sul1		X	TCACCGAGGACTCCTTCTTC	AATATCGGGATAGAGCGCAG	Published	Walker et al MDR (2001) 7:13-21.
V	EURL GEN-2.2	sul3		X	ACCGATAGTTTTTCCGATGG	TGCGGAGATAATCTGCACCT	In-house	
V	EURL GEN-2.2	tetA		X	CATAGATCGCCGTGAAGAGG	CATAGATCGCCGTGAAGAGG	Published	Ng et al AAC (1999) 43:3018-3021.
V	EURL GEN-2.2	tetC		X	CTTGAGAGCCTTCAACCCAG	ATGGTCGTCATCTACCTGCC	Published	Ng et al AAC (1999) 43:3018-3021.
V	EURL GEN-2.2	tetD		X	AAACCATTACGGCATTCTGC	GACCGGATACACCATCCATC	Published	Ng et al AAC (1999) 43:3018-3021.
V	EURL GEN-2.2	CTX	-M-14		ATGGTGACAAAGAGAGTGCAAC	TTACAGCCCTTCGGCGATG	Published	Batchelor et al AAC (2005) 49:1319-1322.
V	EURL GEN-2.2	OXA	-30		ATATCTCTACTGTTGCATCTCC	AAACCCTTCAAACCATCC	Published	Colom et al FEMS Micro Lett (2003) 223(2):147-5
V	EURL GEN-2.2	catA1			CGCCTGATGAATGCTCATCCG	CCTGCCACTCATCGCAGTAC	Published	Aarestrup et al JAC (2003) 52:715-718.
V	EURL GEN-2.2	gyrA	-83		TGTCCGAGATGGCCTGAAGC	TACCGTCATAGTTATCCACG	Published	Griggs et al AAC (1996) 40(4):1009-13
V	EURL GEN-2.2	aadA			TATCAGAGGTAGTTGGCGTCAT	GTTCCATAGCGTTAAGGTTTCATT	Published	Randall et al JAC (2004) 53:208-216.
V	EURL GEN-2.2	strA			CAACTGGCAGGAGGAACA	CGCAGATAGAAGGCAAGG	Published	Hopkins et al MDR (2007) 13:281-288.
V	EURL GEN-2.2	strB			TTCTCATTGCGGACACCT	GGCATTGCTCATCATTTG	In-house	
V	EURL GEN-2.2	sul2			CCGTCTCGCTCGACAGTTAT	GTGTGTGCGGATGAAGTCAG	In-house	
V	EURL GEN-2.2	tetB			TTGGTTAGGGGCAAGTTTTG	GTAATGGGCCAATAACACCG	Published	Ng et al AAC (1999) 43:3018-3021.

Legend:
Fields shaded grey indicate that the detected gene was expected
Genes in bold were detected but not expected
*CTX-M-9-group recorded (which also includes the CTX-M-14 variant)

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