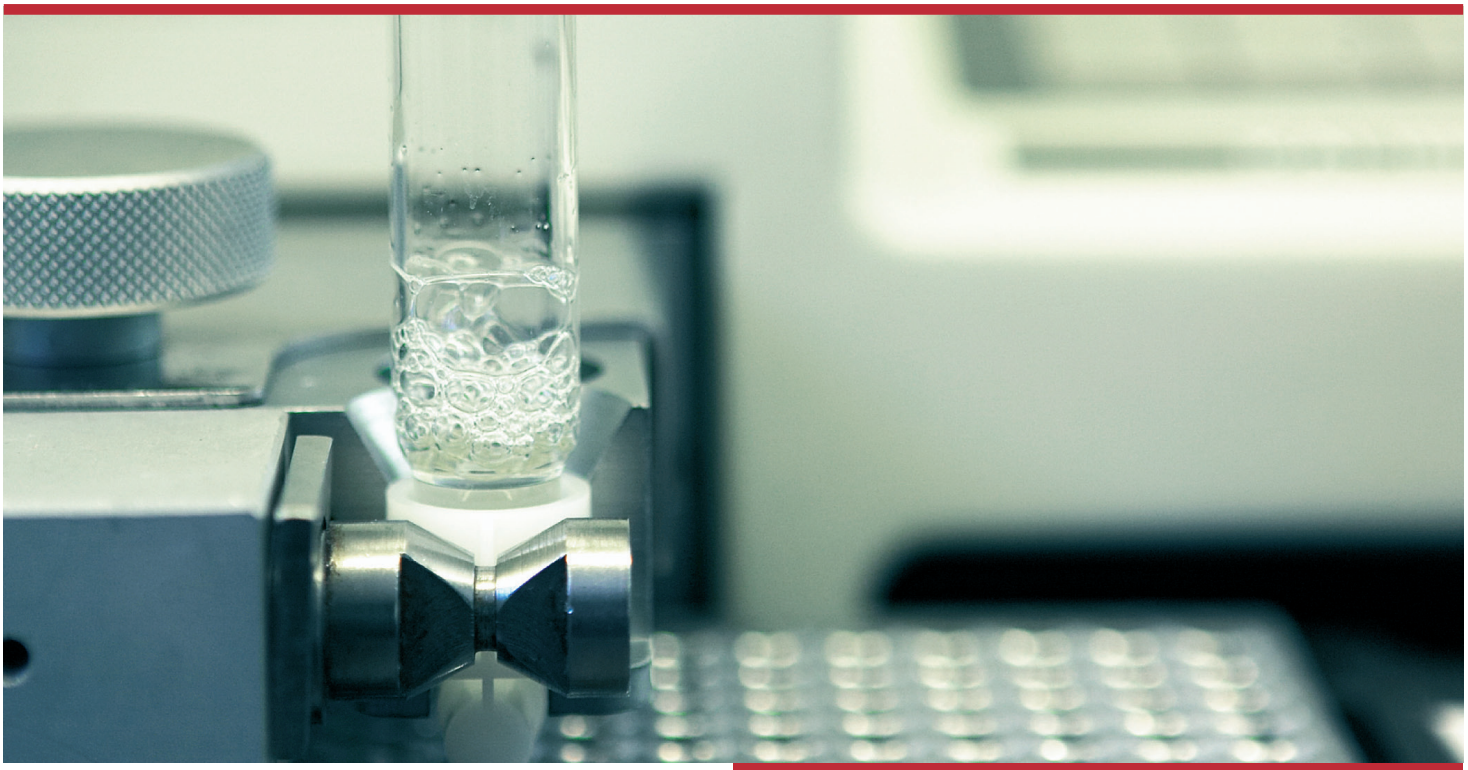
The 11th EURL-AR Proficiency Testing *Salmonella* and *Campylobacter* 2011



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The 11th EURL-AR Proficiency Testing
Salmonella and *Campylobacter* 2011

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1. Introduction

In this report, results are summarised from the eleventh 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 sixth External Quality Assurance System (EQAS) conducted for these microorganisms (the first was EQAS 2006). In addition, the proficiency test for the third time includes an optional element consisting of genotypic characterization by PCR/sequencing of antimicrobial resistance genes of a selected *Staphylococcus aureus* and *Salmonella* spp. isolate.

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

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

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

The AST data reported to EFSA by the Member States (MS) is based on the interpretation of the AST results. The basis for this EQAS evaluation is the interpretation of the AST result; as is also stated in the protocol, the “main objective of this EQAS is to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by the different NRLs”. In addition, the participants of an EQAS should evaluate their own results and introduce corrective actions if necessary. The categorization of an uploaded interpretation as incorrect in the EURL-AR EQAS should induce the participant to perform a self-evaluation. This self-evaluation could very well include a comment on the fact that an acceptable deviation for MIC-determination is ± one dilution step, which in some cases may affect the interpretation of the result. Therefore, the self-evaluation may lead to arguments which can defend the obtained results internally, yet, incorrect interpretations based on a one step dilution difference 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 7th July 2011 by e-mail to the 40 NRLs in the EURL-AR-network (including Croatia, 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 *S. aureus* and *Salmonella* spp. isolate was offered. The pre-notification was sent to NRLs in all EU countries except Luxemburg, where no NRL has been designated. One laboratory was not participating as they had neither *Salmonella* nor *Campylobacter* as their field of responsibility. In addition, Iceland did not participate in this iteration.

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

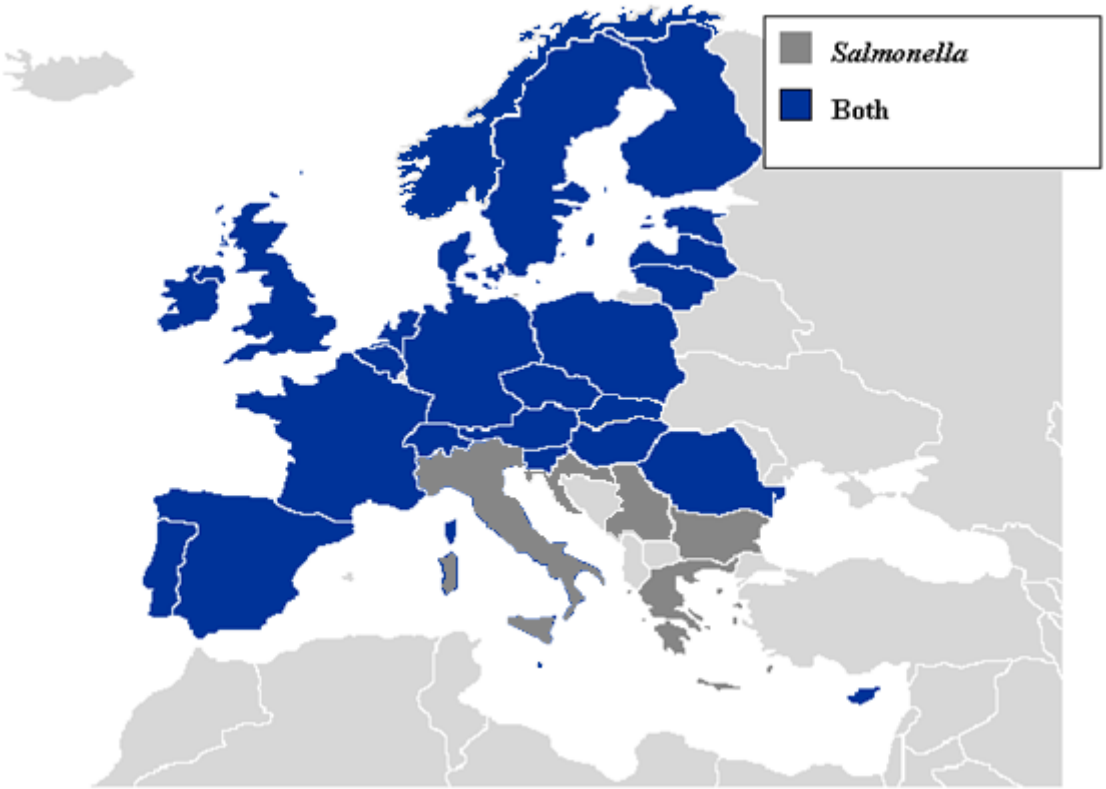

Figure 1: Participating countries that performed antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter.*

The results from the NRLs designated by the MS are presented and evaluated in this report in addition to national reference laboratory in affiliated non-MS; i.e. results from 30 countries consisting of 34 sets of *Salmonella* results and 26 sets of *Campylobacter* results (i.e. results

from the three laboratories not designated by the MS but enrolled on equal terms as these are not further presented or evaluated in this report). Figure 1 illustrates that out of the participating countries, five, for various reasons, uploaded *Salmonella* results only for evaluation (Bulgaria, Croatia, Greece, Italy and Serbia), whereas 26 tested both *Salmonella* and *Campylobacter*. Six laboratories participated in the optional genotypic characterisation of the *S. aureus* and/or the *Salmonella* 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, AST was performed on the *Salmonella* and *Campylobacter* strains 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* and furthermore, chloramphenicol and streptomycin for *Campylobacter*.

The test strains included for optional genotypic characterisation were an *S. aureus* and (EURL GEN 3.1) exhibiting resistance to cefoxitin, penicillin, streptomycin, tetracycline, and tiamulin, and a *Salmonella* spp. (EURL GEN 3.2) exhibiting resistance to amoxicillin-clavulanic acid, ampicillin, cefazolin, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cephalothin, cefpodoxime, ceftiofur, chloramphenicol, florphenicol, gentamicin, spectinomycin, streptomycin, sulfamethoxazole, and tetracycline (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 MIC determination by microbroth dilution (panel code ESB1F), and in addition, for the antimicrobials cefotaxime/clavulanic acid, cefoxitin, ceftazidime/clavulanic acid, tests were performed using E-test from AB-Biodisk, Sweden. The method guidelines used were according to the Clinical and Laboratory Standards Institute (CLSI) document M7-A8 (2009), "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically" (Approved Standard - Eigth Edition), document M100-S21 (2011) "Performance Standards for Antimicrobial Susceptibility Testing" (Twenty-First 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-A2 (2010) "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria" (Approved Guideline – Second Edition) and M31-A3 (2008) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals" (Approved Standard – Third Edition).

2.4 Distribution

On October 24th, 2011, 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, participant receiving a ATCC reference strain were requested to save and maintain this for future proficiency tests.

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

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

Participants using disk diffusion (DD) and E-test were recommended to interpret their results according to their individual routine, categorising the test strains into the terms resistant and susceptible. A categorisation as 'intermediate' was not accepted. The breakpoints used were submitted to the web based database. Breakpoints for 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 the database through a secured individual login. Alternatively, the record sheets from the protocol could be sent by fax to DTU Food. The website was open for data entry in the period from the 25th of October 2011 to the 22nd of December 2011.

Detection of ESBL-producing strains should be performed and interpreted according to recommendations by EUCAST described in the protocol. Concerning the cephalosporins used when detecting ESBL-producing strains 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; i.e. the CLSI documents M31-A3 (2008) or M100-S21 (2011); The Sensititre System (Trek Diagnostic Systems Ltd, UK); or E-tests (AB-Biodisk, Sweden) (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 by the database. 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 mutation site located 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 were obtained by miniaturized microarray; for the Gram-positive strain Identibac *S. aureus* Genotyping test was performed by Alere Technologies GmbH, Germany, detecting 333 genetic markers including mecA and antimicrobial resistance

genes groups such as betalactamases, macrolides, lincosamides, streptogramins, aminoglycosides, tetracycline, chloramphenicol, vancomycin and others. The expected results for the Gram-negative strain were obtained at the EURL-AR by using 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 positive identifications of genes have not been verified elsewhere.

Subsequent to the submission deadline, the laboratories were instructed to login to the secured database once again to retrieve the database generated, individual evaluation report. The evaluation 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-6.2/streptomycin and for S-6.6/streptomycin with a level of agreement with the expected results at 55% and 48%, respectively (Appendix 9a and 9b present the total number of correct/incorrect results for each strain/antimicrobial-combinations).

In both cases with streptomycin, 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 the test strain S-6.2 the presence of *aadA2* 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-6.2/streptomycin and S-6.6/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.

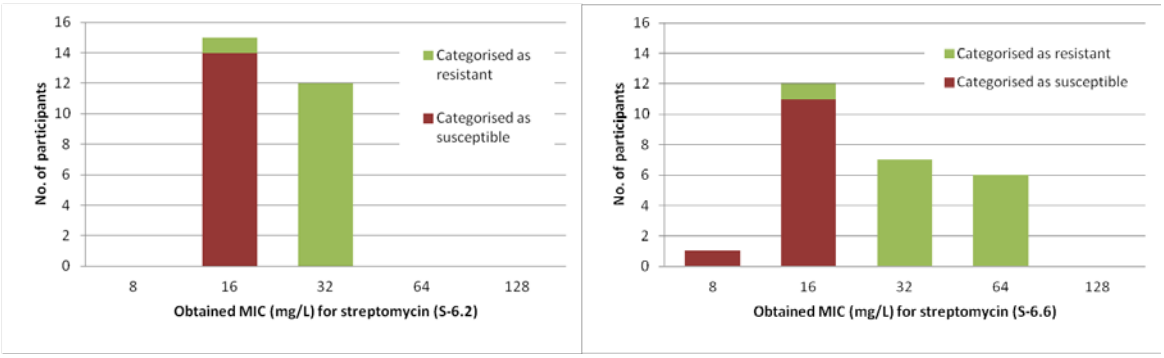

Figure 2: Distribution of the different MIC values obtained by participants performing MIC for the combination S-6.2/streptomycin and S-6.6/streptomycin.

Based on the facts 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, these two strain/antimicrobial combinations have been excluded from the evaluation.

3.1 Methods used by EQAS-participants

In the *Salmonella* trial, 28 laboratories used MIC determination, and six laboratories used disk diffusion. For the *Campylobacter* trial, all 26 included laboratories reported the use of MIC determination (microbroth or agar dilution), in addition, one laboratory submitted disk diffusion results for *Campylobacter* which have been disregarded in this report.

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, 98.1% of the AST's were interpreted correctly. For the *Campylobacter* strains, 97.4% 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). The deviation level in 2011 is acceptable for both the *Salmonella* and the *Campylobacter* trials.

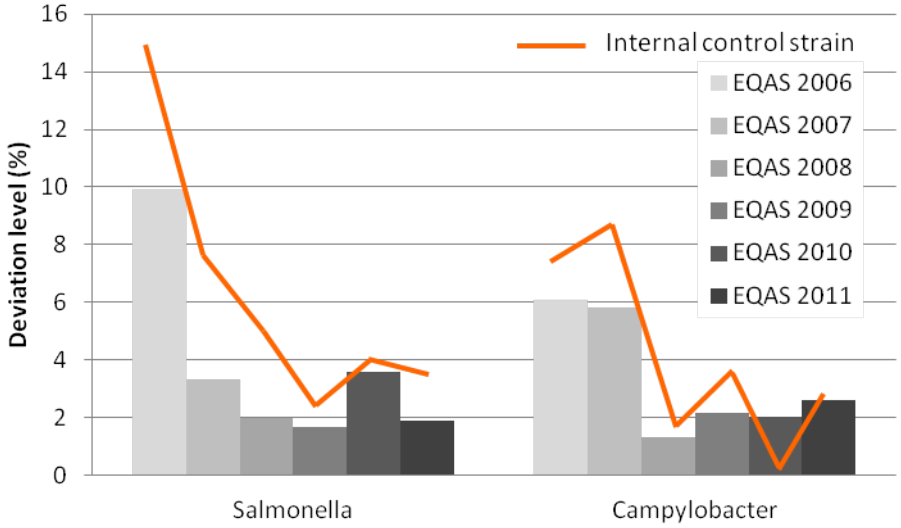

Figure 3: A comparison between the EURL-AR EQAS's since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing performed by participating laboratories

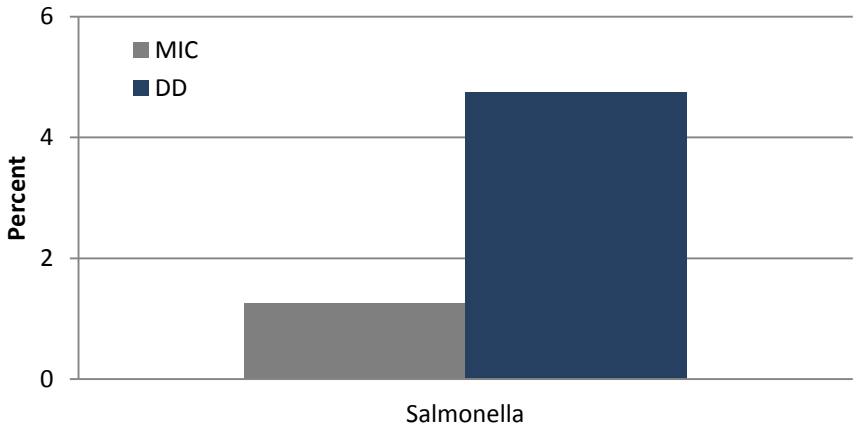

Figure 4: The total percentage of deviations for AST's performed using MIC-methods as opposed to disk diffusion.

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.

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 94.6-99.7% for *Salmonella* and from 96.3-100% for *Campylobacter*.

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 (94.3%) which could be attributed to the strains S-6.3 and S-6.7 which both exhibit reduced susceptibility towards this antimicrobial.

EQAS 2011 – *Salmonella*			EQAS 2011 – *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-6.1	371	99.7	C-6.1 (*C. jejuni*)	169	97.6
S-6.2	337	99.1	C-6.2 (*C. jejuni*)	163	96.3
S-6.3	371	96.5	C-6.3 (*C. jejuni*)	170	96.5
S-6.4	369	96.5	C-6.4 (*C. jejuni*)	169	98.2
S-6.5	371	99.7	C-6.5 (*C. coli*)	176	97.2
S-6.6	335	99.4	C-6.6 (*C. coli*)	176	96.6
S-6.7	369	94.6	C-6.7 (*C. coli*)	176	97.7
S-6.8	370	99.7	C-6.8 (*C. coli*)	176	100.0

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

Sulfamethoxazole also exhibits a low level of correct results, which to a large extent can be attributed to S-6.7 with an expected MIC at 64 mg/L (susceptible), and with a cut-off value at 256 mg/L. The incorrect interpretations as resistant are caused by a three steps (or more) difference from the reference value which is likely to be caused by the fact that this antimicrobial in contrast to other antimicrobial has a bacteriostatic effect and therefore the MIC or the zone diameter result should be read where 80% of the growth is inhibited.

EQAS 2011 Antimicrobial	% correct	
	Salmonella	*Campylobacter*
Ampicillin, AMP	100.0	-
Cefotaxime, CTX	98.9	-
Ceftazidime, CAZ	97.8	-
Ceftiofur, XNL	98.4	-
Chloramphenicol, CHL	100.0	100.0
Ciprofloxacin, CIP	94.3	99.0
Erythromycin, ERY	-	98.0
Gentamicin, GEN	98.9	99.0
Nalidixic acid, NAL	97.7	95.5
Streptomycin, STR	97.0	93.1
Sulphonamides, SMX	95.4	-
Tetracycline, TET	99.3	98.5
Trimethoprim, TMP	99.6	-

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

For *Campylobacter*, streptomycin had the lowest deviation level which was interestingly for all deviations caused by susceptible results identified as resistant. Two laboratories (#4 and #19) each had five of the 14 deviating results from this antimicrobial, with laboratory #4 in fact in four of the five cases obtaining a level of MIC incorrectly categorized as resistant.

ESBL-producing *Salmonella* test strains

It was decided on the EURL-AR workshop 2008 that the testing of ESBL production in *Salmonella* should be mandatory. The laboratories were asked to detect the ESBL-producing

Salmonella strains and to perform confirmatory testing on all relevant strains resistant to cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL) according to the protocol (App. 4b).

The four test strains; S-6.1, S-6.3, S-6.4 and S-6.8 were ESBL-producers, which was confirmed by the majority of the 34 laboratories participating in the *Salmonella* EQAS. As the ESBL detection part is mandatory in this EQAS, all results are evaluated below.

Three of the ESBL-producing strains were so-called 'true ESBLs, harbouring *bla*$_{\text{TEM-52}}$ (S-6.1), *bla*$_{\text{CTX M-15}}$ and *bla*$_{\text{SHV-12}}$ (S-6.3) and *bla*$_{\text{CTX M-15-like}}$ (S-6.4), whereas one was and ampC-producing strain harbouring *bla*$_{\text{CMY-2}}$ (S-6.8) (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-6.1 (CTX M-52)	**Strain S-6.3 (CTX M-15/ SHV-12)**	**Strain S-6.4 (CTX M-15 like)**	**Strain S-6.8 (CMY-2)**
Proportion of laboratories succesfully using different cephalosporins for screening (correct confirmation of ESBL production)	**CTX, CAZ, XNL**	6/7 (86%)	6/7 (86%)	5/7 (71%)	5/7 (71%)
	CTX, CAZ	21/22 (95%)	21/22 (95%)	22/22 (100%)	21/22 (95%)
	CTX, XNL	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
	CTX	4/4 (100%)	4/4 (100%)	4/4 (100%)	3/4 (75%)
Confirmed ESBL-producer		32/34 (94%)	32/34 (94%)	32/34 (94%)	*2/34 (6%)*
FOX^{R}		-	-	-	29/34 (85%)
ampC confirmed		-	-	-	30/34 (88%)
ampC not confirmed		34/34 (100%)	34/34 (100%)	34/34 (100%)	*4/34 (12%)*

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. Fields shaded in grey indicate an unexpected result.

In ten occasions, the ESBL-producing strain was not detected. Four of these deviations were due to one laboratory which did not perform the confirmatory testing (laboratory #54). Additional three deviations were laboratories #21, #26 and #56 failing to detect the strains S-6.1, S-6.2 and S-6.4, respectively, and the remaining cases were laboratories #38, #39 and #41 failing to detect S-6.8 as an ampC-producer. In two occasions, the ampC-producer, S-6.8, was incorrectly confirmed as an ESBL-producer (laboratories #4 and #22).

Eleven laboratories uploaded an MIC-ratio as a result, and 17 uploaded the increase of inhibition zone diameter, additionally, three laboratories uploaded both an MIC and an inhibition zone diameter result.

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.

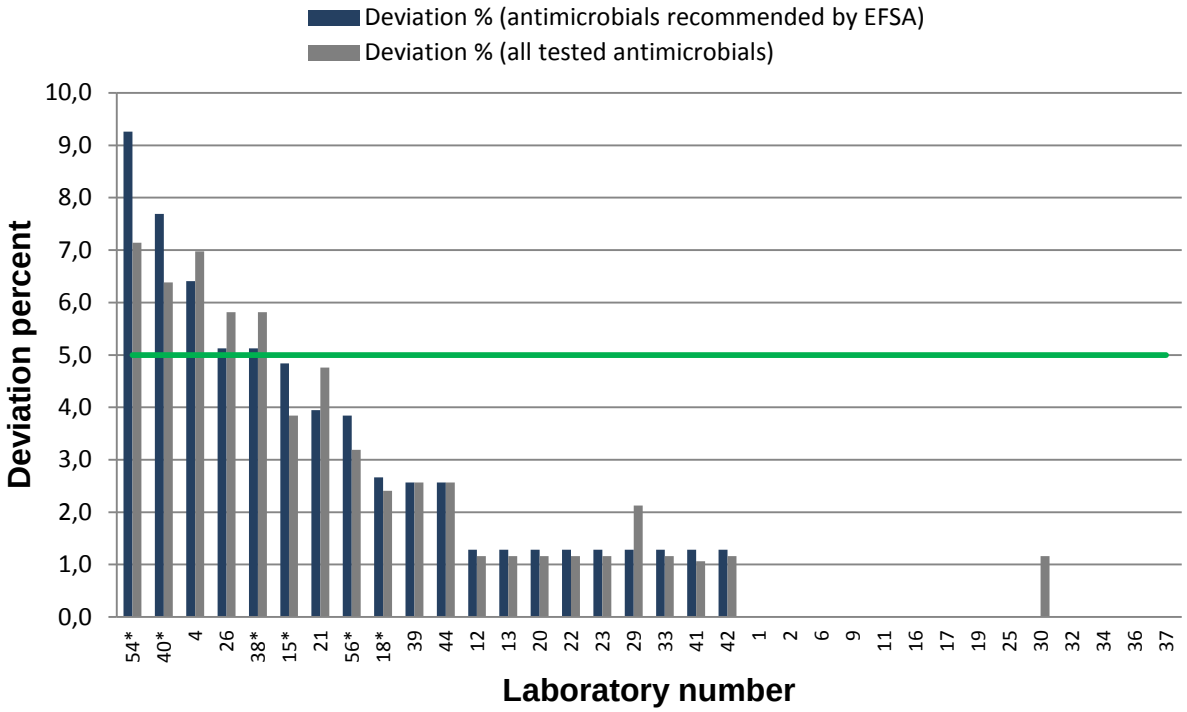

Figure 5: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory performed AST using disk diffusion

3.3.1 *Salmonella* trial

Twenty-nine of the laboratories obtained a result within the acceptance limit at 5% deviations for the *Salmonella* strains. The maximum percentage of deviations was 9.3%.

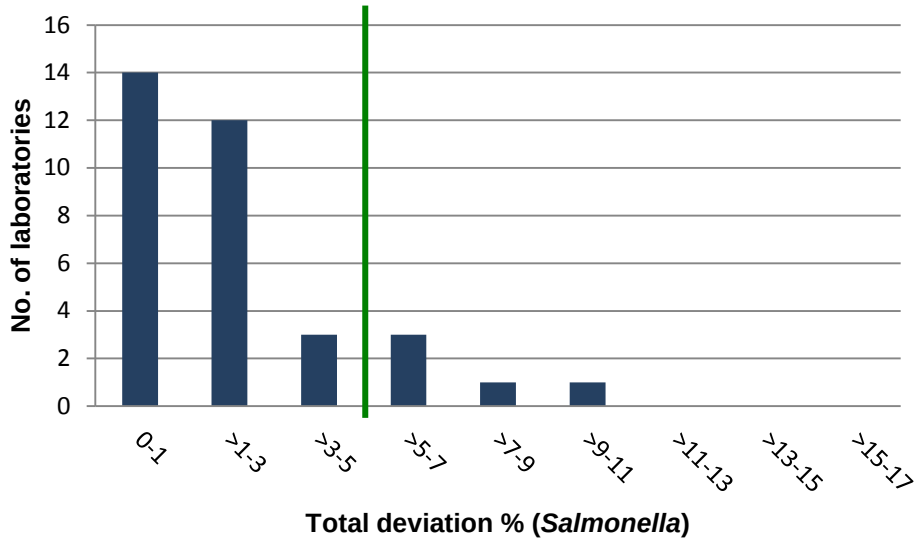

Figure 6: The number of laboratories listed in intervals of percent of total deviations. The green line marks the 5% acceptance limit set by the EURL-AR

Figure 6 illustrates that the performance of five (17%) laboratories resulted in a deviation level above the level of performance expected by the EURL-AR (#4, #26, #38, #40, and #54), however, none of the laboratories are regarded as outliers. As illustrated in Figure 5, deviation levels including all antimicrobials mentioned in the protocol to some extent varies from the deviation levels regarding EFSA-antimicrobials, only.

3.3.2 *Campylobacter* trial

In the *Campylobacter* trial most laboratories performed very well. Applying the 5% acceptance threshold, 23 of 26 participating laboratories performed acceptably, with 20 laboratories having no deviations (Figure 7 and 8). Three laboratories present a deviation level above the 5% acceptance level (#4, #19, and #39) and are regarded as outliers.

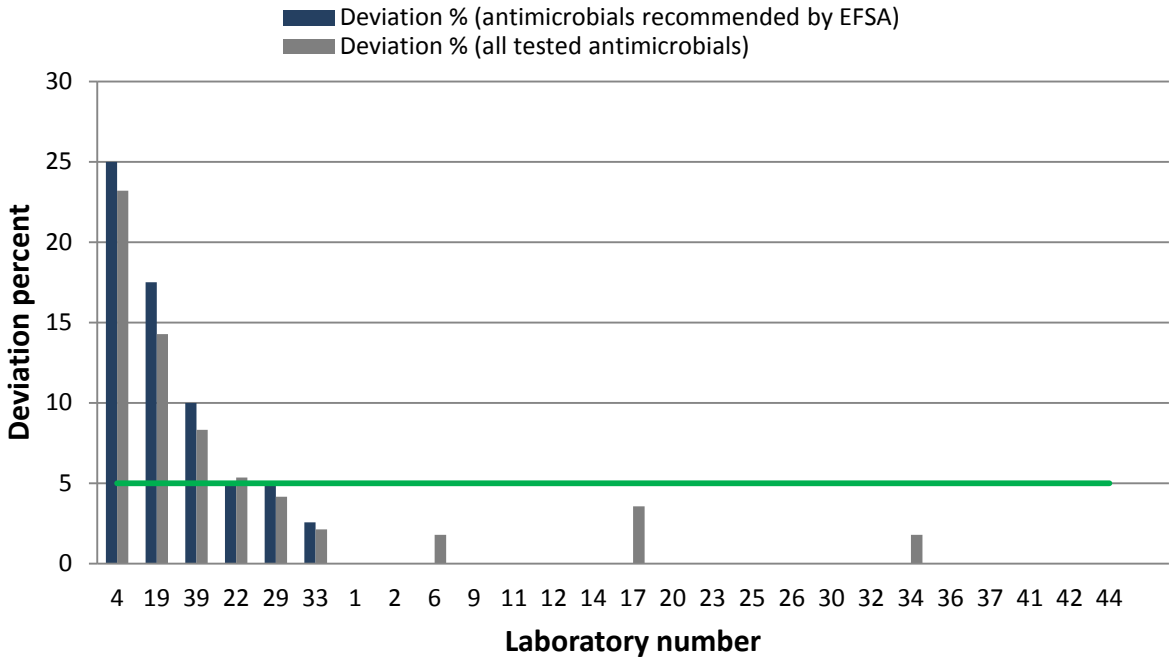

Figure 7: Individual participants' deviations in percent of their total number of *Campylobacter* AST's.

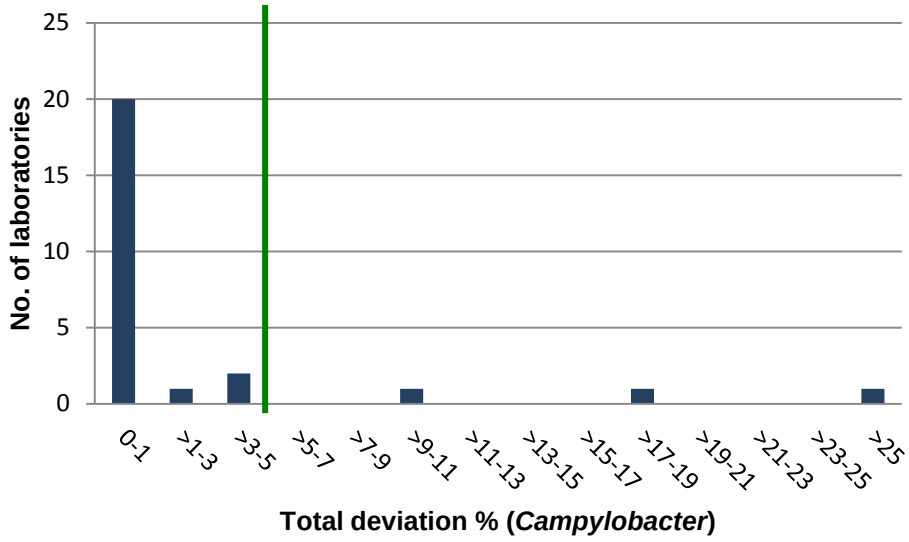

Figure 8: The number of laboratories listed in intervals of percent of total deviations.

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

3.4 Deviations by reference strains

In the following section, deviations are defined as results of antimicrobial susceptibility tests on the reference strain that are outside the quality control (QC) acceptance intervals (App. 7). Values from the participants' testing of the QC strains are listed in Appendix 6a and 6b, and in

Tables 4, 5 and 6 which summarize results from the laboratories' quality control. For the *Salmonella* trial, all laboratories but one (#54) performed QC testing of the reference strain. For the *Campylobacter* trial, all 26 participating laboratories uploaded data from QC-testing on the reference strain.

Table 4 presents the number of laboratories performing disk diffusion to test the *E. coli* reference strain (ATCC 25922). All obtained values were within the QC-range.

EQAS 2011	**Disk diffusion *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in mm inhibition zones (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/4 (0%)	-	-
Cefotaxime, CTX	0/5 (0%)	-	-
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/5 (0%)	-	-
Ceftiofur, XNL	0/4 (0%)	-	-
Chloramphenicol, CHL	0/5 (0%)	-	-
Ciprofloxacin, CIP	0/4 (0%)	-	-
Gentamicin, GEN	0/5 (0%)	-	-
Imipenem, IMI	0/2 (0%)	-	-
Nalidixic acid, NAL	0/5 (0%)	-	-
Streptomycin, STR	0/5 (0%)	-	-
Sulphonamides, SMX	0/3 (0%)	-	-
Tetracycline, TET	0/5 (0%)	-	-
Trimethoprim, TMP	0/4 (0%)	-	-

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

The use of MIC determination for AST of the reference strain *E. coli* ATCC 25922 resulted in submission of data from 28 laboratories, five of which produced one value each outside the QC-limit as illustrated in Table 5.

EQAS 2011	**MIC determination *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/28 (0%)	-	-
Cefotaxime, CTX	0/28 (0%)	-	-
Cefoxitin, FOX	0/6 (0%)	-	-
Ceftazidime, CAZ	0/22 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/28 (0%)	-	-
Ciprofloxacin, CIP	4/28 (14%)	-	1 step
Gentamicin, GEN	0/28 (0%)	-	-
Imipenem, IMI	0/3 (0%)	-	-
Nalidixic acid, NAL	0/28 (0%)	-	-
Streptomycin, STR	0/27 (0%)	-	-
Sulphonamides, SMX	1/20 (5%)	-	1 step
Tetracycline, TET	0/28 (0%)	-	-
Trimethoprim, TMP	0/28 (0%)	-	-

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

EQAS 2011 Antimicrobial	**MIC determination *C. jejuni* ATCC 33560**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	0/19 (0%)	-	-
Ciprofloxacin, CIP	0/25 (0%)	-	-
Erythromycin, ERY	0/25 (8%)	-	-
Gentamicin, GEN	3/24 (13%)	1 step	3 steps
Nalidixic acid, NAL	1/25 (0%)	2 steps	-
Tetracycline, TET	2/25 (8%)	-	1 step

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

All 26 participating 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. Six deviations were seen, divided between five laboratories.

3.5 Genotypic characterisation

For the optional PCR-testing of selected isolates, four and six laboratories performed genotypic characterization on the Gram positive test strain GEN 3.1 and the Gram negative test strain GEN 3.2, respectively. In Appendix 11, information is collected on detected genes, genes which were tested but not detected, primers used, and references for the method used. For all the uploaded results, Table 7 shows very good correlation with the expected genes.

Originally, GEN 3.2 was expected to harbour TEM-1b. This was not verified by any of the participating laboratories. Subsequent to the EURL-AR receiving the results, the GEN 3.2 was retested for TEM-1b, and the presence of the gene could not be verified. The expected results were adjusted and the relevant participating laboratories received direct information about the update.

For the Gram positive bacteria, laboratory III used the MRSA array from Alere (ClonDiag) and PCR-primers described by Argudin et al, 2011 AEM. Laboratory IV also used this microarray and confirmed the results with PCR.

For the Gram negative bacteria, laboratory I, IV and VIII used the Arraytube system from Identibac (AMR-ve genotyping) Alere Technologies GmbH for detection of most of the antimicrobial resistance genes. Laboratory I indicated that if the result for an antimicrobial was found through the Arraytube, no PCR-method was submitted (App. 11).

In addition to the results mentioned in Appendix 11, for GEN 3.1, laboratory VII found the *S. aureus* test strain to be spa-type t011 (repeats 08-16-02-25-34-24-25). For GEN 3.2, laboratory III commented that no mutations conferring quinolone resistance were detected whereas laboratory VIII detected integrase int1.

			Lab I		Lab III		Lab IV	Lab VI	Lab VII	Lab VIII
EURL GEN 3.1	Aminoglycosides	aadE or aad(6)*	NT		NT		NT		NT	
	Betalactamases	blaI	NT		1		1		NT	
	Betalactamases	blaR	NT		1		1		NT	
	Betalactamases	blaZ	1	IH	1		1		NT	
	Betalactamases	mecA	1	IH	1		1		1	
	Streptogramins	vga(A)	1	IH	1		NT		NT	
	Tetracycline	tet(K)	1	IH	1		NT		NT	
	Tetracycline	tet(M)	1	IH	1		1		NT	
EURL GEN 3.2	Betalactams	CMY-2	1/1	IH	1/1	P	1/1	NT	NT	1/1
	Chloramphenicol	floR	1		1	P	1	NT	NT	1
	Streptomycin	strA	NT		1	P	1	NT	NT	1
	Streptomycin	strB	1		1	P	1	NT	NT	1
	Streptomycin	aadA	1		1	P	1	NT	NT	1
	Sulfamethoxazole	sul1	1	IH	1	P	1	NT	NT	1
	Sulfamethoxazole	sul2	1		1	P	1	NT	NT	1
	Tetracycline	tetA	1		1	P	1	NT	NT	1
	Additional info								AmpC Cit	

Table 7: Results from genotypic characterisation.
Legend:
* aadE and aad(6) are synonyms for the same aminoglycoside resistance gene
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
IH indicates that an in-house protocol was used
Shaded fields indicate that no results were uploaded for the test strain.
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 former EQAS iterations.

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct antimicrobial susceptibility test results of *Salmonella* was 98.1%. The majority (n=29) 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 2011-EQAS would appear to be at the same level compared to the performance in the former four iterations.

Salmonella test strain S-6.7 was susceptible to sulphonamides with an MIC at 64µg/mL but appeared to render a number of incorrect interpretations as resistant (8/32). The reason for this is likely to be that this drug is bacteriostatic and not bacteriocidal, why the reading of the MIC or the inhibition zone should be where 80% of the full growth is inhibited. Interestingly, other strains had the same expected MIC with no indication of the same problem.

Ciprofloxacin appeared to cause problems for two of the strains (S-6.3 and S-6.7) which each harbour a plasmid mediated resistance gene; *qnrB* and *qnrD*, respectively, and thus exhibit low-level ciprofloxacin resistance and nalidixic acid susceptibility. The participants generally found these isolates sensitive to nalidixic acid (88% and 94%), whereas a lower number found the strains resistant to ciprofloxacin (82% and 82%). In total for both of the test strains in questions, twelve deviating results have been submitted for ciprofloxacin. Of these, ten were submitted by laboratories performing disk diffusion for AST. These laboratories (#18, #38, #40, #54 and #56) could benefit from referring to the recommendations published by Cavaco and Aarestrup (2009). All four ciprofloxacin-resistant *Salmonella* test strains exhibit reduced susceptibility to this antimicrobial why the use of a 1µg ciprofloxacin disk is recommended when performing DD. Applying the interpretative criteria recommended by Cavaco and Aarestrup to the results obtained by laboratory #54, produce a categorisation as resistant for all the strains in question.

Three (#38, #40, and #54) of the five laboratories exhibiting a deviation level higher than 5% performed disk diffusion for AST and obtained deviation levels at 5.1%, 7.7%, and 9.3% , respectively. The additional two laboratories (#4 and #26) performed MIC for AST and obtained deviation levels at 5.1% and 6.4%, respectively. None of these were defined as outliers.

For laboratory #4, five of the six deviations on *Salmonella* test strains would have been eliminated if the interpretation of the MIC's had followed the cut-off values presented in the protocol. This would have rendered a percentage of correct results at 98.8% for this laboratory.

Laboratories #38, #40, and #54 are recommended to follow the recommendations published by Cavaco and Aarestrup (2009) and in addition, laboratory #54 is recommended to test the *E. coli* QC reference strain and also to submit these values for assessment in future EURL EQAS.

Laboratory #26 obtained 4 deviations on the one test strain (S-6.3) which after investigating this in the laboratory could be attributed to a technical error causing test strain S-6.2 to be tested twice; once as S-6.2 and once as S-6.3 and thereby also ruling out the possibility of detecting the ESBL-production of the S-6.3.

The relatively low performance regarding ciprofloxacin presented in Table 2 (94.3% correct results), was mainly caused by the five laboratories mentioned above (#18, #38, #40, #54 and #56). 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 all laboratories performing disk diffusion for AST except one (#40).

For the *E. coli* reference strain, the obtained results were in general in agreement with the CLSI recommendations. The number of laboratories performing AST on *Salmonella* by the use of disk diffusion was six. Five of these uploaded data for the testing of the reference strain with a total of 100% within range. For the laboratories performing AST on *Salmonella* by an MIC-method, all laboratories uploaded QC-results to the database. The proportion of values within the expected range was 98.4%.

Laboratories #15, #18, #39, #40 and #41 which had a deviation level above the acceptance limit in EQAS 2010 with values of 11%, 8.5%, 11%, 13.4% and 7.3% in 2010, respectively, have

increased their performance considerably with 4.8%, 2.7%, 2.6%, 7.7% and 1.3% deviations, respectively, in the 2011-iteration.

ESBL-producing *Salmonella* test strains

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

Four of the *Salmonella* test strains were ESBL-producers (S-6.1, S-6.3, S-6.4 and S-6.8), and the participants were asked to interpret their results according to the description in the protocol. Of the 34 laboratories which tested *Salmonella*, two one not submit results for confirmatory testing of ESBL-production which resulted in an evaluation as incorrect. The 33 laboratories which uploaded results exhibited in all eight incorrect interpretations, consisting of both false positive and false negative results with the overall proportion of laboratories correctly confirming S-6.1, S-6.3, S-6.4 and S-6.8 as ESBL- or ampC-producers being 94%, 94%, 94% and 88%, 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.

Laboratories #21, #26 and #56 failing to detect the strains S-6.1, S-6.2 and S-6.4, respectively, as ESBL-producers could be attributed to lack of confirmatory testing in spite of resistant screening results (#21 and #56) and a switch of strains in the laboratory (#26).

The laboratories #38, #39 and #41 which failed to detect S-6.8 as an ampC-producer all found the test strain resistant to the respective cephalosporin(s) but did not submit results for cefoxitin. No indication was seen that there was a reaction to the detected cephalosporin resistance.

In the two occasions where the ampC-producer, S-6.8, was incorrectly confirmed as an ESBL-producer (laboratories #4 and #22), the strain was at the same time correctly confirmed as an ampC-producer. Both screening results and results from confirmatory testing were as expected.

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

Laboratory #54 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 97.5%. The performance varied from no deviations to 25% deviations, with 23 laboratories

performing satisfactorily according to the established acceptance ranges. The three laboratories (#4, #19, and #39) with deviation levels above 5% were all defined as outliers.

The deviation levels above 5% appear to be caused by different reasons: The results obtained by laboratory #4 are for five of the 13 deviations interpreted incorrectly when applying the interpretative criteria given in the protocol (Appendix 4). When assessing the results obtained when testing the *C. jejuni* reference strain, all results are within the QC range, however, for all deviating results except one, the obtained MIC is up to four and five folds higher than expected.

Laboratory #19 incorrectly detected streptomycin resistance in five of the *Campylobacter* test strains, which was also the case in last year's EQAS for three of the test strains. No reference values are available for streptomycin for the *C. jejuni* reference strain, and it is suggested that the laboratory collect information in-house to follow the trend of the MIC of the QC-strain towards this antimicrobial.

Laboratory #39 exhibit a deviation level at 10%. The deviations indicate a possibility that two strains have been exchanged by mistake.

The proportion of results for the *C. jejuni* reference strain within the QC intervals was 95.8% which is a little higher than in EQAS 2010. In this year's trial, all participating laboratories uploaded data from tests performed on the reference strain. The six values outside the QC intervals were obtained by five laboratories, of which four performed well (under the 5% acceptance level). The remaining one had a deviation level at 17.5% (laboratory #19).

Laboratories #19, #21, #22, #30 and #44 which had a deviation level above the acceptance limit in EQAS 2010 showed values of 10%, 11.1%, 11.4%, 7.5% and 7.5% in 2010, respectively, where laboratory #19 exhibits a decrease in performance (to 17.5%), one laboratory did not participate in this part of the EQAS this year and the remaining three laboratories (#22, #30 and #44) exhibit an improvement to 5%, 0% and 0%, respectively.

One of the *Campylobacter* test strains exhibits the rare antimicrobial resistance profile of ciprofloxacin resistance and nalidixic acid susceptibility. This strain was isolated from food in Denmark and tested at DTU Food with the EURL for *Campylobacter* at SVA in Sweden kindly confirming the species identification. A number of the participants commented on the rare phenotype, one laboratory chose not to upload the result for nalidixic acid and four laboratories submitted an interpretation as resistant for nalidixic acid/C-6.3; all four obtained MIC values at 32 or 64 µg/mL. The ciprofloxacin result did not cause any problems (100% correct results).

4.3 Optional genotypic characterisation

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 (98.1%) when compared to the results from the EQAS 2009 and 2010 (98.4% and 97.8%). Regarding *Campylobacter* AST's, the level of deviation also appears to be stable with a level at 1.9% in 2011 compared to 2.2% and 2.0% in 2009 and 2010.

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

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

Cavaco LM and **Aarestrup** FM. (2009). Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac*(6')*Ib-cr*, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. J Clin Microbiol. 2009 Sep;47(9):2751-8

Schwarz S, Silley P, Simjee S, Woodford N, van DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 65: 601-604

DTU Food
National Food Institute

European Union Reference Laboratory Antimicrobial Resistance

EURL-AR EQAS pre-notification

DFVF- M00-06-001/21.05.2010

EQAS 2011 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 *Salmonella* spp. and one MRSA). 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, laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. Participation is free of charge for all designated NRL-AR's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
Please remember to provide the EQAS coordinator with documents or other information that can simplify customs procedures (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".

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped in October 2011. The protocol will be available on the website (www.eurl-ar.eu).

Submission of results: Results must be submitted to the National Food Institute no later than December 16th 2011 via the password-protected website. Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

EQAS report: 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 performed in June 2012.

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

Sincerely,

Susanne Karlsmose (EQAS-Coordinator)

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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	-	-	Croatian Veterinary Institut	Croatia
X	X	-	Veterinary Services	Cyprus
X	X	-	State Veterinary Institute Praha	Czech Republic
X	X	X	The National Food Institute	Denmark
X	X	-	Estonian Veterinary and Food Laboratory	Estonia
X	X	-	Finnish Food Safety Authority EVIRA	Finland
X	-	-	ANSES Maisons Alfort	France
-	X	-	ANSES Ploufragan	France
X	-	-	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
X	X	-	Central Veterinary Research Laboratory	Ireland
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 Food and Veterinary Risk Assessment Institute	Lithuania
X	X	-	Public Health Laboratory	Malta
X	X	-	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	-	Veterinærinstituttet	Norway
X	X	-	National Veterinary Research Institute	Poland
X	X	-	Laboratorio National de Investigacáo Veterinaria	Portugal
X	X	X	National Institute of Research-Development for Microbiology and Immunology "Cantacuzino"	Romania
X	X	-	Institute for Hygiene and Veterinary Public Health	Romania
X	(X)	-	Institute of Veterinary Medicine of Serbia	Serbia
X	X	-	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	-	National Veterinary Institute	Slovenia
-	-	-	Laboratorio Central de Sanidad, Animal de Santa Fe (only Staph)	Spain
X	X	X	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	-	VISAVET Health Surveillance Center, Complutense University	Spain
X	-	-	Centro nacional de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricio	Spain
X	X	X	National Veterinary Institute, SVA	Sweden
X	X	X	Vetsuisse faculty Bern: Institute of veterinary bacteriology	Switzerland
X	X	-	The Veterinary Laboratory Agency	United Kingdom
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 CTX:CTX/Cl	Ceftazidime CAZ		ESBL 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-6.1	> 32	RESIST	> 4	RESIST	>8 / phantom	= 8	RESIST	>8 / phantom	<= 4	SUSC	> 8	RESIST	= 4	SUSC	= 0.06	SUSC	= 1	SUSC	<= 0.5	SUSC	= 4	SUSC	= 16	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-6.2	> 32	RESIST	<= 0.12	SUSC		= 0.25	SUSC				<= 0.5	SUSC	= 64	RESIST	= 0.25	RESIST	= 1	SUSC			> 64	RESIST	= 32	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-6.3	> 32	RESIST	> 4	RESIST	>8	= 128	RESIST	>8	<= 4	SUSC	> 8	RESIST	= 64	RESIST	= 0.25	RESIST	> 16	RESIST	<= 0.5	SUSC	= 8	SUSC	> 128	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-6.4	> 32	RESIST	> 4	RESIST	>8	= 1	SUSC	<8	<= 4	SUSC	> 8	RESIST	= 4	SUSC	= 0.5	RESIST	= 1	SUSC	<= 0.5	SUSC	> 64	RESIST	= 16	SUSC	= 64	SUSC	> 32	RESIST	<= 1	SUSC
EURL S-6.5	<= 1	SUSC	<= 0.12	SUSC		= 0.12	SUSC				= 1	SUSC	= 4	SUSC	= 0.03	SUSC	= 0.5	SUSC			= 4	SUSC	= 16	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-6.6	<= 1	SUSC	<= 0.12	SUSC		= 0.25	SUSC				= 1	SUSC	= 4	SUSC	= 0.03	SUSC	= 1	SUSC			= 4	SUSC	= 32	RESIST	> 1024	RESIST	> 32	RESIST	> 32	RESIST
EURL S-6.7	<= 1	SUSC	<= 0.12	SUSC		= 0.5	SUSC				= 1	SUSC	= 8	SUSC	= 0.25	RESIST	= 0.5	SUSC			= 8	SUSC	= 16	SUSC	= 64	SUSC	<= 2	SUSC	<= 1	SUSC
EURL S-6.8	> 32	RESIST	> 4	RESIST	<8 / ND	= 16	RESIST	<8 / ND	= 32	RESIST	> 8	RESIST	> 64	RESIST	= 0.03	SUSC	= 1	SUSC	<= 0.5	SUSC	= 4	SUSC	> 128	RESIST	> 1024	RESIST	> 32	RESIST	<= 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-6.1	<= 2	SUSC	= 0.12	SUSC	= 0.5	SUSC	= 0.25	SUSC	= 4	SUSC	<= 1	SUSC	= 0.25	SUSC
C. jejuni	EURL C-6.2	= 4	SUSC	= 0.12	SUSC	= 1	SUSC	= 0.25	SUSC	= 4	SUSC	<= 1	SUSC	= 64	RESIST
C. jejuni	EURL C-6.3	= 4	SUSC	= 8	RESIST	= 0.5	SUSC	= 0.125	SUSC	<= 2	SUSC	<= 1	SUSC	= 0.5	SUSC
C. jejuni	EURL C-6.4	= 4	SUSC	= 8	RESIST	= 1	SUSC	= 0.125	SUSC	> 64	RESIST	<= 1	SUSC	= 32	RESIST
C. coli	EURL C-6.5	= 8	SUSC	= 64	RESIST	> 64	RESIST	= 0.25	SUSC	> 64	RESIST	<= 1	SUSC	> 64	RESIST
C. coli	EURL C-6.6	= 4	SUSC	= 16	RESIST	= 1	SUSC	= 0.5	SUSC	> 64	RESIST	= 4	SUSC	= 0.25	SUSC
C. coli	EURL C-6.7	= 4	SUSC	= 0.12	SUSC	> 64	RESIST	= 0.5	SUSC	= 4	SUSC	= 2	SUSC	= 0.25	SUSC
C. coli	EURL C-6.8	= 8	SUSC	= 0.06	SUSC	> 64	RESIST	= 0.5	SUSC	= 8	SUSC	> 16	RESIST	= 0.25	SUSC

Resistant

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DFVF- M00-06-001/21.05.2010

European Union Reference Laboratory Antimicrobial Resistance

EURL-AR External Quality Assurance System 2011

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

Id: «Lab_no»
«Institute»
«Country»

Kgs. Lyngby, October 2011

Dear «Name»,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2011.

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. Participants receiving the additional strains, EURL GEN 3.1 and EURL GEN 3.2, have the option to characterise them genotypically. 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 16th, 2011**.

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) 2011

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PROTOCOL

For antimicrobial 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 antimicrobial susceptibility testing (AST) of *Salmonella* and *Campylobacter*. The *Salmonella* and *Campylobacter* EQAS 2011 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 EURL-AR network who have not already received the mentioned reference strains, these are included in the parcel. The reference strains will not be included in the years to come. The reference strains are original certified cultures and are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the manual ‘Subculture and Maintenance of QC Strains’. Please use them for future internal quality control for susceptibility testing in your laboratory.

For this EQAS, members of the Food- and Waterborne Diseases and Zoonoses Programme (FWD) based at ECDC are also participating, however for these participants the EQAS has been slightly adjusted. Description of this can be found in this protocol, i.e. that QC reference strains are not offered, and that for antimicrobial susceptibility testing (AST) of *Campylobacter*, results obtained by in-house methods like disk diffusion or E-test are also accepted.

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

2 OBJECTIVES

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

3 OUTLINE OF THE EQAS 2011

3.1 Shipping, receipt and storage of strains

In October 2011, the EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing eight *Salmonella*, eight *Campylobacter* strains and two additional strain(s) for optional PCR (one *Salmonella* and one *Staphylococcus*). Reference strains will be included for participants who have not previously received these. 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.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

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

3.3 Susceptibility testing

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

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

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

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

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

3.3.1 *Salmonella*

Testing of gentamicin 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.

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)

Also, when following EUCAST epidemiological cut-off values, *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin. When using disc diffusion and CLSI

clinical breakpoints this connection between nalidixic acid and ciprofloxacin is not taken into account. Thus, the result in this situation with regard to ciprofloxacin will deviate from the expected result in this EQAS.

Important notes*: beta-lactam resistance:*

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

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

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

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

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

3.3.2 *Campylobacter*

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

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

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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

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

3.4 Optional genotypic characterisation

An optional PCR-testing of a selected *S. aureus* (EURL GEN 3.1) as well as a *Salmonella* (EURL GEN 3.2) 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 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 EURL-AR.

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Antimicrobial	Gene
Aminoglycosides	addD
	aphA3
	aacA-aphD
	aadE
Betalactamases	blaI
	blaR
	blaZ
	mecA
Chloramphenicol	cat
Glycopeptides	vanA
	vanB
	vanZ
Lincosamides	lnu(A)
Macrolides	erm(A)
	erm(B)
	erm(C)
	mef(A)
	mph(C)
	msr(A)
Quarternary ammonium compounds	qacA
Steroid antibacterial	far1
Streptogramin	vat(A)
	vat(B)
	vga(A)
	vgB(A)
Streptothricin	sat
Tetracycline	tet(K)
	tet(M)
	tet(O)
Trimethoprim	dfrA

Table 3: Genes included in the test of the *S. aureus*-strain (EURL GEN 3.1)

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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
Sulfonamides	-	sul1
	-	sul2
	-	sul3
Tetracycline	-	tetA
	-	tetB
	-	tetC
	-	tetD
	-	tetE
	-	tetF
	-	tetG

Table 4: Genes included in the test of the *Salmonella*-strain (EURL GEN 3.2)

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4 REPORTING OF RESULTS AND EVALUATION

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

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than 16th December 2011.** After the deadline, the database will be closed and you will be able to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

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

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

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

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

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

Susanne Karlsmose
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby
Denmark
Tel: +45 3588 6601
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 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

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

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

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

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

Brand of microdilution plate, strips or disks:

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

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

Comments or additional information:

TEST FORM

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

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

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

TEST FORM

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

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

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

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

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

Brand of broth/agar:

Additional comments:

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

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

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

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

TEST FORM

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

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

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

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

Comments:

TEST FORM

Susceptibility testing of *E. coli* referencestrain ATCC 25922

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

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

TEST FORM

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

TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

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

For Agar dilution:

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

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

TEST FORM – genotypic characterisation

Genotypic characterisation of the test strains

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

Comments:

INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

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

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

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

Please note that:

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

SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1.1 Purpose

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

1.2 References

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

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

1.3 Definition of Terms

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

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

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

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

1.4 Important Considerations

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

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

1.5 Storage of Reference Strains

Preparation of stock cultures

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

Working cultures

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

1.6 Frequency of Testing

Weekly vs. daily testing

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

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

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

Corrective Actions

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

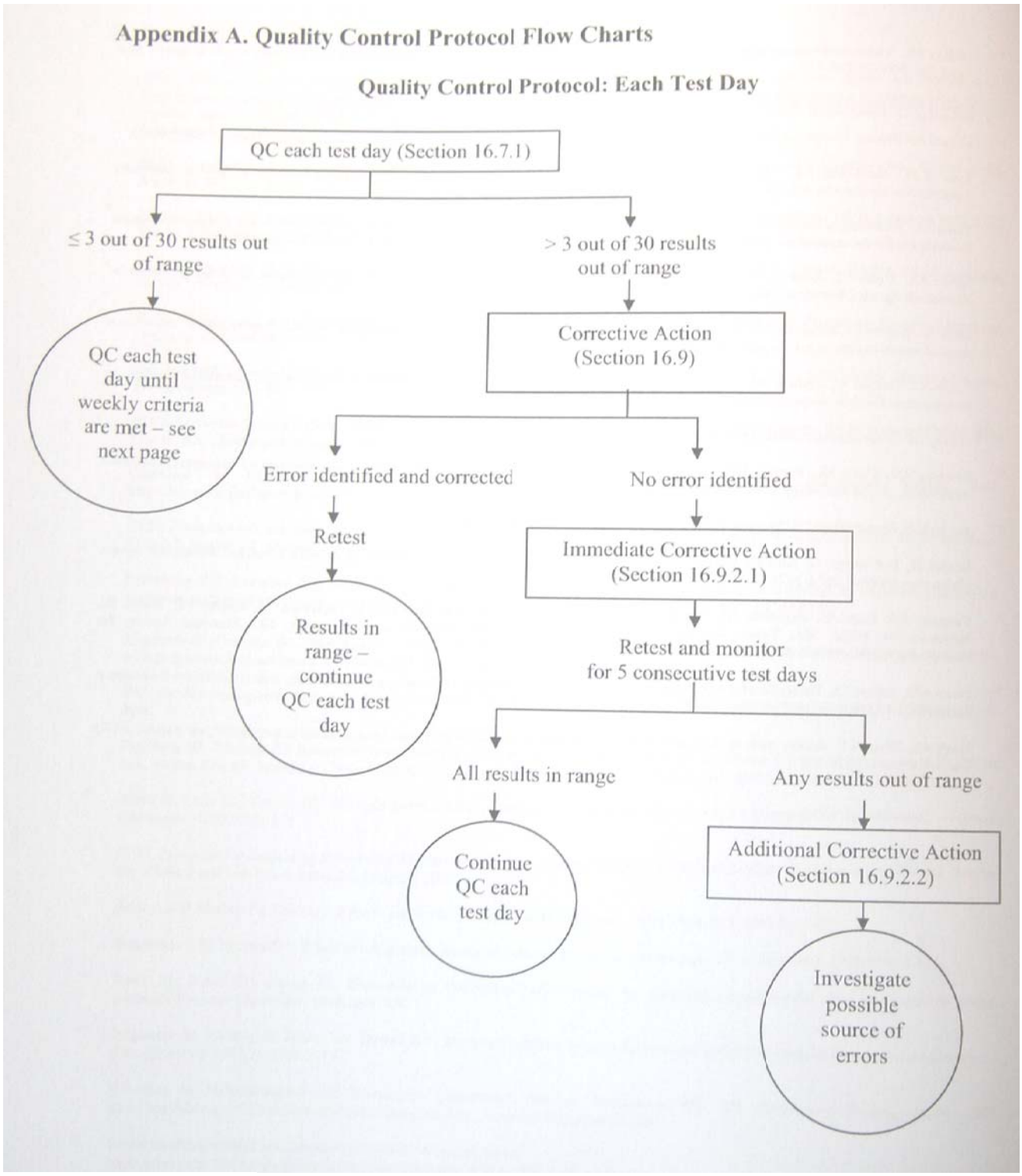
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

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

Repeat the 30 days validation before resuming weekly testing.

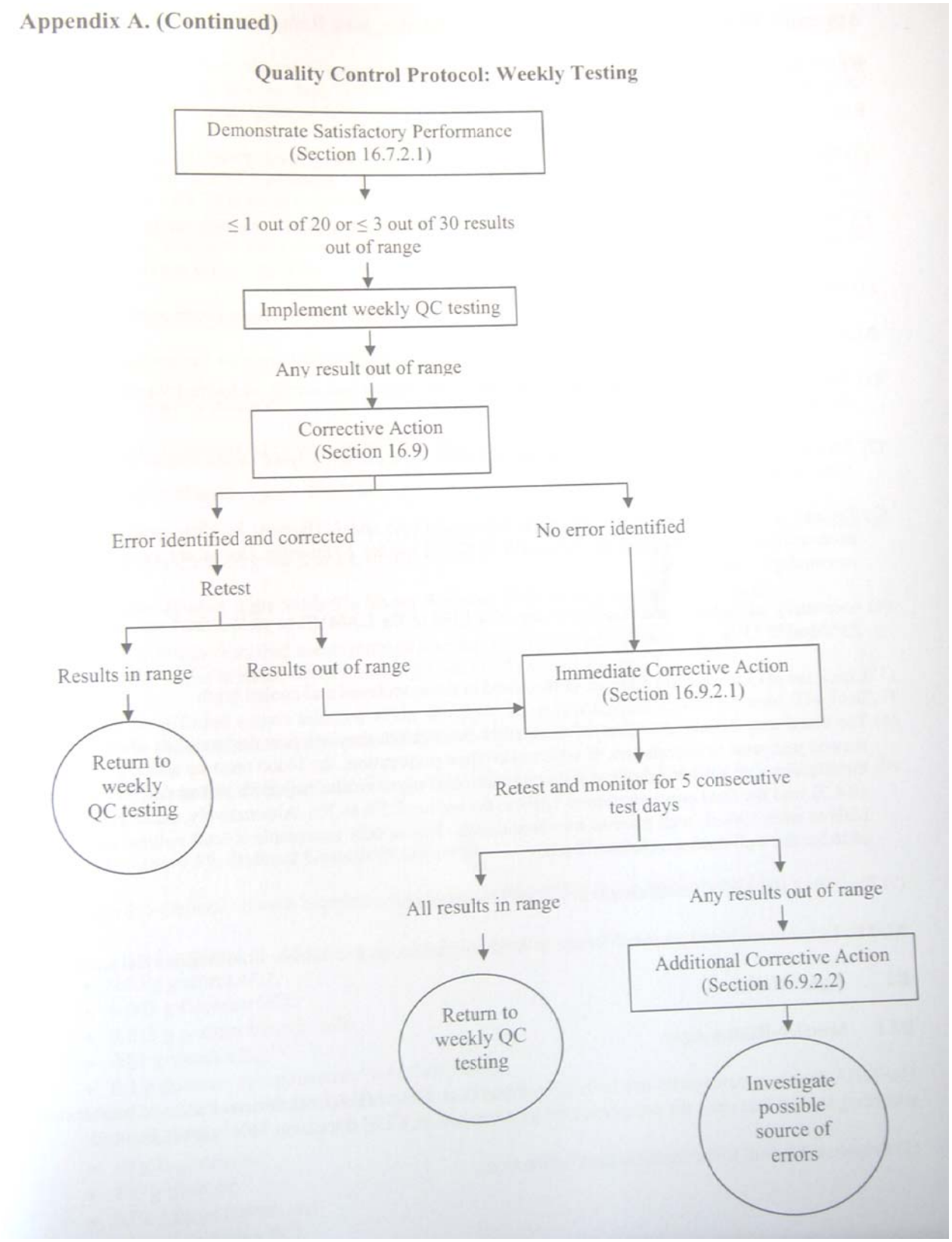
DAILY MIC QC CHART



Reference: CLSI M7-A8, page 44

WEEKLY MIC QC CHART

Appendix A. (Continued)



Reference: CLSI M7-A8, page 45

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

Antimicrobial	Lab No	Disk content (ug)	R <= (mm)	I = (mm)	S >= (mm)
Ampicillin, AMP	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	<=	0.12	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	<=	0.5	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	=	2	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	=	16	8	32	1	MIC
2	Tetracycline, TET	<=	1	0,5	2	1	MIC
2	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
4	Ampicillin, AMP	=	2	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	=	0.5	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	=	16	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	<	0.25	0,25	1	1	MIC
6	Nalidixic acid, NAL	<	4	1	4	1	MIC
6	Streptomycin, STR	=	4	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	Ceftiofur, XNL	=	0.5	0,25	1	1	MIC
9	Chloramphenicol, CHL	=	4	2	8	1	MIC
9	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
9	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
9	Imipenem, IMI	=	0.12	0,06	0,25	1	MIC
9	Nalidixic acid, NAL	=	4	1	4	1	MIC
9	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	=	2	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	<=	2	1	4	1	MIC
11	Streptomycin, STR	=	4	4	16	1	MIC
11	Sulfisoxazole, FIS	<=	8	8	32	1	MIC
11	Tetracycline, TET	=	1	0,5	2	1	MIC
11	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
12	Ampicillin, AMP	=	4	2	8	1	MIC
12	Cefotaxime, CTX	=	0.12	0,03	0,125	1	MIC
12	Ceftazidime, CAZ	=	0.5	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	=	8	4	16	1	MIC
12	Tetracycline, TET	<=	1	0,5	2	1	MIC
12	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
13	Ampicillin, AMP	=	4	2	8	1	MIC
13	Cefotaxime, CTX	<=	0.06	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	=	8	4	16	1	MIC
13	Sulfisoxazole, FIS	=	32	8	32	1	MIC
13	Tetracycline, TET	<=	1	0,5	2	1	MIC
13	Trimethoprim, TMP	=	1	0,5	2	1	MIC
15	Cefotaxime, CTX	=	35	29	35	1	DD
15	Cefoxitin, FOX	=	27	23	29	1	DD
15	Ceftazidime, CAZ	=	32	25	32	1	DD
15	Ceftiofur, XNL	=	28	26	31	1	DD
15	Chloramphenicol, CHL	=	27	21	27	1	DD
15	Gentamicin, GEN	=	26	19	26	1	DD
15	Nalidixic acid, NAL	=	24	22	28	1	DD
15	Streptomycin, STR	=	20	12	20	1	DD
15	Tetracycline, TET	=	25	18	25	1	DD
15	Trimethoprim, TMP	=	24	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	=	1	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	=	2	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	=	0.015	0,004	0,016	1	MIC
17	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
17	Nalidixic acid, NAL	<=	4	1	4	1	MIC
17	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	=	1	0,5	2	1	MIC
18	Ampicillin, AMP	=	19	16	22	1	DD
18	Cefotaxime, CTX	=	32	29	35	1	DD
18	Cefoxitin, FOX	=	27	23	29	1	DD
18	Ceftazidime, CAZ	=	29	25	32	1	DD
18	Chloramphenicol, CHL	=	25	21	27	1	DD
18	Ciprofloxacin, CIP	=	35	30	40	1	DD
18	Gentamicin, GEN	=	22	19	26	1	DD
18	Nalidixic acid, NAL	=	24	22	28	1	DD
18	Streptomycin, STR	=	15	12	20	1	DD
18	Sulfisoxazole, FIS	=	21	15	23	1	DD
18	Tetracycline, TET	=	23	18	25	1	DD
19	Ampicillin, AMP	=	2	2	8	1	MIC
19	Cefotaxime, CTX	=	0.06	0,03	0,125	1	MIC
19	Ceftazidime, CAZ	=	0.25	0,06	0,5	1	MIC
19	Chloramphenicol, CHL	=	8	2	8	1	MIC
19	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
19	Gentamicin, GEN	=	1	0,25	1	1	MIC
19	Nalidixic acid, NAL	=	4	1	4	1	MIC
19	Streptomycin, STR	=	16	4	16	1	MIC
19	Sulfisoxazole, FIS	=	32	8	32	1	MIC
19	Tetracycline, TET	=	1	0,5	2	1	MIC
19	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
20	Ampicillin, AMP	=	4	2	8	1	MIC
20	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
20	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
20	Chloramphenicol, CHL	=	8	2	8	1	MIC
20	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
20	Gentamicin, GEN	=	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	=	32	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	=	0.06	0,03	0,125	1	MIC
21	Ceftazidime, CAZ	=	0.25	0,06	0,5	1	MIC
21	Chloramphenicol, CHL	=	4	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
21	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	16	8	32	1	MIC
21	Tetracycline, TET	=	2	0,5	2	1	MIC
21	Trimethoprim, TMP	=	1	0,5	2	1	MIC

22	Ampicillin, AMP	=	4	2	8	1	MIC
22	Cefotaxime, CTX	<	0.06	0,03	0,125	1	MIC
22	Ceftazidime, CAZ	<	0.25	0,06	0,5	1	MIC
22	Chloramphenicol, CHL	=	4	2	8	1	MIC
22	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
22	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
22	Nalidixic acid, NAL	<	4	1	4	1	MIC
22	Streptomycin, STR	=	8	4	16	1	MIC
22	Sulfisoxazole, FIS	=	32	8	32	1	MIC
22	Tetracycline, TET	<	1	0,5	2	1	MIC
22	Trimethoprim, TMP	=	1	0,5	2	1	MIC
23	Ampicillin, AMP	=	4	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	<	0.008	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
25	Ampicillin, AMP	=	4	2	8	1	MIC
25	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
25	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
25	Chloramphenicol, CHL	=	4	2	8	1	MIC
25	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
25	Gentamicin, GEN	=	0.5	0,25	1	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	=	4	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	=	4	2	8	1	MIC
29	Cefotaxime, CTX	<	0.06	0,03	0,125	1	MIC
29	Ceftazidime, CAZ	=	0.06	0,06	0,5	1	MIC
29	Ceftiofur, XNL	=	27	26	31	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	=	2	0,5	2	1	MIC
29	Trimethoprim, TMP	=	1	0,5	2	1	MIC

30	Ampicillin, AMP	=	2	2	8	1	MIC
30	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
30	Cefoxitin, FOX	<=	4	2	8	1	MIC
30	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
30	Chloramphenicol, CHL	=	4	2	8	1	MIC
30	Ciprofloxacin, CIP	<=	0.008	0,004	0,016	1	MIC
30	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
30	Imipenem, IMI	<=	0.5	0,06	0,25	1	MIC
30	Nalidixic acid, NAL	<=	4	1	4	1	MIC
30	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	=	4	2	8	1	MIC
32	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
32	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
32	Chloramphenicol, CHL	=	4	2	8	1	MIC
32	Ciprofloxacin, CIP	<=	0.008	0,004	0,016	1	MIC
32	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
32	Nalidixic acid, NAL	<=	4	1	4	1	MIC
32	Streptomycin, STR	=	8	4	16	1	MIC
32	Sulfisoxazole, FIS	=	16	8	32	1	MIC
32	Tetracycline, TET	<=	1	0,5	2	1	MIC
32	Trimethoprim, TMP	<=	0.5	0,5	2	1	MIC
33	Ampicillin, AMP	=	4	2	8	1	MIC
33	Cefotaxime, CTX	=	0.12	0,03	0,125	1	MIC
33	Cefoxitin, FOX	=	4	2	8	1	MIC
33	Ceftazidime, CAZ	=	0.5	0,06	0,5	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.03	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	=	8	4	16	1	MIC
33	Sulfisoxazole, FIS	=	16	8	32	1	MIC
33	Tetracycline, TET	=	2	0,5	2	1	MIC
33	Trimethoprim, TMP	=	1	0,5	2	1	MIC
34	Ampicillin, AMP	=	2	2	8	1	MIC
34	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
34	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
34	Chloramphenicol, CHL	=	4	2	8	1	MIC
34	Ciprofloxacin, CIP	<=	0.008	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	=	4	4	16	1	MIC
34	Sulfisoxazole, FIS	=	64	8	32	**0**	MIC
34	Tetracycline, TET	<=	1	0,5	2	1	MIC
34	Trimethoprim, TMP	=	1	0,5	2	1	MIC
36	Ampicillin, AMP	=	2	2	8	1	MIC
36	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
36	Chloramphenicol, CHL	=	4	2	8	1	MIC
36	Ciprofloxacin, CIP	=	0.03	0,004	0,016	**0**	MIC
36	Gentamicin, GEN	=	1	0,25	1	1	MIC
36	Nalidixic acid, NAL	<=	2	1	4	1	MIC
36	Streptomycin, STR	=	8	4	16	1	MIC
36	Tetracycline, TET	=	1	0,5	2	1	MIC
36	Trimethoprim, TMP	=	0.5	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	=	1	0,25	1	1	AGA
37	Nalidixic acid, NAL	<=	2	1	4	1	AGA
37	Streptomycin, STR	=	4	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	=	16.3	16	22	1	DD
38	Cefotaxime, CTX	=	33.7	29	35	1	DD
38	Cefoxitin, FOX	=	26.3	23	29	1	DD
38	Ceftazidime, CAZ	=	29.5	25	32	1	DD
38	Chloramphenicol, CHL	=	23.6	21	27	1	DD
38	Ciprofloxacin, CIP	=	38.1	30	40	1	DD
38	Gentamicin, GEN	=	24.0	19	26	1	DD
38	Imipenem, IMI	=	29.2	26	32	1	DD
38	Nalidixic acid, NAL	=	22.0	22	28	1	DD
38	Streptomycin, STR	=	14.9	12	20	1	DD
38	Tetracycline, TET	=	22.6	18	25	1	DD
38	Trimethoprim, TMP	=	21.0	21	28	1	DD
39	Ampicillin, AMP	=	4	2	8	1	MIC
39	Cefotaxime, CTX	=	0.12	0,03	0,125	1	MIC
39	Chloramphenicol, CHL	=	4	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.03	0,004	0,016	**0**	MIC
39	Gentamicin, GEN	=	1	0,25	1	1	MIC
39	Nalidixic acid, NAL	<=	2	1	4	1	MIC
39	Streptomycin, STR	=	8	4	16	1	MIC
39	Sulfisoxazole, FIS	=	32	8	32	1	MIC
39	Tetracycline, TET	=	1	0,5	2	1	MIC
39	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
40	Ampicillin, AMP	=	22	16	22	1	DD
40	Cefotaxime, CTX	=	32	29	35	1	DD
40	Cefoxitin, FOX	=	24	23	29	1	DD
40	Ceftazidime, CAZ	=	30	25	32	1	DD
40	Ceftiofur, XNL	=	26	26	31	1	DD
40	Chloramphenicol, CHL	=	24	21	27	1	DD
40	Ciprofloxacin, CIP	=	31	30	40	1	DD
40	Gentamicin, GEN	=	23	19	26	1	DD
40	Imipenem, IMI	=	29	26	32	1	DD
40	Nalidixic acid, NAL	=	26	22	28	1	DD
40	Streptomycin, STR	=	17	12	20	1	DD
40	Sulfisoxazole, FIS	=	22	15	23	1	DD
40	Tetracycline, TET	=	23	18	25	1	DD
40	Trimethoprim, TMP	=	27	21	28	1	DD
41	Ampicillin, AMP	=	4	2	8	1	MIC
41	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
41	Cefoxitin, FOX	=	4	2	8	1	MIC
41	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
41	Ceftiofur, XNL	=	0.5	0,25	1	1	MIC
41	Chloramphenicol, CHL	=	8	2	8	1	MIC
41	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
41	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
41	Nalidixic acid, NAL	=	2	1	4	1	MIC
41	Streptomycin, STR	=	8	4	16	1	MIC
41	Tetracycline, TET	<=	1	0,5	2	1	MIC
41	Trimethoprim, TMP	=	0.5	0,5	2	1	MIC
42	Ampicillin, AMP	=	8	2	8	1	MIC

42	Cefotaxime, CTX	<=	0.06	0,03	0,125	1	MIC
42	Ceftazidime, CAZ	<=	0.25	0,06	0,5	1	MIC
42	Chloramphenicol, CHL	=	8	2	8	1	MIC
42	Ciprofloxacin, CIP	=	0.015	0,004	0,016	1	MIC
42	Gentamicin, GEN	=	0.5	0,25	1	1	MIC
42	Nalidixic acid, NAL	<=	4	1	4	1	MIC
42	Streptomycin, STR	=	4	4	16	1	MIC
42	Sulfisoxazole, FIS	=	32	8	32	1	MIC
42	Tetracycline, TET	=	2	0,5	2	1	MIC
42	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	Cefoxitin, FOX	=	3	2	8	1	AGA
44	Chloramphenicol, CHL	<=	8	2	8	1	AGA
44	Ciprofloxacin, CIP	=	0.006	0,004	0,016	1	AGA
44	Gentamicin, GEN	<=	4	0,25	1	1	AGA
44	Imipenem, IMI	=	0.125	0,06	0,25	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
56	Ampicillin, AMP	=	22	16	22	1	DD
56	Cefotaxime, CTX	=	33	29	35	1	DD
56	Ceftazidime, CAZ	=	30	25	32	1	DD
56	Ceftiofur, XNL	=	28	26	31	1	DD
56	Chloramphenicol, CHL	=	25	21	27	1	DD
56	Ciprofloxacin, CIP	=	35	30	40	1	DD
56	Gentamicin, GEN	=	23	19	26	1	DD
56	Nalidixic acid, NAL	=	27	22	28	1	DD
56	Streptomycin, STR	=	17	12	20	1	DD
56	Sulfisoxazole, FIS	=	23	15	23	1	DD
56	Tetracycline, TET	=	25	18	25	1	DD
56	Trimethoprim, TMP	=	25	21	28	1	DD

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	=	4	0,25	2	0	MIC	X	
2	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
2	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
2	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
2	Gentamicin, GEN	=	0.25	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	=	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	=	2	1	4	1	MIC		X
6	Ciprofloxacin, CIP	=	0.12	0,03	0,125	1	MIC		X
6	Erythromycin, ERY	=	1	0,25	2	1	MIC		X
6	Gentamicin, GEN	=	0.5	0,25	2	1	MIC		X
6	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
6	Tetracycline, TET	=	0.5	0,25	1	1	MIC		X
9	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
9	Ciprofloxacin, CIP	=	0.12	0,06	0,25	1	MIC	X	
9	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
9	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
9	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
9	Tetracycline, TET	=	0.5	0,25	2	1	MIC	X	
11	Ciprofloxacin, CIP	=	0.12	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	<=	0.5	0,5	2	1	MIC	X	
12	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
12	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
12	Tetracycline, TET	=	1	0,25	2	1	MIC	X	
14	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
14	Ciprofloxacin, CIP	=	0.125	0,03	0,125	1	MIC		X
14	Erythromycin, ERY	=	1	0,25	2	1	MIC		X
14	Gentamicin, GEN	=	1	0,25	2	1	MIC		X
14	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
14	Tetracycline, TET	=	1	0,25	1	1	MIC		X
17	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
17	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
17	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
17	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
17	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
17	Tetracycline, TET	=	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	=	4	1	8	1	MIC	X	
20	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
20	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
20	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
20	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
20	Tetracycline, TET	=	2	0,25	2	1	MIC	X	
22	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
22	Ciprofloxacin, CIP	=	0.12	0,03	0,125	1	MIC		X
22	Erythromycin, ERY	=	1	0,25	2	1	MIC		X
22	Gentamicin, GEN	=	1	0,25	2	1	MIC		X
22	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
22	Tetracycline, TET	=	1	0,25	1	1	MIC		X
23	Chloramphenicol, CHL	<	2	1	4	1	MIC		X
23	Ciprofloxacin, CIP	=	0.12	0,03	0,125	1	MIC		X
23	Erythromycin, ERY	<	0.5	0,25	2	1	MIC		X
23	Gentamicin, GEN	=	1	0,25	2	1	MIC		X
23	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
23	Tetracycline, TET	=	1	0,25	1	1	MIC		X
25	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
25	Ciprofloxacin, CIP	<=	0.12	0,06	0,25	1	MIC	X	
25	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
25	Gentamicin, GEN	<=	0.25	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	=	4	1	8	1	MIC	X	
26	Ciprofloxacin, CIP	=	0.12	0,06	0,25	1	MIC	X	
26	Erythromycin, ERY	=	1	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	=	2	0,25	2	1	MIC	X	
29	Ciprofloxacin, CIP	=	0.12	0,06	0,25	1	MIC	X	
29	Erythromycin, ERY	=	0.50	0,5	2	1	MIC	X	
29	Gentamicin, GEN	=	16	0,5	2	0	MIC	X	
29	Nalidixic acid, NAL	=	1	4	16	0	MIC	X	
29	Tetracycline, TET	=	0.5	0,25	2	1	MIC	X	
30	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
30	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
30	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
30	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
30	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
30	Tetracycline, TET	=	2	0,25	2	1	MIC	X	
32	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
32	Ciprofloxacin, CIP	=	0.125	0,03	0,125	1	MIC		X
32	Erythromycin, ERY	<=	0.5	0,25	2	1	MIC		X
32	Gentamicin, GEN	=	0.5	0,25	2	1	MIC		X
32	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
32	Tetracycline, TET	=	1	0,25	1	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	=	2	0,25	2	1	MIC	X	
34	Chloramphenicol, CHL	=	8	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	
36	Ciprofloxacin, CIP	=	0.25	0,06	0,25	1	MIC	X	
36	Erythromycin, ERY	=	1	0,5	2	1	MIC	X	
36	Gentamicin, GEN	=	1	0,5	2	1	MIC	X	
36	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
36	Tetracycline, TET	=	1	0,25	2	1	MIC	X	
37	Chloramphenicol, CHL	=	4	0	256	0	AGA	X	
37	Ciprofloxacin, CIP	=	0.125	0,12	1	1	AGA	X	
37	Erythromycin, ERY	=	2	1	8	1	AGA	X	
37	Gentamicin, GEN	=	2	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	=	0.25	0,06	0,25	1	MIC	X	
39	Erythromycin, ERY	=	2	0,5	2	1	MIC	X	
39	Gentamicin, GEN	=	0.5	0,5	2	1	MIC	X	
39	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
39	Tetracycline, TET	=	2	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
42	Chloramphenicol, CHL	=	8				MIC	37°C 24 h	
42	Ciprofloxacin, CIP	=	0.25				MIC	37°C 24 h	
42	Erythromycin, ERY	=	4				MIC	37°C 24 h	
42	Gentamicin, GEN	<=	0.12				MIC	37°C 24 h	
42	Nalidixic acid, NAL	=	16				MIC	37°C 24 h	
42	Tetracycline, TET	=	4				MIC	37°C 24 h	
44	Chloramphenicol, CHL	<	8	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	<	1	0,5	2	1	AGA	X	
44	Nalidixic acid, NAL	<	16	0	256	0	AGA	X	
44	Tetracycline, TET	<	2	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 S21 with the following exceptions: The MIC range for streptomycin is according to Sensititre and the range for ceftiofur is according to M31-A3. Additionally, the range for ciprofloxacin is extended to include 0.016 as well.

E-test ranges are according to AB-Biodisk

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
22	MIC	0.5-32
22	MIC	0.5-32
23	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
36	MIC	0.5-64
37	AGA	0.5-64
39	MIC	0.5-64
41	MIC	0.5-32
42	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
22	MIC	0.06-4
22	MIC	0.06-4
23	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
36	MIC	0.06-8
37	AGA	0.06-8
39	MIC	0.06-8
41	MIC	0.06-4
42	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
22	MIC	0.25-16
22	MIC	0.25-16
23	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
42	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
22	MIC	2-64
22	MIC	2-64
23	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
36	MIC	2-256
37	AGA	2-256
39	MIC	2-256
41	MIC	2-64
42	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
22	MIC	0.008-8
22	MIC	0.008-8
23	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
36	MIC	0.008-1
37	AGA	0.008-8
39	MIC	0.008-1
41	MIC	0.008-8
42	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
22	MIC	0.25-32
22	MIC	0.25-32
23	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
36	MIC	0.25-32
37	AGA	0.25-32
39	MIC	0.25-32
41	MIC	0.25-32
42	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
22	MIC	4-64
22	MIC	4-64
23	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
36	MIC	2-256
37	AGA	2-512
39	MIC	2-256
41	MIC	4-64
42	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
22	MIC	2-128
22	MIC	2-128
23	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
36	MIC	2-256
37	AGA	2-512
39	MIC	2-256
41	MIC	2-128
42	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
22	MIC	8-1024
22	MIC	8-1024
23	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
36	MIC	8-1024
37	AGA	8-1024
39	MIC	8-1024
41	MIC	8-1024
42	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
22	MIC	1-64
22	MIC	1-64
23	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
36	MIC	0.5-64
37	AGA	0.5-64
39	MIC	0.5-64
41	MIC	1-64
42	MIC	1-64
44	AGA	8 and 128
Trimethoprim, TMP		
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
22	MIC	0.5-32
22	MIC	0.5-32
23	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
36	MIC	0.25-32
37	AGA	0.25-32
39	MIC	0.25-32
41	MIC	0.5-32
42	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
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
42	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
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
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
36	MIC	0.06-8
37	AGA	0.06-8
39	MIC	0.06-8
41	MIC	0.06-4
42	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
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
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
36	MIC	0,5-64
37	AGA	0.5-64
39	MIC	0.5-64
41	MIC	0.5-32
42	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
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
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
36	MIC	0,12-16
37	AGA	0.125-16
39	MIC	0.12-16
41	MIC	0.12-16
42	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
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
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
36	MIC	1-64
37	AGA	2-256
39	MIC	1-64
41	MIC	2-64
42	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
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
36	MIC	0,5-64
37	AGA	0.5-32
39	MIC	0.5-64
41	MIC	1-16
42	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
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
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
36	MIC	0,12-16
37	AGA	0.125-16
39	MIC	0.12-16
41	MIC	0.25-16
42	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-6.1	R	100	0	33	0
	EURL S-6.2	R	100	0	33	0
	EURL S-6.3	R	100	0	33	0
	EURL S-6.4	R	100	0	33	0
	EURL S-6.5	S	0	100	33	0
	EURL S-6.6	S	0	100	33	0
	EURL S-6.7	S	0	100	33	0
	EURL S-6.8	R	100	0	33	0
Cefotaxime, CTX	EURL S-6.1	R	100	0	34	0
	EURL S-6.2	S	0	100	34	0
	EURL S-6.3	R	97	3	33	1
	EURL S-6.4	R	97	3	33	1
	EURL S-6.5	S	0	100	34	0
	EURL S-6.6	S	0	100	34	0
	EURL S-6.7	S	0	100	34	0
	EURL S-6.8	R	97	3	33	1
Ceftazidime, CAZ	EURL S-6.1	R	96	4	27	1
	EURL S-6.2	S	0	100	28	0
	EURL S-6.3	R	97	3	28	1
	EURL S-6.4	S	11	89	25	3
	EURL S-6.5	S	0	100	28	0
	EURL S-6.6	S	0	100	28	0
	EURL S-6.7	S	0	100	28	0
	EURL S-6.8	R	100	0	28	0
Ceftiofur, XNL	EURL S-6.1	R	100	0	8	0
	EURL S-6.2	S	0	100	8	0
	EURL S-6.3	R	100	0	8	0
	EURL S-6.4	R	88	13	7	1
	EURL S-6.5	S	0	100	8	0
	EURL S-6.6	S	0	100	8	0
	EURL S-6.7	S	0	100	8	0
	EURL S-6.8	R	100	0	8	0
Chloramphenicol, CHL	EURL S-6.1	S	0	100	34	0
	EURL S-6.2	R	100	0	34	0
	EURL S-6.3	R	100	0	33	0
	EURL S-6.4	S	0	100	33	0
	EURL S-6.5	S	0	100	34	0
	EURL S-6.6	S	0	100	33	0
	EURL S-6.7	S	0	100	34	0
	EURL S-6.8	R	100	0	34	0
Ciprofloxacin, CIP	EURL S-6.1	S	0	100	33	0
	EURL S-6.2	R	94	6	31	2
	EURL S-6.3	R	82	18	27	6
	EURL S-6.4	R	97	3	32	1
	EURL S-6.5	S	0	100	33	0
	EURL S-6.6	S	0	100	33	0
	EURL S-6.7	R	82	18	27	6
	EURL S-6.8	S	0	100	33	0

Gentamicin, GEN	EURL S-6.1	S	0	100	34	0
	EURL S-6.2	S	0	100	34	0
	EURL S-6.3	R	97	3	33	1
	EURL S-6.4	S	3	97	33	1
	EURL S-6.5	S	0	100	34	0
	EURL S-6.6	S	0	100	34	0
	EURL S-6.7	S	3	97	33	1
	EURL S-6.8	S	0	100	34	0
Nalidixic acid, NAL	EURL S-6.1	S	0	100	33	0
	EURL S-6.2	R	100	0	33	0
	EURL S-6.3	S	12	88	29	4
	EURL S-6.4	R	100	0	33	0
	EURL S-6.5	S	0	100	33	0
	EURL S-6.6	S	0	100	33	0
	EURL S-6.7	S	6	94	31	2
	EURL S-6.8	S	0	100	33	0
Streptomycin, STR	EURL S-6.1	S	0	100	34	0
	*EURL S-6.2**	*R*	*55*	*45*	*18*	*15*
	EURL S-6.3	R	100	0	34	0
	EURL S-6.4	S	9	91	30	3
	EURL S-6.5	S	0	100	34	0
	*EURL S-6.6**	*R*	*48*	*52*	*16*	*17*
	EURL S-6.7	S	9	91	31	3
	EURL S-6.8	R	100	0	34	0
Sulphonamides, SMX	EURL S-6.1	S	0	100	33	0
	EURL S-6.2	R	100	0	33	0
	EURL S-6.3	R	100	0	33	0
	EURL S-6.4	S	3	97	32	1
	EURL S-6.5	S	3	97	32	1
	EURL S-6.6	R	94	6	31	2
	EURL S-6.7	S	25	75	24	8
	EURL S-6.8	R	100	0	33	0
Tetracycline, TET	EURL S-6.1	S	0	100	34	0
	EURL S-6.2	R	97	3	33	1
	EURL S-6.3	R	100	0	34	0
	EURL S-6.4	R	97	3	33	1
	EURL S-6.5	S	0	100	34	0
	EURL S-6.6	R	100	0	34	0
	EURL S-6.7	S	0	100	34	0
	EURL S-6.8	R	100	0	34	0
Trimethoprim, TMP	EURL S-6.1	S	0	100	33	0
	EURL S-6.2	R	100	0	33	0
	EURL S-6.3	R	100	0	33	0
	EURL S-6.4	S	3	97	32	1
	EURL S-6.5	S	0	100	33	0
	EURL S-6.6	R	100	0	32	0
	EURL S-6.7	S	0	100	32	0
	EURL S-6.8	S	0	100	32	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-6.1	S	0	100	20	0
	EURL C-6.2	S	0	100	19	0
	EURL C-6.3	S	0	100	20	0
	EURL C-6.4	S	0	100	20	0
	EURL C-6.5	S	0	100	20	0
	EURL C-6.6	S	0	100	20	0
	EURL C-6.7	S	0	100	20	0
	EURL C-6.8	S	0	100	20	0
Ciprofloxacin, CIP	EURL C-6.1	S	4	96	24	1
	EURL C-6.2	S	4	96	23	1
	EURL C-6.3	R	100	0	25	0
	EURL C-6.4	R	100	0	25	0
	EURL C-6.5	R	100	0	26	0
	EURL C-6.6	R	100	0	26	0
	EURL C-6.7	S	0	100	26	0
	EURL C-6.8	S	0	100	26	0
Erythromycin, ERY	EURL C-6.1	S	0	100	25	0
	EURL C-6.2	S	4	96	23	1
	EURL C-6.3	S	0	100	25	0
	EURL C-6.4	S	0	100	25	0
	EURL C-6.5	R	92	8	24	2
	EURL C-6.6	S	4	96	25	1
	EURL C-6.7	R	100	0	26	0
	EURL C-6.8	R	100	0	26	0
Gentamicin, GEN	EURL C-6.1	S	4	96	23	1
	EURL C-6.2	S	0	100	24	0
	EURL C-6.3	S	0	100	25	0
	EURL C-6.4	S	0	100	25	0
	EURL C-6.5	S	0	100	26	0
	EURL C-6.6	S	4	96	25	1
	EURL C-6.7	S	0	100	26	0
	EURL C-6.8	S	0	100	26	0
Nalidixic acid, NAL	EURL C-6.1	S	4	96	24	1
	EURL C-6.2	S	4	96	23	1
	EURL C-6.3	S	16	84	21	4
	EURL C-6.4	R	96	4	23	1
	EURL C-6.5	R	96	4	25	1
	EURL C-6.6	R	96	4	25	1
	EURL C-6.7	S	0	100	26	0
	EURL C-6.8	S	0	100	26	0
Streptomycin, STR	EURL C-6.1	S	4	96	24	1
	EURL C-6.2	S	13	88	21	3
	EURL C-6.3	S	8	92	23	2
	EURL C-6.4	S	4	96	24	1
	EURL C-6.5	S	4	96	25	1
	EURL C-6.6	S	8	92	24	2
	EURL C-6.7	S	15	85	22	4
	EURL C-6.8	R	100	0	26	0
Tetracycline, TET	EURL C-6.1	S	0	100	25	0
	EURL C-6.2	R	100	0	24	0
	EURL C-6.3	S	0	100	25	0
	EURL C-6.4	R	96	4	24	1
	EURL C-6.5	R	96	4	25	1
	EURL C-6.6	S	4	96	25	1
	EURL C-6.7	S	0	100	26	0
	EURL C-6.8	S	0	100	26	0

Deviations - *Salmonella*

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
4	EURL S-6.3	Nalidixic acid, NAL	R	8	S	= 8	MIC
4	EURL S-6.4	Ceftazidime, CAZ	R	0.5	S	= 1	MIC
4	EURL S-6.7	Gentamicin, GEN	R	2	S	= 0.5	MIC
4	EURL S-6.7	Nalidixic acid, NAL	R	8	S	= 8	MIC
4	EURL S-6.7	Streptomycin, STR	R	16	S	= 16	MIC
4	EURL S-6.7	Sulfamethoxazole, SMX	R	1024	S	= 64	MIC
4	EURL S-6.8	Confirmed ESBL	Yes		No		MIC
12	EURL S-6.6	Sulfamethoxazole, SMX	S	256	R	> 1024	MIC
13	EURL S-6.4	Streptomycin, STR	R	8	S	= 16	MIC
15	EURL S-6.3	Nalidixic acid, NAL	R	15	S	= 8	DD
15	EURL S-6.4	Cefotaxime, CTX	S	20	R	> 4	DD
15	EURL S-6.7	Nalidixic acid, NAL	R	15	S	= 8	DD
18	EURL S-6.3	Ciprofloxacin, CIP	S	32	R	= 0.25	DD
18	EURL S-6.7	Ciprofloxacin, CIP	S	31	R	= 0.25	DD
20	EURL S-6.7	Streptomycin, STR	R	32	S	= 16	MIC
21	EURL S-6.1	Confirmed ESBL	No		Yes		MIC
21	EURL S-6.3	Nalidixic acid, NAL	R	64	S	= 8	MIC
21	EURL S-6.4	Ceftazidime, CAZ	R	0.5	S	= 1	MIC
21	EURL S-6.4	Sulfamethoxazole, SMX	R	1024	S	= 64	MIC
21	EURL S-6.4	Trimethoprim, TMP	R	>32	S	<= 1	MIC
22	EURL S-6.7	Sulfamethoxazole, SMX	R	>1024	S	= 64	MIC
22	EURL S-6.8	Confirmed ESBL	Yes		No		MIC
23	EURL S-6.7	Sulfamethoxazole, SMX	R	512	S	= 64	MIC
26	EURL S-6.3	Cefotaxime, CTX	S	<=0.06	R	> 4	MIC
26	EURL S-6.3	Ceftazidime, CAZ	S	<=0.25	R	= 128	MIC
26	EURL S-6.3	Confirmed ESBL	No		Yes		MIC
26	EURL S-6.3	Gentamicin, GEN	S	1	R	> 16	MIC
26	EURL S-6.3	Nalidixic acid, NAL	R	64	S	= 8	MIC
26	EURL S-6.4	Streptomycin, STR	R	32	S	= 16	MIC
29	EURL S-6.4	Ceftiofur, XNL	S	22mm	R	> 8	MIC
29	EURL S-6.7	Sulfamethoxazole, SMX	R	512	S	= 64	MIC
30	EURL S-6.4	Ceftazidime, CAZ	R	1	S	= 1	MIC
33	EURL S-6.4	Gentamicin, GEN	R	4	S	= 1	MIC
33	EURL S-6.7	Confirmed AmpC	Yes		No		MIC
38	EURL S-6.1	Ceftazidime, CAZ	S	17.5 mm	R	= 8	DD
38	EURL S-6.3	Ciprofloxacin, CIP	S	31.8 mm	R	= 0.25	DD
38	EURL S-6.4	Streptomycin, STR	R	9.4 mm	S	= 16	DD
38	EURL S-6.7	Ciprofloxacin, CIP	S	33.4 mm	R	= 0.25	DD
38	EURL S-6.7	Streptomycin, STR	R	10.9 mm	S	= 16	DD
38	EURL S-6.8	Confirmed AmpC	No		Yes		DD
39	EURL S-6.5	Sulfamethoxazole, SMX	R	1024	S	= 64	MIC
39	EURL S-6.7	Sulfamethoxazole, SMX	R	1024	S	= 64	MIC
39	EURL S-6.8	Confirmed AmpC	No		Yes		MIC
40	EURL S-6.2	Ciprofloxacin, CIP	S	32	R	= 0.25	DD
40	EURL S-6.2	Tetracycline, TET	S	13	R	> 32	DD
40	EURL S-6.3	Ciprofloxacin, CIP	S	31	R	= 0.25	DD
40	EURL S-6.4	Tetracycline, TET	S	13	R	> 32	DD
40	EURL S-6.6	Sulfamethoxazole, SMX	S	27	R	> 1024	DD
40	EURL S-6.7	Ciprofloxacin, CIP	S	33	R	= 0.25	DD

41	EURL S-6.7	Sulfamethoxazole, SMX	R	512	S	= 64	MIC
41	EURL S-6.8	Confirmed AmpC	No		Yes		MIC
42	EURL S-6.7	Sulfamethoxazole, SMX	R	512	S	= 64	MIC
44	EURL S-6.3	Ciprofloxacin, CIP	S	<=0.125	R	= 0.25	AGA
44	EURL S-6.7	Ciprofloxacin, CIP	S	<=0.125	R	= 0.25	AGA
54	EURL S-6.1	Confirmed ESBL	No		Yes		DD
54	EURL S-6.2	Ciprofloxacin, CIP	S	17	R	= 0.25	DD
54	EURL S-6.3	Ciprofloxacin, CIP	S	19	R	= 0.25	DD
54	EURL S-6.3	Confirmed ESBL	No		Yes		DD
54	EURL S-6.4	Ciprofloxacin, CIP	S	17	R	= 0.5	DD
54	EURL S-6.4	Confirmed ESBL	No		Yes		DD
54	EURL S-6.7	Ciprofloxacin, CIP	S	20	R	= 0.25	DD
54	EURL S-6.8	Cefotaxime, CTX	S	16	R	> 4	DD
54	EURL S-6.8	Confirmed AmpC	No		Yes		DD
56	EURL S-6.3	Ciprofloxacin, CIP	S	27	R	= 0.25	DD
56	EURL S-6.4	Confirmed ESBL	No		Yes		DD
56	EURL S-6.7	Ciprofloxacin, CIP	S	27	R	= 0.25	DD
56	EURL S-6.7	Sulfamethoxazole, SMX	R	6	S	= 64	DD

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
4	EURL C-6.1	Ciprofloxacin, CIP	R	4	S	= 0.12	MIC
4	EURL C-6.1	Gentamicin, GEN	R	1	S	= 0.25	MIC
4	EURL C-6.1	Nalidixic acid, NAL	R	64	S	= 4	MIC
4	EURL C-6.2	Ciprofloxacin, CIP	R	4	S	= 0.12	MIC
4	EURL C-6.2	Erythromycin, ERY	R	8	S	= 1	MIC
4	EURL C-6.2	Nalidixic acid, NAL	R	32	S	= 4	MIC
4	EURL C-6.2	Streptomycin, STR	R	2	S	<= 1	MIC
4	EURL C-6.3	Nalidixic acid, NAL	R	32	S	<= 2	MIC
4	EURL C-6.3	Streptomycin, STR	R	2	S	<= 1	MIC
4	EURL C-6.4	Streptomycin, STR	R	2	S	<= 1	MIC
4	EURL C-6.4	Tetracycline, TET	S	0.25	R	= 32	MIC
4	EURL C-6.6	Streptomycin, STR	R	4	S	= 4	MIC
4	EURL C-6.7	Streptomycin, STR	R	8	S	= 2	MIC
6	EURL C-6.6	Nalidixic acid, NAL	S	=32	R	> 64	MIC
17	EURL C-6.4	Nalidixic acid, NAL	S	16	R	> 64	MIC
17	EURL C-6.5	Nalidixic acid, NAL	S	32	R	> 64	MIC
19	EURL C-6.1	Streptomycin, STR	R	4	S	<= 1	MIC
19	EURL C-6.2	Streptomycin, STR	R	8	S	<= 1	MIC
19	EURL C-6.3	Nalidixic acid, NAL	R	64	S	<= 2	MIC
19	EURL C-6.3	Streptomycin, STR	R	4	S	<= 1	MIC
19	EURL C-6.5	Erythromycin, ERY	S	32	R	> 64	MIC
19	EURL C-6.6	Gentamicin, GEN	R	4	S	= 0.5	MIC
19	EURL C-6.6	Streptomycin, STR	R	16	S	= 4	MIC
19	EURL C-6.7	Streptomycin, STR	R	8	S	= 2	MIC
22	EURL C-6.2	Streptomycin, STR	R	4	S	<= 1	MIC
22	EURL C-6.3	Nalidixic acid, NAL	R	64	S	<= 2	MIC
22	EURL C-6.7	Streptomycin, STR	R	8	S	= 2	MIC
29	EURL C-6.5	Streptomycin, STR	R	8	S	<= 1	MIC
33	EURL C-6.7	Streptomycin, STR	R	8	S	= 2	MIC
34	EURL C-6.3	Nalidixic acid, NAL	R	32	S	<= 2	MIC
39	EURL C-6.5	Erythromycin, ERY	S	4	R	> 64	MIC
39	EURL C-6.5	Tetracycline, TET	S	0.5	R	> 64	MIC
39	EURL C-6.6	Erythromycin, ERY	R	>64	S	= 1	MIC
39	EURL C-6.6	Tetracycline, TET	R	>16	S	= 0.25	MIC

AGA Agar dilution
MIC Microbroth dilution

Optional genotypic characterisation

Lab no.	Strain	Gene tested		Not detected	Primer used 5'→3'	Primer used 3'→5'	PCR-method	Reference
I	EURL GEN-3.1	blaZ					In-house	
I	EURL GEN-3.1	mecA					In-house	
I	EURL GEN-3.1	vga(A)					In-house	
I	EURL GEN-3.1	tet(K)					In-house	
I	EURL GEN-3.1	tet(M)					In-house	
I	EURL GEN-3.1	aacA-aphD		X	5'-TAATCCAAGAGCAATAAGGGC-3'	5'-GCCACACTATCATAACCACTA-3'	In-house	
I	EURL GEN-3.1	cat		X	5'-GGATATGAAATTTATCCCTC-3'	5'-CAATCATCTACCCTATGAAT-3'	In-house	
I	EURL GEN-3.1	tet(O)		X			In-house	
I	EURL GEN-3.2	CMY	-2				In-house	
I	EURL GEN-3.2	floR						
I	EURL GEN-3.2	aadA						
I	EURL GEN-3.2	strB						
I	EURL GEN-3.2	sul1					In-house	
I	EURL GEN-3.2	sul2						
I	EURL GEN-3.2	tetA						
I	EURL GEN-3.2	aac(3)-II		X				
I	EURL GEN-3.2	aac(3)-IV		X				
I	EURL GEN-3.2	ant(2'')-I		X				
III	EURL GEN-3.1	blaI						
III	EURL GEN-3.1	blaR						
III	EURL GEN-3.1	blaZ						
III	EURL GEN-3.1	mecA						
III	EURL GEN-3.1	tet(K)						
III	EURL GEN-3.1	tet(M)						
III	EURL GEN-3.1	vga(A)						
III	EURL GEN-3.1	aacA-aphD		X				
III	EURL GEN-3.1	aadD		X				
III	EURL GEN-3.1	aphA3		X				
III	EURL GEN-3.1	cat		X				
III	EURL GEN-3.1	vanA		X				
III	EURL GEN-3.1	vanB		X				
III	EURL GEN-3.1	vanZ		X				
III	EURL GEN-3.1	lnu(A)		X			Published	Van Hoek et al, 2011
III	EURL GEN-3.1	erm(A)		X				
III	EURL GEN-3.1	erm(B)		X				
III	EURL GEN-3.1	erm(C)		X				
III	EURL GEN-3.1	mef(A)		X				
III	EURL GEN-3.1	msr(A)		X				
III	EURL GEN-3.1	qacA		X				
III	EURL GEN-3.1	far1		X				
III	EURL GEN-3.1	vat(A)		X				
III	EURL GEN-3.1	vat(B)		X				
III	EURL GEN-3.1	vgB(A)		X				
III	EURL GEN-3.1	sat		X				
III	EURL GEN-3.1	dfrA		X				

III	EURL GEN-3.2	CMY	-2				Published	Zhao et al., 2001
III	EURL GEN-3.2	floR					Published	Ng et al, 1999, AAC
III	EURL GEN-3.2	aadA					Published	Sandvang et al, 1997
III	EURL GEN-3.2	strA					Published	Madsen et al, 2000
III	EURL GEN-3.2	strB					Published	Madsen et al, 2000
III	EURL GEN-3.2	sul1					Published	Sandvang et al, 1997
III	EURL GEN-3.2	sul2					Published	Chu et al, 2001
III	EURL GEN-3.2	tetA					Published	Ng et al., 1999, MCP
III	EURL GEN-3.2	CTX		X			Published	Carattoli et al. 2008
III	EURL GEN-3.2	OXA		X			Published	Guerra et al., 2000
III	EURL GEN-3.2	SHV		X			Published	Weill et al. 2004
III	EURL GEN-3.2	TEM		X			Published	Guerra et al. 2001; Olesen et al. 2004
III	EURL GEN-3.2	catA1		X			Published	Guerra et al. 2001
III	EURL GEN-3.2	cmlA		X			Published	Guerra et al. 2001
III	EURL GEN-3.2	aac(3)-II		X			Published	Beutlich et al., 2011
III	EURL GEN-3.2	aac(3)-IV		X			Published	Guerra et al. 2001
III	EURL GEN-3.2	aac(3)-Ie		X				
III	EURL GEN-3.2	ant(2")-I		X			Published	Frana et al, 2001
III	EURL GEN-3.2	aph(3')-I		X			Published	Frana et al., 2001
III	EURL GEN-3.2	aph(3')-II		X			Published	Frana et al., 2001
III	EURL GEN-3.2	aph(3')-III		X			Published	Gibreel et al.
III	EURL GEN-3.2	gyrA-83		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	gyrA-87		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	parC-57		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	parC-78		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	parC-80		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	parC-84		X			Published	Malorny et al. 2003
III	EURL GEN-3.2	qnrA		X			Published	Wang et al., 2003
III	EURL GEN-3.2	qnrB		X			Published	Jacoby et al., 2006
III	EURL GEN-3.2	qnrC		X			Published	Wang et al., 2009
III	EURL GEN-3.2	qnrD		X			Published	Cavaco et al, 2009
III	EURL GEN-3.2	qnrS		X			Published	Gay et al., 2006
III	EURL GEN-3.2	sul3		X			Published	Perreten & Boerlin, 2003
III	EURL GEN-3.2	tetB		X			Published	Ng et al., 1999, MCP
III	EURL GEN-3.2	tetC		X			Published	Ng et al., 1999, MCP
III	EURL GEN-3.2	tetD		X			Published	Ng et al., 1999, MCP
III	EURL GEN-3.2	tetE		X			Published	Ng et al., 1999, MCP
III	EURL GEN-3.2	tetG		X			Published	Ng et al., 1999, MCP
IV	EURL GEN-3.1	blaI						
IV	EURL GEN-3.1	blaR						
IV	EURL GEN-3.1	blaZ						
IV	EURL GEN-3.1	mecA						
IV	EURL GEN-3.1	tet(M)						
IV	EURL GEN-3.2	CMY	-2					
IV	EURL GEN-3.2	floR						
IV	EURL GEN-3.2	aadA						
IV	EURL GEN-3.2	strA						
IV	EURL GEN-3.2	strB						
IV	EURL GEN-3.2	sul1						
IV	EURL GEN-3.2	sul2						
IV	EURL GEN-3.2	tetA						
VI	EURL GEN-3.2	qnrA		X	GGATGCCAGTTTCGAGGA	TGCCAGGCACAGATCTTG	Published	PCR qnr genes CRL-AR 1th Ed. January 2009
VI	EURL GEN-3.2	qnrB		X	GGMATHGAAATTCGCCACTG	TTTGCYGYYCGCCAGTCGAA	Published	PCR qnr genes CRL-AR 1th Ed. January 2009
VI	EURL GEN-3.2	qnrC		X	GGGTTGTACATTTATTGAATC	TCCACTTTACGAGGTTCT	Published	Antimicrob.AgentsChemother, May2099, 1892-1897
VI	EURL GEN-3.2	qnrD		X	CGAGATCAATTTACGGGGAATA	AACAAGCTGAAGCGCCTG	Published	PCR qnr genes CRL-AR 1th Ed. January 2009
VI	EURL GEN-3.2	qnrS		X	TCGACGTGCTAACTTGCG	GATCTAAACCGTCGAGTTCGG	Published	PCR qnr genes CRL-AR 1th Ed. January 2009
VI	EURL GEN-3.2	qepA		X				
VI	EURL GEN-3.2	aac(6")Ib-cr		X				

VII	EURL GEN-3.1	mecA					Published	Batchelor et al AAC (2005) 49:1319-1322.
VII	EURL GEN-3.2							Salmonella Amp-C Cit-group
VIII	EURL GEN-3.2	CMY						AMR-ve 0.5m
VIII	EURL GEN-3.2	floR						AMR-ve 0.5m
VIII	EURL GEN-3.2	aadA						AMR-ve 0.5m
VIII	EURL GEN-3.2	strA						AMR-ve 0.5m
VIII	EURL GEN-3.2	strB						AMR-ve 0.5m
VIII	EURL GEN-3.2	sul1						AMR-ve 0.5m
VIII	EURL GEN-3.2	sul2						AMR-ve 0.5m
VIII	EURL GEN-3.2	tetA						AMR-ve 0.5m
VIII	EURL GEN-3.2	CTX		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	OXA		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	SHV		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	TEM		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	catA1		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	cmlA		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	aac(3)-II		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	aac(3)-IV		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	ant(2")-I		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	qnrA		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	qnrB		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	qnrS		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	sul3		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	tetB		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	tetC		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	tetD		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	tetE		X				AMR-ve 0.5m
VIII	EURL GEN-3.2	tetG		X				AMR-ve 0.5m

Legend:

Fields shaded grey indicate that the gene was expected

Genes in bold were detected but not expected

Field with an unexpected result are framed

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