



THE 1ST PROFICIENCY TESTING - 2006

ISSUED THE 14 MARCH 2007

SALMONELLA AND CAMPYLOBACTER

Community Reference Laboratory – Antimicrobial Resistance

National Food Institute

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ISBN: 978-87-92158-49-9

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1. Introduction

In this report, results of the first proficiency test trial – the External Quality Assurance System (EQAS) - regarding *Salmonella* and *Campylobacter* conducted by the National Food Institute (FOOD-DTU) appointed as community reference laboratory (CRL) by the European Union (EU) are summarised. The objective is to monitor the quality of the antimicrobial susceptibility data produced and pin point areas or laboratories, which need guidance or assistance to produce reliable susceptibility data. The goal is having all laboratories perform susceptibility testing within the range of either of the following: a maximum of 5 % very major / major and 5 % minor errors, or a maximum of 10 % minor errors.

2. Materials and Methods

2.1 Participants

An invitation to participate in the External Quality Assurance System (EQAS) on susceptibility testing of *Salmonella* and *Campylobacter* through the European Union community reference laboratory on antimicrobial resistance (CRL-AR) was distributed on the 13th of October, 2006 by e-mail to the 30 national reference laboratories (NRL) within the EU (App.1). This includes all EU countries but to one laboratory no contact was established (Malta) (App.2). All 30 laboratories responded. 23 of the NRLs were appointed by the individual member states. The remaining seven NRLs were not designated yet but enrolled on equal terms as the designated NRLs based on their participation in an EU funded concerned action (FAIR5-QLK2-2002-01146), ARBAO II project (Antibiotic resistance in bacteria of animal origin). Figure 1 show that 26 member states participated (29 NRLs). Three countries

tested only the *Salmonella* strains. One designated NRL from Belgium appointed by Luxembourg declined to participate. The Commission has been alerted about this situation.

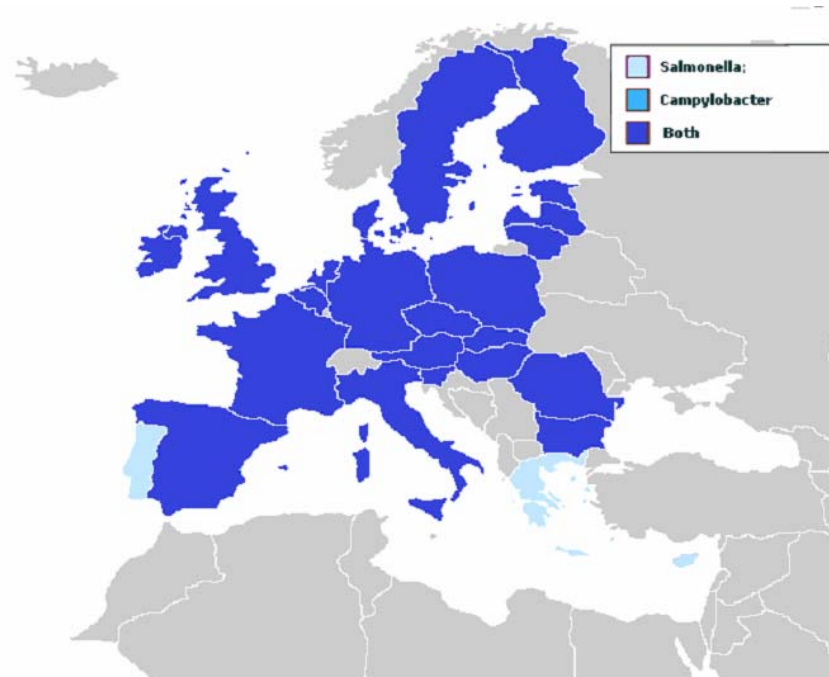


Figure 1. Participating member states.

2.2 Strains

Eight strains of *Salmonella* and eight strains of *Campylobacter* were selected for this trial among isolates from the National Food Institute. Individual sets of the *Salmonella* strains were inoculated as agar stab cultures and the *Campylobacter* strains were lyophilised in glass vials. The susceptibility patterns for all the strains were verified by the US Food and Drug Administration (FDA) prior to distribution. Furthermore, laboratories were provided with lyophilised international reference strains for susceptibility testing; *E. coli* CCM 3954 ~ ATCC 25922 and *Campylobacter jejuni* CCM 6214 ~ ATCC 33560 purchased at the Czech Collection of Micro-organisms (CCM); The Czech Republic.

2.3 Antimicrobials

Antimicrobial susceptibility testing (AST) on the *Salmonella* and *Campylobacter* strains were performed at the National Food Institute and the obtained MIC values serve as reference for

both *Salmonella* and *Campylobacter*. The following antimicrobials were used in the trial for *Salmonella*: ampicillin / amoxicillin; amoxicillin + clavulanic acid; cefotaxime; cefotaxime + clavulanic acid; cefoxitin; cefpodoxime; ceftazidime; ceftazidime + clavulanic acid; ceftiofur; chloramphenicol; ciprofloxacin / enrofloxacin; florfenicol; gentamicin; imipenem; imipenem + EDTA; nalidixic acid; streptomycin; sulphonamides; tetracycline; trimethoprim and trimethoprim + sulphonamides (App. 3).

MIC determination was performed using Sensititre systems from Trek diagnostics Ltd with the exception of cefotaxime + clavulanic acid, cefoxitin, ceftazidime + clavulanic acid, imipenem, imipenem + EDTA and trimethoprim + sulphonamides. These exceptions were tested using E-test from AB-Biodisk. Guidelines and breakpoints were according to the Clinical and Laboratory Standards Institute (CLSI) document M07-A7 (2006) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically”; Approved Standard - Seventh Edition, document M100-S17 (2007) “Performance Standards for Antimicrobial Susceptibility Testing”; Seventeenth Informational Supplement and document M31-A2 (2002) “Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals”; Approved Standard - Second Edition. Exceptions were the following antimicrobials where different breakpoints were used: ciprofloxacin, florfenicol, gentamicin (according to www.eucast.org) and streptomycin (according to FOOD-DTU) (App.5). Twelve out of 16 reference breakpoints differ from the resistance cut-off values recommended by the European Food safety Authority (EFSA). The four antimicrobials where the breakpoints are equal to the ones suggested by EFSA are: ciprofloxacin; nalidixic acid; sulfonamides and tetracycline (App. 6).

For *Campylobacter* the following antimicrobials were included: chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulphonamides and tetracycline (App. 3). MIC determination was performed using Sensititre systems from Trek diagnostics Ltd according to guidelines and breakpoints from the Clinical and Laboratory Standards Institute (CLSI) document M45-A (2006) “Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria”; Approved Guideline with the only exception of the breakpoint used for streptomycin which was according to FOOD-DTU (App. 5). All reference breakpoints differ from the resistance cut-off values recommended by EFSA with the exception of erythromycin for *C. coli* (App. 6).

2.4 Distribution

The cultures and documents (App. 4a,b,c,d,e) downloaded on a diskette were enclosed in double pack containers (class UN 6,2) and sent the 24th of October 2006 to the selected laboratories according to the International Air Transport Association (IATA) regulations as dangerous goods UN3373. Prior to shipping each laboratory was informed about the dispatched parcels and the air way bill (AWB) number for tracking of the parcel and pick up at the airport. Import permit was necessary for shipping the parcel to Romania.

2.5 Procedure

The laboratories were instructed to follow the protocol and subculture the strains prior to performing the antimicrobial susceptibility test using the method routinely used by the laboratory e.g. MIC determination, E-test or disk diffusion tests. Furthermore, they were requested to save and maintain the ATCC reference strains for future proficiency tests according to App. 4c.

The laboratories were instructed to enter the obtained MIC values or zone-diameter in millimetres and the susceptibility categories to an electronic record sheet in the CRL-AR web based database through a secured individual login and passwords or alternatively send the record sheets from the enclosed protocol by fax to FOOD-DTU. The website was open for entry in the period from the 22nd of November 2006 to the 6th of February 2007.

The strains were categorised as resistant, intermediate or sensitive against the tested antimicrobials. Only antimicrobials used for detection of ESBL should be interpreted clinically according to recommendations from CLSI. Laboratories were instructed to use antimicrobials used in their daily routine methods for performing susceptibility testing. In addition, they should use the daily used breakpoints for categorising the susceptibility data obtained. The breakpoints used were submitted to the web based database (App. 5).

All laboratories entered either the zone diameter in millimetres or MIC value for the *E. coli* (ATCC 25922) and *C. jejuni* (ATCC 33560) reference strains. The results were individually compared to the quality control ranges according to: CLSI documents M31-A2 (2002) / M100-S17 (2007) / M45-A (2006); The Sensititre System, Trek Diagnostic; Neo-Sensitabs, Rosco; or E-tests, AB-Biodisk (App. 7).

After submitting the data the laboratories were instructed to retrieve an instant generated individual report from the secured web site evaluating the submitted results. All deviations

from the expected were reported along with suggestions of how to either solve or investigate the problem. Deviations are categorised as minor, major or very major. Minor deviations are defined as an intermediate result that was determined as sensitive, resistant or vice versa (i.e. $I \leftrightarrow S$ or $I \leftrightarrow R$). When a sensitive strain was classified as resistant it was regarded as a major deviation (i.e. $S \rightarrow R$). When a resistant strain was classified as sensitive it was regarded as a very major deviation (i.e. $R \rightarrow S$). The questionnaire (App 4e) and the evaluation form (App 4d) enclosed with the strains were later collected and summarised (App.8, 9).

3. Results

In the description of results, we have tried to avoid defining arbitrary thresholds of quality limits. We are expressing results purely as correct, minor, major and very major deviations as described above.

3.1 Methods used by EQAS-participants.

Participating laboratories all used their routine methods for performing AST.

In the *Salmonella* trials, 14 laboratories used MIC determination included four which used E-test. Fourteen laboratories used disk diffusion whereas one laboratory used both methods in combination. The majority of laboratories (n:20) used MIC determination for the *Campylobacter* including three which used E-test (#4, #15 and #19) whereas five laboratories (#22, #26, #28, #29 and #34) used disk diffusion. The disk diffusion method and the use of E-test have not been recommended by CLSI for testing of *Campylobacter* and no interpretation guidelines or breakpoints are available.

3.2 Deviations by strain and antibiotic

Figure 2 shows the percentage minor, major and very major deviations from the expected results of AST performed by participating laboratories. For the *Salmonella* strains, 90.1 % of the AST's were interpreted correct. Of the 9.9 % of results that deviated from the expected results, 5.4 % were classified as minor, 1.4 % as major and 3.1 % as very major.

For the *Campylobacter* strains, 93.9 % of AST's were done correctly. Of the 6.1 % of results that deviated from expected results, 1.2 % was classified as minor, 2.0 % as major and 2.9 % as very major.

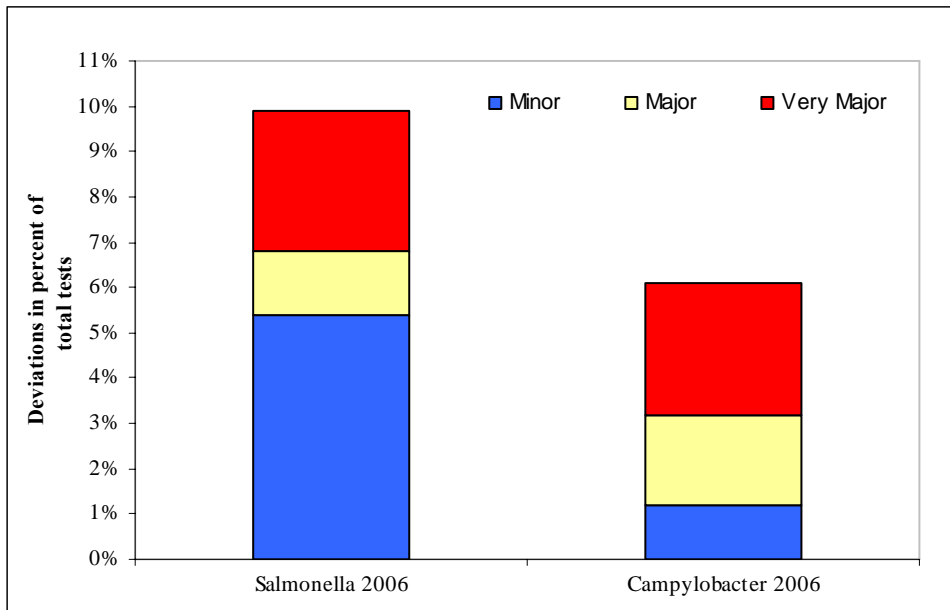


Figure 2. Deviations in percentages minor, major and very major for antimicrobial susceptibility testing performed by participating laboratories in the first CRL-AR proficiency test trial – (EQAS) regarding *Salmonella* and *Campylobacter*.

The number of AST performed and the percentage of correct results for the individual *Salmonella* and *Campylobacter* strains in the EQAS are listed in Table 1. There is a large variation between strains of the same species. For *Salmonella* strains, major difficulty was observed for strains #3 (85.3 % correct) which is multi-resistant to the following antimicrobials: ampicillin, cefotaxime, cefotaxime + clavulanic acid, cefpodoxime, ceftazidime, ceftiofur, ciprofloxacin, nalidixic acid and tetracycline and for strain #2 (79.5 % correct) resistant to ampicillin, ciprofloxacin, gentamicin, nalidixic acid, streptomycin, sulphonamides and intermediate to amoxicillin + clavulanic acid and chloramphenicol. Also for *Salmonella* strains #1 and #8 some difficulty was observed with percentages of and 89.8 % and 90.4 % corrects AST (Table 1).

For *Campylobacter*, strain #1 and strain #2 posed some problems. Strain #1 (87.9 % correct) is resistant to ciprofloxacin, nalidixic acid and tetracycline and strain #2 (90.9 %) is resistant to the same antimicrobials as strain #1 with the addition of erythromycin (Table 1).

Year Species Strain	2006			
	<i>Salmonella</i>		<i>Campylobacter</i>	
	AST in total	% correct	AST in total	% correct
1.1	303	89.8	132	87.9
1.2	356	79.5	132	90.9
1.3	368	85.3	112	98.2
1.4	368	92.4	121	92.6
1.5	358	93.9	137	96.4
1.6	371	93.8	132	95.5
1.7	344	96.5	135	92.3
1.8	355	90.4	132	99.2

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

In Table 2 the percentage of correct AST per antibiotic by species is shown. Many of the antimicrobials seem to pose a common problem for many laboratories when testing *Salmonella* whereas for *Campylobacter* the problem seems more moderate. Especially, amoxicillin + clavulanic acid, cefpodoxime, chloramphenicol, ciprofloxacin, florfenicol, streptomycin and tetracycline, seem to cause problems when testing *Salmonella* (App.10) The two lowest percentages for correctly tested antimicrobials against *Salmonella* is amoxicillin + clavulanic acid with 70.0 % and streptomycin with 76.1 %. The problem seems not to be as profound when testing *Campylobacter* (App.10) where the result reflects no major problems with the exception of streptomycin (87.4 % correct).

ESBL producing organisms is an emerging problem worldwide. The laboratories were asked to detect the three ESBL producing *bla_{ctx}* *Salmonella* strains (#3, #4, and #6) according to the clinical guidelines described by CLSI which indicate that all cephalosporins should be interpreted resistant if one is interpreted resistant regardless of the value detected from the results. In Table 3 it seems that the laboratories have problem detecting the ESBL producing strain #3. There are differences in how many cephalosporins the laboratories use in the daily routine ranging between the five which 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 double disk test. Using only cefotaxime or ceftazidime posed a problem for strain #3 where only 50 % of the laboratories found the strain to be an ESBL. The best result for strain

#3 was obtained when using cefotaxime, ceftazidime and ceftiofur in combination (83 %) or just ceftiofur alone (100 %). These data should of course be interpreted with care as only eight participants used the opportunity to test for ESBL. No major problems were observed for strain #4 and #6 where almost all laboratories found the strains to be ESBL producing.

Species Antimicrobials	<i>Salmonella</i> % correct	<i>Campylobacter</i> % correct
Amoxicillin / Ampicillin	98.4	-
Amoxicillin + Clavulanic acid	70.0	-
Cefotaxime	93.6	-
Cefpodoxime	87.5	-
Ceftazidime	92.0	-
Ceftiofur	92.3	-
Chloramphenicol	86.1	97.0
Ciprofloxacin / Enrofloxacin	79.8	95.3
Erythromycin	-	91.9
Florphenicol	89.5	-
Gentamicin	90.9	99.4
Nalidixic acid	94.9	96.8
Streptomycin	76.1	87.4
Sulfonamides	96.4	-
Trimethoprim + Sulfonamides	96.3	-
Tetracycline	89.4	90.1
Trimethoprim	100.0	-

Table 2. Percentage correct Antimicrobial Susceptibility Test per antibiotic by species.

Cephalosporines / strains	Strain #3				Strain #4				Strain #6			
	ESBL not detected		ESBL detected		ESBL not detected		ESBL detected		ESBL not detected		ESBL detected	
	Number, n	Percentages, %	Number, n	Percentages, %	Number, n	Percentages, %	Number, n	Percentages, %	Number, n	Percentages, %	Number, n	Percentages, %
CTX, CAZ, XNL	1	17%	5	83%	0	0%	6	100%	0	0%	6	100%
CTX, CAZ	4	50%	4	50%	2	25%	6	75%	0	0%	8	100%
CTX, XNL	2	33%	4	67%	0	0%	5	100%	0	0%	5	100%
CTX	1	33%	2	67%	0	0%	4	100%	0	0%	4	100%
XNL	0	0%	4	100%	0	0%	4	100%	0	0%	5	100%
CTX/Cl:CTX	2	33%	4	67%	1	17%	5	83%	0	0%	6	100%
CAZ/Cl:CAZ	0	0%	0	0%	2	33%	4	67%	0	0%	6	100%

Table 3. Percentages and number of laboratories which detected correctly and incorrectly the three ESBL producing *Salmonella* strains.

3.3 Deviations by laboratory

Figure 3 shows the percentage of deviations and the severity hereof for each participating laboratory by strain. The laboratories are ranked after increasing performance as determined by the percentage minor, major and very major deviations of results. In Figure 4 the total amount of errors (minor, major and very major) in percentages are listed by number of laboratories. In addition, the same figure is showed with exclusion of minor errors in Figure 5.

3.3.1 *Salmonella* trial

None of the laboratories gained a result of 100 % correctly tested *Salmonella* strains. The percentage of errors differed a lot between laboratories with a maximum of 30 % in laboratory #29 and a minimum of 2% in laboratory #25.

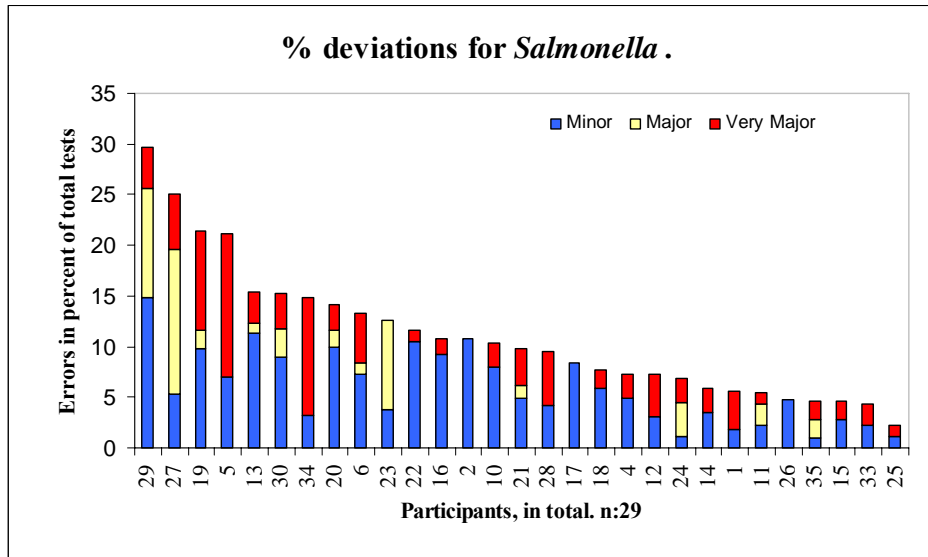


Figure 3. Minor, major and very major errors in percentages of the individual participants.

The majority of the laboratories have errors ranging from 4 % to 16 % with a group of outliers of four laboratories with between 20 – 30 % errors. Only two laboratories (#25, #33) had < 4 % errors.

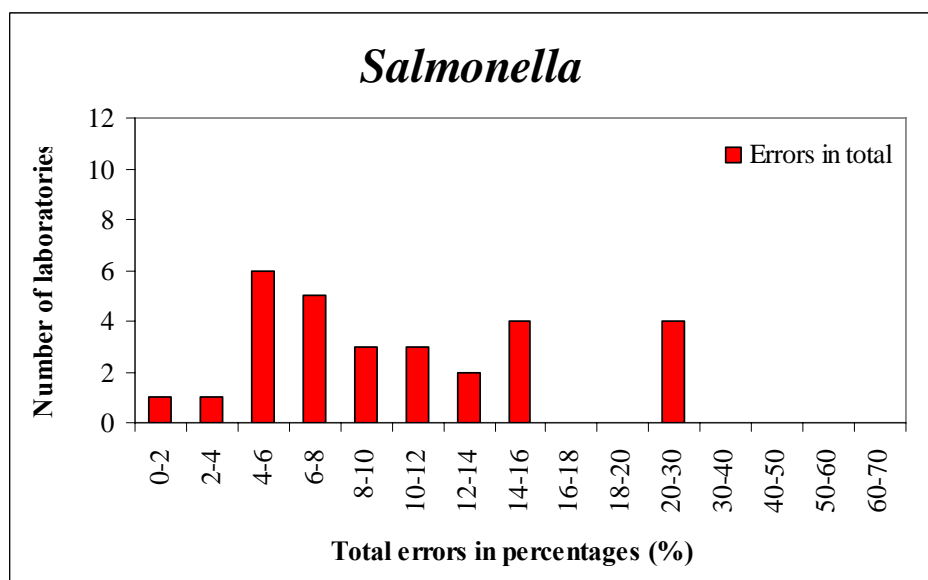


Figure 4. The number of laboratories listed in intervals of percentages per total errors.

When pin pointing the seriousness of the errors it is helpful to exclude the minor errors. Figure 5 illustrate a large group of laboratories which has errors between 0-6 % whereas six laboratories (#5, #19, #23, #27, #29, #34) are outliers with errors ranging from 8 -20 %. The acceptable amount of errors is set to either 5 % very major / major and 5 % minor errors or 10 % minor errors, which were meet by 14 laboratories. The future focus will be on the six laboratories with most errors. In the case where no obvious reason can explain the errors, the laboratories will be offered the possibility to re-test additional *Salmonella* strains.

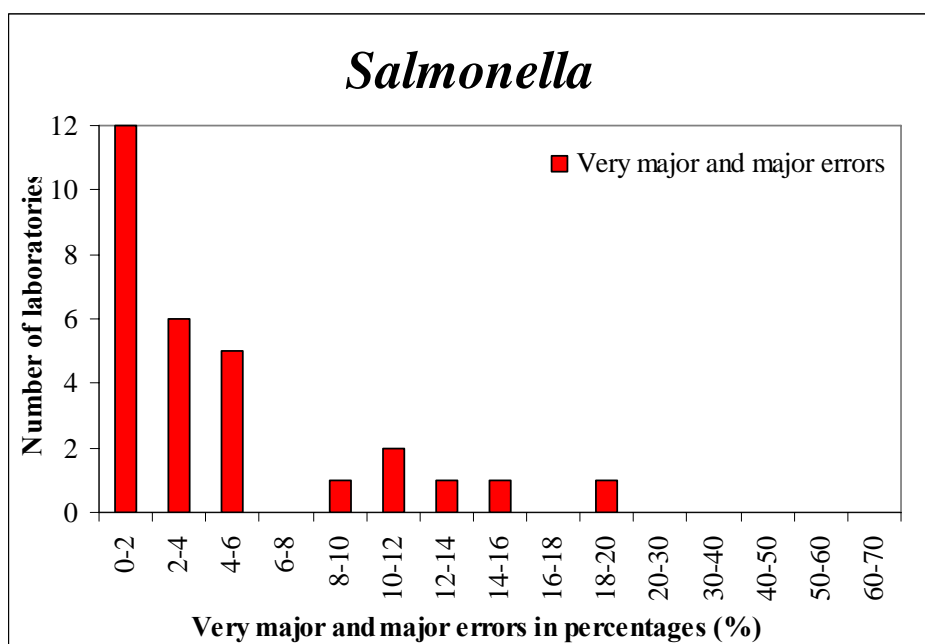


Figure 5. The number of laboratories listed in intervals of percentages per major and very major errors.

Laboratory # 27 using MIC determination accounted for most major and very major deviations (20 %) (Figure 5). The *E. coli* ATCC 25922 was tested satisfactory (App. 11a). The laboratory mainly used breakpoints, which they do not share with the majority of the laboratories. This indicates that it might be the reason for the low score in the test (App.5).

The *E. coli* ATCC 25922 showed some errors due to small zone diameters for a wide range of the same antimicrobials for laboratory # 29 (App. 11a). Furthermore, breakpoints applied were also mainly not used by the majority of the other laboratories (App.5). This indicates that it might be useful for the laboratory to optimise the procedure for disk diffusion and maybe apply internationally used breakpoints.

There is no obvious reason for the deviations by laboratory #5 why it is recommended to go through the disk diffusion methodology to search for indications of failure (App.11a).

The number of antimicrobials causing the deviations for laboratory # 19 was long and accounted for 12 % deviations with no major problems testing *E. coli* ATCC 25922 (App.11a). The reason for all the deviations might lay in the used breakpoints which the laboratory for most antimicrobials does not share with others laboratories (App.5).

Laboratory #34 seems to have a major problem with the quality control strain which should be addressed in order to get reliable data for future monitoring purposes. The laboratory has larger zone diameters for a large number of antimicrobials (App.11a).

Nine percentages of deviations for laboratory #23 was caused mainly by one antimicrobial: amoxicillin + clavulanic acid which five time results in errors (App.11a). The problem testing for amoxicillin + clavulanic acid is also apparent when testing the reference strain and should be addressed before the next trial.

3.3.2 *Campylobacter* trial

Over-all, most laboratories performed well. Applying the same acceptable threshold as described for *Salmonella*, 15 laboratories performed within this threshold.

One laboratory (#26) had 33 % correctly tested *Campylobacter* strains. The rest of the laboratories scored between 75 % and up to 100 % correct AST's (Figure 6). Nine laboratories performed all AST's correct and three did not enter results in the database (#6, #18 and #27).

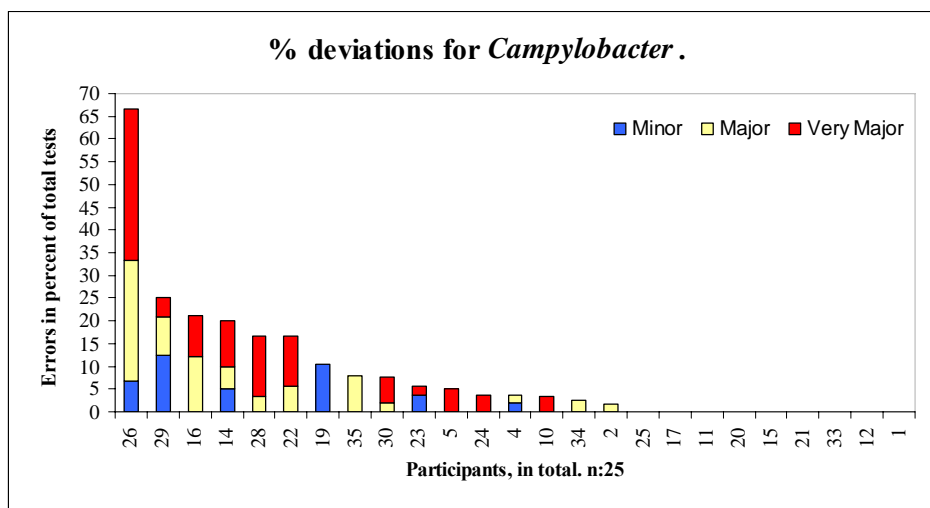


Figure 6. Minor, major and very major errors in percentages of the individual participants.

When grouping laboratories in intervals of total amount of errors in percentages (Figure 7) it is clear that six laboratories have a considerable higher number of errors (from 16 % up to 70 %) than the majority of the participating laboratories.

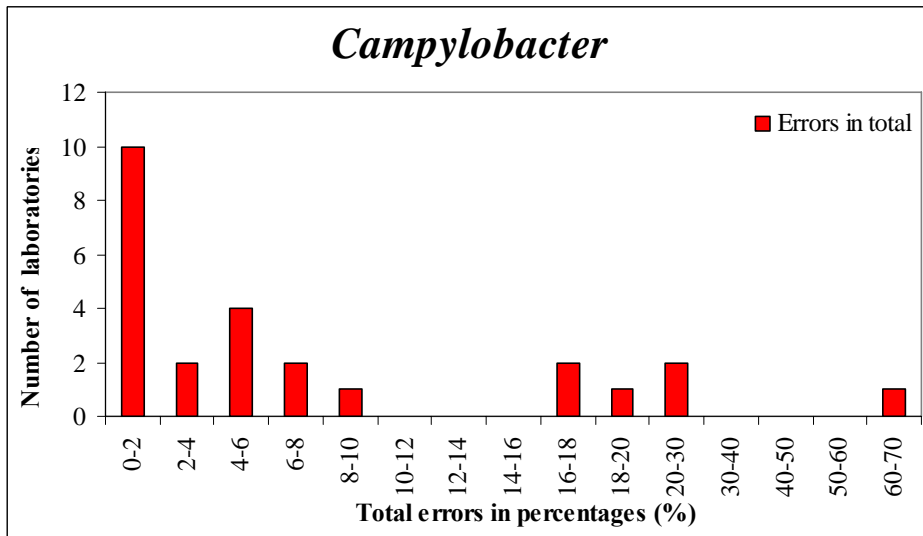


Figure 7. The number of laboratories listed in intervals of percentages per total errors.

The same six laboratories (#14, #16, #22, #26, #28 and #29) are outliers when evaluating errors caused only by major and very major errors shown in Figure 8. The six laboratories have a span of major and very major errors ranging from 12 % to 60 % while the remaining laboratories have errors ranging from 0 % to 8 % with eight laboratories having no errors at all.

Laboratory #16 has deviations in a range of antimicrobials and used MIC determination. Testing the reference strain *C. jejuni* ATCC 33560 caused no errors. The laboratory has not submitted any breakpoints and this could be the reason. The laboratory should focus on the breakpoints described in this test and apply them if possible.

MIC determination was also used by laboratory #14, which had 15% deviations (App.11b). Laboratory #22, #26, #28 and #29 all used disk diffusion.

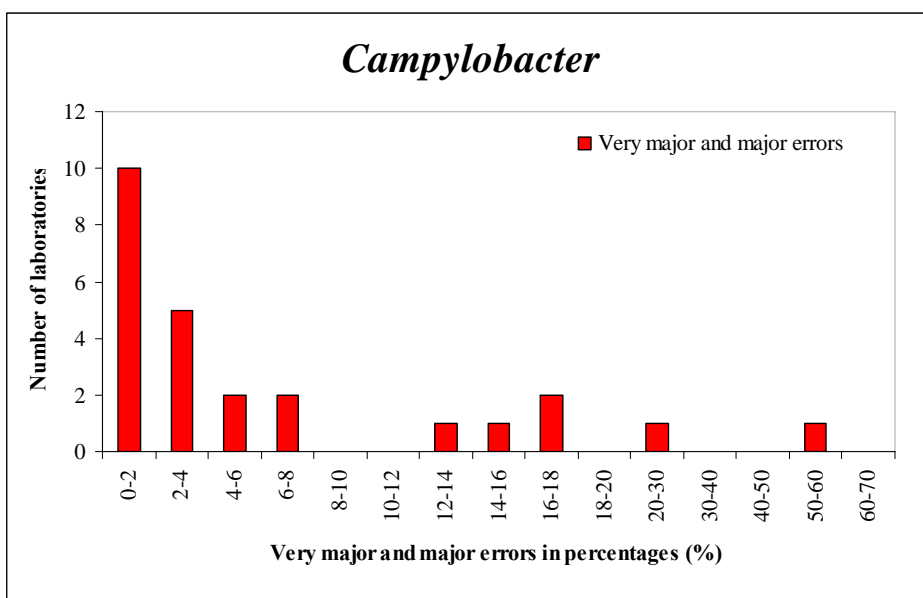


Figure 8. The number of laboratories listed in intervals of percentages per major and very major errors.

3.4 Deviations by reference strains

In this section, deviations are defined as the value with which the quality control (QC) interval limits are exceeded (App.7). The exceeding values of the QC interval are listed in the tables illustrating the laboratories quality control performance when an obtained value (zone millimetre or MIC steps) exceed the lower end or the upper end of the QC interval.

Table 4 shows the proportion of laboratories that obtained values outside the QC interval of reference strain *E. coli* ATCC 25922 using disk diffusion. Fourteen laboratories tested the reference strain using the disk diffusion method. Mistakes at up to four laboratories caused values outside the recommended QC interval for 12 of the 19 antimicrobials in the test. No mistakes were recorded for the following antimicrobials: ampicillin, amoxicillin, cefpodoxime, ciprofloxacin, streptomycin, trimethoprim and ceftazidime. For seven antimicrobials only one laboratory had obtained a value outside the QC interval. Most problems were recorded for the following antimicrobials: amoxicillin + clavulanic acid (40.0 % errors) with a maximum of 10 mm less than the end point of the QC interval and 5 mm greater; tetracycline (40.0 %) with a maximum of 4 mm above the end point of the QC interval and sulphonamides (37.5 %) with a maximum of 9 mm less than the end point of the QC interval and 5 mm greater. Also against cephalosporins problems were detected with a maximum of 6 mm greater than the upper end of QC interval for ceftazidime and 8 mm for cefotaxime.

2006 Antimicrobials	Disk Diffusion ATCC25922		
	Prop. of labs outside QC range (%)	Range of obtained values in mm zones	
		Below lower QC limit	Above upper QC limit
Ampicillin	0 / 9	(0.0)	
Amoxicillin	0 / 4	(0.0)	
Amoxicillin + Clavulanic acid	4 / 10	(40.0)	10 mm 5 mm
Cefpodoxime	0 / 3	(0.0)	
Ceftiofur	2 / 5	(40.0)	1 mm
Ceftazidime	1 / 8	(12.5)	6 mm
Cefotaxime	2 / 11	(18.2)	1 mm 8 mm
Chloramphenicol	1 / 13	(7.7)	1 mm
Ciprofloxacin	0 / 9	(0.0)	
Florphenicol	1 / 6	(16.7)	5 mm
Gentamicin	1 / 13	(7.7)	1 mm
Nalidixic acid	1 / 13	(7.7)	5 mm
Streptomycin	0 / 12	(0.0)	
Sulfonamides	3 / 8	(37.5)	9 mm 5 mm
Tetracycline	4 / 10	(40.0)	4 mm
Trimethoprim	0 / 10	(0.0)	
Trimethoprim + Sulfonamides	1 / 12	(8.3)	5 mm
Cefoxitin	0 / 4	(0.0)	
Imipenem	1 / 3	(33.3)	3 mm

Table 4. Range of obtained values for *E. coli* ATCC 25922 by disk diffusion.

It seems that using MIC determination towards the reference strain *E. coli* ATCC 25922 results in fewer errors. Thirteen laboratories submitted data where only problems were detected against seven antimicrobials of the nineteen. No mistakes were seen in thirteen of the antimicrobials. Most problems were seen against ciprofloxacin (30.0 %) where three out of 10 laboratories had errors ranging from a MIC step less than the recommended QC interval to two MIC steps greater. Also amoxicillin + clavulanic acid caused problems as 25 % had a MIC value less than the lower QC end point.

2006 Antimicrobials	MIC Determinations ATCC25922		
	Prop. of labs outside QC range (%)	Range of obtained values in MIC steps	
		Below lower QC limit	Above upper QC limit
Ampicillin	0 / 8	(0.0)	
Amoxicillin	0 / 4	(0.0)	
Amoxicillin + Clavulanic acid	1 / 4	(25.0)	4 steps
Cefpodoxime	0 / 1	(0.0)	
Ceftiofur	0 / 6	(0.0)	
Ceftazidime	0 / 3	(0.0)	
Cefotaxime	1 / 7	(14.3)	1 step
Chloramphenicol	0 / 12	(0.0)	
Ciprofloxacin	3 / 10	(30.0)	1 step
Florphenicol	0 / 9	(0.0)	
Gentamicin	1 / 12	(8.3)	2 steps
Nalidixic acid	1 / 11	(9.1)	1 step
Streptomycin	0 / 9	(0.0)	
Sulfonamides	0 / 10	(0.0)	
Tetracycline	0 / 12	(0.0)	
Trimethoprim	1 / 10	(10.0)	1 step
Trimethoprim + Sulfonamides	0 / 5	(0.0)	
Cefoxitin	0 / 2	(0.0)	
Imipenem	0 / 1	(0.0)	

Table 5. Range of obtained values for the *E. coli* ATCC 25922 using MIC determination.

Quality control was also performed using MIC determination including three laboratories using E-test against the *C. jejuni* reference strain ATCC 33560 with participation of 18 laboratories. This seems to result in more problems but in many cases with only one MIC step less than the recommended QC interval. In Table 6 most problems were detected against erythromycin (38.5 %) where five out of 13 laboratories had an error. Also ciprofloxacin caused a high amount of errors. Four out of 14 laboratories (28.6 %) had a one MIC step less than the QC interval and a maximum of two MIC steps greater than the QC interval. The three laboratories performing AST using E-tests accounts for one error against ciprofloxacin / erythromycin and two errors for nalidixic acid.

No QC intervals are available for disk diffusion as the method is not recommended to use for testing *Campylobacter*.

2006 Antimicrobials	MIC Determinations ATCC33560			
	Prop. of labs outside QC range (%)		Range of obtained values in MIC steps	
			Below lower QC limit	Above upper QC limit
Chloramphenicol	0 / 8	(0.0)		
Ciprofloxacin	4 / 14	(28.6)	1 step	2 steps
Erythromycin	5 / 13	(38.5)	1 step	1 step
Gentamicin	2 / 12	(16.7)	1 step	
Nalidixic acid	2 / 14	(14.3)	1 step	
Streptomycin	0 / 0	(0.0)		
Tetracycline	2 / 16	(12.5)	1 step	

Table 6. Range of obtained values for the *C.jejuni* ATCC 33560 using MIC determination.

4. Discussion

4.1 *Salmonella* trial

Over-all, the percentage of correct susceptibility testing of *Salmonella* was 90.1 %. Large differences in the performance of the laboratories were observed ranging from 2 % to 30 % incorrect results. There is no international standpoint for what should be considered satisfactory. Internally, the CRL uses a level of acceptance of maximum 2% major and very major errors and maximum 5% minor errors. For the EQAS the CRL have decided to use either a maximum of 5 % very major / major and 5 % minor errors or a maximum of 10 % minor errors to estimate the satisfactory level. Fifteen laboratories performed unsatisfactory according to the acceptable ranges established by the CRL. It was obvious that six of the laboratories were outliers. This indicates a clear need for harmonisation of the susceptibility testing of *Salmonella*. However, it is important to determine the factors which cause the errors. The factors could be incorrect breakpoints, demanding test strains, difficult reading of the antimicrobials or the methodology.

Amoxicillin + clavulanic acid, ciprofloxacin, streptomycin, gentamicin and tetracycline caused unsatisfactory results.

Problems associated with amoxicillin + clavulanic acid is not only due to a breakpoint phenomenon but also related to the methodology, as 40 % (MIC determination) and 25 % (disk diffusion) of the laboratories had difficulties with the quality control towards the reference strain *E. coli* ATCC 25922.

The reference breakpoint used to determine resistance against ciprofloxacin may have resulted in the many errors against this antimicrobial.

Streptomycin often poses a challenge in susceptibility testing, as many strains are borderliners and balance between resistance / intermediate or intermediate / sensitive.

In strain #3 some laboratories had difficulties in testing for ESBL as only 50 % have tested cefotaxime and ceftazidime in combination correct. The problem is also illustrated in table 3. Being able to detect ESBL producing strains is paramount for the public health and it should be of a high priority for the NRL's to be able to detect it.

4.2 *Campylobacter* trial

The amount of errors testing *Campylobacter* was less than for *Salmonella* with an over-all performance of 94% correct results. The performance of the laboratories differed from 0% up to 67% of incorrect results. Eight laboratories had no errors at all and seventeen laboratories performed satisfactory according to the acceptable ranges established by the CRL.

Six laboratories (#14, #16, # 22, #26, #28 and #29) did not perform as well as the other laboratories.

Laboratories #26, #22, #28 and #29 have all used the methodology based on disk diffusion and had 60 %, 17 %, 17 % and 13 % of incorrect susceptibility tests, respectively.

Diffusion tests are not international recommended for susceptibility testing of *Campylobacter* as there are no international breakpoints or quality control intervals available.

5. Conclusion

The goal is to have all laboratories perform susceptibility testing of *Salmonella* and *Campylobacter* with a deviation margin below 5 % very major / major and 5 % minor errors or below 10 % minor errors. This seems some time away but within reach.

The recommendation mentioned above for the specific laboratories should be followed in order to be able to submit better results in the next trial and produce reliable data. The Laboratories which did not perform satisfactory will be contacted and expected to perform a re-test of two strains. Furthermore, it is expected that they will participate in a discussion / investigation of the reasons behind the unsatisfactory performance. In addition, the CRL expect to launch training courses for selected laboratories in 2008.

Harmonising breakpoints, antimicrobials and ranges of these will be important issues to address in the future. Also, attention should be addressed to the problem of detecting ESBL



producing strains. In general, the laboratories seemed content about the way the trial was launched and the system (App.8), The CRL-AR will take into consideration the suggestions and issues raised by the participating laboratories.

Appendix 1.: Invitation letter.

Fra: Rene Sjøgren Henriksen (DFVF)
Sendt: 13. oktober 2006 14:05
Til: Rene Sjøgren Henriksen (DFVF)
Emne: EU CRL AR: first task, proficiency test

Vedhæftede filer: Work plan for 2006 - CRL Antimicrobial Resistance1.doc
Dear potential NRL colleagues.

I am contacting you as our institute have been appointed as community reference laboratory for EU. We haven't yet been notified by EU whether you will be appointed as national reference laboratory for antimicrobial resistance in bacteria originating from either food, feed and animals or combinations. Nevertheless, we think it might be possible that your institute will be appointed why we invite you to participate in this proficiency testing.

According to work plan accepted by EU we have planned to launch the first proficiency test (EQAS) aimed for Salmonella and Campylobacter. We are sending eight strains of both species.

Please let me know if you want to participate, if I need somehow an import permit for shipping the strains and please let me know the full address and phone number of the person who should receive the parcel.

Attached you will find the work plan for 2006. All necessary documents for the EQAS will be enclosed in the parcel.

I dispatch the parcels if you accept in week 43. The deadline for submitting the results are 1st of Dec. 2006.

I have only in mind of sending one parcel - please indicate to whom it should be as all three institutes (LERAP, LERQAP, LERMVD) have been appointed.



Work plan for 2006
- CRL Antim...

Regards,

René Sjøgren Henriksen
Laboratoriefølgemægter.

The Danish Institute for Food and Veterinary Research.
Institute of Food Safety.
Dept. of Microbiology and Risk assessment.
Section of Diagnostic and Antimicrobial Resistance.
Bülowsvej 27.
DK-1790 Copenhagen V.
Denmark.
Ph: 0045 72346288
Fax: 0045 72346001
e-mail:rsh@dfvf.dk

Appendix 2.: Participant list and origin of samples.

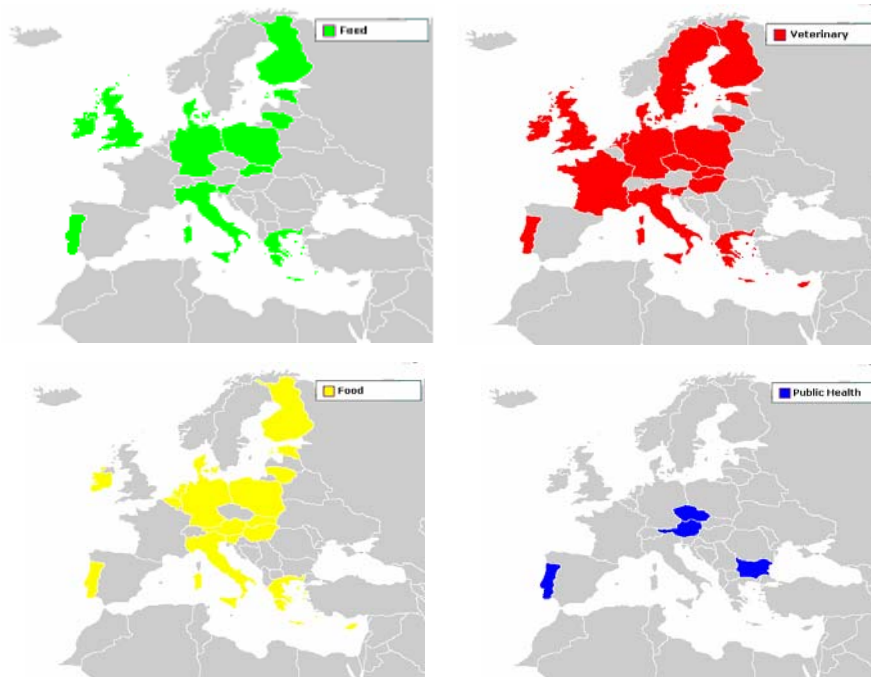
Institute	Country	Area of interest			
		Food	Feed	Vet	Public
Austrian Agency for Health and Food Safety	Austria	X			X
Institute of public Health	Belgium	X			
<i>Université de Liège</i>	<i>Belgium, (Luxembourg)</i>	<i>X</i>			
National Center of Infectious and Parasitic Diseases	Bulgaria				X
Veterinary Services, Bacteriology - Serology Laboratory (BSL)	Cyprus	X		X	
<i>Food Microbiology Laboratory, State General Laboratory</i>	<i>Cyprus</i>				
<i>State Veterinary Institute Praha</i>	<i>Czech Republic</i>				
National Institute of Public Health	Czech Republic				X
National food Institute	Denmark	X	X	X	
Estonian Veterinary and Food Laboratory	Estonia	X	X	X	
Finnish Food Safety Authority EVIRA	Finland	X	X	X	
AFSSA LERQAP - Pôle HQSA Unité CEB	France			X	
AFSSA Ploufragan - LERAP - Unité Mycoplasmodologie-Bactériologie	France			X	
AFSSA Lyon - Unité Bactériologie Bovine et Sécurité des Viandes	France			X	
AFSSA Fougères LERMVD, Unité pharmacocinétique-pharmacodynamie La Haute Marche - Javené	France			X	
Federal Institute for Risk Assessment, Center of Infectiology and Pathogen Characterization	Germany	X	X	X	
Veterinary Laboratory of Chalkis	Greece	X	X	X	
National Veterinary Institute	Hungary	X		X	
Central Veterinary Research Laboratory, Department of Agriculture and Food	Ireland	X	X	X	
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy	X	X	X	
National Diagnostic Centre of Food and Veterinary Service	Latvia				
National Veterinary Laboratory	Lithuania	X	X	X	
Food and Consumer Product Safety Authority (VWA)	Netherlands	X			
Central Institute for Animal Disease Control (CIDC-Lelystad)	Netherlands			X	
National Veterinary Research Institute	Poland	X	X	X	
Instituto Nacional de Saude (INSA)	Portugal				
National Institute of Research-Development for Microbiology and Immunology "Cantacuzino"	Romania				
State Veterinary and Food Institute (SVFI)	Slovakia	X	X	X	
National Veterinary Institute	Slovenia	X	X	X	
<i>Laboratorio Central de Sanidad, Animal de Santa Fe</i>	<i>Spain</i>				
<i>Laboratorio Central de Sanidad, Animal de Algete</i>	<i>Spain</i>				
Complutense University of Madrid	Spain	X		X	
National Veterinary Institute, SVA	Sweden			X	
The Veterinary Laboratory Agency	United Kingdom		X	X	

Institutes marked in italic were designated too late to participate.

Institutes marked in light gray participated but have not been appointed by the member states.

The University de Liège in Belgium declined to participate – appointed by Luxembourg.

Origin of samples.



Appendix 3; Strain collection and reference values in MIC.

CRL EQAS strain	AMP/AMX	AUG	POD	XNL	CAZ	CTX	CHL	CIP/ENRO	ERY	FFN	GEN	NAL	STR	SMX	TET	TMP	SXT	CAZ/CL	CTX/CL	FX	ESBL gene
CRL S.1,1	>32	8/4	0,5	2	0,75	0,38	>64	0,06	-	>64	2	8	>64	>1024	>32	<=4	0,5/9,5	MIC ratio <8	MIC ratio <8	6	-
CRL S.1,2	>32	16/8 32/16	0,5	2	1	0,38	16	1	-	8	16	>64	64	>1024	4	<=4	0,25/4,75	MIC ratio <8	MIC ratio <8	6	-
CRL S.1,3	>32	8/4	>4	>8	0,75	8	4	0,5	-	4	<=1	>64	8	<=64	32	<=4	<=0,12/2,38	MIC ratio <8	MIC ratio =>8	4	CTX-M9
CRL S.1,4	>32	8/4	>4	>8	4	>256	>64	<=0,03	-	4	<=1	<=4	32	>1024	>32	>32	>4/76	MIC ratio =>8	MIC ratio =>8	6	CTX-14
CRL S.1,5	4	<=2/1	1	2	1	0,5	8	<=0,03	-	8	>32	<=4	64	>1024	<=2	<=4	<=0,12/2,38	MIC ratio <8	MIC ratio <8	16	-
CRL S.1,6	>32	8/4	>4	>8	8	>256	>64	<=0,03	-	>64	<=1	<=4	32	>1024	<=2	>32	>4/76	MIC ratio =>9	MIC ratio =>8	3	CTX-1
CRL S.1,7	>32	8/4	0,25	1	0,38	0,125	>64	<=0,03	-	64	>32	<=4	>64	>1024	>32	>32	>4/76	MIC ratio <8	MIC ratio <8	3	-
CRL S.1,8	2	<=2/1	0,25	1	0,25	0,094	8	>4	-	8	<=1	>64	16	>1024	>32	>32	>4/76	MIC ratio <8	MIC ratio <8	3	-
CRL C.1,1	-	-	-	-	-	-	4	>4	1	-	0,5	>64	2	-	>16	-	-	-	-	-	-
CRL C.1,2	-	-	-	-	-	-	4	>4	>32	-	0,25	>64	<=2	-	>16	-	-	-	-	-	-
CRL C.1,3	-	-	-	-	-	-	8	>4	>32	-	>16	>64	>16	-	>16	-	-	-	-	-	-
CRL C.1,4	-	-	-	-	-	-	4	>4	1	-	0,25	>64	<=2	-	>16	-	-	-	-	-	-
CRL C.1,5	-	-	-	-	-	-	4	0,125	1	-	<=0,125	4	<=2	-	>16	-	-	-	-	-	-
CRL C.1,6	-	-	-	-	-	-	<=2	0,06	<=0,5	-	0,25	4	<=2	-	<=0,25	-	-	-	-	-	-
CRL C.1,7	-	-	-	-	-	-	8	0,25	2	-	0,5	16	16	-	1	-	-	-	-	-	-
CRL C.1,8	-	-	-	-	-	-	<=2	0,06	>32	-	0,25	8	>16	-	0,5	-	-	-	-	-	-

AMP, ampicillin / AMX, amoxicillin, AUG, amoxicillin + clavulanic acid, CTX, cefotaxime, CTX/CL, cefotaxime + clavulanic acid, FX, ceftiofur, POD, cefepime, CAZ, ceftazidime, CAZ/CL, ceftazidime + clavulanic acid, XNL, ceftiofur, CHL, chloramphenicol, CIP, ciprofloxacin / ENRO, enrofloxacin, FFN, florphenicol, GEN, gentamicin, IMI, imipenem, IME, imipenem + EDTA, NAL, nalidixic acid, STR, streptomycin, SMX, sulphonamides, TET, tetracycline, TMP, trimethoprim and SXT, trimethoprim + sulphonamides

CRL EQAS strain	AMP/AMX	AUG	POD	XNL	CAZ	CTX	CHL	CIP/ENRO	ERY	FFN	GEN	NAL	STR	SMX	TET	TMP	SXT	CAZ/CL	CTX/CL	ESBL gene	FX	IP/IFE
CRL S.1,1	R	S	S	S	S	S	R	S	S	R	S	S	R	R	R	S	S	none ESBL	none ESBL	-	none ampC	none Metallo beta lactamase
CRL S.1,2	R	I/R	S	S	S	S	I	R	S	S	R	R	R	R	S	S	S	none ESBL	none ESBL	-	none ampC	none Metallo beta lactamase
CRL S.1,3	R	S	R	R	R	R	S	R	S	S	S	R	S	S	R	S	S	none ESBL	ESBL	CTX-M9	none ampC	none Metallo beta lactamase
CRL S.1,4	R	S	R	R	R	R	R	S	S	S	S	S	R	R	R	R	R	ESBL	ESBL	CTX-14	none ampC	none Metallo beta lactamase
CRL S.1,5	S	S	I	S	S	S	S	S	S	S	R	S	R	R	S	S	S	none ESBL	none ESBL	-	none ampC	none Metallo beta lactamase
CRL S.1,6	R	S	R	R	R	R	R	S	S	R	S	S	R	R	S	S	R	ESBL	ESBL	CTX-1	none ampC	none Metallo beta lactamase
CRL S.1,7	R	S	S	S	S	S	R	S	S	R	R	S	R	R	R	R	R	none ESBL	none ESBL	-	none ampC	none Metallo beta lactamase
CRL S.1,8	S	S	S	S	S	S	S	R	S	S	S	R	I	R	R	R	R	none ESBL	none ESBL	-	none ampC	none Metallo beta lactamase
CRL C.1,1	-	-	-	-	-	-	S	R	S	-	S	R	S	S	R	-	-	-	-	-	-	-
CRL C.1,2	-	-	-	-	-	-	S	R	R	-	S	R	S	S	R	-	-	-	-	-	-	-
CRL C.1,3	-	-	-	-	-	-	S	R	R	-	R	R	R	-	R	-	-	-	-	-	-	-
CRL C.1,4	-	-	-	-	-	-	S	R	S	-	S	R	S	-	R	-	-	-	-	-	-	-
CRL C.1,5	-	-	-	-	-	-	S	S	S	-	S	S	S	-	R	-	-	-	-	-	-	-
CRL C.1,6	-	-	-	-	-	-	S	S	S	-	S	S	S	-	S	-	-	-	-	-	-	-
CRL C.1,7	-	-	-	-	-	-	S	S	S	-	S	S	R	-	S	-	-	-	-	-	-	-
CRL C.1,8	-	-	-	-	-	-	S	S	R	-	S	S	R	-	S	-	-	-	-	-	-	-

R: Resistance (yellow), I: Intermediate (green), S: Sensitive (colourless), Values marked in blue are sensitive but clinical interpreted as resistant due to resistance to other cephalosporines.

**EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2006**

Appendix 4a.: Documents

Dear CRL AR EQAS 2006 participant!

Please find enclosed the bacterial strains for the CRL AR EQAS 2006 together with the following documents:

**Protocol for 2006
Manual for Opening, Reviving and Maintaining of Freeze-dried Cultures
Evaluation form**

In the protocol you will find detailed description of how to testing the strains. In the guidelines for submitting the data you will find a description of how to enter your results into the interactive web database. For the data entry you need a username and a password. Please keep this document. Your username and password will not appear in other documents.

Your username:

Your password:

We are looking forward to this first trial of the CRL AR EQAS.

For further information, please don't hesitate to contact:

Rene Hendriksen
Telephone +45 7234 6288
E-mail: rsh@dfvf.dk

- *the Danish Institute for Food and Veterinary Research*
Bülowsvej 27, DK-1790 Copenhagen V
Denmark
Fax: +45 7234 6341



Appendix 4b

PROTOCOL

Susceptibility testing of *Salmonella* and *Campylobacter*

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 2006 will include susceptibility testing of eight *Salmonella* and eight *Campylobacter* strains together with susceptibility testing of the reference strains ATCC 25922 *E. coli* and ATCC 33560 *Campylobacter jejuni*. All the strains you will receive are non-toxin producing human pathogens of Class II.

The reference strains included are original CERTIFIED cultures of the ATCC 25922 (CCM 3954) *E. coli* and the ATCC 33560 (CCM 6214) *Campylobacter jejuni*. These original certified strains are free of charge. Please take proper care of the strains. Handle and maintain them as suggested in the enclosed manual. Please use them for future internal quality control for susceptibility testing in your laboratory. The reference strains will not be included in the years to come.

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 by different laboratories on *Salmonella* and *Campylobacter* and to harmonise the breakpoints used within the EU.

Outline of the EQAS 2006

Shipping, receipt and storage of strains

In October 2006 all EU appointed National Reference Laboratories would receive a parcel containing eight *Salmonella* and eight *Campylobacter* strains as well as the reference strains *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560 from DFVF. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material. The strains are shipped as stab cultures except for the *Campylobacter* strains, which are lyophilised. Please keep strains refrigerated. On arrival, the cultures must be subcultured and ensured proper storage conditions until testing. A suggested procedure for reconstitution of lyophilized *Campylobacter* is presented below.

Suggested procedure for reconstitution of the lyophilized *Campylobacter* strains:

- 1) Open, take out some of the material and dissolve in 1/2 ml broth. Leave it for 10 minutes. Spread 1 loop or 1 swab of the solution on blood agar. Incubate microaerophilic for 24-48 h at 37°C or 42°C.
- 2) Take rest of the broth (with the dissolved material) and incubate microaerophilic as mentioned above with parafilm on top. After incubation spread on blood agar and incubate microaerophilic again.
- 3) If you don't succeed with 1) or 2) take rest of the lyophilized material, and shake it directly onto blood agar. Add a little saline, and spread properly with a triangle or hockey stick. Incubate microaerophilic as mentioned above



Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the methods routinely used in the laboratory. *Salmonella*: Ampicillin or Amoxicillin (AMP/AMX), Amoxicillin + clavulanic acid (AUG), Cefotaxime (CTX), Ceftazidime (CAZ), Cefpodoxime (POD), Ceftiofur (XNL), Chloramphenicol (CHL), Ciprofloxacin or Enrofloxacin (CIP/ENRO), Florphenicol (FFN), Gentamicin (GEN), Nalidixic acid (NAL), Streptomycin (STR), Sulphonamide (SMX), Tetracycline (TET), Trimethoprim (TMP) and the combination of Sulphonamide and Trimethoprim (SXT).

All strains classified reduced susceptibility against CTX or CAZ (MIC >1) or resistance against XNL (MIC=>8) could be confirmed by confirmatory tests for ESBL production. The confirmatory tests for ESBL (CTX, CAZ) include tests for AmpC (FOX) and metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic and a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in the two cases (E-test) or a MIC ration =>8 or an increase in zone diameter =>5mm or signs of synergy the test is confirmed ESBL positive.

For *Campylobacter*: Chloramphenicol (CHL), Ciprofloxacin or Enrofloxacin (CIP/ENRO), Erythromycin (ERY), Gentamicin (GEN), Nalidixic acid (NAL), Streptomycin (STR) and Tetracycline (TET).

You may use Amoxicillin instead of Ampicillin, and another fluoroquinolones as substitute for Ciprofloxacin.

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.

No reference to an interpretation guidelines have been enclosed as we wish you to use the breakpoints routinely used to determine the susceptibility categories in your laboratories. Please fill in the breakpoints used in the designated form at the end of the protocol.

In general we use the CLSI interpretation guidelines M100-S16 and M31-A2 or in special cases breakpoints developed by Eucast (www.eucast.org) or based on population distribution when determined the reference data values of this EQAS. These antimicrobials are Cefpodoxime (POD) S<=0,5; I = 1; R=>2. Streptomycin (STR) S<=8; I = 16; R=>32. Florfenicol (FFN) S<=8; I=16; R=>32. Ceftiofur (XNL) S<=2; I=4; R=>8. Please follow the guidelines according to CLSI M100-S16 table 2A when testing cephalosporins.

Reporting of results and evaluation

Fill in your results in the enclosed test form. Please enter your results into the interactive web database <http://thor.dfvf.dk/crl> 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. Enclosed you will find a detailed description of how to enter the results into the web database. Please read the description before entering the web database. You can find your username and password in the letter following the parcel. **Please submit results by latest December the 1st 2006.**



If you do not have access to the Internet or experience difficulties entering the data, please return results by e-mail, fax or mail to DFVF. Finally, a summary report with all results will be performed and made available. Finally, a summary report with all results will be performed and made available

If you have any questions, please don't hesitate to contact me:

Rene Hendriksen
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27 Bülowsvej, DK-1790 Copenhagen V
Denmark
Tel: +45 7234 6288
Fax: +45 7234 6001
E-mail: RSH@DFVF.DK



Description of how to enter results etc. in the interactive database

Please read these two pages 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 forth and back by using the forward and back keys or click on the CRL logo.

- 1) You enter the EU CRL AR EQAS 2006 start web page (<http://thor.dfvf.dk/crl>) then
 - Write your username and password in low cases and press enter. *You can find your username and password in the letter following your parcel. Your username and password will be the same in future trials.*
 - Click on either “*Salmonella* test results” or “*Campylobacter* test results” depending on the extend of your results. The below description is aimed at *Salmonella* entry but are the exact the same as for *Campylobacter* entry
 - Click on “Start of Data Entry - Methods and Breakpoints for Salm.”

- 2) In the next page you navigate to fields with the Tab-key and mouse
 - Fill in what kind of method have been used for the susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays etc.
 - Fill in whether you used the NCCLS guidelines and breakpoints or not.
 - Fill in the actually breakpoints used in this test to determine the susceptibility category. Remember to use the operator keys in order to show – equal to, less than, less or equal to, greater than or greater or equal to

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"**

- 3) In the data entry pages for each *Salmonella* and *Campylobacter* strain, you enter
 - The read value and the interpretation as R, I or S.
 - **Click on "save and go to next page"**

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

- 4) In the data entry page of the reference strains “*E. coli* ATCC 22925 reference strain” and “*C.jejuni* ATCC 33560 reference strain”:

- Enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc.
- **click on "Save and go to next page"**

- 5) This page is a menu, from where you can review the input pages, approve your input and finally

see and print the evaluated results:

- Go through the pages 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.*



- 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 EU CRL AR EQAS 2006 interactive database, but allows you to see the evaluated results.**
- See the evaluated results. You can print each page. *You may have to choose a smaller text size to print the whole screen on one piece of paper. In the Internet Explorer (or the Internet program you may have), you click on "view", "text size" and e.g. "smallest".*

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#18	1. Strains	CRL S.1,2	Ceftiofur, XNL	I	S	Minor
			Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,3	Cefotaxime, CTX	I	R	Minor
			Ceftiofur, XNL	I	R	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,5	Ceftiofur, XNL	I	S	Minor
		CRL S.1,8	Streptomycin, STR	R	I	Minor
	2. Refstr.	ATCC 25922	Ceftiofur, XNL	22	26-31	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance		
#19	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	I	S	Minor		
			Chloramphenicol, CHL	S	R	Very major		
			Florphenicol, FFN	I	R	Minor		
			TMP+SMX, SXT	I	S	Minor		
			CRL S.1,2	Cefotaxime, CTX	R	S	Major	
				Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major	
				Florphenicol, FFN	I	S	Minor	
				Gentamicin, GEN	S	R	Very major	
				Tetracycline, TET	R	S	Major	
				CRL S.1,3	Amoxicillin cl., AUG	I	S	Minor
					Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
					CTX/CL:CTX incr. in zone dia.		ESBL	Very major
				CRL S.1,4	Amoxicillin cl., AUG	I	S	Minor
					Streptomycin, STR	S	R	Very major
					Tetracycline, TET	S	R	Very major
				CRL S.1,5	Cefpodoxime, POD	S	I	Minor
					Tetracycline, TET	I	S	Minor
				CRL S.1,6	Amoxicillin cl., AUG	I	S	Minor
					Streptomycin, STR	I	R	Minor
				CRL S.1,7	Florphenicol, FFN	S	R	Very major
					Gentamicin, GEN	S	R	Very major
				CRL S.1,8	Streptomycin, STR	S	I	Minor
					Sulfonamides, SMX	S	R	Very major
					TMP+SMX, SXT	S	R	Very major

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#20	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	R	S	Major
			TMP+SMX, SXT	I	S	Minor
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Florphenicol, FFN	I	S	Minor
			Gentamicin, GEN	I	R	Minor
			Tetracycline, TET	I	S	Minor
		CRL S.1,3	Amoxicillin cl., AUG	I	S	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,4	Amoxicillin cl., AUG	I	S	Minor
			Streptomycin, STR	S	R	Very major
		CRL S.1,5	Cefpodoxime, POD	S	I	Minor
			Tetracycline, TET	I	S	Minor
		CRL S.1,6	Amoxicillin cl., AUG	R	S	Major
			Streptomycin, STR	I	R	Minor
		CRL S.1,7	Amoxicillin cl., AUG	I	S	Minor
CRL S.1,8	Streptomycin, STR	S	I	Minor		

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#21	1. Strains	CRL S.1,2	Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,3	Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Streptomycin, STR	R	S	Major
		CRL S.1,6	Amoxicillin cl., AUG	I	S	Minor
			TMP+SMX, SXT	S	R	Very major
		CRL S.1,7	Amoxicillin cl., AUG	I	S	Minor
		CRL S.1,8	Chloramphenicol, CHL	I	S	Minor
			Streptomycin, STR	R	I	Minor
		2. Refstr.	ATCC 25922	Gentamicin, GEN	4	,25-1

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#22	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	I	S	Minor
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
			Gentamicin, GEN	S	R	Very major
		CRL S.1,3	Cefotaxime, CTX	I	R	Minor
			Ceftiofur, XNL	I	R	Minor
		CRL S.1,4	Amoxicillin cl., AUG	I	S	Minor
			Streptomycin, STR	I	R	Minor
		CRL S.1,6	Amoxicillin cl., AUG	I	S	Minor
			Streptomycin, STR	I	R	Minor
		CRL S.1,7	Amoxicillin cl., AUG	I	S	Minor

User	Method	Strain	Antibiotic	Obtained	Expected	Importance	
#23	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	R	S	Major	
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor	
			Tetracycline, TET	R	S	Major	
		CRL S.1,3	Amoxicillin cl., AUG	R	S	Major	
			Streptomycin, STR	I	S	Minor	
		CRL S.1,4	Amoxicillin cl., AUG	R	S	Major	
			Cipro- enrofloxacin, CIP/ENRO	R	S	Major	
			Streptomycin, STR	I	R	Minor	
		CRL S.1,6	Amoxicillin cl., AUG	R	S	Major	
		CRL S.1,7	Amoxicillin cl., AUG	R	S	Major	
		2. Refstr.	ATCC 25922	Amoxicillin cl., AUG	8	18-24	
				Ceftiofur, XNL	25	26-31	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#24	1. Strains	CRL S.1,1	Chloramphenicol, CHL	S	R	Very major
			Cipro- enrofloxacin, CIP/ENRO	R	S	Major
			Florphenicol, FFN	S	R	Very major
			Gentamicin, GEN	R	S	Major
			Nalidixic acid, NAL	R	S	Major
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#25	1. Strains	CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
		CRL S.1,4	CAZ/CL:CAZ mic ratio		ESBL	Very major

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#26	1. Strains	CRL S.1,2	Cipro- /enrofloxacin, CIP/ENRO	I	R	Minor
			Gentamicin, GEN	I	R	Minor
		CRL S.1,3	Cipro- /enrofloxacin, CIP/ENRO	I	R	Minor
		CRL S.1,8	Chloramphenicol, CHL	I	S	Minor

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#27	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	R	S	Major
		CRL S.1,2	Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,3	Amoxicillin cl., AUG	I	S	Minor
			Cefotaxime, CTX	I	R	Minor
			Ceftazidime, CAZ	S	R	Very major
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,4	Amoxicillin cl., AUG	I	S	Minor
			Gentamicin, GEN	R	S	Major
		CRL S.1,5	Cefotaxime, CTX	R	S	Major
			Ceftazidime, CAZ	R	S	Major
			CTX/CL:CTX mic ratio	ESBL		Major
		CRL S.1,6	Amoxicillin cl., AUG	R	S	Major
			Gentamicin, GEN	R	S	Major
		CRL S.1,7	Amoxicillin cl., AUG	R	S	Major
	2. Refstr.	ATCC 25922	Cefotaxime, CTX	0.125	,03-,12	
			Trimethoprim, TMP	0.25	,5-2	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#28	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	I	S	Minor
			Chloramphenicol, CHL	S	R	Very major
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,3	Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,4	Streptomycin, STR	I	R	Minor
			Tetracycline, TET	S	R	Very major
		CRL S.1,6	Amoxicillin cl., AUG	I	S	Minor
		CRL S.1,8	Tetracycline, TET	S	R	Very major

User	Method	Strain	Antibiotic	Obtained	Expected	Importance	
#29	1. Strains	CRL S.1,1	Ceftiofur, XNL	I	S	Minor	
			Nalidixic acid, NAL	R	S	Major	
				TMP+SMX, SXT	I	S	Minor
			CRL S.1,2	Florphenicol, FFN	R	S	Major
				Tetracycline, TET	R	S	Major
			CRL S.1,3	Chloramphenicol, CHL	I	S	Minor
				Florphenicol, FFN	R	S	Major
				Streptomycin, STR	I	S	Minor
				Sulfonamides, SMX	R	S	Major
			CRL S.1,4	Florphenicol, FFN	R	S	Major
				Nalidixic acid, NAL	I	S	Minor
			CRL S.1,5	Ceftiofur, XNL	I	S	Minor
				Nalidixic acid, NAL	I	S	Minor
				Tetracycline, TET	R	S	Major
			CRL S.1,6	Cefotaxime, CTX	S	R	Very major
				Ceftazidime, CAZ	S	R	Very major
				Ceftiofur, XNL	S	R	Very major
				Nalidixic acid, NAL	I	S	Minor
				Tetracycline, TET	R	S	Major
			CRL S.1,7	Nalidixic acid, NAL	I	S	Minor
	CRL S.1,8	Florphenicol, FFN	I	S	Minor		
		Streptomycin, STR	R	I	Minor		
	2. Refstr.	ATCC 25922	Ceftiofur, XNL	25	26-31		
			Cefotaxime, CTX	28	29-35		
			Chloramphenicol, CHL	20	21-27		
			Florphenicol, FFN	17	22-28		
			Sulphonamides, SMX	6	15-23		

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#30	1. Strains	CRL S.1,1	Amoxicillin cl., AUG	I	S	Minor
			Nalidixic acid, NAL	I	S	Minor
		CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Florphenicol, FFN	I	S	Minor
			Tetracycline, TET	I	S	Minor
		CRL S.1,3	Amoxicillin cl., AUG	R	S	Major
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Streptomycin, STR	I	S	Minor
		CRL S.1,4	Amoxicillin cl., AUG	R	S	Major
			Streptomycin, STR	I	R	Minor
		CRL S.1,5	Cefpodoxime, POD	S	I	Minor
			Tetracycline, TET	I	S	Minor
		CRL S.1,6	Amoxicillin cl., AUG	R	S	Major
		CRL S.1,8	Streptomycin, STR	R	I	Minor
			Sulfonamides, SMX	S	R	Very major
			TMP+SMX, SXT	S	R	Very major

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#33	1. Strains	CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
		CRL S.1,4	Streptomycin, STR	S	R	Very major
		CRL S.1,8	Streptomycin, STR	S	I	Minor
			Sulfonamides, SMX	S	R	Very major
	2. Refstr.	ATCC 25922	Cipro/enroflox., CIP/ENRO	0.03	,004-,015	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#34	1. Strains	CRL S.1,2	Amoxicillin cl., AUG	S	I	Minor
			Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Gentamicin, GEN	S	R	Very major
			Streptomycin, STR	S	R	Very major
		CRL S.1,3	Cefotaxime, CTX	S	R	Very major
			Ceftazidime, CAZ	S	R	Very major
			Ceftiofur, XNL	S	R	Very major
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,4	Streptomycin, STR	S	R	Very major
		CRL S.1,5	Streptomycin, STR	S	R	Very major
		CRL S.1,6	Streptomycin, STR	S	R	Very major
		CRL S.1,7	Gentamicin, GEN	S	R	Very major
		CRL S.1,8	Streptomycin, STR	S	I	Minor
2. Refstr.	ATCC 25922	Amoxicillin cl., AUG	29	18-24		
		Ceftazidime, CAZ	38	25-32		
		Cefotaxime, CTX	43	29-35		
		Gentamicin, GEN	27	19-26		
		Nalidixic acid, NAL	33	22-28		
		Sulphonamides, SMX	28	15-23		
		Tetracycline, TET	29	18-25		
		TMP+SMX, SXT	34	23-29		

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#35	1. Strains	CRL S.1,2	Chloramphenicol, CHL	S	I	Minor
			Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
		CRL S.1,3	Cipro- /enrofloxacin, CIP/ENRO	S	R	Very major
			Streptomycin, STR	R	S	Major
		CRL S.1,5	Amoxi-/Ampicil. AMX/AMP	R	S	Major
	2. Refstr.	ATCC 25922	Amoxicillin cl., AUG	22	2-8	
			Ceftazidime, CAZ	31	,06-,5	
			Trimethoprim, TMP	26	,5-2	
			Cefoxitin, FOX	26	2-8	
			Imipenem, IMI	31	,06-,25	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#29	1. Strains	CRL C.1,1	Erythromycin, ERY	R	S	Major
			Streptomycin, STR	R	S	Major
		CRL C.1,2	Streptomycin, STR	I	S	Minor
		CRL C.1,3	Streptomycin, STR	S	R	Very major
		CRL C.1,6	Erythromycin, ERY	I	S	Minor
			Streptomycin, STR	I	S	Minor
2. Refstr.	ATCC 33560		Chloramphenicol, CHL	30	1-8	
			Erythromycin, ERY	19	,5-2	
			Gentamicin, GEN	14	,5-2	
			Nalidixic acid, NAL	20	4-16	
			Tetracycline, TET	30	,25-2	

User	Method	Strain	Antibiotic	Obtained	Expected	Importance	
#30	1. Strains	CRL C.1,1	Cipro/enrofloxacin, CIP/ENRO	S	R	Very major	
		CRL C.1,7	Streptomycin, STR	S	R	Very major	
			Tetracycline, TET	R	S	Major	
		CRL C.1,8	Erythromycin, ERY	S	R	Very major	
	2. Refstr.	ATCC 33560		Chloramphenicol, CHL	34	1-8	
				Cipro/enrofloxacin, CIP/ENRO	26	,06-,25	
				Erythromycin, ERY	30	,5-2	
				Nalidixic acid, NAL	24	4-16	
			Tetracycline, TET	34	,25-2		

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#34	1. Strains	CRL C.1,6	Streptomycin, STR	R	S	Major

User	Method	Strain	Antibiotic	Obtained	Expected	Importance
#35	1. Strains	CRL C.1,6	Chloramphenicol, CHL	R	S	Major
		CRL C.1,7	Chloramphenicol, CHL	R	S	Major