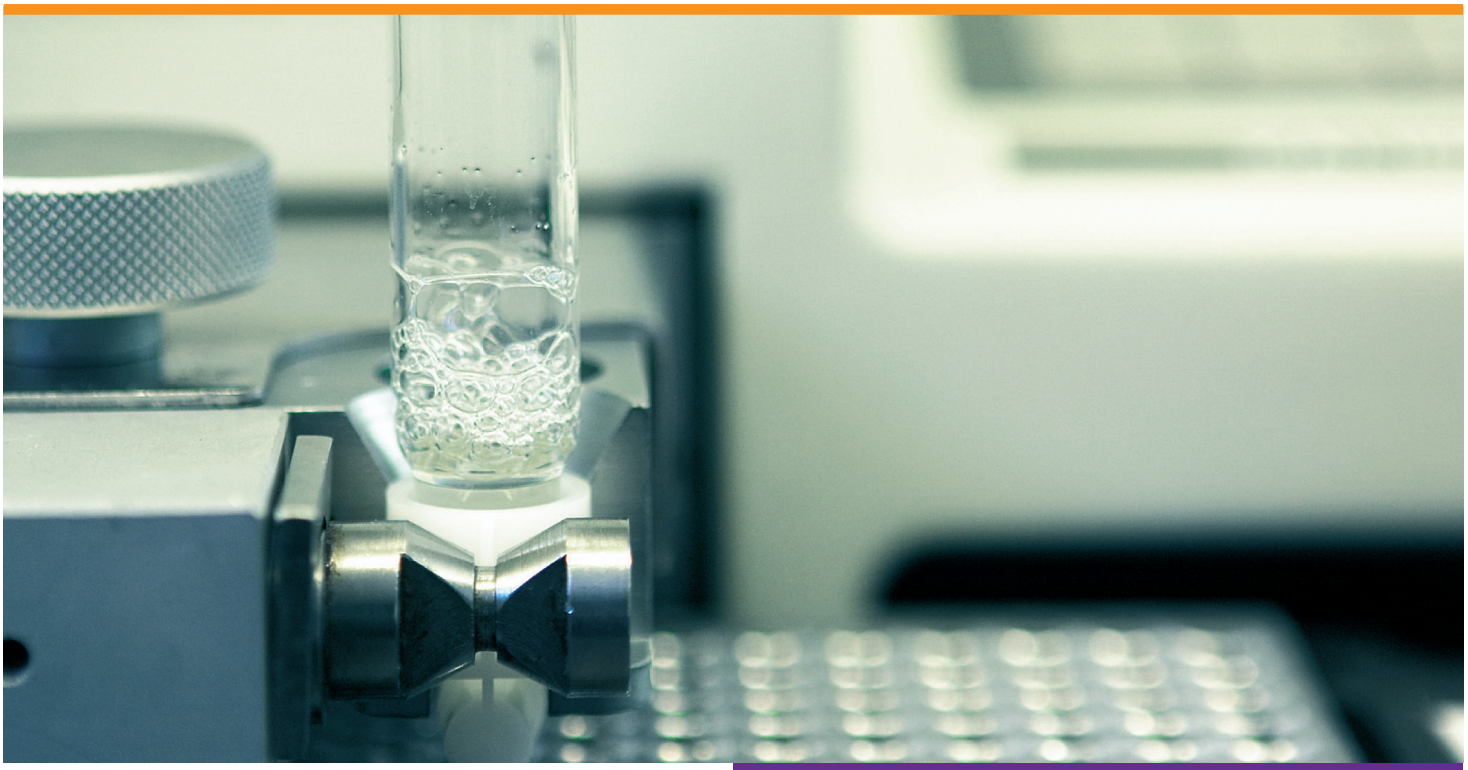
The 7th EURL-AR Proficiency Testing *Salmonella* and *Campylobacter* 2009



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The 7th EURL-AR Proficiency Testing
Salmonella and *Campylobacter* 2009

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1. Introduction

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

The objective of the EQAS is to monitor the quality of the antimicrobial susceptibility data produced 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 having 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 having each laboratory performing AST with less than 5% incorrect interpretations (interpretations deviating from the expected results).

The data in this report are presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the EURL-AR and the EU Commission. All conclusions are public. The technical advisory group for the EURL-AR EQAS scheme consists of competent representatives from all National Reference Laboratories (NRLs), who meet once a year at the EURL-AR workshop.

The EURL-AR is accredited by DANAK 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 21st of August 2009 by e-mail to the 37 NRLs in the EURL-AR-network (including Norway and Switzerland). In addition, to the AST of *Salmonella* and *Campylobacter*, an optional PCR-testing and sequencing of resistance genes aiming to genotypically characterize a selected *Salmonella* isolate was offered. The pre-notification was sent to NRLs in all EU countries except Luxemburg, where no NRL has been designated. All 37 laboratories responded. One laboratory declined to participate as they had neither *Salmonella* nor *Campylobacter* as their field of responsibility. In addition, Norway did not participate in this iteration.

Appendix 2 shows that 33 of the 35 participating NRLs were appointed by the individual member states. Two NRLs had not been appointed, but had – along with Norway and Switzerland – been 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 laboratory in Switzerland was charged a fee for their participation in the EQAS, whereas the NRLs from EU member states participated free of charge.

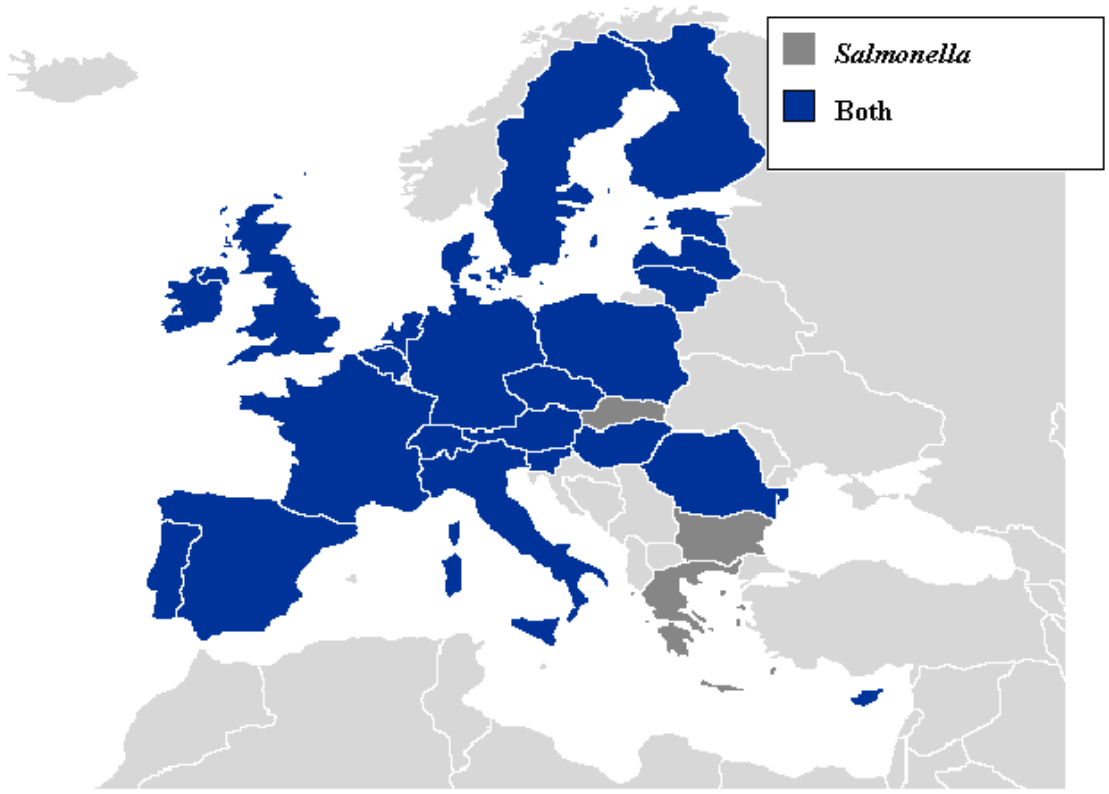

Figure 1: Participating countries that perform antimicrobial susceptibility testing of *Salmonella* or both *Salmonella* and *Campylobacter*.

Figure 1 shows that out of 28 participating countries, three uploaded the *Salmonella* results only (Slovakia, Bulgaria and Greece), whereas 25 tested both *Salmonella* and *Campylobacter*. The results from the designated NRLs are being presented and evaluated in this report; i.e. results from 25 countries consisting of 31 sets of *Salmonella* results and 26 sets of *Campylobacter* results. Five laboratories participated in the optional genotypic characterisation of the additional *Salmonella* test strain (not illustrated on 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 inoculated as agar stab cultures and the *Campylobacter* strains as charcoal swabs.

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

Prior to distribution of the strains, antimicrobial susceptibility testing (AST) on the *Salmonella* and *Campylobacter* strains was performed at DTU Food and verified by the US Food and Drug Administration (FDA). The obtained MIC values served as reference for the test strains (App. 3a and 3b). However, results from the following antimicrobials were not verified by FDA:

cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, imipenem, imipenem/EDTA, and trimethoprim for *Salmonella*, furthermore, streptomycin and chloramphenicol for *Campylobacter*.

The test strain offered for optional genotypic characterisation was a multiple resistant *Salmonella* Concord exhibiting resistance to ampicillin, cephalothin, cefpodoxime, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim (selection of antimicrobials was different from those used for the AST in this EQAS).

2.3 Antimicrobials

The antimicrobials used in the EQAS are listed in the protocol (App. 4b) and were included mainly according to the recommendations in the 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, e.g. due to the detection of ESBL-production being included.

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 testing, the antimicrobials cefotaxime + clavulanic acid, cefoxitin, ceftazidime + clavulanic acid were additionally tested using E-test from AB-Biodisk, Sweden. In addition to the E-test, the ESBL confirmatory testing was also performed by microbroth dilution MIC determination. This test also included imipenem. The method guidelines used were according to the Clinical and Laboratory Standards Institute (CLSI) document M7-A7 (2006), “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically” (Approved Standard - Seventh Edition), document M100-S19 (2009) “Performance Standards for Antimicrobial Susceptibility Testing” (Ninteenth Informational Supplement) and document M31-A3 (2008) “Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacterial Isolated From Animals” (Approved Standard – Third Edition).

For *Campylobacter* the following antimicrobials were included: chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline. MIC determination was performed using the Sensititre systems from Trek Diagnostic Systems Ltd, UK, according to guidelines from the CLSI document M45-A (2006) “Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria” (Approved Guideline).

2.4 Distribution

On October 6th 2009, 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

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

It is the aim that MIC methods only should be 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 upon all NRLs working 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 streptomycin and sulphonamides, where values from DTU Food and CLSI, respectively, were used according to the description in the protocol (App. 4b).

Participants using disk diffusion and E-test were recommended to interpret the results according to their individual routine, categorising the test strains into the terms resistant and sensitive. A categorisation as 'intermediate' was not accepted. The breakpoints used were submitted to the web based database, from which the relevant breakpoints (disk diffusion for *Salmonella*) are listed in Appendix 5.

It should be noted that for AST of *Campylobacter* only MIC methods are recommendable, i.e. broth or agar dilution methods. The EURL-AR does not recommend the use of neither disk diffusion nor 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 would be accepted as of the EQAS 2009.

The laboratories were instructed to upload the obtained MIC values (µg/mL) or zone-diameter in millimetres and the susceptibility categories (resistant or sensitive) to an electronic record sheet in the EURL-AR web based database through a secured individual login. Alternatively, the record sheets from the protocol could be sent by fax to DTU Food. The website was open for data entry in the period from the 30th of October 2009 to the 13th of January 2010.

Detection of ESBL-producing strains should be performed and interpreted according to recommendations in the protocol: If a microorganism is resistant to one or two of the antimicrobials cefotaxime, ceftazidime and/or ceftiofur, it should be regarded as resistant to all three (this does not include cefoxitin, as ampC's are resistant to cefoxitin and 'true ESBLs' are not).

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 zone diameter in millimetres. The results should be in agreement with the quality control ranges according to the relevant guidelines; the CLSI documents M31-A3 (2008) / M100-S19 (2009) / M45-A (2006); The Sensititre System (Trek Diagnostic Systems Ltd, UK); or E-tests (AB-Biodisk, Sweden) (App. 7).

For the optional PCR-testing of a selected *Salmonella* 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 extra genes, not listed in the protocol would not be evaluated. The results were evaluated based on the actual genes identified. The groups of TEM-, CTX-, SHV-, CMY-, OXA-genes as well as the gyrA-mutations and parC-mutations were additionally evaluated on the group selected. For gyrA and parC, the codon of the point of mutation 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 the EURL-AR by using miniaturized microarrays (Identibac Amr-ve array tubes; New Haw, Addlestone, Surrey, United Kingdom) containing probes for most relevant Gram-negative antimicrobial gene groups such as quinolone, sulfonamide, tetracycline, class 1/2 integrase, aminoglycoside, carbenicillinase, chloramphenicol exporter/acetyltransferase, florfenicol, trimethoprim, plasmidic AmpC, and beta-lactam groups. Analysis was performed as described by the manufacturer. PCR was run for confirmation of weak array results. The results have not been verified elsewhere (due to this optional test being a pilot study).

After submitting the data, the laboratories were instructed to retrieve the instantly generated, individual evaluation report from the secured web site. The evaluation reports assessed the submitted results, describing all deviations from the expected. Deviations in the interpretation as resistant of susceptible were categorised as 'incorrect', as was also deviations in categorisations as ESBL 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 resistance, 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. These were collected and summarised (App. 8, 9).

3. Results

The participants were asked to report results, including MIC values or disk diffusion diameters together with the categorisation as resistant or sensitive. 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 only 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 was not the case for any strain/antimicrobial combinations (App. 10a and 10b), and no results have been omitted.

3.1 Methods used by EQAS-participants

In the *Salmonella* trial, 26 laboratories used MIC determination, and five laboratories used disk diffusion. For the *Campylobacter* trial, all 26 laboratories reported the use of MIC determination (microbroth or agar dilution). Moreover, two sets of results for the *Campylobacter* trial were excluded from the evaluation; one set of results was obtained by disk diffusion, and the remaining one was requested to be extracted by the NRL due to problems with the introduction of a new method. Follow-up is being carried out by the EURL-AR regarding this last issue.

3.2 Deviations by strain and antimicrobial

The list of deviations is shown in Appendix 11a and 11b. Figure 2 shows the total percentage of deviations from the expected results of AST performed by participating laboratories. For the

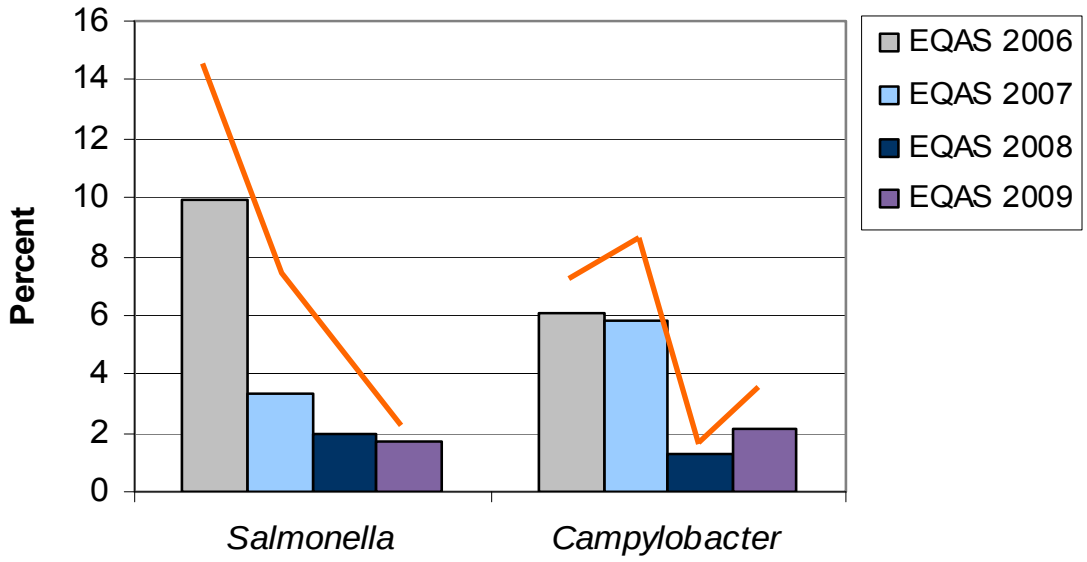

Figure 2: A comparison between the four EURL-AR EQAS's since 2006, showing the percent of deviations in total for antimicrobial susceptibility testing performed by participating laboratories

Salmonella strains, 98.3% of the AST's were interpreted correctly. For the *Campylobacter* strains, 97.8% of AST's were correctly tested. The deviation level is acceptable for both the *Salmonella* and the *Campylobacter* trials.

Figure 3 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 percent is significantly higher ($p<0.01$) when performed by disk diffusion compared to a MIC-method.

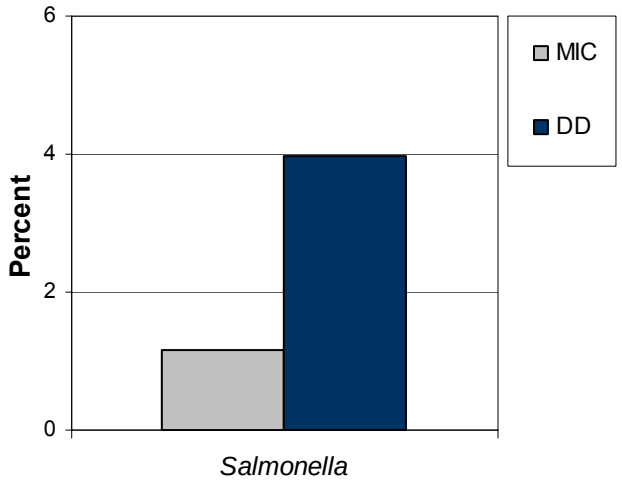

Figure 3: The percent of deviations in total for EQAS 2009 for AST's comparing the results when using MIC-methods as opposed to disk diffusion.

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 were observed between strains of the same species, ranging from 96.6-99.7% for *Salmonella* and from 95.8-99.4% for *Campylobacter*.

EQAS 2009 – *Salmonella*			EQAS 2009 – *Campylobacter*		
Test strain	AST in total	% correct	Test strain	AST in total	% correct
S-4.1	331	99.7	C-4.1 (*C. jejuni*)	168	97.0
S-4.2	330	98.5	C-4.2 (*C. jejuni*)	161	97.5
S-4.3	329	99.7	C-4.3 (*C. coli*)	168	97.6
S-4.4	328	98.2	C-4.4 (*C. jejuni*)	161	96.3
S-4.5	320	97.8	C-4.5 (*C. coli*)	168	95.8
S-4.6	326	96.9	C-4.6 (*C. coli*)	168	99.4
S-4.7	329	99.1	C-4.7 (*C. coli*)	168	99.4
S-4.8	327	96.6	C-4.8 (*C. jejuni*)	168	99.4

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

For *Salmonella*, the test strain S-4.5 (97.8% correct results) was also included in former EQAS's as internal reference strain, with 85.3%, 92.3% and 94.7% correct results, respectively. This strain is resistant to ampicillin, cefotaxime, ceftiofur, ciprofloxacin, nalidixic acid, and

tetracycline. Additionally, for ceftazidime the MIC value is 0.5, but as the strain is ESBL-producing, it should be regarded resistant towards this antimicrobial as well (interpretation of cephalosporins described in the protocol).

Table 2 illustrates the percentage of correct AST per antimicrobial by species. When testing *Salmonella*, it appeared that the antimicrobial with the lowest percentage of correct AST was ciprofloxacin (94.3%). In former EQAS's this has also been the case with even lower percentages of correct results: 79.8%, 90.0% and 90.5%, respectively. Thus, this year there appears to be an improvement in performance regarding this issue.

EQAS 2009 Antimicrobial	% correct	
	Salmonella	*Campylobacter*
Ampicillin, AMP	99.6	-
Cefotaxime, CTX	99.2	-
Ceftazidime, CAZ	98.3	-
Ceftiofur, XNL	100.0	-
Chloramphenicol, CHL	99.2	99.4
Ciprofloxacin, CIP	94.3	98.5
Erythromycin, ERY	-	97.0
Gentamicin, GEN	99.6	98.5
Nalidixic acid, NAL	99.6	96.5
Streptomycin, STR	95.3	98.9
Sulphonamides, SMX	98.7	-
Tetracycline, TET	97.1	96.5
Trimethoprim, TMP	100.0	-

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

For *Campylobacter*, it appears that none of the antimicrobials have a notably different deviation percent compared to the other antimicrobials on the list.

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 according to the description in the protocol: ESBL producing strains that are resistant to one or two of the antimicrobials cefotaxime (CTX), ceftazidime (CAZ) and/or ceftiofur (XNL), should be regarded resistant to all three (this does not include cefoxitin, as ampC's are resistant to cefoxitin (FOX) and 'true ESBLs' are not).

The three test strains S-4.1, S-4.2 and S-4.5 were ESBL-producers, and this was confirmed by the majority of the 31 laboratories participating in the *Salmonella* EQAS. The ESBL detection part is mandatory in this EQAS, therefore all results are evaluated below.

All ESBL-producing strains were so-called 'true ESBLs' harbouring $bla_{\text{CTX M-15}}$ (S-4.1 and S-4.2) and CTX M-15-like-gene (S-4.5) (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-4.1 (CTX M-15)	**Strain S-4.2 (CTX M-15)**	**Strain S-4.5 (CTX M-15 like)**
CTX, CAZ, XNL	5/5 (100%)	5/5 (100%)	5/5 (100%)
CTX, CAZ	17/18 (94%)	17/18 (94%)	15/18 (83%)
CTX, XNL	3/3 (100%)	3/3 (100%)	3/3 (100%)
CTX	3/5 (60%)	3/5 (60%)	2/5 (40%)
Confirmed ESBL	28/31 (90%)	28/31 (90%)	25/31 (81%)
FOX^{S}	31/31 (100%)	31/31 (100%)	31/31 (100%)
AmpC not confirmed	31/31 (100%)	31/31 (100%)	31/31 (100%)

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

In twelve occasions, the ESBL producing strain was not detected. Nine of these deviations were due to three laboratories which had not performed the confirmatory testing (laboratory no. 38, 39 and 40). The remaining three cases were observed for the test strain S-4.5 which was also included in the former EQAS's. In former EQAS's, this test strain also caused problems as this test strain did not show resistance towards ceftazidime (MIC <0.5), but it should be regarded resistant to this cephalosporin also. Consequently, it appears that the laboratories quite confidently detected and confirmed two of the ESBL-producers (S-4.1 and S-4.2; 100% when disregarding the three laboratories which did not perform the confirmatory testing). Three further laboratories in addition to laboratory no. 38, 39 and 40 did not detect the test strain S-4.5 as ESBL-producing.

In Table 4, the results obtained when comparing the different methods for ESBL confirmatory testing are shown. Eleven laboratories uploaded an MIC-ratio as a result, and 15 uploaded the increase of zone diameter (data shown refer to all three ESBL-producing strains). For the laboratories that obtained an MIC-result, all conclusions were correct, whereas the laboratories that performed disk diffusion failed to confirm ESBL-production in four cases.

		Increase in zone diameter	MIC-ratio
Expected result / no. of results in total	CAZ:CAZ/Cl	41/41 (100%)	33/33 (100%)
	CTX:CTX/Cl	44/45 (98%)	37/37 (100%)

Table 4: Comparison of obtained results when performing confirmatory tests by either of the two methods: measurement of zone diameters (disk diffusion) or by obtaining a MIC-ratio (E-test). Results compiled for all three ESBL-producing strains.

Two laboratories reported the use of cefpodoxime for screening and confirmatory testing, one laboratory reported the use of ertapenem instead of imipenem and one laboratory mentioned that they do not routinely screen for ESBL. However, they observed a synergy zone between amoxycillin-clavulanic acid and ceftiofur using Neo-sensitabs. Therefore, this strain was considered to be an ESBL producing strain.

According to the expected, none of the laboratories reported resistance towards cephalosporins for any of the non-ESBL's. However, two laboratories confirmed test strains S-4.8 as being an AmpC-type based on the fact that they found this strain exhibiting resistance to cefoxitin.

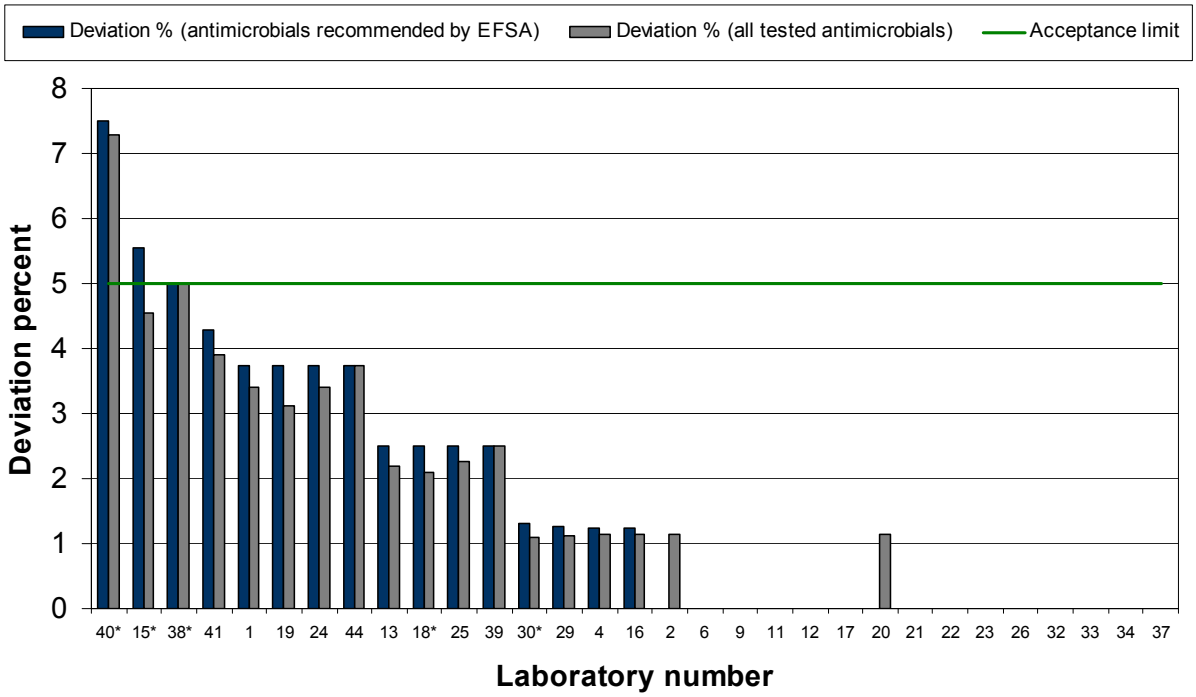

Figure 4: Individual participants' deviations in percent of their total number of *Salmonella* AST's. An asterisk indicates that the laboratory has performed AST using disk diffusion

3.3 Deviations by laboratory

Figure 4 and 6 illustrate the percentage of deviations for each participating laboratory. The laboratories are ranked according to their performance determined by the percentage of deviating results in tests with antimicrobials recommended by EFSA. These results will be the focus of the evaluation in the following sections. Obtained results including all antimicrobials mentioned in the protocol are additionally indicated. In Figure 5 and 7, the total amount of deviations in percentages is illustrated by number of laboratories.

3.3.1 *Salmonella* trial

Fifteen of the laboratories obtained a result of 100% correct AST in the Salmonella strains. The maximum percentage of deviations was 7.5%.

The vast majority of the laboratories have a deviation percentage below 5, and none of the laboratories can be categorized as outliers. In all, 29 of the 31 (94%) participating laboratories achieved the level of performance expected by the EURL-AR.

Figure 5 also illustrates that the majority of laboratories had less than 5% deviation, whereas two laboratories (#15 and #40) obtained levels of deviations slightly above the acceptance limit.

Deviation levels including all antimicrobials mentioned in the protocol do not vary much from the deviation levels regarding EFSA-antimicrobials, only. These will not be further analysed.

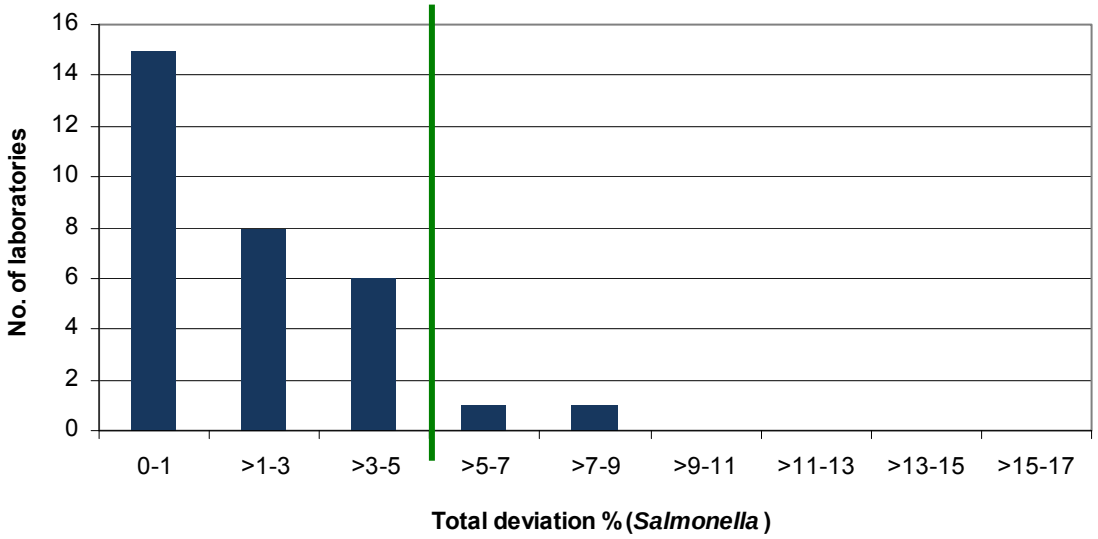

Figure 5: The number of laboratories listed in intervals of percent of total deviations. The green line marks the 5% acceptance limit set by the EURL-AR

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 18 laboratories having

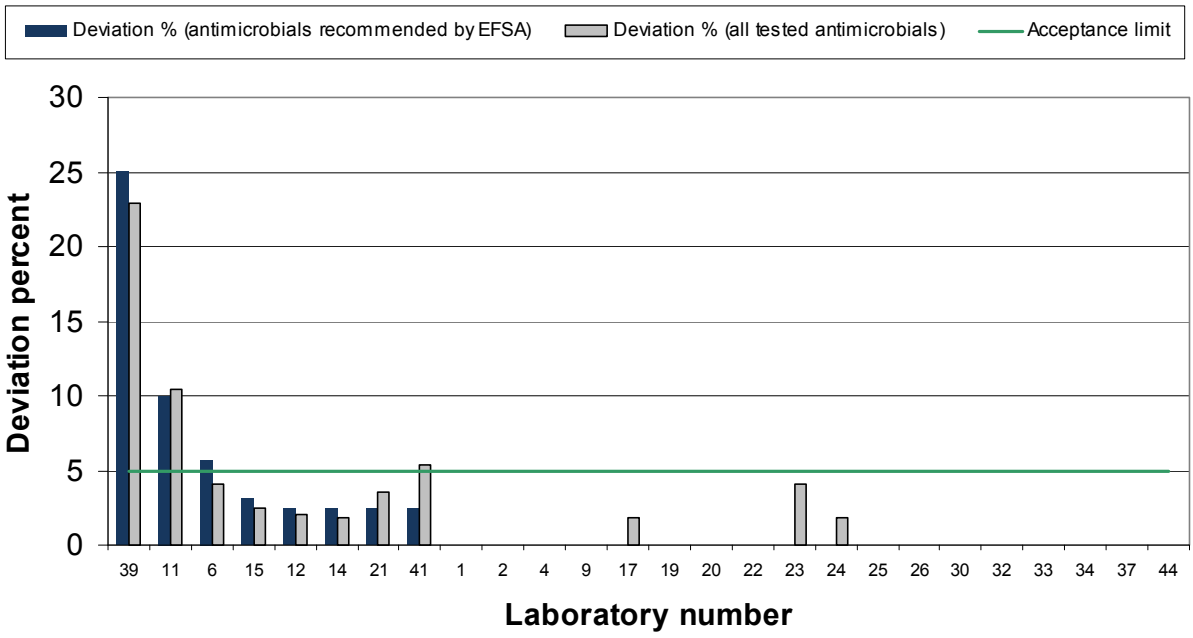

Figure 6: Individual participants' deviations in percent of their total number of *Campylobacter* AST's.

no deviations at all. One laboratory (#39) had a very high level of deviation (25%) and is considered an outlier (Figure 6 and 7). Laboratory #39 has recently introduced MIC-methods for AST of *Campylobacter*, and is working on improving this technique.

Deviation levels including all antimicrobials mentioned in the protocol vary to a somewhat high extent from the deviation levels regarding EFSA-antimicrobials for especially two laboratories; #23 and #41. For the full selection of antimicrobials, both laboratories obtain deviation levels close to 5%.

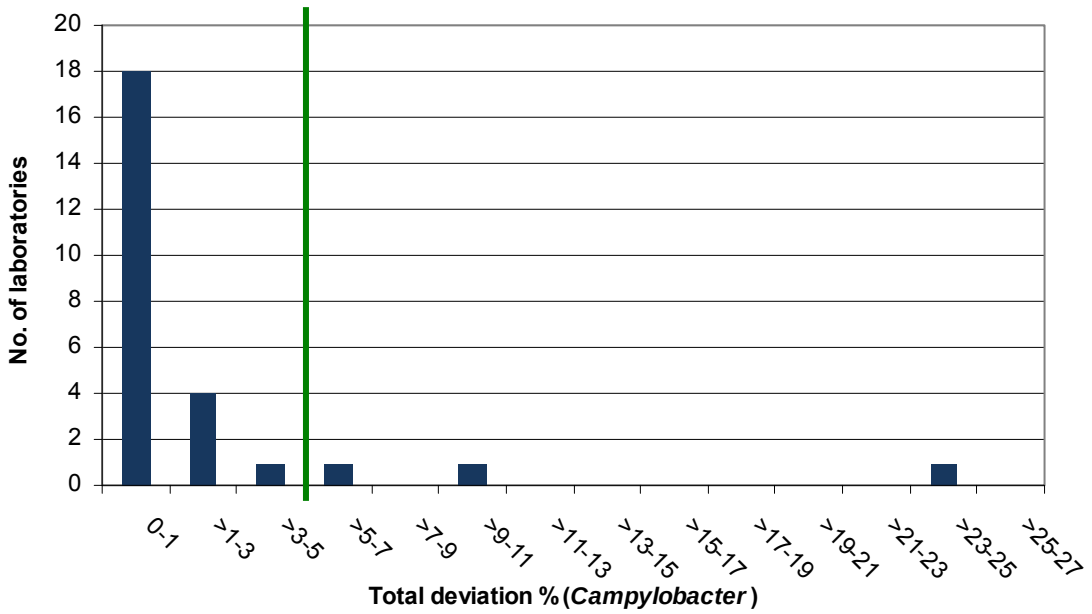

Figure 7: The number of laboratories listed in intervals of percent of total deviations.

EQAS 2009	**Disk diffusion *E. coli* ATCC 25922**		
	Proportion of labs outside QC range	Obtained values in mm zones (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/5	-	-
Cefotaxime, CTX	1/5	-	1
Cefoxitin, FOX	0/5	-	-
Ceftazidime, CAZ	0/3	-	-
Ceftiofur, XNL	1/3	1	-
Chloramphenicol, CHL	0/5	-	-
Ciprofloxacin, CIP	0/4	-	-
Gentamicin, GEN	1/5	-	1
Imipenem, IMI	1/4	-	8
Nalidixic acid, NAL	0/5	-	-
Streptomycin, STR	0/5	-	-
Sulphonamides, SMX	0/3	-	-
Tetracycline, TET	0/5	-	-
Trimethoprim, TMP	0/5	-	-

Table 5: Obtained values for reference testing of *E. coli* ATCC 25922 by disk diffusion.

3.4 Deviations by reference strains

In this section, deviations are defined as results from tests on the reference strain that exceed the quality control (QC) acceptance intervals (App. 7). Values from the participants' testing of the QC strains are listed in Appendix 6a and 6b, along with Tables 5, 6 and 7 which summarize results from the laboratories' quality control. For the *Salmonella* trial, all laboratories performed QC testing of the reference strain. For the *Campylobacter* trial, 24 of the 26 participating laboratories performed QC-testing on the reference strain.

Table 5 presents the proportion of laboratories that obtained values out of range for the *E. coli* reference strain (ATCC 25922), when performing disk diffusion. For four out of 14 antimicrobials, a value outside the QC-range was obtained. Three of these are uploaded by one laboratory (#15).

EQAS 2009	**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	1/26 (4%)	-	1 step
Cefotaxime, CTX	1/26 (4%)	-	2 steps
Cefoxitin, FOX	2/4 (50%)	2 steps	1 step
Ceftazidime, CAZ	1/19 (5%)	-	3 steps
Ceftiofur, XNL	0/5 (0%)	-	-
Chloramphenicol, CHL	0/26 (0%)	-	-
Ciprofloxacin, CIP	3/26 (12%)	-	1 step
Gentamicin, GEN	1/26 (4%)	-	1 step
Nalidixic acid, NAL	0/26 (0%)	-	-
Streptomycin, STR	0/25 (0%)	-	-
Sulphonamides, SMX	1/13 (8%)	3 steps	-
Tetracycline, TET	1/26 (4%)	-	1 step
Trimethoprim, TMP	0/25 (0%)	-	-

Table 6: Obtained values for reference testing of *E. coli* ATCC 25922 by MIC determination

The use of MIC determination towards the reference strain *E. coli* ATCC 25922 resulted in the outcome presented in Table 6. Twenty-six laboratories submitted MIC data. No mistakes were seen for five antimicrobials, whereas for eight antimicrobials MIC-values up to three steps lower or higher than the QC interval limit was detected. Four different laboratories have contributed to the 11 MIC-values outside the QC-ranges, of which one laboratory has seven deviations.

EQAS 2009	**MIC determination *C. jejuni* ATCC 33560**		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
Antimicrobial		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	0/18 (0%)	-	-
Ciprofloxacin, CIP	2/24 (8%)	-	2 steps
Erythromycin, ERY	1/24 (4%)	1 step	-
Gentamicin, GEN	3/24 (13%)	2 steps	-
Nalidixic acid, NAL	2/22 (9%)	1 step	2 steps
Tetracycline, TET	1/22 (5%)	-	1 step

Table 7: Obtained values for reference testing of *C. jejuni* ATCC 33560 using MIC determination

Twenty four laboratories performed MIC determination against the *C. jejuni* reference strain ATCC 33560. Table 7 presents the proportion of the laboratories with results from the QC strain below or above the QC interval. Deviations were seen for all antimicrobials except for chloramphenicol. The highest level of deviation was seen for gentamicin.

3.4 Genotypic characterisation

For the optional PCR-testing of a selected *Salmonella* isolate, five laboratories performed the genotypic characterization. Table 8 shows that four of the five laboratories correctly detected 10, 11, 11 and 11 of the expected genes. Two laboratories have obtained information as to the specific bla-gene. Additionally, one laboratory appears to have detected the aac(3)-Ie-gene in the isolate, one detected the dfrA1-gene, and one did not detect the qnrB-gene. Three of the five laboratories performed PCR-methods which were all published, whereas two made use of in-house methods.

		Lab I		Lab II		Lab III		Lab IV		Lab V	
Betalactams	CTX M-15	1/-	P	1/-		1/1	P	1/1	P	1/-	P
Betalactams	SHV-12	1/-	P	1/-		1/1	P	1/1	P	1/-	P
Betalactams	TEM-1b	1/-	P	1/-		1/1	P	1/1	P	1/-	P
Florphenicol	floR	1	P	-	-	1	P	1		1	P
Quinolones	qnrB	1	P	-	-	1	P	1		-	-
Streptomycin	strA	1	P	-	-	1	P	1	P	1	P
Streptomycin	strB	1	P	-	-	1	P	1	P	1	P
Sulfamethoxazole	sul1	1	P	-	-	1	P	1		1	P
Sulfamethoxazole	sul2	1	P	-	-	1	P	1		1	P
Tetracycline	tetA	1	P	-	-	1	P	1		1	P
Tetracycline	tetD	1	P	-	-	1	P	1		1	P
Additional genes detected		dfrA1	P	-	-	aac(3)-Ie	P	-		-	-

Table 8: Results from genotypic characterisation. Laboratory numbers are not consistent with the numbers otherwise used in this report. Identification in accordance with the expected is indicated with '1'. Identification not performed is indicated with '-'. For CTX, SHV and TEM, the result, 1/1, indicates 'correctly identified gene or gene group'/'specific gene correctly identified'. The result, 1/-, indicates that the PCR-product was not sequenced to obtain a specific gene number. 'P' indicates that a published PCR-method was used.

Two laboratories informed of the use of a microarray (Clondiag, VLA, UK; Identibac). One of these laboratories (IV) used this as a pre-screening and subsequently performed PCR for typing of the betalactam resistance genes and for detection of mutations in the gyrA and parC genes. Additionally, this laboratory performed strA and strB PCR for confirmation of weak array results for strA. The other laboratory (I) did not perform confirmation of the microarray results.

4. Discussion

4.1 *Salmonella* trial

Overall, the percentage of correct susceptibility test results of *Salmonella* was 98.4%. The majority of participants (29) 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.

Compared to the performance in 2006, 2007 and 2008 iteration (90.1%, 96.7% and 98.0% correct results, respectively), it would therefore appear that the quality of the results is increasingly satisfactory.

Two laboratories had a deviation level slightly higher than 5% (#15 and #40) with values of 5.6% and 7.5%, respectively. These laboratories performed AST by disk diffusion, and none of the deviations were caused by antimicrobials for which the reference strain was out of range.

The relatively low performance regarding ciprofloxacin (94.3% correct results), was in part caused by two laboratories performing MIC but failing to interpret the MIC value according to the low cut off value. In addition, when performing disk diffusion, the issue regarding the low cut off value for ciprofloxacin is addressed in the protocol '*Salmonella* strains resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin'. These guidelines were followed by four of the five laboratories performing disk diffusion.

The test strain S-4.6 was a *Salmonella* strain which harbours a plasmid mediated quinolone resitance gene; *qnrB*. This *qnr*-gene confers low-level resistance to ciprofloxacin (MIC=0.25µg/mL), but not to nalidixic acid (MIC=8µg/mL). The participants generally found this isolate sensitive to nalidixic acid (97%), whereas only 76% found the isolate resistant to ciprofloxacin. The low-level ciprofloxacin resistance caused by a *qnr*-gene is difficult to detect when performing disk diffusion as the usual association between ciprofloxacin and nalidixic acid is not seen. All three laboratories performing disk diffusion on this strain/antimicrobial-combination obtained a diffusion zone indicating that the test strain is sensitive. Furthermore, four laboratories performing MIC-determination found S-4.6 sensitive to ciprofloxacin, albeit two of these obtained an MIC-value above the cut off-value.

The *Salmonella* test strain the S-4.5 obtained 97.8% correct results and was the ESBL-producing isolate which had an MIC value for ceftazidime below the cut off value. The strain was resistant towards cefotaxime and ceftiofur, which according to the guidelines would render an interpretation as resistant to ceftazidime, also. Five laboratories reported either cefotaxime or ceftazidime sensitive, and all five had registered at least one of the remaining two relevant cephalosporins resistant. The deviation level of the cephalosporins could have been zero when disregarding these misinterpretations.

For the *E. coli* reference strain, the results obtained were in general in agreement with the CLSI recommendations. The number of laboratories performing AST on *Salmonella* by the use of disk diffusion was five. All of these laboratories uploaded data for the testing of the reference strain, and a total of 93.5% were within range. For the laboratories performing AST on *Salmonella* by an MIC-method, all laboratories uploaded QC-results to the database. The proportion of values within the expected range was 96.0%.

A follow-up on the laboratories which had a deviation level above the acceptance limit in EQAS 2008 showed considerable improvement. This concerns the laboratories #29, #38 and #40, with values of 10.9%, 10.0% and 8.6%deviations in 2008 which have improved to 1.3%, 5.0% and 7.5% deviations, respectively, this year.

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.

Three of the *Salmonella* test strains were ESBL producing (S-4.1, S-4.2 and S-4.5), and the participants were asked to interpret their results according to the description in the protocol. Of the 31 laboratories which tested *Salmonella*, three did not upload results from confirmatory testing of ESBL-production which resulted in an evaluation as incorrect. The proportion of laboratories that could confirm S-4.1, S-4.2 and S-4.5 as ESBL-producers was 90%, 90% and 81%, respectively.

For the detection of an ESBL-producing *Salmonella* when initially screening the isolate, it is recommended that more than one cephalosporin is used. In this EQAS, it appeared that laboratories performing the initial screening with cefotaxime only, had problems with detecting the ESBL-production of the test strain. Also for the combination of cefotaxime and ceftazidime this appeared to be a problem (Table 3).

4.2 *Campylobacter* trial

The percentage of correct susceptibility test results of *Campylobacter* was 97.8%. The performance varied from no deviations to 25% deviations, with 23 laboratories performing satisfactorily according to the established acceptance ranges.

One laboratory (#39) was identified as an outlier (25% deviations). This laboratory has introduced the MIC-determination for AST of *Campylobacter* and is working on improving this method.

Laboratories #23 and #41 had deviation levels close to 5% when including results from all antimicrobials mentioned in the protocol. Both laboratories had two deviations for nalidixic acid; therefore, it appeared that there could be a problem in testing of nalidixic acid.

The proportion of results for the *C. jejuni* reference strain within the QC intervals was 93.2% which was an increase in comparison to EQAS 2008, where the proportion was 89.2%. In this year's trial, 24 of 26 participating laboratories uploaded data from tests performed on the reference strain. Three laboratories each obtained two values outside the QC intervals, one of these laboratories is the outlier (laboratory #39). Follow-up on the outlier will include discussions regarding methodical issues.

A follow-up on the laboratory which was an outlier in the *Campylobacter* trial in EQAS 2008 (#40) is not possible, as unfortunately this laboratory has not had the opportunity to apply MIC methods for *Campylobacter* AST.

4.3 Optional genotypic characterisation of selected *Salmonella* test strain

As the focus on molecular aspects appear to be increasing, it is likely that genotypic characterisation of relevant bacterial isolates in the future will gain further interest. The genotypic characterisation offered as an optional supplementary part of this EQAS was performed by five laboratories. All participating laboratories obtained satisfying results.

5. Conclusions

The goal of the EURL-AR EQAS is having all participating NRLs performing susceptibility testing of *Salmonella* and *Campylobacter* with a deviation level less than 5%. This seems within reach for *Salmonella*, and also for *Campylobacter*. However, for *Campylobacter* one laboratory would need to validate the methodological changes to be able to improve the quality of the results.

The performance of the NRL's appear to have improved for *Salmonella* AST's in this EQAS (98.4%) when compared to the results from the EQAS 2007 and 2008 (96.7% and 98.0%). Regarding *Campylobacter* AST's, it appears that the level of deviation has increased slightly from 1.3% in 2008 to 2.2% in 2009 with mainly one outlier presenting 25% deviation. This laboratory took part in a training course focussing on *Campylobacter* AST by MIC-methods in February 2009, and additional follow-up will be carried out including discussions regarding methodical issues.

Laboratories which have not yet introduced tests to detect ESBL-producing Enterobacteriaceae, should prioritize this area, as these challenging 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 it is likely to be repeated.

6. References

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

CRL-AR EQAS pre-notification

DFVF- M00-06-001/31.10.2008

EQAS 2009 FOR *SALMONELLA* AND *CAMPYLOBACTER*

The CRL 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 and eight *Campylobacter* isolates. New participants will be offered the following QC strains: *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214). Additionally, optional PCR-testing of a selected *Salmonella* isolate will be offered.

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

Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

Please remember to provide the EQAS coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the contents of the parcel are "Biological Substance Category B": Nine *Salmonella* isolates, eight *Campylobacter* isolates and for new participants also the QC strains mentioned above. The strains are expected to arrive at your laboratory in October 2009.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped in October 2009. The protocol will be provided electronically.

Returning of results: Results must be returned to the National Food Institute, by December 31st, 2009. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

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

Next EQAS: The next CRL EQAS that we will have is on antimicrobial susceptibility testing of *E. coli*, enterococci and staphylococci which will be carried out in June 2009.

Any comments regarding the EQAS, please contact me by e-mail (suska@food.dtu.dk) or by fax (+45 3588 6001).

Sincerely,

Susanne Karlsmose
EQAS-Coordinator

EU Community Reference Laboratory, Antimicrobial Resistance, Bülowsvej 27, DK-1790, Copenhagen V, Denmark
Ph: +45 3588 6601, Fax: +45 3588 6001, e-mail: suska@food.dtu.dk

Participant list

Campylobacter	*Salmonella*	Genetypic characterisation	Institute	Country
X	X	-	Austrian Agency for Health and Food Safety	Austria
X	X	-	Institute of Public Health	Belgium
-	X	-	Nacional Diagnostic and Research Veterinary Institute	Bulgaria
X	X	-	Veterinary Services	Cyprus
X	X	-	State Veterinary Institute Praha	Czech Republic
X	X	X	The National Food Institute	Denmark
X	X	-	Estonian Veterinary and Food Laboratory	Estonia
X	X	-	Finnish Food Safety Authority EVIRA	Finland
-	X	X	AFSSA LERQAP Maisons Alfort	France
X	-	-	AFSSA Ploufragan - LERAP	France
X	X	-	AFSSA Lyon	France
-	X	-	AFSSA Fougères LERMVD	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	X	-	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	-	National Diagnostic Centre of Food and Veterinary Service	Latvia
X	X	-	National Veterinary Laboratory	Lithuania
X	X	-	Public Health Laboratory	Malta
X	X	-	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
-	-	-	Veterinærinstituttet	Norway
X	X	-	National Veterinary Research Institute	Poland
X	X	-	Laboratorio National de Investigacáo Veterinaria)	Portugal
X	X	-	National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
X	X	-	Institute for Hygiene and Veterinary Public Health	Romania
-	X	-	State Veterinary and Food Institute (SVFI)	Slovakia
X	X	-	National Veterinary Institute	Slovenia
-	-	-	Laboratorio Central de Sanidad, Animal de Santa Fe (only Staph)	Spain
X	X	-	Laboratorio Central de Sanidad, Animal de Algete	Spain
X	X	-	Complutense University of Madrid	Spain
-	X	-	Centro nacional de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricio	Spain
X	X	-	National Veterinary Institute, SVA	Sweden
X	X	-	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
X	X	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 CRL
Not a Member State of the EU

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

	Ampicillin AMP	CTX	Cefotaxime		ESBL-confirmation CTX:CTX/Cl		Ceftazidime CAZ		ESBL-confirmation CAZ:CAZ/Cl		Cefoxitin		Ceftiofur XNL		Chloramphenicol CHL		Ciprofloxacin CIP		Gentamicin GEN	IMI	Imipenem		Nalidixic acid NAL		Streptomycin STR		Sulfamethoxazole SMX		Tetracycline TET		Trimethoprim TMP	
CRL S-4.1	>32	RESIST	>4	RESIST	>8	ESBL	64	RESIST	>8	ESBL	8	SUSC	>8	RESIST	4	SUSC	<=0.015	SUSC	0.5	SUSC	<=0.5	SUSC	4	SUSC	<8	SUSC	<64	SUSC	<2	SUSC	<1	SUSC
CRL S-4.2	>32	RESIST	>4	RESIST	>8	ESBL	32	RESIST	>8	ESBL	4	SUSC	>8	RESIST	>64	RESIST	0.03	SUSC	>16	RESIST	<=0.5	SUSC	4	SUSC	128	RESIST	>1024	RESIST	16	RESIST	>32	RESIST
CRL S-4.3	2	SUSC	<=0.12	SUSC			0.25	SUSC			2	SUSC	1	SUSC	8	SUSC	0.03	SUSC	0.5	SUSC			4	SUSC	<=8	SUSC	<=64	SUSC	<=2	SUSC	<=1	SUSC
CRL S-4.4	>32	RESIST	<=0.12	SUSC			0.25	SUSC			2	SUSC	<=0.5	SUSC	>64	RESIST	0.12**	RESIST**	0.5	SUSC			>64	RESIST	16	SUSC	>1024	RESIST	32	RESIST	>32	RESIST
CRL S-4.5	>32	RESIST	>4	RESIST	>8	ESBL	0.50*	RESIST*	<8	ESBL	2	SUSC	>8	RESIST	8	SUSC	0.25**	RESIST**	0.5	SUSC	<=0.5	SUSC	>64	RESIST	<=8	SUSC	<=64	SUSC	32	RESIST	<=1	SUSC
CRL S-4.6	<=1	SUSC	<=0.12	SUSC			0.5	SUSC			2	SUSC	<=0.5	SUSC	4	SUSC	0.25**	RESIST**	0.5	SUSC			8	SUSC	<=8	SUSC	128	SUSC	<=2	SUSC	<=1	SUSC
CRL S-4.7	>32	RESIST	<=0.12	SUSC			0.25	SUSC			2	SUSC	1	SUSC	4	SUSC	0.03	SUSC	0.5	SUSC			8	SUSC	>128	RESIST	>1024	RESIST	>32	RESIST	<=1	SUSC
CRL S-4.8	>32	RESIST	0.25	SUSC			0.5	SUSC			16	SUSC	2	SUSC	8	SUSC	1	RESIST	<0.25	SUSC			>64	RESIST	32	RESIST	>1024	RESIST	4	SUSC	>32	RESIST

*MIC value is not resistant, but due to the rule about cephalosporins the interpretation should be resistant
** Low-level cip-resistance

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	CRL C-4.1	<=2	SUSC	16	RESIST	>64	RESIST	0.25	SUSC	>64	RESIST	<=1	SUSC	0.5	SUSC
C. jejuni	CRL C-4.2	8	SUSC	32	RESIST	>64	RESIST	>32	RESIST	>64	RESIST	>16	RESIST	>64	RESIST
C. coli	CRL C-4.3	8	SUSC	32	RESIST	4	SUSC	0.25	SUSC	>64	RESIST	>16	RESIST	>64	RESIST
C. jejuni	CRL C-4.4	4	SUSC	16	RESIST	1	SUSC	0.25	SUSC	>64	RESIST	<=1	SUSC	64	RESIST
C. coli	CRL C-4.5	4	SUSC	8	RESIST	>64	RESIST	0.5	SUSC	>64	RESIST	>16	RESIST	16	RESIST
C. coli	CRL C-4.6	4	SUSC	0.06	SUSC	1	SUSC	0.25	SUSC	4	SUSC	<=1	SUSC	<=0.25	SUSC
C. coli	CRL C-4.7	4	SUSC	0.06	SUSC	2	SUSC	0.25	SUSC	8	SUSC	16	RESIST	0.5	SUSC
C. jejuni	CRL C-4.8	<=2	SUSC	8	RESIST	2	SUSC	0.25	SUSC	>64	RESIST	>16	RESIST	0.12	SUSC

Resistant

DFVF-M00-06-001/15.03.2009

CRL-AR Inter-laboratory Proficiency Test 2009
- *Salmonella, Campylobacter* and optional PCR

Copenhagen, October 2009

Dear >>name<<,

Please find enclosed the bacterial strains for the CRL AR EQAS 2009.

On the CRL-website (www.crl-ar.eu) the following documents relevant for the CRL EQAS are available:

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

We ask you to examine the eight *Salmonella* and the eight *Campylobacter* strains that we send to you by performing antimicrobial susceptibility testing. The additional strain (CRL GEN 1.1) is included for optional genotypic characterisation. In the protocol you will find detailed description of how to test the strains. Additionally, you will find a description of how to enter your results into the interactive web database. For entering data you need this username and password.

Your username: >>username<<

Your password: >>password<<

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

After receipt, the strains should be stored dark and at 4°C for stabs, and dark and cool for freeze-dried strains. The *Campylobacter* charcoal swabs must be sub-cultured straight away!

The results should be returned to us no later than December 31st 2009.

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 Community Reference Laboratory, Antimicrobial Resistance, Bülowsvej 27, DK-1790, Copenhagen V, Denmark
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EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

PROTOCOL

For susceptibility testing of *Salmonella* and *Campylobacter*

1 INTRODUCTION

One of the tasks as the EU Community Reference Laboratory for Antimicrobial Resistance is to organise and conduct an External Quality Assurance System (EQAS) on susceptibility testing of *Salmonella* and *Campylobacter*. The *Salmonella* and *Campylobacter* EQAS 2009 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 *Salmonella* isolate is offered (pilot).

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

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

2 OBJECTIVES

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

3 OUTLINE OF THE EQAS 2009

3.1 Shipping, receipt and storage of strains

In October 2009 the EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing nine *Salmonella* and eight *Campylobacter* strains. Reference strains will be included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material.

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

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please see the document 'Instructions for opening and reviving lyophilised cultures' on the CRL-website (see www.crl-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 3.3.1 and 3.3.2 should be used. The epidemiological cut-off values allow two categories of characterisation – resistant or sensitive.

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

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

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

3.3.1 *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.

Also, when following EUCAST epidemiological cut-off values, *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin. When using disc diffusion and CLSI clinical breakpoints this connection between nalidixic acid and ciprofloxacin is not taken into account. Thus, the result in this situation with regard to ciprofloxacin will deviate from the expected result in this EQAS.

Antimicrobials for *Salmonella*	MIC (µg/mL) **R is >**
Ampicillin (AMP)	4
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)*	32
Sulphonamides (SMX)**	256
Tetracycline (TET)	8
Trimethoprim (TMP)	2

* ARBAO ** CLSI

*** Not part of the EFSA monitoring programme (used for confirmatory tests for ESBL production)

ESBL production

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

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

(CLSI M100 Table 2A; enterobacteriaceae). If the test shows signs of synergy it is an indication of the presence of ESBL.

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

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

Concerning **cefotaxime, ceftazidime and/or ceftiofur** used when detecting ESBL-producing strains in the EQAS: If a microorganism is resistant to one or two of these drugs, it should be regarded resistant to all three (this does not include cefoxitin, as ampC's are resistant to cefoxitin and 'true ESBLs' are not).

3.3.2 *Campylobacter*

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

*Not part of the EFSA monitoring programme

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

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

3.4 Optional genotypic characterisation

An optional PCR-testing of a selected *Salmonella* isolate is offered (test strain label: CRL GEN 1.1). If performing the genotypic characterisation of this test strain, the results requested are the genes harboured in the test strain. The genes listed in the table below are those included in the test.

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The test strain may harbour resistance genes not present on this list, these will not be evaluated by the database, but may be mentioned in the comments-field. When uploading the results in the

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

database, the identified genes will be evaluated against the expected results. The results will be evaluated on the actual gene identified. The groups of TEM-, CTX-, SHV-, CMY-, OXA-genes as well as the gyrA-mutations and parC-mutations will additionally be evaluated on the group selected. For gyrA and parC the codon-no of the site of mutation will be evaluated in the same way as the genes.

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

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the test forms, and enter your results into the interactive web database. Please read the detailed description below before entering your results. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest December 31st, 2009.

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

All results will be summarized in a report which will be made available to all participants. The data in the report will be presented with laboratory codes. A laboratory code is known to the individual laboratory, whereas the entire list of laboratories and their codes is confidential and known only to the CRL and the EU Commission. All conclusions are public.

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

Susanne Karlsmose

National Food Institute

Technical University of Denmark

27 Bülowsvej, DK-1790 Copenhagen V

Denmark

Tel: +45 3588 6601

Fax: +45 3588 6001

E-mail: suska@food.dtu.dk

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 CRL logo.

You enter the EU CRL-AR EQAS 2009 start web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

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

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

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

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

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

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

Click on "save and go to next page"

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

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

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

Click on "save and go to next page"

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

Click on "save and go to next page"

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

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

Please fill in the evaluation form.

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

If you have performed the optional genotypic characterisation:

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

TEST FORMS

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

Comments:

TEST FORM

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

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

Brand**:**

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

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

Comments or additional information:

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

TEST FORM

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

☐ MIC – Microtitre
☐ MIC – Agar dilution

Brand**:**

Incubation conditions: °C/ h

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

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

Comments or additional information:

Antimicrobial	General info The relevant information should be filled in below
	Test-range for MIC (µg/mL)
Chloramphenicol	
Ciprofloxacin	
Erythromycin	
Gentamicin	
Nalidixic Acid	
Streptomycin	
Tetracycline	

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Salmonella CRL S. 4.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 : CTX/CL 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 : CAZ/CL 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 : IMI/E 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	

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter CRL C. 4.1 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.2 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.3 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.4 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter CRL C. 4.5 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.6 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.7 *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		
Campylobacter CRL C. 4.8 *C. jejuni*	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	Nalidixic Acid		
	Streptomycin		
	Tetracycline		

TEST FORM

For Microbroth:

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)	
		36-37 °C/48 hours	42 °C/24 hours
C. jejuni ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Erythromycin		
	Gentamicin		
	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	Chloramphenicol	
	Ciprofloxacin	
	Erythromycin	
	Gentamicin	
	Nalidixic Acid	
	Tetracycline	

TEST FORM – genotypic characterisation

Genotypic characterisation of the test strain CRL GEN 1.1

The test strain was found to harbour the following genes:

Strain	Gene found (e.g. strA, TEM-52)	PCR-method used
CRL GEN 1.1		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method
		☐ Published method ☐ In-house method

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

M7-A7, January 2006 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard)

1.3 Definition of Terms

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

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

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

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

1.4 Important Considerations

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

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

1.5 Storage of Reference Strains

Preparation of stock cultures

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

Working cultures

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

1.6 Frequency of Testing

Weekly vs. daily testing

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

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

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

Corrective Actions

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

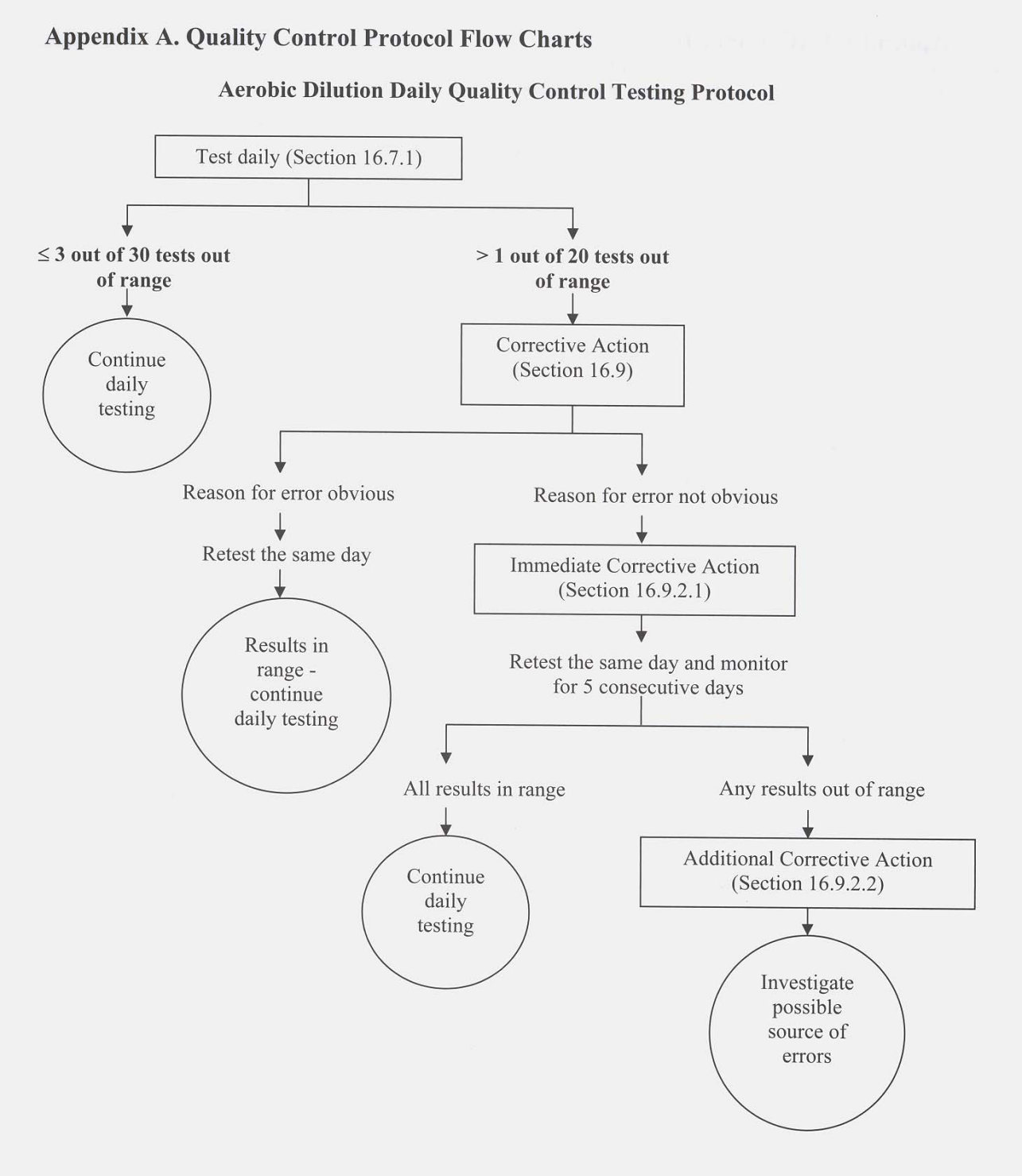
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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

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

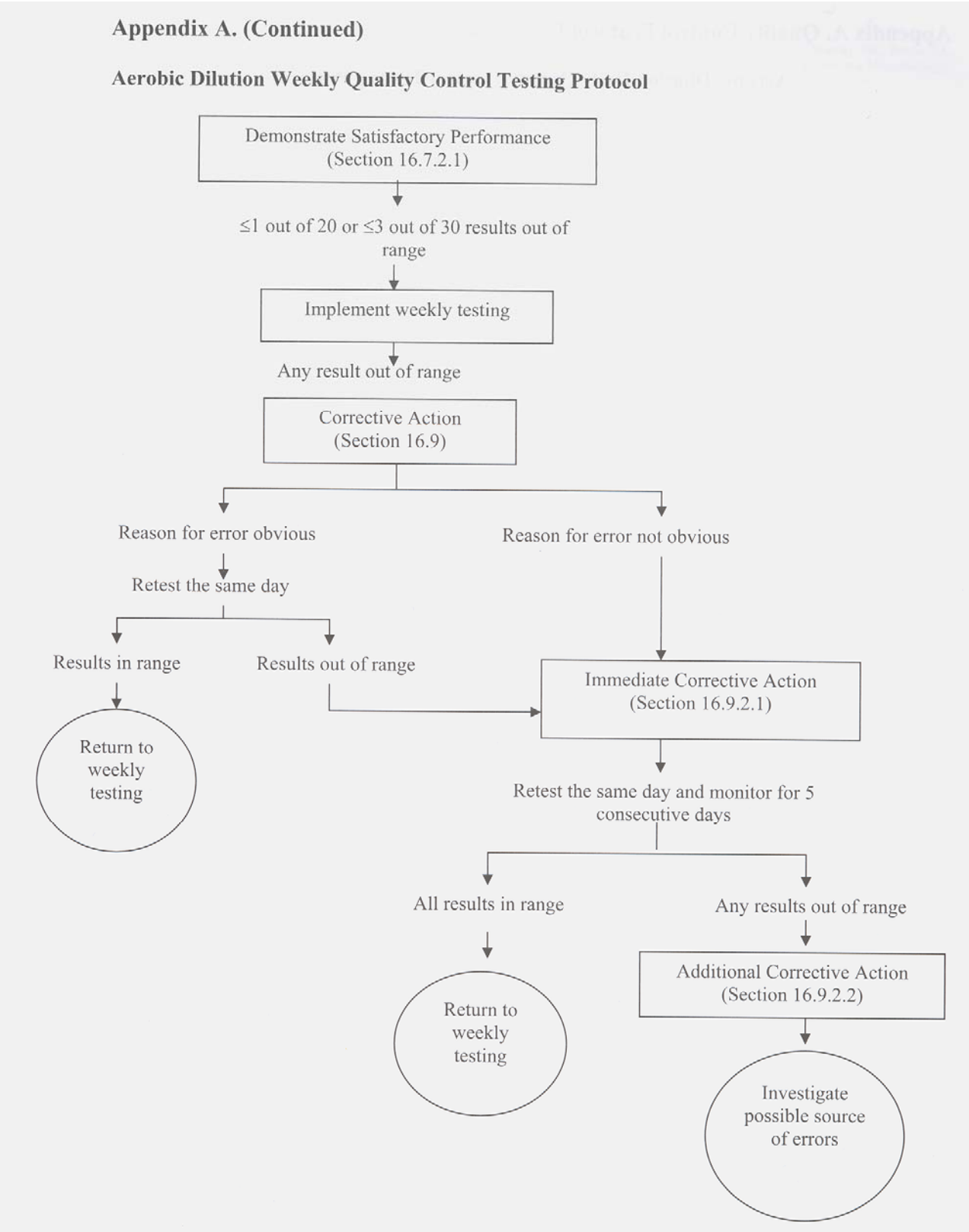
Repeat the 30 days validation before resuming weekly testing.

DAILY MIC QC CHART



Reference: CLSI M7-A7, page 39

WEEKLY MIC QC CHART



Reference: CLSI M7-A7, page 40

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

Antimicrobial	Lab No	Disk content (ug)	R <= (mm)	I = (mm)	S >= (mm)
Ampicillin, AMP	15	25	13	14-20	21
Ampicillin, AMP	18	10	13	14-16	17
Ampicillin, AMP	30	10	13	14-16	17
Ampicillin, AMP	38	10	13	14-16	17
Cefotaxime, CTX	15	30	22	23-25	26
Cefotaxime, CTX	18	30	26		27
Cefotaxime, CTX	30	30	14	15-22	23
Cefotaxime, CTX	38	30	14	15-22	23
Ceftazidime, CAZ	15	30	18	19-25	26
Ceftazidime, CAZ	18	30	21		22
Ceftazidime, CAZ	30	30	14	15-17	18
Ceftiofur, XNL	15	30	17	18-20	21
Ceftiofur, XNL	30	30	16	17-19	20
Chloramphenicol, CHL	15	30	18	19-21	22
Chloramphenicol, CHL	18	30	12	13-17	18
Chloramphenicol, CHL	30	30	12	13-17	18
Chloramphenicol, CHL	38	30	12	13-17	18
Ciprofloxacin, CIP	15		16	17-21	22
Ciprofloxacin, CIP	18	5	15	16-20	21
Ciprofloxacin, CIP	30	5	15	16-20	21
Ciprofloxacin, CIP	38	5	15	16-20	21
Gentamicin, GEN	15	15	15	16-17	18
Gentamicin, GEN	18	10	12	13-14	15
Gentamicin, GEN	30	10	12	13-14	15
Gentamicin, GEN	38	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	30	30	13	14-18	19
Nalidixic acid, NAL	38	30	13	14-18	19
Streptomycin, STR	15	10 UI	12	13-14	15
Streptomycin, STR	18	10	11	12-14	15
Streptomycin, STR	30	10	11	12-14	15
Streptomycin, STR	38	10	11	12-14	15
Sulfamethoxazole, SMX	15	200	11	12-16	17
Sulfamethoxazole, SMX	18	300	12	13-16	17
Sulfamethoxazole, SMX	30	250-300	12	13-16	17
Tetracycline,TET	15	30 UI	16	17-18	19
Tetracycline,TET	18	30	11	12-14	15
Tetracycline,TET	30	30	11	12-14	15
Tetracycline,TET	38	30	11	12-14	15
Trimethoprim, TMP	15	5	11	12-15	16
Trimethoprim, TMP	18	5	10	11-15	16
Trimethoprim, TMP	30	5	10	11-15	16
Trimethoprim, TMP	38	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	=	8	2	8	1	MIC
1	Cefotaxime, CTX	<=	0.125	0.03	0.125	1	MIC
1	Ceftiofur, XNL	<=	0.5	0.25	1	1	MIC
1	Chloramphenicol, CHL	=	4	2	8	1	MIC
1	Ciprofloxacin, CIP	<=	0.015	0.004	0.016	1	MIC
1	Gentamicin, GEN	<=	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	=	4	2	8	1	MIC
2	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
2	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
2	Chloramphenicol, CHL	=	4	2	8	1	MIC
2	Ciprofloxacin, CIP	<=	0.008	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	=	4	4	16	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	=	1	0.25	1	1	MIC
4	Nalidixic acid, NAL	<=	4	1	4	1	MIC
4	Streptomycin, STR	=	8	4	16	1	MIC
4	Tetracycline, TET	<=	1	0.5	2	1	MIC
4	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
6	Ampicillin, AMP	=	8	2	8	1	MIC
6	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
6	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
6	Chloramphenicol, CHL	=	4	2	8	1	MIC
6	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
6	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
6	Nalidixic acid, NAL	<	4	1	4	1	MIC
6	Streptomycin, STR	=	8	4	16	1	MIC
6	Tetracycline, TET	=	2	0.5	2	1	MIC
6	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
9	Ampicillin, AMP	=	8	2	8	1	MIC
9	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
9	Cefoxitin, FOX	=	4	2	8	1	MIC
9	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
9	Chloramphenicol, CHL	=	4	2	8	1	MIC
9	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
9	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
9	Nalidixic acid, NAL	<=	4	1	4	1	MIC
9	Streptomycin, STR	=	8	4	16	1	MIC
9	Sulfisoxazole, FIS	=	32	8	32	1	MIC
9	Tetracycline, TET	=	1	0.5	2	1	MIC
9	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
11	Ampicillin, AMP	=	4	2	8	1	MIC
11	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
11	Cefoxitin, FOX	=	25	23	29	1	DD
11	Chloramphenicol, CHL	=	4	2	8	1	MIC
11	Ciprofloxacin, CIP	=	0.016	0.004	0.016	1	MIC
11	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
11	Nalidixic acid, NAL	=	4	1	4	1	MIC
11	Streptomycin, STR	=	16	4	16	1	MIC
11	Sulfisoxazole, FIS	=	16	8	32	1	MIC
11	Tetracycline, TET	=	1	0.5	2	1	MIC
11	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
12	Ampicillin, AMP	=	2	2	8	1	MIC
12	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
12	Ceftiofur, XNL	=	0.25	0.25	1	1	MIC
12	Chloramphenicol, CHL	=	2	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	=	8	2	8	1	MIC
13	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
13	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
13	Chloramphenicol, CHL	=	4	2	8	1	MIC
13	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	MIC
13	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
13	Nalidixic acid, NAL	<=	4	1	4	1	MIC
13	Streptomycin, STR	=	4	4	16	1	MIC
13	Tetracycline, TET	<=	1	0.5	2	1	MIC
13	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
15	Ampicillin, AMP	=	22	16	22	1	DD
15	Cefotaxime, CTX	=	36	29	35	**0**	DD
15	Cefoxitin, FOX	=	28	23	29	1	DD
15	Ceftazidime, CAZ	=	32	25	32	1	DD
15	Ceftiofur, XNL	=	29	26	31	1	DD
15	Chloramphenicol, CHL	=	25	21	27	1	DD
15	Gentamicin, GEN	=	27	19	26	**0**	DD
15	Imipenem, IMI	=	40	26	32	**0**	DD
15	Nalidixic acid, NAL	=	25	22	28	1	DD
15	Streptomycin, STR	=	18	12	20	1	DD
15	Sulfisoxazole, FIS	=	19	15	23	1	DD
15	Tetracycline, TET	=	22	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	=	4	2	8	1	MIC
16	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
16	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
16	Nalidixic acid, NAL	=	2	1	4	1	MIC
16	Streptomycin, STR	=	8	4	16	1	MIC
16	Sulfisoxazole, FIS	=	32	8	32	1	MIC
16	Tetracycline, TET	<=	1	0.5	2	1	MIC
16	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
17	Ampicillin, AMP	=	2	2	8	1	MIC
17	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
17	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
17	Chloramphenicol, CHL	=	4	2	8	1	MIC
17	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	MIC
17	Gentamicin, GEN	<=	0.25	0.25	1	1	MIC
17	Nalidixic acid, NAL	<=	4	1	4	1	MIC
17	Streptomycin, STR	=	4	4	16	1	MIC
17	Tetracycline, TET	<=	1	0.5	2	1	MIC
17	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
18	Ampicillin, AMP	=	18	16	22	1	DD
18	Cefotaxime, CTX	=	34	29	35	1	DD
18	Ceftiofur, XNL	=	28	26	31	1	DD
18	Chloramphenicol, CHL	=	22.7	21	27	1	DD
18	Ciprofloxacin, CIP	=	33.7	30	40	1	DD
18	Gentamicin, GEN	=	23.4	19	26	1	DD
18	Nalidixic acid, NAL	=	26	22	28	1	DD
18	Streptomycin, STR	=	16	12	20	1	DD
18	Sulfisoxazole, FIS	=	23	15	23	1	DD
18	Tetracycline, TET	=	24	18	25	1	DD
18	Trimethoprim, TMP	=	24	21	28	1	DD
19	Ampicillin, AMP	=	4	2	8	1	MIC
19	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
19	Cefoxitin, FOX	=	2	2	8	1	MIC
19	Ceftazidime, CAZ	=	0.25	0.06	0.5	1	MIC
19	Chloramphenicol, CHL	=	4	2	8	1	MIC
19	Ciprofloxacin, CIP	=	0.008	0.004	0.016	1	MIC
19	Gentamicin, GEN	=	0.25	0.25	1	1	MIC
19	Nalidixic acid, NAL	=	2	1	4	1	MIC
19	Streptomycin, STR	=	8	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	=	4	2	8	1	MIC
20	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
20	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
20	Nalidixic acid, NAL	<=	4	1	4	1	MIC
20	Streptomycin, STR	=	8	4	16	1	MIC
20	Sulfisoxazole, FIS	=	16	8	32	1	MIC
20	Tetracycline, TET	<=	1	0.5	2	1	MIC
20	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC
21	Ampicillin, AMP	=	2	2	8	1	MIC
21	Cefotaxime, CTX	=	0.06	0.03	0.125	1	MIC
21	Ceftazidime, CAZ	=	0.5	0.06	0.5	1	MIC
21	Chloramphenicol, CHL	=	2	2	8	1	MIC
21	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
21	Gentamicin, GEN	=	0.25	0.25	1	1	MIC
21	Nalidixic acid, NAL	=	4	1	4	1	MIC
21	Sulfisoxazole, FIS	=	32	8	32	1	MIC
21	Tetracycline, TET	=	2	0.5	2	1	MIC
21	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
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	=	4	4	16	1	MIC
22	Sulfisoxazole, FIS	=	16	8	32	1	MIC
22	Tetracycline, TET	<	1	0.5	2	1	MIC
22	Trimethoprim, TMP	=	0.5	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	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	=	8	8	32	1	MIC
23	Tetracycline, TET	=	1	0.5	2	1	MIC
23	Trimethoprim, TMP	=	0.5	0.5	2	1	MIC
24	Ampicillin, AMP	=	4	2	8	1	MIC
24	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
24	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
24	Chloramphenicol, CHL	=	8	2	8	1	MIC
24	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
24	Gentamicin, GEN	=	0.5	0.25	1	1	MIC
24	Nalidixic acid, NAL	<=	4	1	4	1	MIC
24	Streptomycin, STR	=	4	4	16	1	MIC
24	Tetracycline, TET	<=	1	0.5	2	1	MIC
24	Trimethoprim, TMP	=	1	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	=	2	2	8	1	MIC
26	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	MIC
26	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
26	Chloramphenicol, CHL	=	4	2	8	1	MIC
26	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	MIC
26	Gentamicin, GEN	<=	0.25	0.25	1	1	MIC
26	Nalidixic acid, NAL	<=	4	1	4	1	MIC
26	Streptomycin, STR	=	4	4	16	1	MIC
26	Tetracycline, TET	<=	1	0.5	2	1	MIC
26	Trimethoprim, TMP	<=	0.5	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
29	Ampicillin, AMP	=	2	2	8	1	MIC
29	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
29	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
29	Chloramphenicol, CHL	=	4	2	8	1	MIC
29	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
29	Gentamicin, GEN	=	1	0.25	1	1	MIC
29	Nalidixic acid, NAL	=	4	1	4	1	MIC
29	Streptomycin, STR	=	4	4	16	1	MIC
29	Tetracycline, TET	=	2	0.5	2	1	MIC
30	Ampicillin, AMP	=	19	16	22	1	DD
30	Cefotaxime, CTX	=	33	29	35	1	DD
30	Cefoxitin, FOX	=	24	23	29	1	DD
30	Ceftazidime, CAZ	=	28	25	32	1	DD
30	Ceftiofur, XNL	=	25	26	31	**0**	DD
30	Chloramphenicol, CHL	=	21	21	27	1	DD
30	Ciprofloxacin, CIP	=	32	30	40	1	DD
30	Gentamicin, GEN	=	22	19	26	1	DD
30	Imipenem, IMI	=	30	26	32	1	DD
30	Nalidixic acid, NAL	=	26	22	28	1	DD
30	Streptomycin, STR	=	15	12	20	1	DD
30	Sulfisoxazole, FIS	=	21	15	23	1	DD
30	Tetracycline, TET	=	24	18	25	1	DD
30	Trimethoprim, TMP	=	23	21	28	1	DD
32	Ampicillin, AMP	=	4	2	8	1	MIC
32	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
32	Ceftazidime, CAZ	<	0.25	0.06	0.5	1	MIC
32	Chloramphenicol, CHL	=	8	2	8	1	MIC
32	Ciprofloxacin, CIP	=	0.015	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	=	4	4	16	1	MIC
32	Tetracycline, TET	=	2	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	=	16	2	8	**0**	MIC
33	Ceftiofur, XNL	=	0.5	0.25	1	1	MIC
33	Chloramphenicol, CHL	=	4	2	8	1	MIC
33	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	MIC
33	Gentamicin, GEN	=	1	0.25	1	1	MIC
33	Nalidixic acid, NAL	=	2	1	4	1	MIC
33	Streptomycin, STR	=	16	4	16	1	MIC
33	Sulfisoxazole, FIS	=	32	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	=	8	2	8	1	MIC
34	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
34	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
34	Chloramphenicol, CHL	=	8	2	8	1	MIC
34	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
34	Gentamicin, GEN	<=	0.25	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	=	32	8	32	1	MIC
34	Tetracycline, TET	=	2	0.5	2	1	MIC
34	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
37	Ampicillin, AMP	=	8	2	8	1	AGA
37	Cefotaxime, CTX	<=	0.06	0.03	0.125	1	AGA
37	Chloramphenicol, CHL	=	4	2	8	1	AGA
37	Ciprofloxacin, CIP	<=	0.008	0.004	0.016	1	AGA
37	Gentamicin, GEN	=	0.5	0.25	1	1	AGA
37	Nalidixic acid, NAL	<=	2	1	4	1	AGA
37	Streptomycin, STR	=	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	=	16.5	16	22	1	DD
38	Cefotaxime, CTX	=	30.5	29	35	1	DD
38	Cefoxitin, FOX	=	25.2	23	29	1	DD
38	Chloramphenicol, CHL	=	24.8	21	27	1	DD
38	Ciprofloxacin, CIP	=	33.6	30	40	1	DD
38	Gentamicin, GEN	=	20	19	26	1	DD
38	Imipenem, IMI	=	28.7	26	32	1	DD
38	Nalidixic acid, NAL	=	23	22	28	1	DD
38	Streptomycin, STR	=	14.4	12	20	1	DD
38	Tetracycline, TET	=	21	18	25	1	DD
38	Trimethoprim, TMP	=	23.7	21	28	1	DD
39	Ampicillin, AMP	=	16	2	8	**0**	MIC
39	Cefotaxime, CTX	=	0.5	0.03	0.125	**0**	MIC
39	Cefoxitin, FOX	=	0.5	2	8	**0**	MIC
39	Ceftazidime, CAZ	=	4	0.06	0.5	**0**	MIC
39	Ceftiofur, XNL	<	4	0.25	1	1	MIC
39	Chloramphenicol, CHL	=	4	2	8	1	MIC
39	Ciprofloxacin, CIP	=	0.03	0.004	0.016	**0**	MIC
39	Gentamicin, GEN	=	2	0.25	1	**0**	MIC
39	Nalidixic acid, NAL	=	2	1	4	1	MIC
39	Streptomycin, STR	=	8	4	16	1	MIC
39	Sulfisoxazole, FIS	=	1	8	32	**0**	MIC
39	Tetracycline, TET	=	1	0.5	2	1	MIC
39	Trimethoprim, TMP	=	1	0.5	2	1	MIC
40	Ampicillin, AMP	=	20	16	22	1	DD
40	Cefotaxime, CTX	=	29	29	35	1	DD
40	Cefoxitin, FOX	=	27	23	29	1	DD
40	Ceftazidime, CAZ	=	27	25	32	1	DD
40	Chloramphenicol, CHL	=	24	21	27	1	DD
40	Ciprofloxacin, CIP	=	34	30	40	1	DD
40	Gentamicin, GEN	=	20	19	26	1	DD
40	Imipenem, IMI	=	27	26	32	1	DD
40	Nalidixic acid, NAL	=	26	22	28	1	DD
40	Streptomycin, STR	=	17	12	20	1	DD
40	Tetracycline, TET	=	20	18	25	1	DD
40	Trimethoprim, TMP	=	27	21	28	1	DD
41	Ampicillin, AMP	=	8	2	8	1	MIC
41	Cefotaxime, CTX	=	0.12	0.03	0.125	1	MIC
41	Ceftazidime, CAZ	<=	0.25	0.06	0.5	1	MIC
41	Chloramphenicol, CHL	=	8	2	8	1	MIC
41	Ciprofloxacin, CIP	=	0.015	0.004	0.016	1	MIC
41	Gentamicin, GEN	=	1	0.25	1	1	MIC
41	Nalidixic acid, NAL	<=	4	1	4	1	MIC
41	Streptomycin, STR	=	8	4	16	1	MIC
41	Tetracycline, TET	=	4	0.5	2	**0**	MIC
41	Trimethoprim, TMP	=	1	0.5	2	1	MIC

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method
44	Ampicillin, AMP	<	8	2	8	1	AGA
44	Cefotaxime, CTX	<	1	0.03	0.125	1	AGA
44	Chloramphenicol, CHL	<	8	2	8	1	AGA
44	Ciprofloxacin, CIP	<	0.125	0.004	0.016	1	AGA
44	Gentamicin, GEN	<	4	0.25	1	1	AGA
44	Nalidixic acid, NAL	<	16	1	4	1	AGA
44	Streptomycin, STR	<	8	4	16	1	AGA
44	Sulfisoxazole, FIS	<	64	8	32	1	AGA
44	Tetracycline, TET	<	8	0.5	2	1	AGA
44	Trimethoprim, TMP	<	2	0.5	2	1	AGA

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

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
1	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
1	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
1	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
1	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
1	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
1	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
2	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
2	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
2	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
2	Gentamicin, GEN	=	0.25	0.5	2	0	MIC	X	
2	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
2	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
4	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
4	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
4	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
4	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
4	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
4	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
6	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
6	Ciprofloxacin, CIP	=	0.12	0.03	0.12	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	=	1	0.25	2	1	MIC	X	
11	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
11	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
11	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
11	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
11	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
12	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
12	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
12	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
12	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
12	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
14	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
14	Ciprofloxacin, CIP	<=	0.06	0.03	0.12	1	MIC		X
14	Erythromycin, ERY	=	1	0.25	2	1	MIC		X
14	Gentamicin, GEN	=	0.5	0.25	2	1	MIC		X
14	Nalidixic acid, NAL	=	4	4	16	1	MIC		X
14	Tetracycline, TET	=	0.5	0.25	1	1	MIC		X
15	Ciprofloxacin, CIP	=	0.094	0.06	0.5	1	AGA		X
15	Erythromycin, ERY	=	0.75	1	4	0	AGA		X
15	Gentamicin, GEN	=	0.38	0.5	4	0	AGA		X
15	Nalidixic acid, NAL	=	4				AGA		X
15	Tetracycline, TET	=	0.75				AGA		X

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
17	Chloramphenicol, CHL	<=	2	1	8	1	MIC	X	
17	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
17	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
17	Gentamicin, GEN	=	0.5	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	=	0.06	0.03	0.12	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	=	16	4	16	1	MIC		X
19	Tetracycline, TET	=	1	0.25	1	1	MIC		X
20	Chloramphenicol, CHL	<	2	1	8	1	MIC	X	
20	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
20	Erythromycin, ERY	=	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	=	0.25	0.03	0.12	0	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	=	16	4	16	1	MIC		X
22	Tetracycline, TET	=	2	0.25	1	0	MIC		X
23	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
23	Ciprofloxacin, CIP	=	0.12	0.03	0.12	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	=	4	4	16	1	MIC		X
23	Tetracycline, TET	=	1	0.25	1	1	MIC		X
24	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
24	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
24	Erythromycin, ERY	=	2	0.5	2	1	MIC	X	
24	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
24	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
24	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
25	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
25	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
25	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
25	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
25	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
25	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
26	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
26	Ciprofloxacin, CIP	=	0.25	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	
30	Chloramphenicol, CHL	=	4	1	8	1	MIC	X	
30	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
30	Erythromycin, ERY	<=	0.5	0.5	2	1	MIC	X	
30	Gentamicin, GEN	=	0.5	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	

Lab no.	Antimicrobial	Operator	Value	Low limit	High limit	Mark	Method	36-37ºC/48h	42ºC/24h
32	Chloramphenicol, CHL	=	2	1	4	1	MIC		X
32	Ciprofloxacin, CIP	=	0.5	0.03	0.12	0	MIC		X
32	Erythromycin, ERY	<	0.25	0.25	2	1	MIC		X
32	Gentamicin, GEN	=	0.5	0.25	2	1	MIC		X
32	Nalidixic acid, NAL	=	8	4	16	1	MIC		X
32	Tetracycline, TET	=	0.25	0.25	1	1	MIC		X
33	Ciprofloxacin, CIP	=	0.25	0.06	0.25	1	MIC	X	
33	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
33	Gentamicin, GEN	=	1	0.5	2	1	MIC	X	
33	Nalidixic acid, NAL	=	16	4	16	1	MIC	X	
33	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
34	Chloramphenicol, CHL	=	8	1	8	1	MIC	X	
34	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
34	Erythromycin, ERY	=	1	0.5	2	1	MIC	X	
34	Gentamicin, GEN	=	0.5	0.5	2	1	MIC	X	
34	Nalidixic acid, NAL	=	8	4	16	1	MIC	X	
34	Tetracycline, TET	=	2	0.25	2	1	MIC	X	
37	Chloramphenicol, CHL	=	4				AGA	X	
37	Ciprofloxacin, CIP	=	0.25	0.12	1	1	AGA	X	
37	Erythromycin, ERY	=	1	1	8	1	AGA	X	
37	Gentamicin, GEN	=	1	0.5	2	1	AGA	X	
37	Nalidixic acid, NAL	=	8				AGA	X	
37	Tetracycline, TET	=	1				AGA	X	
39	Ciprofloxacin, CIP	=	0.12	0.06	0.25	1	MIC	X	
39	Erythromycin, ERY	<	0.5	0.5	2	1	MIC	X	
39	Gentamicin, GEN	<	0.12	0.5	2	0	MIC	X	
39	Nalidixic acid, NAL	>	64	4	16	0	MIC	X	
39	Tetracycline, TET	=	1	0.25	2	1	MIC	X	
41	Chloramphenicol, CHL	<=	2	1	4	1	MIC		X
41	Ciprofloxacin, CIP	<=	0.06	0.03	0.12	1	MIC		X
41	Erythromycin, ERY	<=	0.5	0.25	2	1	MIC		X
41	Gentamicin, GEN	=	0.25	0.25	2	1	MIC		X
41	Nalidixic acid, NAL	<=	2	4	16	0	MIC		X
41	Tetracycline, TET	=	0.5	0.25	1	1	MIC		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 S19 with the following exceptions: The MIC range for streptomycin is according to Sensititre and the ranges for ceftiofur and florphenicol 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)

Evaluation comments, summarised

Participants' evaluation of the CRL EQAS Salm/Camp 2009

As means of improving the quality and usefulness of the EQAS, the participants of the CRL EQAS Salm/Camp were asked to fill in an evaluation form in the database.

This year, three participants have uploaded their evaluation of the CRL EQAS Salm/Camp to the evaluation form in the database. The evaluations were all 'good' or 'very good' and will not be further summarised. None of the participants mentioned limitations or other comments

The questionnaire in the database included the following questions:

Information received during the CRL AR EQAS 2009 and how the EQAS was performed:	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general					
The EQAS welcome letter (the letter in the parcel)					
The EQAS protocol and test forms					
The distribution of the samples					
What is your overall impression of the interactive web database					
The evaluation report					
How did participation in this EQAS meet your expectations					

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

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Ampicillin, AMP	1	MIC	1-32
	2	MIC	0.5-32
	6	MIC	0.5-32
	9	MIC	0.5-32
	11	MIC	0.5-64
	12	MIC	0.25-32
	13	MIC	0.5-32
	17	MIC	0.5-32
	19	MIC	4
	20	MIC	0.5-32
	22	MIC	4
	25	MIC	0.5-32
	26	MIC	0.5-32
	32	MIC	0.5-32
	33	MIC	0.5-64
	39	AGA	8 and 128
Cefotaxime, CTX	1	MIC	0.125-4
	2	MIC	0.06-4
	6	MIC	0.06-4
	9	MIC	0.06-4
	11	MIC	0.06-8
	12	MIC	0.06-2
	13	MIC	0.06-4
	17	MIC	0.06-4
	19	MIC	0.5
	20	MIC	0.06-4
	22	MIC	0.5
	25	MIC	0.06-4
	26	MIC	0.06-4
	32	MIC	0.006-4
	33	MIC	0.06-8
	37	AGA	0.015-512
	39	AGA	1
Ceftazidime, CAZ	1	MIC	0.25-128
	2	MIC	0.25-16
	6	MIC	0.25-16
	9	MIC	0.25-16
	13	MIC	0.25-16
	17	MIC	0.25-16
	19	MIC	2
	20	MIC	0.25-16
	22	MIC	2
	25	MIC	0.25-16
	26	MIC	0.25-16
	32	MIC	0.25-16
	39	AGA	1
Ceftiofur, XNL	1	MIC	0.5-8
	12	MIC	0.12-16
	22	MIC	2
	33	MIC	0.12-16
Chloramphenicol, CHL	1	MIC	2-64
	2	MIC	2-64
	6	MIC	2-64
	9	MIC	2-64
	11	MIC	2-256
	12	MIC	1-128
	13	MIC	2-64
	17	MIC	2-64
	19	MIC	16
	20	MIC	2-64
	22	MIC	16
	25	MIC	2-64
	26	MIC	2-64
	32	MIC	2-64
	33	MIC	2-256
	39	AGA	8
Ciprofloxacin, CIP	1	MIC	0.015-4
	2	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
	17	MIC	0.008-8
	19	MIC	0.06
	20	MIC	0.008-8
	22	MIC	0.06
	25	MIC	0.008-8
	26	MIC	0.008-8
	32	MIC	0.008-8
	33	MIC	0.008-8
	37	AGA	0.015-512
	39	AGA	0.125 and 1
Gentamicin, GEN	1	MIC	0.5-16
	2	MIC	0.25-32
	6	MIC	0.25-32
	9	MIC	0.25-32
	11	MIC	0.25-32
	12	MIC	0.5-64
	13	MIC	0.25-32
	17	MIC	0.25-32
	19	MIC	2
	20	MIC	0.25-32
	22	MIC	2
	25	MIC	0.25-32
	26	MIC	0.25-32
	32	MIC	0.25-32
	33	MIC	0.25-32
	37	AGA	0.015-512
	39	AGA	4

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Nalidixic acid, NAL	1	MIC	4-64
	2	MIC	4-64
	6	MIC	4-64
	9	MIC	4-64
	11	MIC	2-256
	12	MIC	1-128
	13	MIC	8-64
	17	MIC	4-64
	19	MIC	16
	20	MIC	4-64
	22	MIC	16
	25	MIC	4-64
	26	MIC	4-64
	32	MIC	4-64
	33	MIC	2-256
	37	AGA	0.015-512
	39	AGA	16
Streptomycin, STR	1	MIC	8-128
	2	MIC	2-128
	6	MIC	2-128
	9	MIC	2-128
	11	MIC	2-256
	12	MIC	2-256
	13	MIC	2-128
	17	MIC	2-128
	19	MIC	32
	20	MIC	2-128
	22	MIC	32
	25	MIC	2-128
	26	MIC	2-128
	32	MIC	2-128
	33	MIC	2-256
	37	AGA	0.015-512
	39	AGA	8 and 128
Sulfamethoxazole, SMX	1	MIC	64-1024
	2	MIC	8-1024
	6	MIC	8-1024
	9	MIC	8-1024
	11	MIC	8-1024
	12	MIC	16-2048
	13	MIC	8-1024
	17	MIC	8-1024
	19	MIC	256
	20	MIC	8-1024
	22	MIC	256
	25	MIC	8-1024
	26	MIC	8-1024
	32	MIC	8-1024
	33	MIC	8-1024
	37	AGA	0.015-512
	39	AGA	64
Tetracycline,TET	1	MIC	2-32
	2	MIC	1-64
	6	MIC	1-64
	9	MIC	1-64
	11	MIC	0.5-32
	12	MIC	0.5-64
	13	MIC	1-64
	17	MIC	1-64
	19	MIC	8
	20	MIC	1-64
	22	MIC	8
	25	MIC	1-64
	26	MIC	1-64
	32	MIC	1-64
	33	MIC	0.5-64
	37	AGA	0.015-512
	39	AGA	8 and 128
Trimethoprim, TMP	1	MIC	1-32
	2	MIC	0.5-32
	6	MIC	0.5-32
	9	MIC	0.5-32
	11	MIC	0.25-32
	12	MIC	0.25-32
	13	MIC	0.5-32
	17	MIC	0.5-32
	19	MIC	2
	20	MIC	0.5-32
	22	MIC	2
	25	MIC	0.5-32
	26	MIC	0.5-32
	32	MIC	0.5-32
	33	MIC	0.25-32
	37	AGA	0.015-512
	39	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

Questionnaire, summarised - test range for MIC (µg/mL) - *Campylobacter*

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Chloramphenicol, CHL	1	MIC	2-32
	2	MIC	2-64
	6	MIC	2-32
	9	MIC	2-32
	17	MIC	2-32
	19	MIC	16
	20	MIC	2-32
	21	MIC	1-32
	22	MIC	16
	25	MIC	2-128
	26	MIC	2-32
	29	MIC	0.125-256
	32	MIC	0.5-32
	33	MIC	0.12-16
	34	MIC	1-32
	39	AGA	8
Ciprofloxacin, CIP	1	MIC	0.06-4
	2	MIC	0.06-32
	6	MIC	0.06-4
	9	MIC	0.06-4
	11	MIC	0.06-8
	12	MIC	0.06-8
	14	AGA	0.06-8
	17	MIC	0.06-4
	19	MIC	1
	20	MIC	0.06-4
	21	MIC	0.06-128
	22	MIC	1
	25	MIC	0.12-16
	26	MIC	0.06-4
	29	MIC	0.01-32
	32	MIC	0.06-8
	33	MIC	0.06-8
	34	MIC	0.032-32
	37	AGA	0.015-512
	39	AGA	1
Erythromycin, ERY	1	MIC	0.5-32
	2	MIC	0.25-128
	6	MIC	0.5-32
	9	MIC	0.5-32
	11	MIC	0.5-64
	12	MIC	0.5-64
	14	AGA	0.5-64
	17	MIC	0.5-32
	19	MIC	jejuni:4;coli:16
	20	MIC	0.5-32
	21	MIC	0.12-128
	22	MIC	4/C.coli16
	25	MIC	0.5-64
	26	MIC	0.5-32
	29	MIC	0.125-256
	32	MIC	0.25-64
	33	MIC	0.5-64
	34	MIC	0.125-128
	37	AGA	0.015-512
	39	AGA	4
Gentamicin, GEN	1	MIC	0.125-16
	2	MIC	0.125-16
	6	MIC	0.12-16
	9	MIC	0.12-16
	11	MIC	0.12-16
	12	MIC	0.12-16
	14	AGA	0.125-16
	17	MIC	0.12-16
	19	MIC	j:1; c:2
	20	MIC	0.12-16
	21	MIC	0.12-128
	22	MIC	1/C.coli2
	25	MIC	0.25-32
	26	MIC	0.12-16
	29	MIC	0.03-64
	32	MIC	0.125-16
	33	MIC	0.12-16
	34	MIC	0.125-32
	37	AGA	0.015-512
	39	AGA	4

Antimicrobial	Lab no	Method	Test range for MIC (ug/mL)
Nalidixic acid, NAL	1	MIC	2-64
	2	MIC	2-256
	6	MIC	2-64
	9	MIC	2-64
	11	MIC	1-64
	12	MIC	1-64
	14	AGA	1-256
	17	MIC	2-64
	19	MIC	j:16;c:32
	20	MIC	2-64
	21	MIC	0.12-128
	22	MIC	16/C.coli32
	25	MIC	1-128
	26	MIC	2-64
	32	MIC	2-64
	33	MIC	1-64
	34	MIC	0.5-64
	37	AGA	0.015-512
	39	AGA	16
Streptomycin, STR	1	MIC	1-16
	2	MIC	0.5 -32
	6	MIC	1-16
	9	MIC	1-16
	11	MIC	0.5-64
	12	MIC	0.5-64
	14	AGA	0.5-32
	17	MIC	1-16
	19	MIC	j:2;c:4
	20	MIC	1-16
	21	MIC	0.12-128
	22	MIC	2/C.coli4
	25	MIC	1-128
	26	MIC	1-16
	29	MIC	0.06-128
	32	MIC	0.5-32
	33	MIC	0.5-64
	34	MIC	0.25-64
	37	AGA	0.015-512
Tetracycline,TET	1	MIC	0.25-16
	2	MIC	0.125-64
	6	MIC	0.25-16
	9	MIC	0.25-16
	11	MIC	0.12-16
	12	MIC	0.12-16
	14	AGA	0.125-16
	17	MIC	0.25-16
	19	MIC	2
	20	MIC	0.25-16
	21	MIC	0.12-128
	22	MIC	2
	25	MIC	0.5-64
	26	MIC	0.25-16
	29	MIC	0.125-256
	32	MIC	0.125-16
	33	MIC	0.12-16
	34	MIC	0.125-128
	37	AGA	0.015-512
	39	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	CRL S-4.1	R	100	0	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	100	0	31	0
	CRL S-4.5	R	100	0	30	0
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	R	97	3	30	1
	CRL S-4.8	R	100	0	31	0
Cefotaxime, CTX	CRL S-4.1	R	100	0	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	S	0	100	31	0
	CRL S-4.5	R	93	7	28	2
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	S	0	100	31	0
	CRL S-4.8	S	0	100	31	0
Ceftazidime, CAZ	CRL S-4.1	R	100	0	23	0
	CRL S-4.2	R	100	0	23	0
	CRL S-4.3	S	0	100	23	0
	CRL S-4.4	S	0	100	23	0
	CRL S-4.5	R	86	14	19	3
	CRL S-4.6	S	0	100	23	0
	CRL S-4.7	S	0	100	23	0
	CRL S-4.8	S	0	100	23	0
Ceftiofur, XNL	CRL S-4.1	R	100	0	10	0
	CRL S-4.2	R	100	0	10	0
	CRL S-4.3	S	0	100	8	0
	CRL S-4.4	S	0	100	8	0
	CRL S-4.5	R	100	0	10	0
	CRL S-4.6	S	0	100	8	0
	CRL S-4.7	S	0	100	8	0
	CRL S-4.8	S	0	100	8	0
Chloramphenicol, CHL	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	97	3	30	1
	CRL S-4.5	S	0	100	30	0
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	S	0	100	31	0
	CRL S-4.8	S	3	97	30	1
Ciprofloxacin, CIP	CRL S-4.1	S	3	97	29	1
	CRL S-4.2	S	0	100	30	0
	CRL S-4.3	S	0	100	30	0
	CRL S-4.4	R	93	7	28	2
	CRL S-4.5	R	97	3	28	1
	CRL S-4.6	R	76	24	22	7
	CRL S-4.7	S	0	100	30	0
	CRL S-4.8	R	93	7	28	2

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Gentamicin, GEN	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	97	3	30	1
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	S	0	100	31	0
	CRL S-4.5	S	0	100	30	0
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	S	0	100	31	0
	CRL S-4.8	S	0	100	31	0
Nalidixic acid, NAL	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	S	0	100	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	100	0	31	0
	CRL S-4.5	R	100	0	30	0
	CRL S-4.6	S	3	97	29	1
	CRL S-4.7	S	0	100	31	0
	CRL S-4.8	R	100	0	31	0
Streptomycin, STR	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	3	97	30	1
	CRL S-4.4	S	10	90	27	3
	CRL S-4.5	S	0	100	30	0
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	R	97	3	30	1
	CRL S-4.8	S	21	79	23	6
Sulphonamides, SMX	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	100	0	31	0
	CRL S-4.5	S	0	100	30	0
	CRL S-4.6	S	7	93	28	2
	CRL S-4.7	R	97	3	30	1
	CRL S-4.8	R	100	0	31	0
Tetracycline, TET	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	87	13	26	4
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	100	0	31	0
	CRL S-4.5	R	97	3	29	1
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	R	100	0	31	0
	CRL S-4.8	S	6	94	29	2
Trimethoprim, TMP	CRL S-4.1	S	0	100	31	0
	CRL S-4.2	R	100	0	31	0
	CRL S-4.3	S	0	100	31	0
	CRL S-4.4	R	100	0	31	0
	CRL S-4.5	S	0	100	30	0
	CRL S-4.6	S	0	100	31	0
	CRL S-4.7	S	0	100	31	0
	CRL S-4.8	R	100	0	31	0

Campylobacter - expected and obtained interpretation

Antimicrobial	Strain	Expected	% R	% S	No. correct	No. incorrect
Chloramphenicol, CHL	CRL C-4.1	S	0	100	21	0
	CRL C-4.2	S	0	100	20	0
	CRL C-4.3	S	0	100	21	0
	CRL C-4.4	S	5	95	19	1
	CRL C-4.5	S	0	100	21	0
	CRL C-4.6	S	0	100	21	0
	CRL C-4.7	S	0	100	21	0
	CRL C-4.8	S	0	100	21	0
Ciprofloxacin, CIP	CRL C-4.1	R	96	4	25	1
	CRL C-4.2	R	100	0	25	0
	CRL C-4.3	R	100	0	26	0
	CRL C-4.4	R	92	8	23	2
	CRL C-4.5	R	100	0	26	0
	CRL C-4.6	S	0	100	26	0
	CRL C-4.7	S	0	100	26	0
	CRL C-4.8	R	100	0	26	0
Erythromycin, ERY	CRL C-4.1	R	92	8	24	2
	CRL C-4.2	R	96	4	24	1
	CRL C-4.3	S	8	92	24	2
	CRL C-4.4	S	0	100	25	0
	CRL C-4.5	R	100	0	26	0
	CRL C-4.6	S	0	100	26	0
	CRL C-4.7	S	0	100	26	0
	CRL C-4.8	S	4	96	25	1
Gentamicin, GEN	CRL C-4.1	S	0	100	26	0
	CRL C-4.2	R	100	0	25	0
	CRL C-4.3	S	4	96	25	1
	CRL C-4.4	S	0	100	25	0
	CRL C-4.5	S	4	96	25	1
	CRL C-4.6	S	0	100	26	0
	CRL C-4.7	S	4	96	25	1
	CRL C-4.8	S	0	100	26	0
Nalidixic acid, NAL	CRL C-4.1	R	92	8	24	2
	CRL C-4.2	R	92	8	23	2
	CRL C-4.3	R	96	4	25	1
	CRL C-4.4	R	100	0	25	0
	CRL C-4.5	R	88	12	23	3
	CRL C-4.6	S	0	100	26	0
	CRL C-4.7	S	0	100	26	0
	CRL C-4.8	R	100	0	26	0
Streptomycin, STR	CRL C-4.1	S	0	100	24	0
	CRL C-4.2	R	100	0	23	0
	CRL C-4.3	R	100	0	24	0
	CRL C-4.4	S	4	96	22	1
	CRL C-4.5	R	100	0	24	0
	CRL C-4.6	S	4	96	23	1
	CRL C-4.7	R	100	0	24	0
	CRL C-4.8	R	100	0	24	0
Tetracycline, TET	CRL C-4.1	S	0	100	26	0
	CRL C-4.2	R	96	4	24	1
	CRL C-4.3	R	100	0	26	0
	CRL C-4.4	R	92	8	23	2
	CRL C-4.5	R	85	15	22	4
	CRL C-4.6	S	0	100	26	0
	CRL C-4.7	S	0	100	26	0
	CRL C-4.8	S	0	100	26	0

Deviations - *Salmonella*

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	CRL S-4.7	Sulfamethoxazole, SMX	S	<=64	R	>1024	MIC
1	CRL S-4.7	Streptomycin, STR	S	16	R	>128	MIC
1	CRL S-4.7	Ampicillin, AMP	S	2	R	>32	MIC
2	CRL S-4.5	Confirmed ESBL	No		Yes		MIC
2	CRL S-4.5	Ceftazidime, CAZ	S	1	R	=0.5	MIC
4	CRL S-4.2	Tetracycline, TET	S	8	R	=16	MIC
13	CRL S-4.6	Sulfamethoxazole, SMX	R	512	S	=128	MIC
13	CRL S-4.6	Ciprofloxacin, CIP	S	0.12	R	=0.25	MIC
15	CRL S-4.4	Streptomycin, STR	R	6	S	=16	DD
15	CRL S-4.6	Nalidixic acid, NAL	R	14	S	=8	DD
15	CRL S-4.8	Tetracycline, TET	R	14	S	=4	DD
15	CRL S-4.8	Streptomycin, STR	R	6	S	=32	DD
16	CRL S-4.4	Streptomycin, STR	R	32	S	=16	MIC
18	CRL S-4.6	Ciprofloxacin, CIP	S	32	R	=0.25	DD
18	CRL S-4.8	Streptomycin, STR	R	6	S	=32	DD
19	CRL S-4.6	Sulfamethoxazole, SMX	R	1024	S	=128	MIC
19	CRL S-4.6	Ciprofloxacin, CIP	S	0.06	R	=0.25	MIC
19	CRL S-4.8	Streptomycin, STR	R	64	S	=32	MIC
20	CRL S-4.5	Confirmed ESBL	No		Yes		MIC
20	CRL S-4.5	Ceftazidime, CAZ	S	1	R	=0.5	MIC
22	CRL S-4.8	Confirmed AmpC	Yes		No		MIC
24	CRL S-4.3	Streptomycin, STR	R	64	S	<=8	MIC
24	CRL S-4.5	Tetracycline, TET	S	64	R	=32	MIC
24	CRL S-4.8	Chloramphenicol, CHL	R	16	S	=8	MIC
25	CRL S-4.8	Streptomycin, STR	R	> 128	S	=32	MIC
25	CRL S-4.8	Tetracycline, TET	R	> 64	S	=4	MIC
29	CRL S-4.2	Tetracycline, TET	S	8	R	=16	MIC
30	CRL S-4.2	Tetracycline, TET	S	15	R	=16	DD
34	CRL S-4.8	Confirmed AmpC	Yes		No		MIC
38	CRL S-4.1	Confirmed ESBL	No		Yes		DD
38	CRL S-4.2	Confirmed ESBL	No		Yes		DD
38	CRL S-4.4	Streptomycin, STR	R	9.8	S	=16	DD
38	CRL S-4.5	Confirmed ESBL	No		Yes		DD
38	CRL S-4.5	Cefotaxime, CTX	S	17.3	R	>4	DD
38	CRL S-4.6	Ciprofloxacin, CIP	S	28.7	R	=0.25	DD
38	CRL S-4.8	Streptomycin, STR	R	8.5	S	=32	DD
39	CRL S-4.1	Confirmed ESBL	No		Yes		MIC
39	CRL S-4.1	Ciprofloxacin, CIP	R	0,12	S	<=0.015	MIC
39	CRL S-4.2	Confirmed ESBL	No		Yes		MIC
39	CRL S-4.4	Chloramphenicol, CHL	S	64	R	>64	MIC
39	CRL S-4.5	Confirmed ESBL	No		Yes		MIC
40	CRL S-4.2	Gentamicin, GEN	S	13	R	>16	DD
40	CRL S-4.2	Tetracycline, TET	S	14	R	=16	DD
40	CRL S-4.4	Ciprofloxacin, CIP	S	32	R	=0.125	DD
40	CRL S-4.5	Ciprofloxacin, CIP	S	22	R	=0.25	DD
40	CRL S-4.5	Ceftazidime, CAZ	S	23	R	=0.5	DD
40	CRL S-4.6	Ciprofloxacin, CIP	S	27	R	=0.25	DD
40	CRL S-4.8	Ciprofloxacin, CIP	S	23	R	=1	DD
41	CRL S-4.1	Confirmed ESBL	No		Yes		MIC
41	CRL S-4.2	Confirmed ESBL	No		Yes		MIC
41	CRL S-4.4	Ciprofloxacin, CIP	S	0.12	R	=0.125	MIC
41	CRL S-4.5	Confirmed ESBL	No		Yes		MIC
41	CRL S-4.6	Ciprofloxacin, CIP	S	0.12	R	=0.25	MIC
41	CRL S-4.8	Ciprofloxacin, CIP	S	0.5	R	=1	MIC
44	CRL S-4.5	Confirmed ESBL	No		Yes		AGA
44	CRL S-4.5	Cefotaxime, CTX	S	<1	R	>4	AGA
44	CRL S-4.6	Ciprofloxacin, CIP	S	<0.125	R	=0.25	AGA
44	CRL S-4.8	Streptomycin, STR	R	>8,<128	S	=32	AGA

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
6	CRL C-4.4	Tetracycline, TET	S	0.5	R	=64	MIC
6	CRL C-4.4	Ciprofloxacin, CIP	S	1	R	=16	MIC
11	CRL C-4.1	Erythromycin, ERY	S	<=0.5	R	>64	MIC
11	CRL C-4.2	Tetracycline, TET	S	0.5	R	>64	MIC
11	CRL C-4.2	Nalidixic acid, NAL	S	16	R	>64	MIC
11	CRL C-4.2	Erythromycin, ERY	S	2	R	>64	MIC
11	CRL C-4.4	Streptomycin, STR	R	8	S	<=1	MIC
12	CRL C-4.3	Erythromycin, ERY	R	8	S	=4	MIC
14	CRL C-4.5	Tetracycline, TET	S	2	R	=16	MIC
15	CRL C-4.5	Tetracycline, TET	S	2	R	=16	AGA
17	CRL C-4.5	Nalidixic acid, NAL	S	32	R	>64	MIC
21	CRL C-4.5	Tetracycline, TET	S	2	R	=16	MIC
21	CRL C-4.5	Nalidixic acid, NAL	S	32	R	>64	MIC
23	CRL C-4.1	Nalidixic acid, NAL	S	32	R	>64	MIC
23	CRL C-4.2	Nalidixic acid, NAL	S	8	R	>64	MIC
24	CRL C-4.4	Chloramphenicol, CHL	R	32	S	=4	MIC
39	CRL C-4.1	Ciprofloxacin, CIP	S	<0.06	R	=16	MIC
39	CRL C-4.1	Erythromycin, ERY	S	<0.5	R	>64	MIC
39	CRL C-4.1	Nalidixic acid, NAL	S	<1	R	>64	MIC
39	CRL C-4.3	Gentamicin, GEN	R	2	S	=0.25	MIC
39	CRL C-4.3	Erythromycin, ERY	R	32	S	=4	MIC
39	CRL C-4.4	Ciprofloxacin, CIP	S	<0.06	R	=16	MIC
39	CRL C-4.4	Tetracycline, TET	S	<0.12	R	=64	MIC
39	CRL C-4.5	Gentamicin, GEN	R	2	S	=0.5	MIC
39	CRL C-4.6	Streptomycin, STR	R	4	S	<=1	MIC
39	CRL C-4.7	Gentamicin, GEN	R	2	S	=0.25	MIC
39	CRL C-4.8	Erythromycin, ERY	R	>64	S	=2	MIC
41	CRL C-4.3	Nalidixic acid, NAL	S	16	R	>64	MIC
41	CRL C-4.5	Tetracycline, TET	S	1	R	=16	MIC
41	CRL C-4.5	Nalidixic acid, NAL	S	16	R	>64	MIC

AGA Agar dilution
MIC Microbroth dilution

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