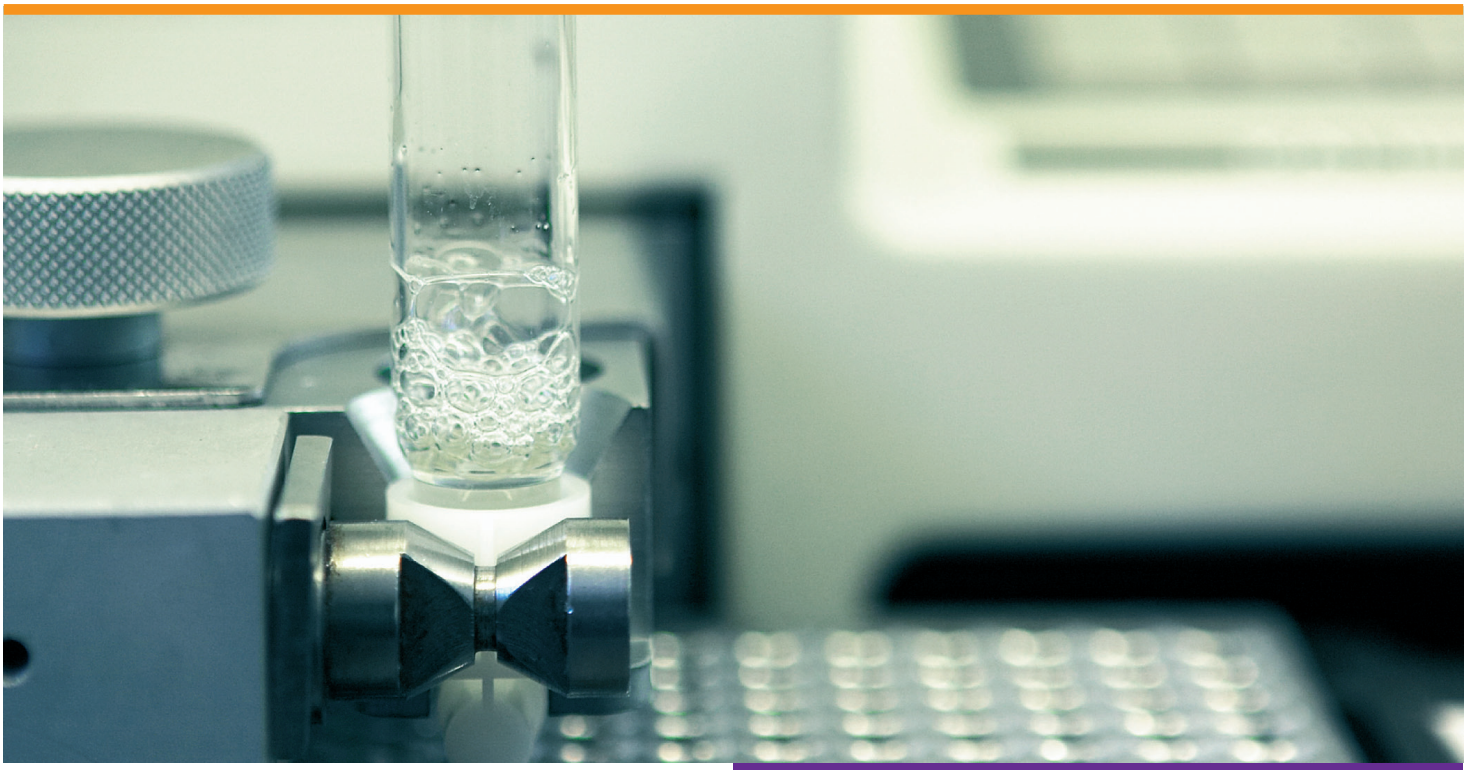
The second CRL Proficiency Testing enterococci, staphylococci and *E. coli* 2007



DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

Community Reference Laboratory – Antimicrobial Resistance

THE SECOND CRL-AR PROFICIENCY TESTING ENTEROCOCCI, STAPHYLOCOCCI AND *E. COLI* - 2007

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1. INTRODUCTION

In this report, results of the proficiency test trial – the External Quality Assurance System (EQAS) 2007- concerning *E. coli*, Enterococci and Staphylococci are summarised. The National Food Institute (DTU Food) appointed as Community Reference Laboratory on Antimicrobial Resistance (CRL-AR) by the European Commission (EC) conducts the EQAS. 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.

In the light of results from former EQAS conducted by the CRL-AR, an acceptance level for each laboratory of a maximum of 7% deviations has been decided.

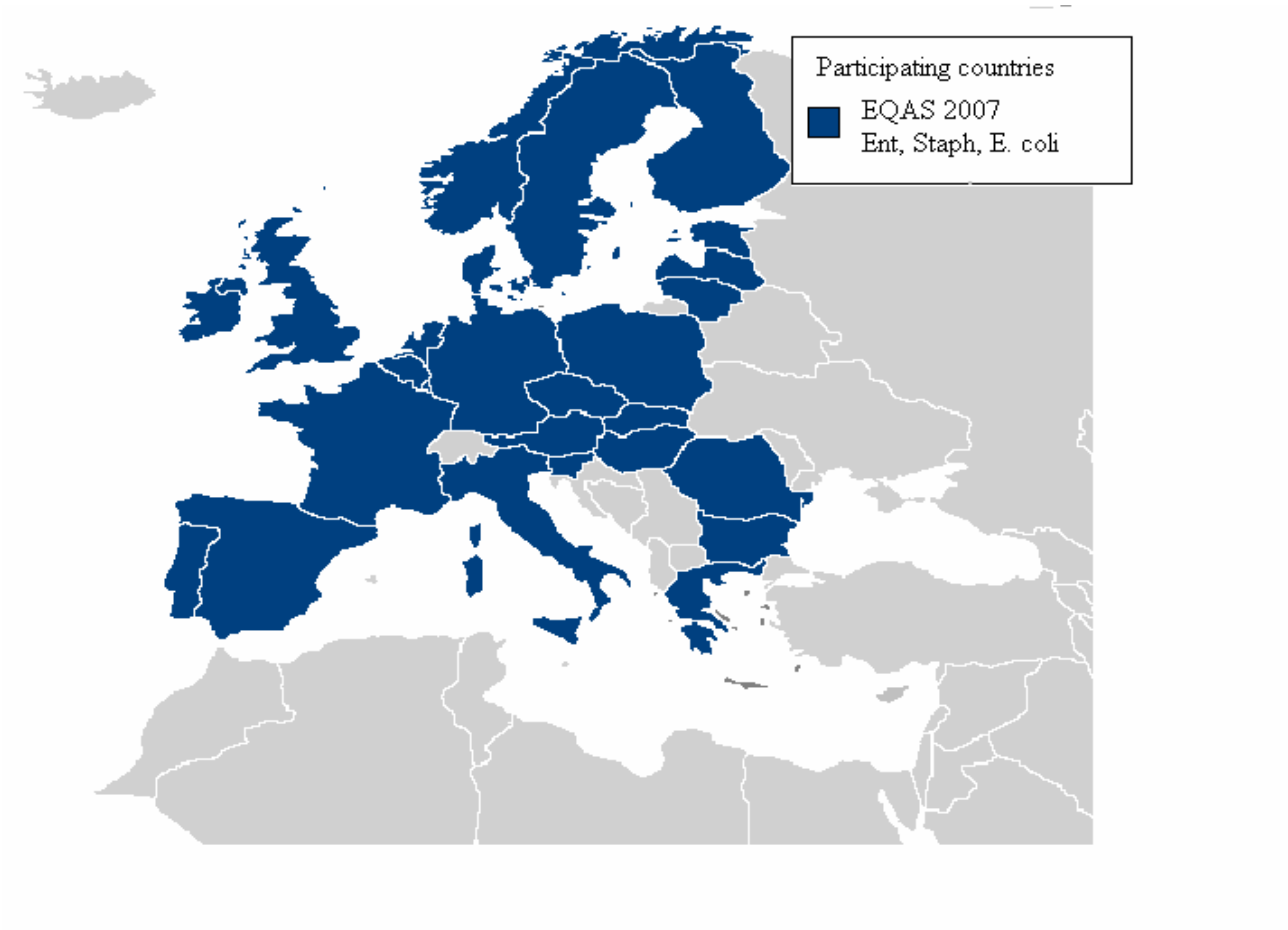
2. MATERIALS AND METHODS

2.1 Participants

A pre-notification to announce the EQAS on susceptibility testing of Enterococci, Staphylococci and *E. coli* was distributed on the 15th of May 2007 by e-mail to the 34 National Reference Laboratories (NRL) within the EU and Norway (App.1). This includes all EU countries except Malta. Luxembourg has designated Belgium as NRL. (App.2). Twenty-seven of the NRLs were appointed by the individual member states. The remaining five NRLs were not designated yet but enrolled on equal terms as the designated NRLs based on their participation in a previous EU funded concerned action (FAIR5-QLK2-2002-01146), ARBAO II project (Antibiotic resistance in bacteria of animal origin). Figure 1 illustrates that 25 member states participated; 26 laboratories analysed the Enterococci strains, 31 the Staphylococci strains and 30 the *E. coli* strains.

Cyprus asked for permission to postpone their participation in CRL-EQAS until their laboratory staff had participated in a workshop at CRL-AR in Copenhagen. The workshop founded by TAIEX was held for three participants from the Bacteriology - Serology Laboratory (BSL), Cyprus in week 38-2007.

Figure 1. Participating countries.



2.2 Strains

Eight strains of Enterococci, eight strains of Staphylococci and eight strains of *E. coli* were selected for this trial from DTU, Food's strain collection. The antimicrobial susceptibility testing (AST) on the strains were performed at DTU Food and the obtained MIC values served as reference for the EQAS. (App. 3). U.S. Food and Drug Administration (FDA), Centre for Veterinary Medicine, verified the susceptibility patterns for the strains prior to distribution. Individual sets of the strains were inoculated as agar stab cultures and subsequently send to the participating laboratories.

All participating laboratories were provided with reference strains *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, and *S. aureus* ATCC 29213. Newly enrolled laboratories were also

provided with reference strain *E. coli* CCM 3954 ~ ATCC 25922, as the participants in the CRL EQAS *Salmonella/Campylobacter* 2006 had received the *E. coli* reference strain previously. The strains were purchased at the Czech Collection of Micro-organisms (CCM); The Czech Republic.

2.3 Antimicrobials

Table 1. The antimicrobials utilized by CRL-AR according to the agreement with the participants at the CRL-workshop in 2007 in Copenhagen.

Enterococci	Staphylococci*	*E. coli*
Ampicillin ¤	Chloramphenicol	Ampicillin ¤
Chloramphenicol ¤	Ciprofloxacin	Amoxicillin + clavulanic acid
Avilamycin	Erythromycin	Cefotaxime ¤
Ciprofloxacin	Florfenicol	Cefotaxime + clavulanic acid
Daptomycin	Gentamicin	Cefoxitin
Erythromycin ¤	Penicillin	Cefpodoxime
Florfenicol	Streptomycin	Ceftazidime
Gentamicin ¤	Sulfonamides	Ceftazidime + clavulanic acid
Linezolid ¤	Tetracycline	Ceftiofur
Streptomycin ¤	Trimethoprim	Chloramphenicol ¤
Quinpristin-dalfopristin ¤		Ciprofloxacin ¤
Tetracycline ¤		Florfenicol
Tigecycline		Gentamicin ¤
Vancomycin ¤		Imipenem
		Imipenem + EDTA
		Nalidixic acid ¤
		Streptomycin ¤
		Sulphonamides ¤
		Tetracycline ¤
		Trimethoprim ¤
		Trimethoprim+ sulphonamides

¤ EFSA recommended antimicrobials to be included in the antimicrobial resistance monitoring.

*EFSA does not recommend specific antimicrobials for resistance monitoring of Staphylococci.

Guidelines for the AST 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.

MIC determinations at the CRL-AR were performed using the Sensititre systems from Trek diagnostics Ltd. For the ESBL analysis of the *E. coli* strains the E-test from AB-Biodisk was used. The cut off values used by CRL-AR in the interpretation of the MIC results are developed by EUCAST (www.eucast.org). and recommended by EFSA. (App.6). Antimicrobials used for detection of ESBL should be interpreted clinically according to recommendations from CLSI.

The participants at the CRL- workshop in Copenhagen in May 2007 decided that the NRLs gradually should harmonise their AST analyses in agreement with the MIC method and the antimicrobial panel and cut-off values recommended by EFSA and used by CRL-AR.

2.4 Distribution

The documents (App. 4a,b, c,d) were send to the participants by email and the cultures send in double pack containers (class UN 6,2) 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 airway bill (AWB) number for tracking of the parcel and pick up at the airport

2.5 Procedure

At receipt, the laboratories were instructed to place the tubes in a refrigerator and subculture the strains, in accordance with the protocol, prior to performing the antimicrobial susceptibility test (App. 4). The laboratories were in this EQAS asked to apply the method currently used. For MIC the participants were asked to use the cut off values listed in the protocol (App. 4b). The results should be categorised only as resistant or sensitive. Furthermore, the laboratories were requested to save and maintain the ATCC reference strains for quality assurance.

The laboratories were instructed to enter the results to an electronic record sheet in the CRL-AR web based database through a secured individual login and passwords. Alternatively to send the

record sheets from the enclosed protocol by fax to CRL-AR. The website was open for entry in the period from the 27th of June 2007 to the 18th of august 2007.

Participants using disk diffusion were recommended to interpret the results according to the individually routinely used breakpoints and categorise the results as resistant or sensitive. The laboratories were also asked to submit the breakpoints used to the web-based database (App. 5).

In addition, the laboratories entered also the zone diameter in millimetres or MIC value of the reference strains. The results were individually compared to the quality control ranges according to: CLSI documents M31-A2 (2002) / M100-S17 (2007); The Sensititre System, Trek Diagnostic or E-tests, AB-Biodisk (App. 7).

After submitting the data, the laboratories were instructed to retrieve an instant generated individual report, which evaluated the submitted results, from the secured web site. All deviations from the expected were reported. The questionnaire and the evaluation form (App. 4) were send by email to CRL-AR and later collected and summarised (App.8, 9).

3. RESULTS

3.1 Methods used by EQAS-participants.

In the Enterococci trials, 15 laboratories used MIC determination and 11 laboratories used disk diffusion. In the Staphylococci trials, 14 laboratories used MIC determination, two used E-test and 15 laboratories used disk diffusion. In the *E. coli* trials, 15 laboratories used MIC determination, one used E-test and 14 laboratories used disk diffusion.

3.2 Deviations by strain and antibiotic

Fig. 2 Deviations per strain

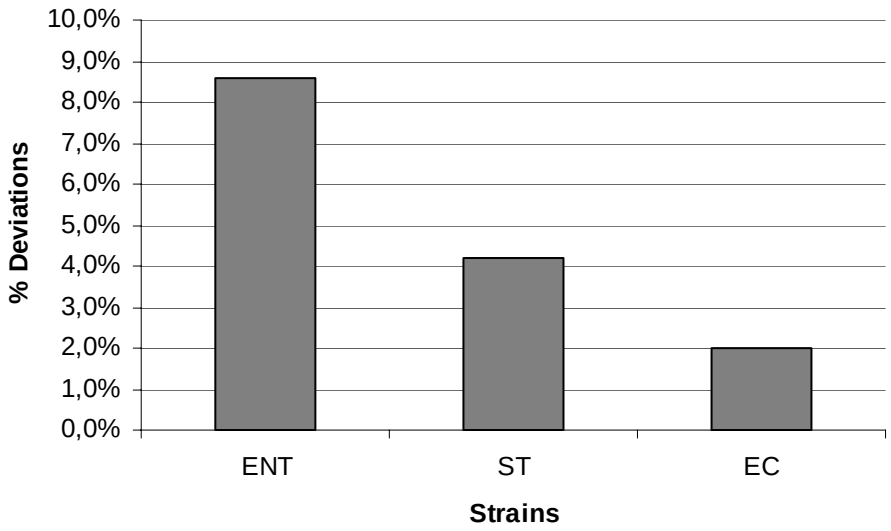
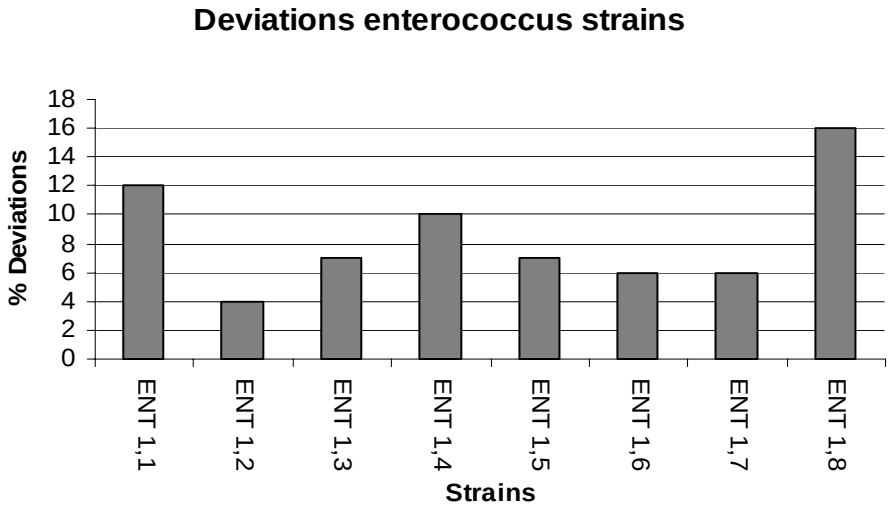


Figure 2 illustrates the percentage of deviations from the expected results of AST performed by participating laboratories. For the Entercocci (ENT) strains 91.4% of AST's were interpreted correct, for the Staphylococci (ST) strains 95.8% of AST's were correct and for the *E. coli* (EC) strains, 98.0% of the AST's were correct.

Enterococci

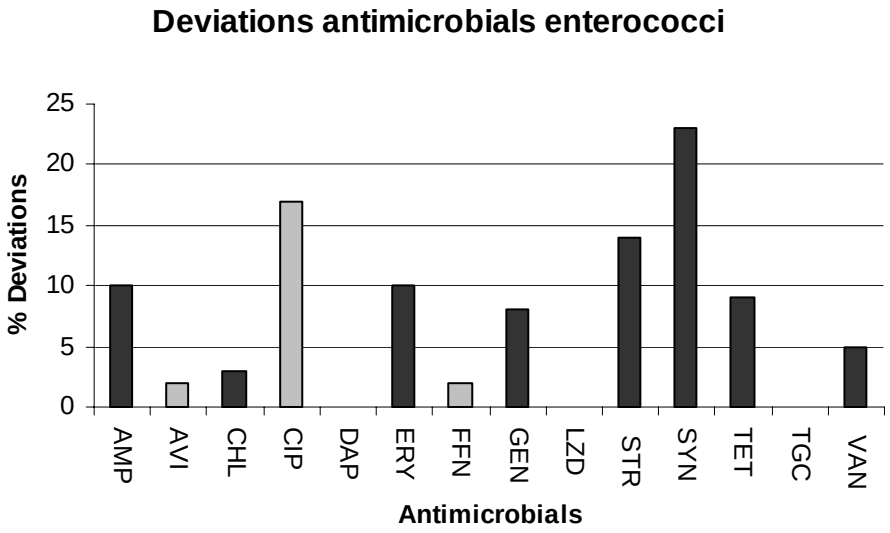
Fig 3.



As illustrated in figure 3, significant deviations (>= 5% for each strain) were observed for seven Enterococci strains:

- ENT 1.1. 12% deviations
- ENT 1.3. 7% deviations
- ENT 1.4. 10% deviations
- ENT 1.5. 7% deviations
- ENT 1.6. 6% deviations
- ENT 1.7. 6% deviations
- ENT 1.8. 16% deviations.

Fig. 4



EFSA recommended antimicrobials to be included in the antimicrobial resistance monitoring

As illustrated in figure 4, significant deviations (>= 5% for each antimicrobial) were observed for eight antimicrobials in the AST for Enterococci. Seven of the antimicrobials are recommended by EFSA for antimicrobial resistance monitoring:

- Ampicillin 10% deviations
- Erythromycin 10% deviations
- Gentamycin 8% deviations
- Streptomycin 14% deviations
- Quinpristin-dalfopristin (SYN) 23 % deviations
- Tetracycline 9% deviations
- Vancomycin 5% deviations

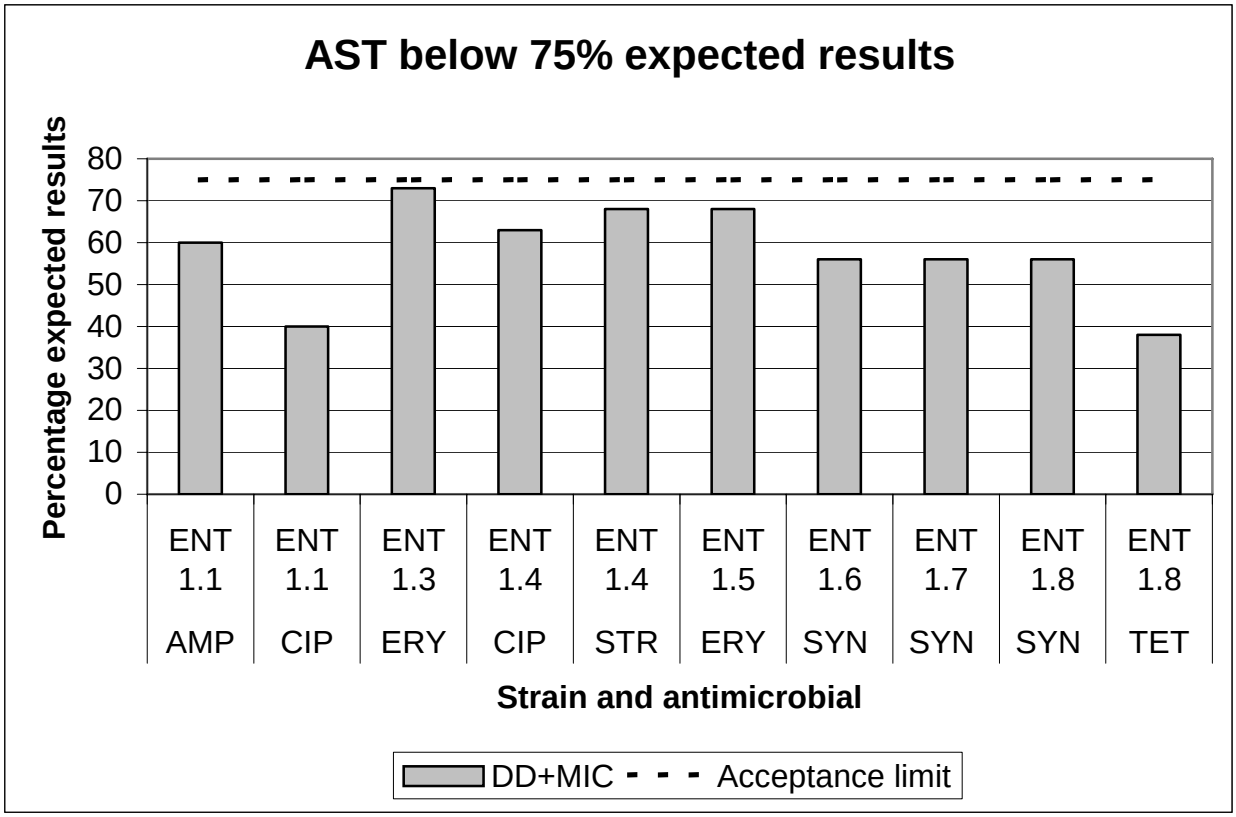
The total numbers of deviations and the value of the deviations concerning ciprofloxacin (not included in the EFSA recommended panel) and quinpristin-dalfopristin (SYN) were very high. The reason might be that ciprofloxacin in some cases could be considered as borderline by disk diffusion and quinpristin-dalfopristin has two different break points, for *E. faecium* and *E. faecalis*, respectively. This might have caused misinterpretations by some of the laboratories.

Ten AST involving seven strains and five different antimicrobials had results below 75% of the expected results as shown in table 2 and figure 5:

Table 2

AST below 75% of the expected results		
Strain	**Antimicrobial**	**% expected results**
ENT 1.1	AMP	60%
	CIP	40%
ENT 1.3	ERY	73%
ENT 1.4	CIP	63%
	STR	68%
ENT 1.5	ERY	68%
ENT 1.6	SYN	56%
ENT 1.7	SYN	56%
ENT 1.8	SYN	56%
	TET	38%

Fig. 5



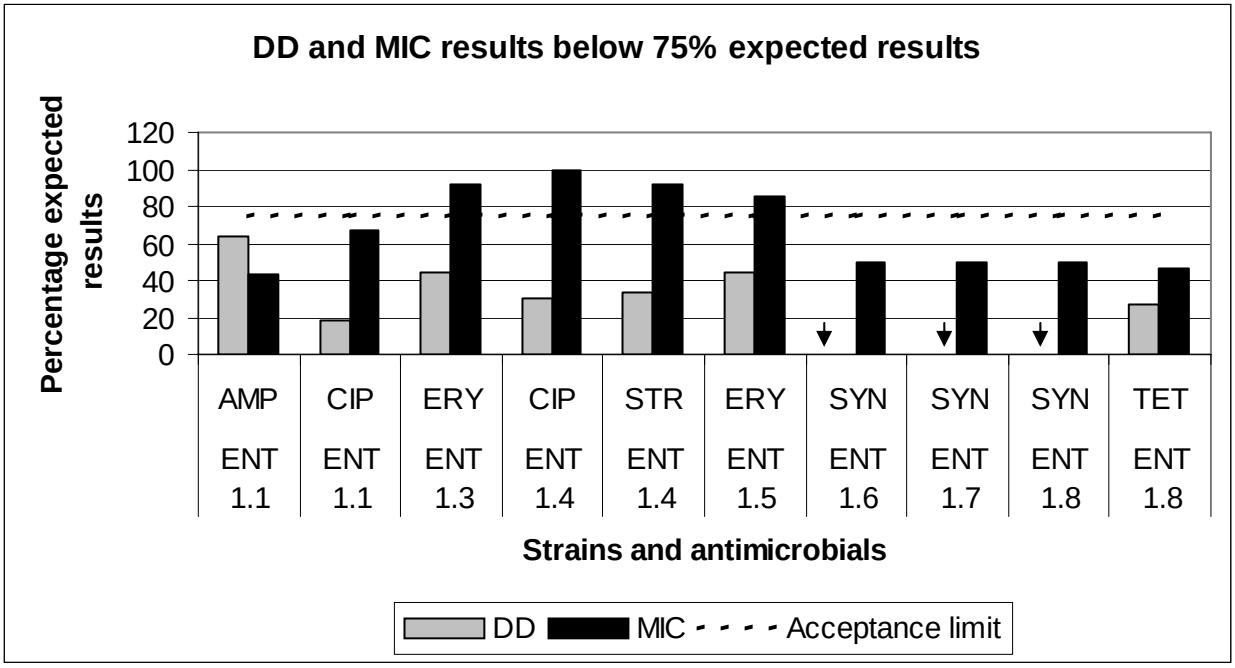
In table 3 and figure 6, the AST are divided in DD and MIC analyses. The arrows indicate that no column has been shown because of only one DD test.

Table 3

DD+MIC tests below 75% expected results					
		% Expected results		Number of tests	
Strain	Antimicrobial	DD	MIC	DD	MIC
ENT 1.1	AMP	64%	43%	11	14
	CIP	18%	67%	11	9
ENT 1.3	ERY	44%	92%	9	13
ENT 1.4	CIP	30%	100%	10	9
	STR	33%	92%	9	13
ENT 1.5	ERY	44%	85%	9	13
ENT 1.6	SYN	- *	50%	1	8
ENT 1.7	SYN	-*	50%	1	8
ENT 1.8	SYN	-*	50%	1	8
	TET	27%	46%	11	15

*Percentage not calculated (only one DD test)

Fig. 6

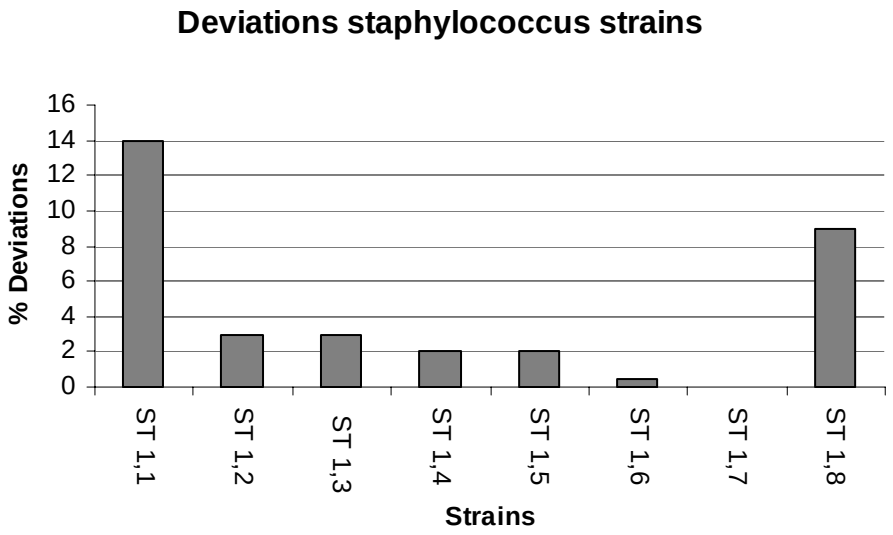


As shown in table 3 and figure 6, all MIC results - except for ampicillin in strain ENT 1.1 - are more in compliance with the expected results than DD. This agrees with the fact that MIC determination of enterococci has proved to be significantly better than disk diffusion (P=0.02) as stated in Paragraph 3.3.

The EQAS' results from AR-CRL showed no deviations from the expected results in any of the AST shown in table 2 and 3.

Staphylococci

Fig. 7

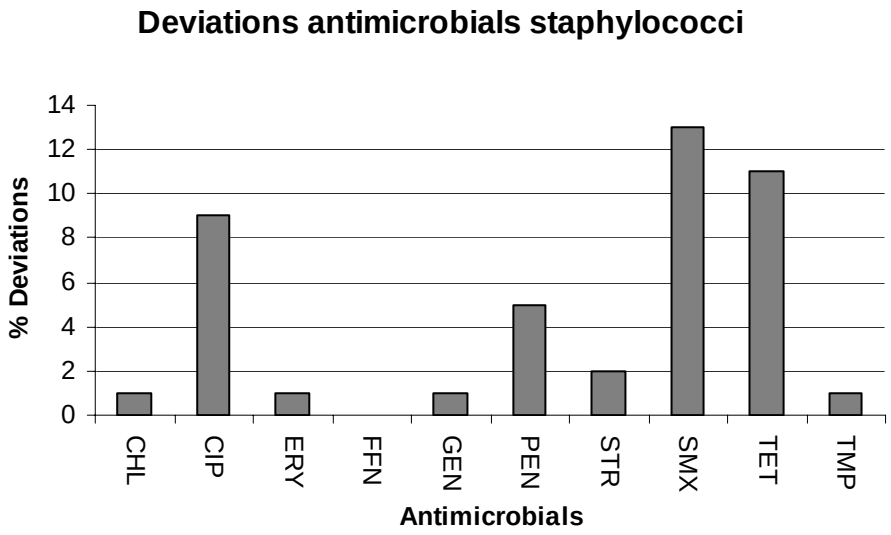


As illustrated in figure 7, significant deviations were observed for two staphylococcus strains in the AST:

- ST 1.1. 14% deviations
- ST 1.8. 9% deviations

The reason for the great deviation in strain ST 1.1. might be that the strain is resistant to methicillin and therefore according to CLSI M100-S17 table 2c should be interpreted resistant to penicillin even though the MIC value was found to be 0.12 (cut off 0.25) by the CRL-AR.

Fig. 8



EFSA does not recommend specific antimicrobials for resistance monitoring of Staphylococci.

As illustrated in figure 8, significant deviations were observed for four antimicrobials in the AST for Staphylococci:

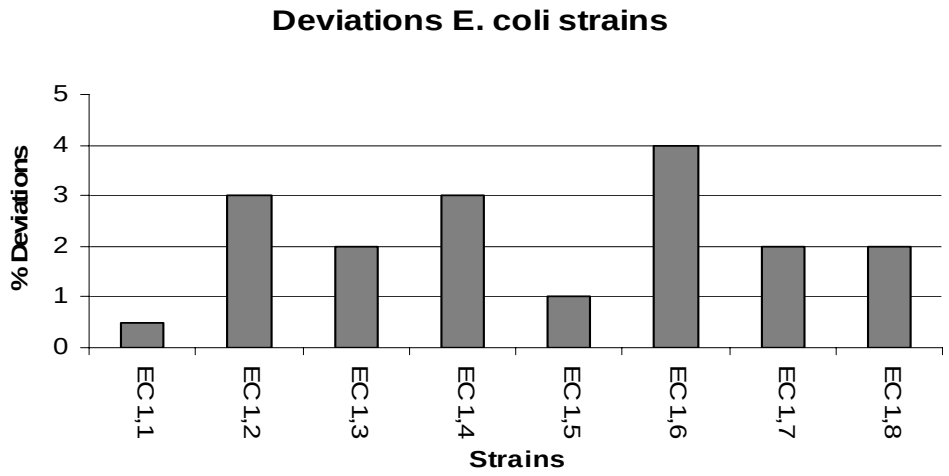
- Ciprofloxacin 9% deviations
- Penicillin 5% deviations
- Sulphonamide 13% deviations
- Tetracycline 11% deviations

The high percentage of deviations in sulphonamide could be observed in five strains, which obtained only between 72%-89% correct results. (App. 10). It is known that the interpretation of results from disk diffusion of sulphonamide is difficult because of false negative resistance and double zone on the agar.

MRSA is an emerging problem in animal farming and the participants could optionally analyse for MRSA positive strains. Strain ST 1.1 and ST 1.8 were MRSA positive. Twenty three laboratories analysed the strains for MRSA and four (17%) laboratories, #15, #17, # 34 and # 35 did not identify strain ST 1.1 or ST 1.8 correctly as MRSA.

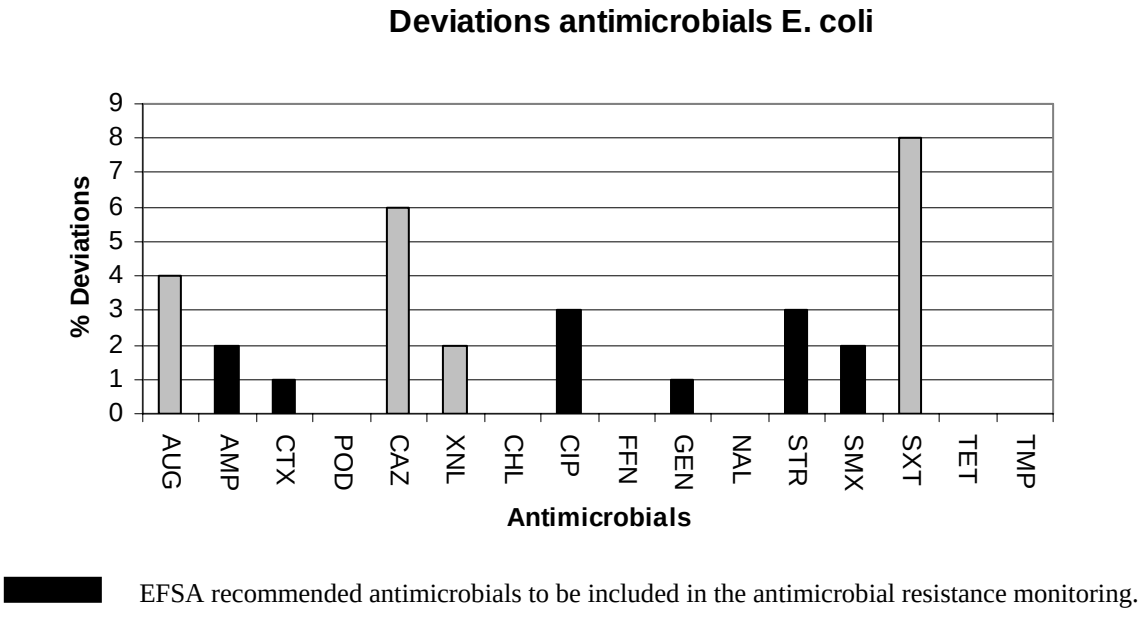
E. coli

Fig. 9



As illustrated in figure 9 no *E. coli* strains exhibited any significant deviations in the AST

Fig. 10



EFSA recommended antimicrobials to be included in the antimicrobial resistance monitoring.

As illustrated in figure 10, no antimicrobials, which EFSA recommends for antimicrobial resistance monitoring, show significant deviations while ceftazidime and the combination of sulphonamide and trimethroprim does.

ESBL producing organisms are also an emerging problem worldwide. The laboratories could optionally analyse for the ESBL producing *E. coli* strains according to the clinical guidelines described by CLSI. The guidelines specify that all the isolates should be interpreted resistant to all cephalosporins if it is interpreted resistant to one, regardless of the obtained results.

Table 4. ESBL producing strains

Lab #	Strain	Antimicrobial	Reading	ESBL	Expected result	Method
9	CRL EC.1,6	CAZ/CL:CAZ incr. in zone dia.	>= 5 mm	Pos	Pos	DD
		CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	
19	CRL EC.1,6	CAZ/CL:CAZ incr. in zone dia.	>= 5 mm	Pos	Pos	DD
		CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	
20	CRL EC.1,6	CAZ/CL:CAZ incr. in zone dia.	>= 5 mm	Pos	Pos	DD
		CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	
21	CRL EC.1,6	CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	DD
22	CRL EC.1,6	CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	DD
24	CRL EC.1,6	CAZ/CL:CAZ mic ratio	>= 8	Pos	Pos	MIC
		CTX/CL:CTX mic ratio	>= 8	Pos	Pos	
	CRL EC.1,7	CAZ/CL:CAZ mic ratio	< 8	Neg	Neg	
		CTX/CL:CTX mic ratio	>= 8	Pos	Neg	
25	CRL EC.1,6	CAZ/CL:CAZ mic ratio	>= 8	Pos	Pos	MIC
		CTX/CL:CTX mic ratio	>= 8	Pos	Pos	
27	CRL EC.1,6	CAZ/CL:CAZ mic ratio	>= 8	Pos	Pos	MIC
		CTX/CL:CTX mic ratio	>= 8	Pos	Pos	
34	CRL EC.1,6	CAZ/CL:CAZ incr. in zone dia.	>= 5 mm	Pos	Pos	DD
		CTX/CL:CTX incr. in zone dia.	>= 5 mm	Pos	Pos	

As shown in Table 4, nine laboratories (six using disk diffusion and three MIC determination) analysed the *E. coli* strains for ESBL production. All nine laboratories correctly identified strain 1,6 as ESBL producing. Lab # 24 identified in addition incorrectly strain EC 1,7 as ESBL producing.

AmpC. Strain EC 1.7 was AmpC positive. Eight out of nine laboratories, which analysed the EC-strains for AmpC, detected correctly the strain as AmpC. Lab # 20 analysed the strain as

non-AmpC. None of the nine laboratories which performed the AmpC analyse described the strain as ESBL producing.

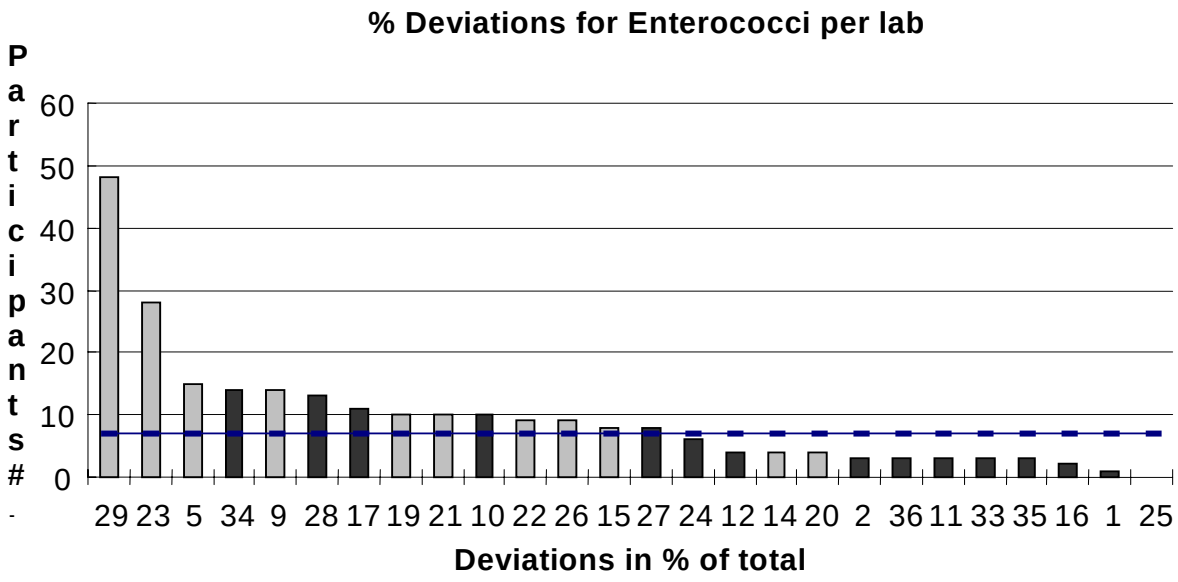
3.3 Deviations by laboratory

Figures 11, 13 and 15 illustrate the percentage of the deviations for each laboratory by strain. The laboratories are ranked after decreasing percentage of deviations.

In figures 12, 14 and 16, the numbers of laboratories are listed in intervals of percentages per total deviations.

Enterococci

Fig. 11



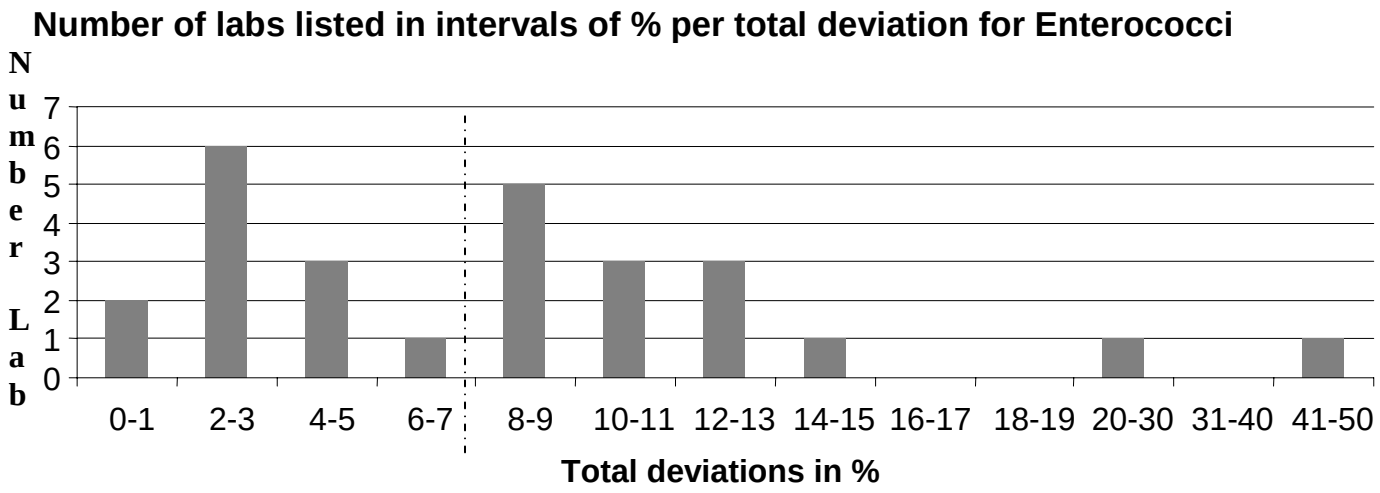
Disk diffusion

MIC determination

As illustrated in figure 11, fourteen laboratories had > 7 % deviations in the AST (dotted line). The percentage of deviations differed widely between the laboratories with a maximum of 48 % deviations in laboratory #29 and to a minimum of 0% deviations in laboratory #25. Nine of eleven laboratories with > 7% deviations used disk diffusion and four of fourteen laboratories

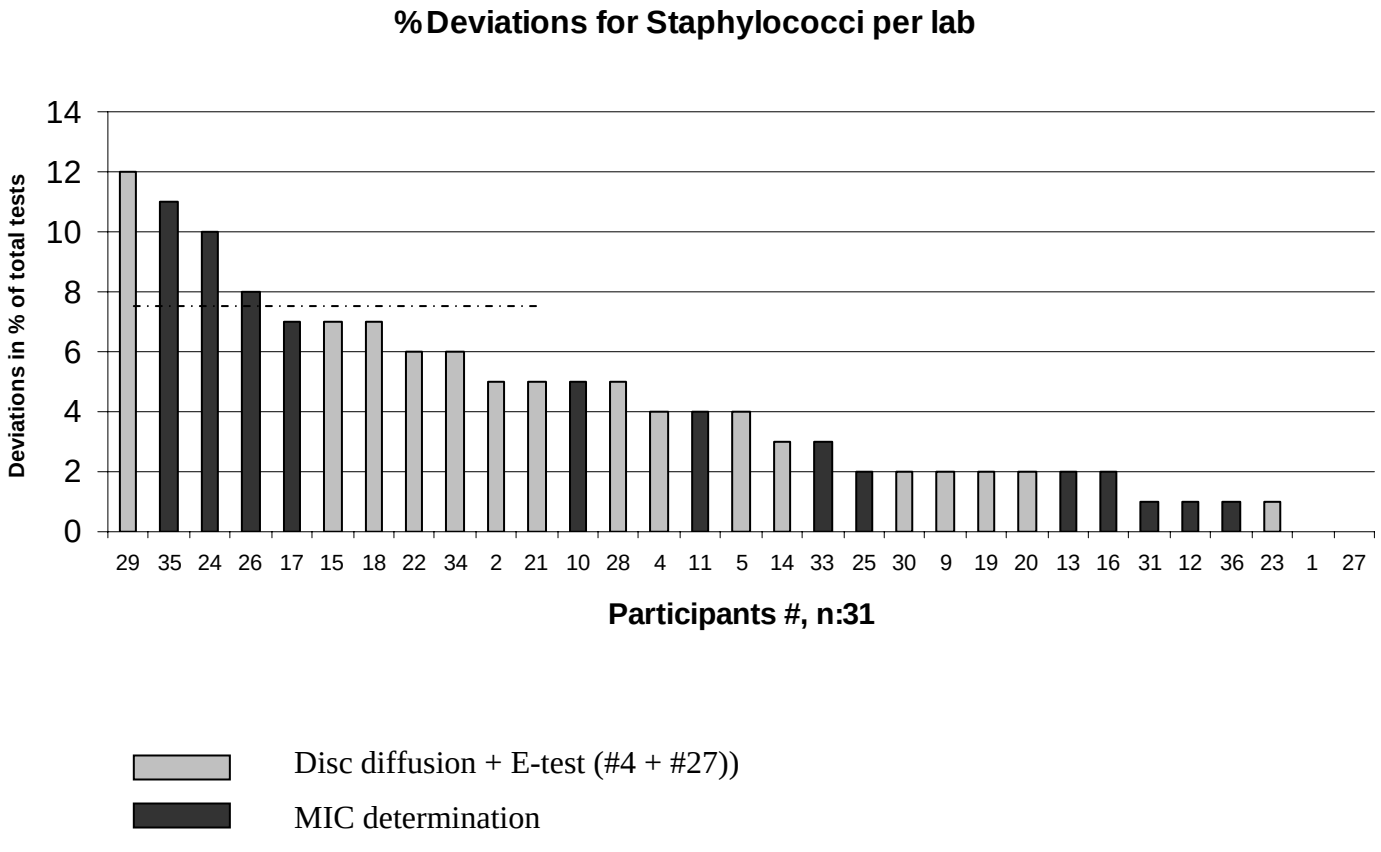
with > 7% deviations. The results obtained by using MIC determination was significantly better than the results from disk diffusion (P=0.02)

Fig. 12



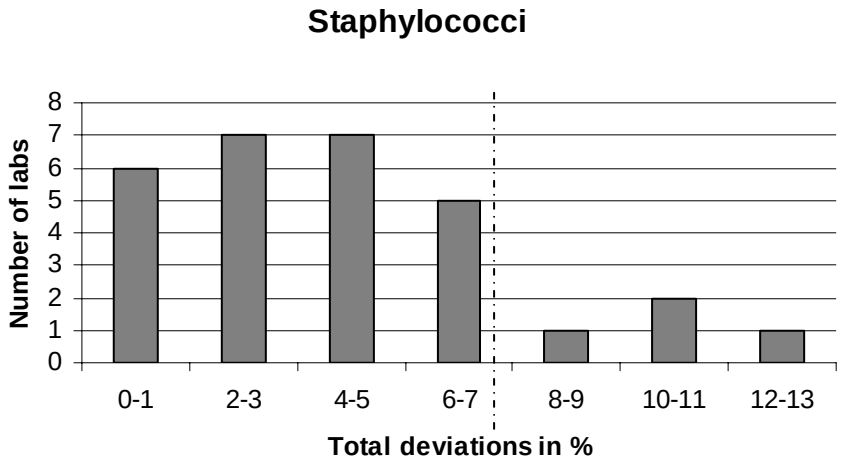
As illustrated in figure 12, two laboratories # 29 and #23 are considered as outliers with 28% and 48 % deviations respectively. Both used disk diffusion.

Staphylococci
Fig. 13



As illustrated in figure 13, four laboratories had > 7% deviations in the AST (dotted line). The percentage of deviations differed between the laboratories from 12 % in laboratory #29 to 0% deviations in laboratory #1 and #27. One of fifteen laboratories with > 7% deviations used disk diffusion and three of fourteen laboratories with > 7% deviations used MIC determination. There is no significant difference between the methods.

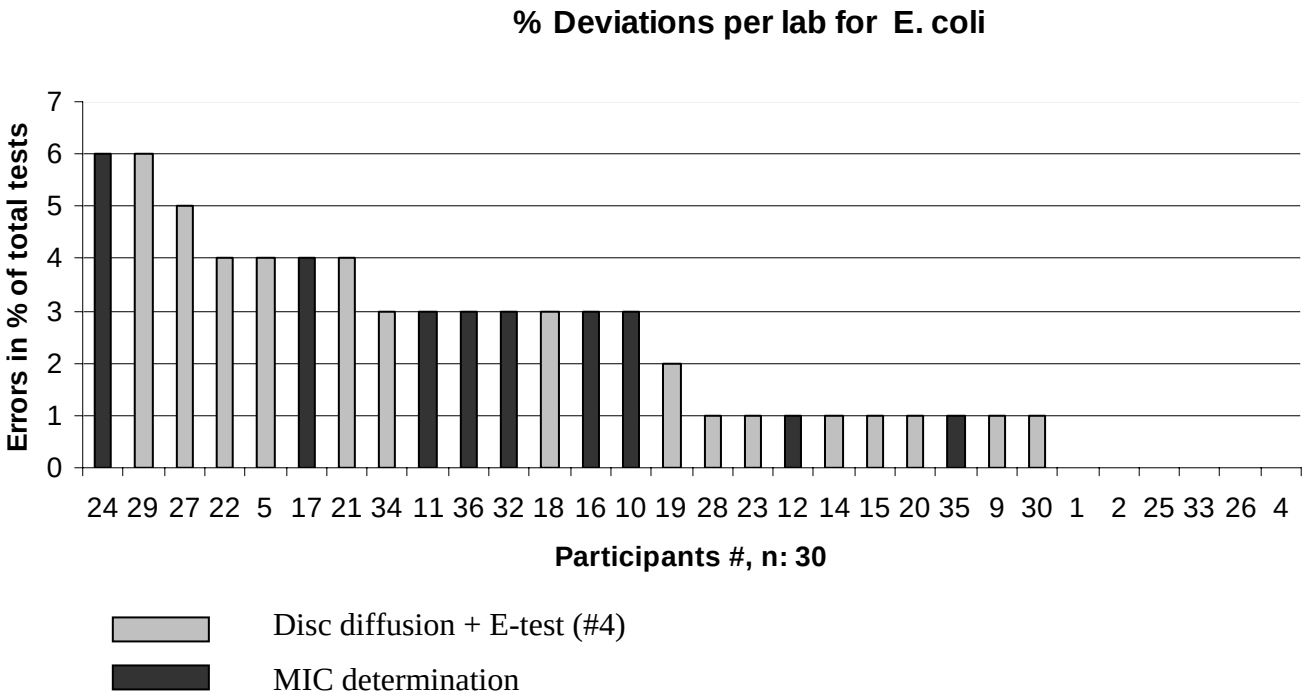
Fig. 14



As illustrated in figure 14, none of the laboratories could be considered as outliers.

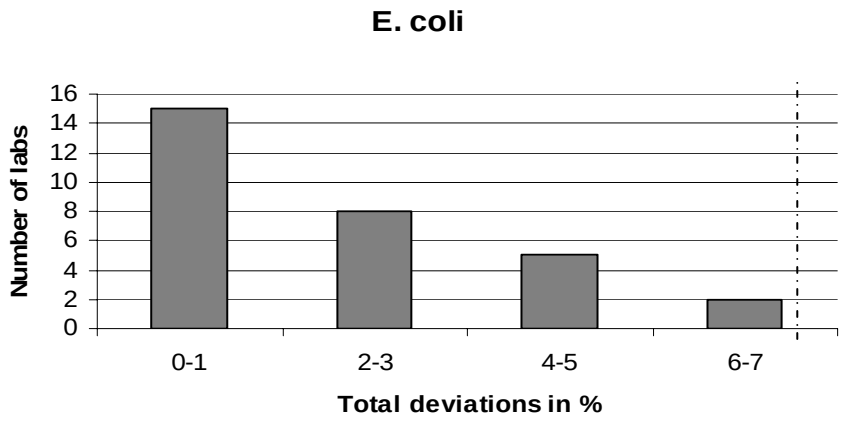
E. coli

Fig. 15



As illustrated in figure 15, six of the laboratories obtained a result of 100 % correctly tested E. *coli* strains. All 30 labs showed <7 % deviations. There is no significant difference between the results obtained by disc diffusion and MIC determination.

Fig. 16



As illustrated in figure 16 none of the laboratories could be considered as outliers.

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. The exceeding values of the QC interval are listed in the tables illustrating the laboratories quality control performance.

Enterococci:

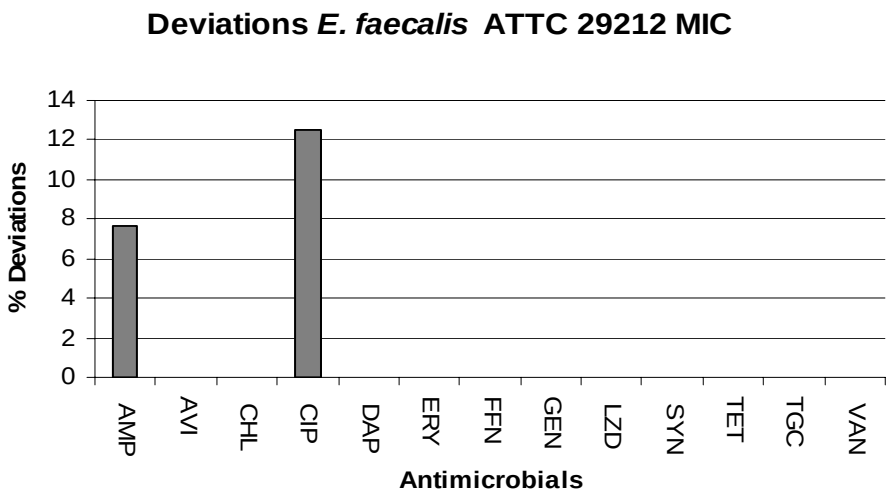
Table 5. Range of obtained values for *E. faecalis* 29212 by MIC determination.

Antimicrobial	QC ranges*	Total tests	Deviations	% Deviations	Min. value	Max. Value
Ampicillin, AMP	.5 – 2	13	1	7.7	0	4
Avilamycin, AVI	.5 – 4	4	0	0	0	0
Chloramphenicol, CHL	4-16	12	0	0	0	0
Ciprofloxacin, CIP	.25 – 2	8	1	12.5	.05	0
Daptomycin, DAP	1-8	1	0	0	0	0
Erythromycin, ERY	1-4	12	0	0	0	0
Florfenicol, FFN	2-8	2	0	0	0	0
Gentamicin, GEN	4-16	13	0	0	0	0
Linezolid, LZD	1-4	10	0	0	0	0
Quinpristin-dalfopristin, SYN	2-8	7	0	0	0	0
Tetracycline, TET	8-32	13	0	0	0	0
Tigecycline, TGC	.03 - .12	2	0	0	0	0
Vancomycin, VAN	1-4	13	0	0	0	0
		Sum 110	Sum 2	Av. 1.8%		

*from CLSI

Table 5 illustrates the laboratories that obtained values outside the QC interval of reference strain *E. faecalis* 29212 using MIC. Thirteen laboratories tested the reference strain using the MIC method. Two labs had in all two deviations against four antimicrobials, in all 1.8 % deviations.

Fig 17. Deviations in % for reference *E. faecalis* 29212 by MIC determination



As illustrated in figure 17 ampicillin caused 7.7 % deviation and ciprofloxacin 12,5 %.

CLSI has not published a QC range for *E. faecalis* 29212 using disc diffusion.

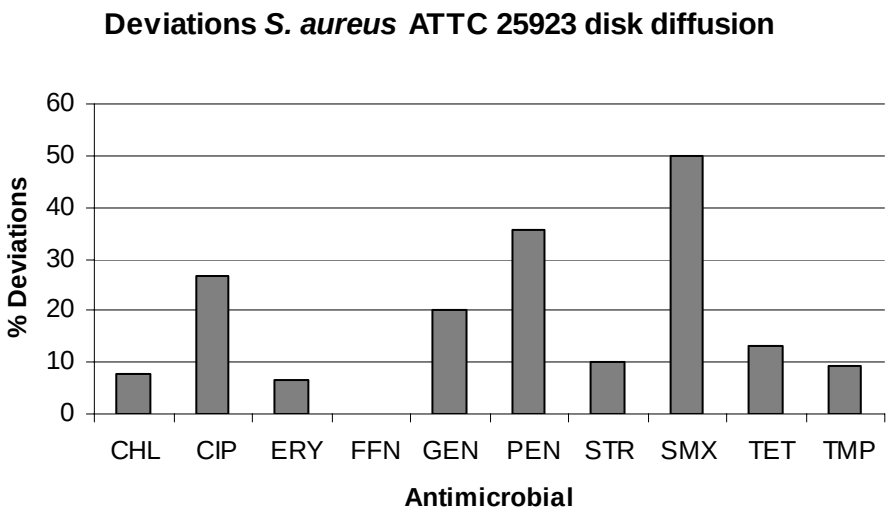
Staphylococci:

Table 6. Range of obtained values for *S. aureus* 25923 by disk diffusion.

Antimicrobial	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Chloramphenicol, CHL	16 - 26	13	1	7.7		27
Ciprofloxacin, CIP	22 - 30	15	4	26.7	20	38
Erythromycin, ERY	22 - 30	15	1	6.7	19	
Gentamicin, GEN	19 - 27	15	3	20.0	17	29
Penicillin, PEN	26 - 37	14	5	35.7	15	39
Streptomycin, STR	14 - 22	10	1	10.0	12	
Suphonamides, SMX	24 - 30	12	6	50.0	0	
Tetracycline, TET	24 - 34	15	2	13.3	20	
Trimethoprim, TMP	19 - 26	11	1	9.1		28
		Sum 131	Sum 24	Av. 18.3%		

Table 6 illustrates the values outside the QC interval of reference strain *S. aureus* 25923 by the fifteen laboratories using disk diffusion. Nine labs had deviations against nine antimicrobials. (App. 11).

Fig 18. Deviations in % for reference strain *S .aureus* ATTC 25923 disk diffusion



When analysing the Staphylococci reference strain by disk diffusion, 50% of the tests deviated on sulphonamide, 36% on ciprofloxacin, 20% on gentamicin and 13% on tetracycline. The rest of the antimicrobials deviated less than 10%.

Table 7. Range of obtained values for *S. aureus* 25923 by E-test

Antimicrobial	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Chloramphenicol, CHL	2-8	2	0	.0		
Ciprofloxacin, CIP	.125 - .5	2	1	50.0		.75
Erythromycin, ERY	.125 - .5	2	0	.0		
Suphonamides, SMX	8-32	1	0	.0		
Tetracycline, TET	.125 - 1	2	0	.0		
Trimethoprim, TMP	.5 - 2	2	0	.0		

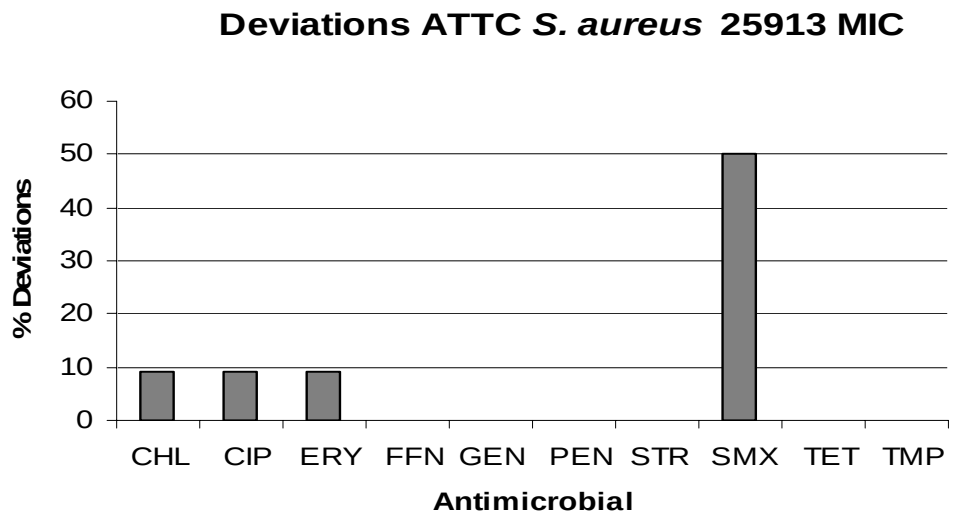
Table 7 illustrates the ratio of values outside the QC interval of reference strain *S. aureus* 25923 using E-test. Two laboratories tested the reference strain using the E-test.

Table 8. Range of obtained values for *S. aureus* 25913 by MIC determination.

Antimicrobial	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Chloramphenicol, CHL	2-8	11	1	9.1		16
Ciprofloxacin, CIP	.12 - .5	11	1	9.1		1
Erythromycin, ERY	.25 - 1	11	1	9.1	.12	
Florfenicol, FFN	2-8	4	0	.0		
Gentamicin, GEN	.12 - 1	9	0	.0		2
Penicillin, PEN	.25 - 2	9	0	.0		
Suphonamides, SMX	32 - 128	4	2	50.0	8	
Tetracycline, TET	.12 - 1	10	0	.0		2
Trimethoprim, TMP	1-4	7	0	.0		
		Sum 85	Sum 5	Av. 5.9 %		

Table 8 illustrates the values outside the QC interval of reference strain *S. aureus* 25913 using MIC. The results have improved significantly compared to disc diffusion. Eleven laboratories tested the reference strain using the MIC method. Results from four labs resulted in values outside the recommended QC interval for four of the nine antimicrobials tested in all 6% deviations.

Fig. 19. Deviations in % for reference strain *S. aureus* ATTC 25913 MIC



As illustrated in figure 19, when analysing the Staphylococci references train by MIC, 50% the reference strain tests deviated on sulphonamide, and 9.1 % on ciprofloxacin, gentamicin and erythromycin. The percentage of deviations by MIC determination is - with the exception of sulphonamides - smaller than with disk diffusion.

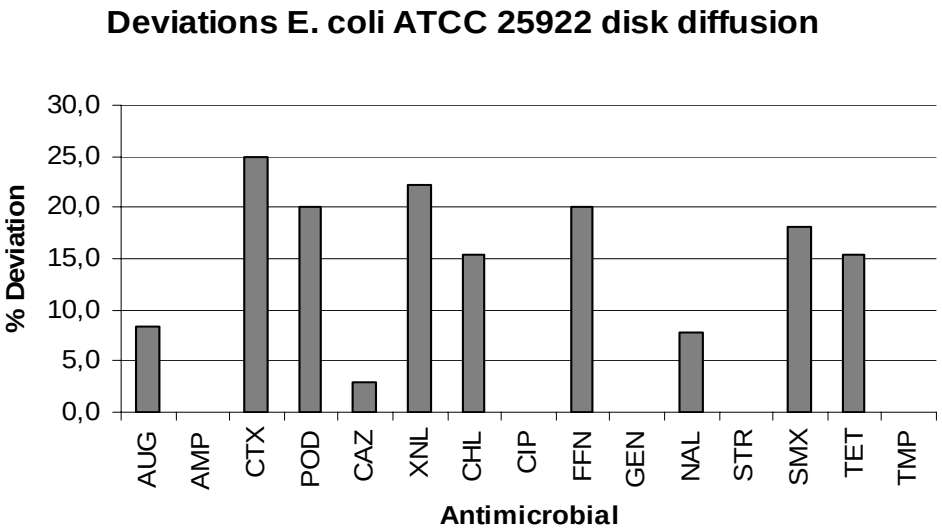
E. coli

Table 9. Range of obtained values for *E. coli* ATCC 25922 by disk diffusion.

Antibiotic	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Amoxicillin cl., AUG	18 - 24	12	1	8.3		25
Amoxicillin, AMX	0 - 50	5	0	.0		
Ampicillin, AMP	16 - 22	11	0	.0		
Cefotaxime, CTX	29 - 35	12	3	25.0	27	38
Cefpodoxime, POD	23 - 28	5	1	20.0		30
Ceftazidime, CAZ	25 - 32	10	3	30.0	23	33
Ceftiofur, XNL	26 - 31	9	2	22.2	25	34
Chloramphenicol, CHL	21 - 27	13	2	15.4	20	29
Ciprofloxacin, CIP	30 - 40	11	0	.0		
Florphenicol, FFN	22 - 28	10	2	20.0		33
Gentamicin, GEN	19 - 26	13	0	.0		
Nalidixic acid, NAL	22 - 28	13	1	7.7		29
Streptomycin, STR	0 - 50	12	0	.0		
Sulphonamides, SMX	15 - 23	11	2	18.2		26
Tetracycline, TET	18 - 25	13	2	15.4		26
Trimethoprim, TMP	21 - 28	11	0	.0		
		Sum 171	Sum 19	Av. 11.1%		

Table 9 illustrates the values outside the QC interval of reference strain *E. coli* ATCC 25922 using disk diffusion. Thirteen laboratories tested the reference strain. Results from five laboratories resulted in values outside the recommended QC interval for ten of the sixteen antimicrobials in the test, in all 11% deviations. (App. 11)

Fig 20.



As illustrated in figure 20 seven of the antimicrobials exhibit deviations of more than10%. This number is considerably higher than the results obtained for the streptococci and Staphylococci reference strains.

Table 10. Range of obtained values for *E. coli* ATCC 25922 by E-test

Antimicrobial	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Ampicillin, AMP	2-8	1	0	.0		
Nalidixic acid, NAL	1-4	1	0	.0		
Streptomycin, STR	2-8	1	0	.0		
Sulphonamides, SMX	32 - 128	1	0	.0		
Tetracycline, TET	.5 - 2	1	1	100.0		6
Trimethoprim, TMP	.5 - 2	1	0	.0		

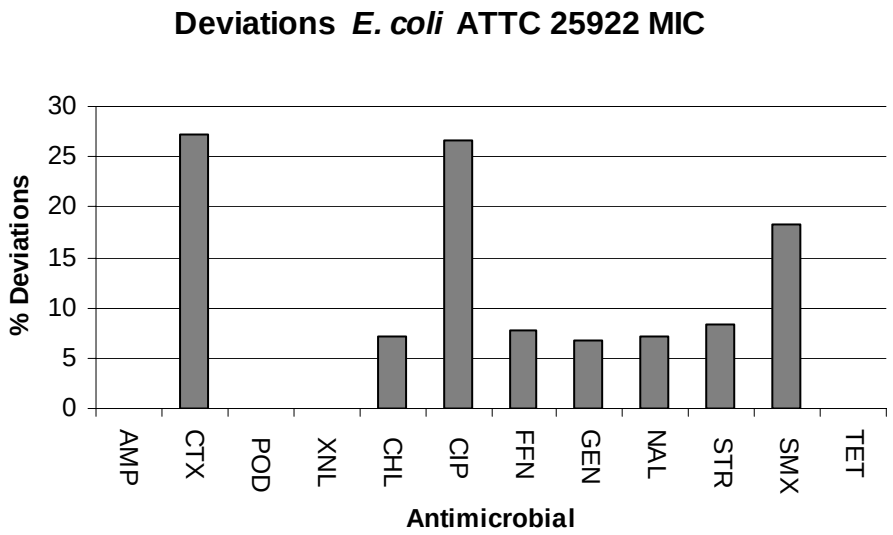
Table 10 illustrates the values outside the QC interval of reference strain *E. coli* ATCC 25922 using E-test. One laboratory tested the reference strain using the E-test.

Table 11. Range of obtained values for the *E. coli* ATCC 25922 using MIC determination.

Antimicrobial	QC ranges	Total tests	Deviations	% Deviations	Min. value	Max. Value
Ampicillin, AMP	2-8	11	0	.0		
Cefotaxime, CTX	.03 - .12	11	3	27.3	.012	.5
Cefpodoxime, POD	.25 - 1	1	0	.0		
Ceftiofur, XNL	.25 - 1	8	0	.0		
Chloramphenicol, CHL	2-8	14	1	7.1		16
Ciprofloxacin, CIP	.004 - .015	15	4	26.7		1
Florphenicol, FFN	2-8	13	1	7.7		16
Gentamicin, GEN	.25 - 1	15	1	6.7		2
Nalidixic acid, NAL	1-4	14	1	7.1		16
Streptomycin, STR	4-16	12	1	8.3	2	
Sulphonamides, SMX	8-32	11	2	18.2		2048
Tetracycline, TET	.5 - 2	14	0	.0		
		Sum 139	Sum 14	Av. 10.0%		

As illustrated in table 11 MIC determinations of the reference strain *E. coli* ATCC 25922 results in the same level of deviations as by disk diffusion. Fifteen laboratories submitted data. Eight labs exhibited deviations against eight antimicrobials of the sixteen used in all 10% deviations. (App. 11)

Fig 21



As illustrated in figure 21, three of the antimicrobials exhibit deviations of more than 10%.

4. DISCUSSION

4.1 Enterococci trial

Strains
Significant deviations were noticed for seven of the eight Enterococci strains tested (fig. 3).

Antimicrobials
Significant deviations were noticed for seven antimicrobials used testing the Enterococci strains: Ampicillin 10% deviations, ciprofloxacin 17%, erythromycin 10%, gentamicin 8%, streptomycin 13%, Quinpristin-dalfopristin 23% and tetracycline 9% (fig. 4). It is noteworthy that 5% deviation was observed for vancomycin. Only one antimicrobial, ciprofloxacin, is not recommended by EFSA to be included in antimicrobial resistance monitoring.

AST of seven strains showed ten results with less than 75% of the expected results (table 2 and fig. 5). MIC determination was more in compliance with the expected results than disk diffusion (table 3 and fig.6).

Laboratories

Over all, the percentage of correct susceptibility testing of Enterococci was 91% (figure 2). Large differences in the performance of the laboratories were observed ranging from 0% to 48% deviating results (figure 9).

Fourteen laboratories had deviations higher than 7% (figure 10) and two of the laboratories (#29 and #23) were outliers with 48% and 28% deviations respectively. Nine of eleven laboratories with > 7% deviations used disk diffusion and four of fourteen laboratories with > 7% deviations MIC determination. Both outliers used disk diffusion. The results obtained by using MIC determination were significantly better than results obtained from disk diffusion (P=0.02)

The number of labs with results outside the acceptance level is unexpectedly high, and the CRL-AR will discuss the results with the participants aiming towards highlighting the reasons why. The results from the next EQAS on Enterococci will determine if a laboratory will require an audition by the CRL-AR.

Reference strain

Analysing the reference strain monitors the quality of the laboratories practice. Analysing the reference strain *E. faecalis* 29212 by MIC determination (table 5 and fig. 15) exhibited very modest deviations from the values published by CLSI. Only 1.8% of the tests deviated.

It was not possible to use disk diffusion in a reference test since no internationally acknowledged QC ranges are available for *E. faecalis* 29212 for disk diffusion.

4.2 Staphylococci trial

Strains

Significant deviations were noticed for two of the eight strains tested (fig. 5).

Antimicrobials
Significant deviations were observed for four antimicrobials used to test Staphylococci strains: Ciprofloxacin (9% deviations), Penicillin (5% deviations), Sulphonamide (13% deviations) and Tetracycline (11% deviations) (fig. 6).

Laboratories
Over all, the percentage of correct susceptibility testing of Staphylococci was 96% (figure 2).

Differences in the performance of the laboratories ranged from 0% to 13% deviating results (figure 11). Four laboratories performed unsatisfactory according to the 7% limit. There is no significant difference between using MIC determination and disk diffusion.

Reference strains
Nine of the fifteen laboratories, which used disk diffusion to test the reference strain *S. aureus* 25923 obtained deviating results against nine of the ten used antimicrobials. The majority of deviations were seen against ciprofloxacin, gentamicin, penicillin and sulphonamides. A remarkably high percentage (18%) of the tests deviated (Table 4 & fig.16).

Eleven laboratories tested the reference strain *S. aureus* 25913 using the MIC method. Five labs (22%) obtained deviating results against four of the eleven antimicrobials used. A total of 6% of the tests deviated (Table 6 & fig. 17).

MRSA is an emerging problem in animal farming and EU has in January 2008 initiated a baseline study in the member states to reveal the magnitude of the problem.

Strain ST 1.1 and ST 1.8 were MRSA positive. Four laboratories of 23 (17%) did not identify strain ST 1.1 or ST 1.8 correctly as MRSA. The result is not satisfactory and can be caused by the difficulty in detecting the MRSA using disk diffusion. The mecA gene might not be expressed during the growth of the *S. aureus* on the agar.

4.3 *E. coli* trial

Strains
The results were satisfactory with no significant deviations.

Antimicrobials

No antimicrobials, which EFSA recommends for antimicrobial resistance monitoring, show significant deviations while ceftazidime (6% deviations) and the combination of sulphonamide and trimethroprim (8% deviations) do.

Laboratories

Over all, the percentage of correct susceptibility tests of *E. coli* was 98.0%. Differences in the performance of the laboratories ranged from 0% to 6% deviating results. No laboratories performed out of the acceptable range.

Reference strain

Remarkably, the laboratories did not keep the high standard analysing the reference strain: Thirteen laboratories tested the reference strain *E. coli* ATCC 25922 using the disk diffusion method. Results from five laboratories resulted in values outside the recommended QC interval for ten of the sixteen antimicrobials in the test. It seems that the participants in particular had problems with determining the AST of cephalosporins, since most problems were recorded for the following antimicrobials: Ceftazidime, cefotaxime, cetiofur, cefpodoxime and florfenicol. A total of 11% of the tests were out of range (table 7 & fig.18).

Using MIC to analyse the reference strain resulted in the same level of deviations. Fifteen laboratories submitted data. Eight labs (53%) obtained deviating results against eight antimicrobials of the twelve used. A total of 10% of the tests were out of range (Table 9 & fig 19).

Strain EC 1.6 was ESBL positive. Nine laboratories analysed for ESBL producing strains and all correctly detected strain EC 1.6 as ESBL producing. One laboratory incorrectly detected strain EC 1.7 as ESBL producing.

Strain EC 1.7 was AmpC positive. Eight out of nine laboratories, which analysed the *E. coli* strains for AmpC, correctly detected the strain as AmpC. None of the laboratories described the strain as ESBL producing.

No acceptance limit has been set as regards to the results from the testing of ESBL and AmpC strains. Even so, the participants' results from the analysis of ESBL and AmpC strains appear to be satisfactory.

In general

It is important for the laboratories to work towards determining the factors that have caused the deviations. A reason for deviating results could be incorrect breakpoints. As shown in Appendix 5, the breakpoints used by the laboratories for disk diffusion varies to great a extend. Demanding test strains (strains with MIC values close to the breakpoint) could also affect the results.

The laboratories should as a part of their quality control check the following on a daily basis:

- The disk temperature before use (should be room temperature)
- The age of the disks
- The disks' concentration of the antimicrobial
- The volume of the agars in the Petri dish. Dishes with < 4mm agar can cause extended inhibition zones.
- That the pH of the media is as listed in the protocol
- Turbidity of the broth (should be McFarland standard 0.5)
- The density of the bacteria layer on the agars
- The age of the plates. Old and dry plates will inhibit growth
- The autoclaving of the media.

5. CONCLUSION

The goal of the CRL-AR is that all laboratories perform susceptibility testing with a deviation margin below 7% in the EQAS and as a consequence that all NRLs generate correct and reliable data on a routine basis.

The performance of AST of Enteroococci needs considerable improvement to reach the goal, while the goal for Staphylococci is closer at hand and is already accomplished for *E. coli*.

For Enterococci, the results obtained by using MIC determination were significantly better than results obtained from disk diffusion (P=0.02) while there were no significant differences for Staphylococci and *E. coli.*

The analyses of the reference strains showed surprisingly divergent results compared to the AST. The AST of the Enterococci strains obtained the greatest deviation on 9% overall while the test of the reference strain obtained 1.8% deviations in MIC testing. The AST of the Staphylococci strains obtained 4% deviations overall while the test of the reference strain obtained 18% deviations in disk diffusion and 6% in MIC testing. The AST of the *E. coli* strains obtained 2% deviations overall while the test of the reference strain obtained 10% deviations in both disk diffusion and MIC. The results illustrates that the tests of the reference strains can be more difficult to perform correctly than the AST of the strains. The reference tests are very useful indicators on errors in the methodology of the laboratories.

It is encouraging that the laboratories which carried out the analyse for ESBL producing *E. coli*-strain performed so well, while there still is room for improvement concerning the MRSA analysis where 17% of the laboratories obtained deviating results. This result cause major concern and will be discussed at the next CRL-workshop in Copenhagen in June 19-20 2008.

The most important issues to address in the future collaboration between the CRL-AR and the NRL's is the harmonisation of breakpoints as well as the choice of antimicrobials and methods. The goal is that the participants in analysing the strains carry out the criteria set by EUCAST/EFSA for AST as agreed on at the CRL-AR Workshop in Copenhagen in May 2007.

The CRL-AR will discuss the deviating results with the relevant laboratories. The results from the next EQAS on Enterococci, Staphylococci and *E. coli* in June 2008 will be the background for a decision to offer the laboratories an audition by the CRL-AR.

APPENDICES

1. CRL-AR EQAS Pre notification
2. Participants list
3. Strain collection and reference values
4.a. Protocol
4.b. Instructions for opening the vials and reviving freeze-dried cultures
4.c. Evaluation form
4.d. EQAS Questionnaire
5. Break points used by the participant (disk diffusion)
6. EUCAST/EFSA cut of values
7. Quality control range reference strains
8. Participants evaluation
9. Antimicrobials and range/disk content used in the daily routine by participants
10. Correct % R-S for antimicrobials and strains
11. Deviations for each laboratory

Appendix 1

Technical University of Denmark DTU

National Food Institute

Copenhagen, May 15th, 2007

CRL-AR EQAS pre-notification

EQAS 2007 FOR *E. COLI*, STAPHYLOCOCCI AND ENTEROCOCCI

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 *E. coli* isolates, eight staphylococci and eight enterococci isolates. Additionally, we will send you following QC strains: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212.

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

Participation is free of charge for all NRL's.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
Please remember to provide the coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the content of the parcel is "Biological Substance Category B": Eight *E. coli*, ten staphylococci, eight enterococci and one *E. faecalis* that are expected to arrive at your laboratory in June 2007.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped at the beginning of June 2007 in the package you will find information about your username and password for entering the results. The protocol will be provided by e-mail.

Returning of results: Results must be returned to the National Food Institute, by July 15th, 2007. 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 *Salmonella* and *Campylobacter* which will be carried out in October, 2007.

Any comments regarding the EQAS, please contact me by e-mail (rsh@food.dtu.dk) or by fax (+45 7234 6001).

Sincerely,

Rene S. Hendriksen
EQAS-Coordinator

Appendix 2 Participants list	
Institute	**Country**
Austrian Agency for Health and Food Safety	Austria
Institute of Public Health	Belgium
National Center of Infectious and Parasitic Diseases	Bulgaria
State Veterinary Institute Praha	Czech Republic
The National Food Institute	Denmark
Estonian Veterinary and Food Laboratory	Estonia
Finnish Food Safety Authority EVIRA	Finland
AFSSA LERQAP	France
AFSSA Ploufragan - LERAP	France
AFSSA Lyon	France
AFSSA Fougères LERMVD	France
Federal Institute for Risk Assessment	Germany
Veterinary Laboratory of Chalkis	Greece
Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
Central Veterinary Research Laboratory	Ireland
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
National Diagnostic Centre of Food and VeterinaryService	Latvia
National Veterinary Laboratory	Lithuania
Food and Consumer Product Safety Authority (VWA)	Netherlands
Central Institute for Animal Disease Control (CIDC-Lelystad)	Netherlands
Veterinærinstituttet	Norway
National Veterinary Research Institute	Poland
Instituto Nacional de Saude (INSA)	Portugal
National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
State Veterinary and Food Institute (SVFI)	Slovakia
National Veterinary Institute	Slovenia
Laboratorio Central de Sanidad, Animal de Santa Fe	Spain
Laboratorio Central de Sanidad, Animal de Algete	Spain
Complutense University of Madrid	Spain
National Veterinary Institute, SVA	Sweden
The Veterinary Laboratory Agency	United Kingdom

Appendix 3

MIC values enterococci

Strain no.	AMP	AVI	CHL	CIP	DAP	ERY	FFN	GEN	KANA	LINEZO	STR	SYN	TET	TGC	VAN
1,1	4	<=2	4	4	2	>32	<=4	<=128	>2048	<=1	>2048	2	>32	0,125	<=2
1,2	<=2	<=2	4	1	2	<=0.5	<=4	<=128	<=128	2	<=128	2	<=1	0,125	<=2
1,3	4	<=2	4	<=0.25	1	8	<=4	<=128	256	2	>2048	2	>32	0,125	<=2
1,4	<=2	<=2	<=2	1	1	1	<=4	<=128	<=128	<=1	512	1	<=1	0,06	<=2
1,5	4	<=2	4	0.5	0,5	4	<=4	<=128	256	2	<=128	2	32	0,125	>32
1,6	<=2	<=2	8	0.5	1	>32	<=4	>2048	>2048	<=1	>2048	8	>32	0,25	<=2
1,7	<=2	<=2	>64	>8	1	>32	<=4	>2048	>2048	<=1	>2048	8	>32	0,25	<=2
1,8	<=2	16	8	1	1	>32	<=4	<=128	<=128	<=1	>2048	8	4	0,125	<=2

S-R Enterococci

Strain no.	AMP	AVI	CHL	CIP	DAP	ERY	FFN	GEN	KANA	LINEZO	STR	SYN	TET	TGC	VAN
1,1	S	S	S	S	S	**R**	S	S	**R**	S	**R**	S	**R**	S	S
1,2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
1,3	S	S	S	S	S	**R**	S	S	S	S	**R**	S	**R**	S	S
1,4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
1,5	S	S	S	S	S	S	S	S	S	S	S	S	**R**	S	**R**
1,6	S	S	S	S	S	**R**	S	**R**	**R**	S	**R**	S	**R**	S	S
1,7	S	S	**R**	**R**	S	**R**	S	**R**	**R**	S	**R**	S	**R**	S	S
1,8	S	**R**	S	S	S	**R**	S	S	S	S	**R**	S	**R**	S	S

App 3 MIC values staphylococci

Strain no.	Chloramphenicol	Ciprofloxacin	Erthromycin	Florfenicol	Gentamicin	MRSA	Penicillin	Streptomycin	Suphonamides	Tetracycline	Trimethoprim
1,1	4	0.25	<=0.12	2	<=1	Positiv	0,12	<=2	256	2	<=1
1,2	8	0.12	>16	4	<=1	Negativ	4	<=2	<=8	>32	<=1
1,3	8	1	>16	4	<=1	Negativ	2	4	16	>32	>32
1,4	4	>8	0.25	2	<=1	Negativ	16	>128	16	>32	>32
1,5	8	0.5	>16	4	<=1	Negativ	16	>128	16	<=0.5	>32
1,6	8	0.5	0.5	4	<=1	Negativ	0,06	4	<=8	1	<=1
1,7	4	0.5	0.25	2	<=1	Negativ	4	<=2	<=8	>32	>32
1,8	4	2	0.25	2	>32	Positiv	>16	>128	256	32	<=1

S-R staphylococci

Strain no.	CHL	CIP	ERY	FFN	GEN	MRSA	PEN	STR	SMX	TET	TMP
1,1	S	S	S	S	**S**	Positiv	**R**	S	S	R	S
1,2	S	S	**R**	S	S	Negativ	**R**	S	S	**R**	S
1,3	S	S	**R**	S	S	Negativ	**R**	S	S	**R**	**R**
1,4	S	**R**	S	S	S	Negativ	**R**	**R**	S	**R**	**R**
1,5	S	S	**R**	S	S	Negativ	**R**	**R**	S	S	**R**
1,6	S	S	S	S	S	Negativ	S	S	S	S	S
1,7	S	S	S	S	S	Negativ	**R**	S	S	**R**	**R**
1,8	S	**R**	S	S	R	Positiv	**R**	**R**	S	**R**	S

Appendix 3 Strain collection and reference value in MIC for *E. coli*

MIC values *E. coli*																				
Strain	**AMP**	**AUG**	**CAZ**	**CAZ/CL**	**CHL**	**CIP**	**CTX**	**CTX/CL**	**ESBL gene**	**FFN**	**FOX**	**GEN**	**IP / IPE**	**NAL**	**POD**	**SMX**	**STR**	**SXT**	**TET**	**TMP**	**XNL**
1,1	>32	8/4	0,25	0,25	>64	<=0.03	0,125	0,125	NONE	>64	8	<=1	MIC ratio <8	<=4	0,5	>1024	>64	>32	>32	>32	<=0.5
1,2	2	<=2/1	0,125	0,125	8	<=0.03	0,064	0,032	NONE	4	4	<=1	MIC ratio <8	<=4	0.25	<=64	64	0.5	<=2	>32	<=0.5
1,3	>32	8/4	0,125	0,125	8	<=0.03	0,064	0,032	NONE	4	4	32	MIC ratio <8	<=4	0.5	<=64	>64	0.25	>32	<=4	<=0.5
1,4	4	8/4	0,25	0,125	16	<=0.03	0,125	0,064	NONE	16	8	<=1	MIC ratio <8	<=4	0.5	<=64	64	0.5	>32	>32	<=0.5
1,5	2	<=2/1	0,25	0,125	8	<=0.03	0,064	0,032	NONE	8	4	<=1	MIC ratio <8	<=4	0.5	<=64	8	0.125	<=2	<=4	<=0.5
1,6	>32	8/4	4	0,25	4	<=0.03	64	0,125	ESBL(CTX-M-1)	4	8	<=1	MIC ratio <8	<=4	>4	<=64	<=4	0.125	<=2	<=4	>8
1,7	>32	32/16	16	>4	>64	<=0.03	8	>1	NONE	4	32	<=1	MIC ratio <8	<=4	>4	>1024	>64	0.5	>32	<=4	8
1,8	>32	8/4	0,25	<=0,064	8	4	0,25	0,064	NONE	8	8	<=1	MIC ratio <8	>64	0.5	>1024	32	>32	>32	>64	<=0.5

S-R *E. coli*																				
Srain	**AMP**	**AUG**	**CAZ**	**CAZ/CL**	**CHL**	**CIP**	**CTX**	**CTX/CL**	**ESBL gene**	**FFN**	**FOX**	**GEN**	**IP/IPE**	**NAL**	**POD**	**SMX**	**STR**	**SXT**	**TET**	**TMP**	**XNL**
1,1	**R**	S	S	MIC ratio <8	**R**	S	S	MIC ratio <8	none ESBL	**R**	none ampC	S	none Metallo b l	S	S	**R**	**R**	**R**	**R**	**R**	S
1,2	S	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	S	none ampC	S	none Metallo b l	S	S	S	**R**	S	S	**R**	S
1,3	**R**	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	S	none ampC	**R**	none Metallo b l	S	S	S	**R**	S	**R**	S	S
1,4	S	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	S	none ampC	S	none Metallo b l	S	S	S	**R**	S	**R**	**R**	S
1,5	S	S	S	MIC ratio <8	S	S	S	MIC ratio <8	none ESBL	S	none ampC	S	none Metallo b l	S	S	S	S	S	S	S	S
1,6	**R**	S	R	MIC ratio <8	S	S	**R**	Ratio >8	ESBL(CTX-M-1)	S	none ampC	S	none Metallo b l	S	**R**	S	S	S	S	S	**R**
1,7	**R**	**R**	**R**	MIC ratio <8	**R**	S	**R**	MIC ratio <8	none ESBL	S	ampC(CMY-2)	S	none Metallo b l	S	**R**	**R**	**R**	S	**R**	S	**R**
1,8	**R**	S	S	MIC ratio <8	S	**R**	S	MIC ratio <8	none ESBL	S	none ampC	S	none Metallo b l	**R**	S	**R**	**R**	**R**	**R**	**R**	S

National Food Institute

PROTOCOL

For susceptibility testing of *E. coli*, enterococci and staphylococci

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 *E. coli*, enterococci and staphylococci. The EC/Ent/Staph EQAS 2007 will include susceptibility testing of eight *E. coli*, eight enterococci and eight staphylococci strains together with susceptibility testing of the reference strains *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 (for disk diffusion) and *S. aureus* ATCC 29213 (for MIC).

The reference strains included are original CERTIFIED cultures. 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.

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 *E. coli*, enterococci and staphylococci. Furthermore, to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported by different laboratories on *E. coli*, enterococci and staphylococci and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2007

3.1 Shipping, receipt and storage of strains

In June 2007 all EU appointed National Reference Laboratories will receive a parcel from the National Food Institute containing eight *E. coli*, eight enterococci and eight staphylococci strains as well as the four reference strains mentioned above. 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, and the test strains are stab cultures. Please keep strains refrigerated. On arrival, the cultures must be subcultured and ensured proper storage conditions until testing.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

a) Open the ampoule. Dissolve the material in 0,5 ml appropriate broth. Leave it for 10 minutes. Inoculate the solution on a non selective agar plate using either a 1 µl loop or a cotton swab. Incubate at 35ºC in ambient air for 16-18 h.

b) Incubate the remaining culture/broth in the vial/ampoule as mentioned above. Seal the vial/ampoule with parafilm if necessary. After incubation re-inoculate the culture using either a 1 µl loop or a cotton swab on none selective agar and incubate.

c) If you do not succeed with a) or b), shake the vial/ampoule and empty it directly onto a none selective agar plate Add a little saline to the plate, and spread the culture properly with a triangle or hockey stick. Incubate as mentioned above.

Please note the document that provides ‘instructions for opening and reviving freeze-dried cultures’.

3.3 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. For MIC please use the cut off values listed in tables 3.3.1; 3.3.2 and 3.3.3. In this EQAS, epidemiological MIC cut off values are used for MIC determination which allow only two categories of characterisation – resistant or sensitive. Participants using disk diffusion are recommended to interpret the results according to the individually daily routinely used breakpoints categorising them into the terms resistant and sensitive. Interpretations in concordance with the expected value will be categorised as ‘correct’, whereas interpretation that deviates 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).

As to the breakpoint that you routinely use in your laboratories to determine the susceptibility category we ask you to please fill in the breakpoints used, in the questionnaire enclosed. Please use

the space in the questionnaire for comments if you use a method that differs from what is mentioned in this protocol (eg. concentration of disks).

When testing cephalosporins, please follow the guidelines according to CLSI M100-S16 table 2A; that when an isolate is found resistant to one cephalosporin, the isolate is regarded resistant to all cephalosporins.

3.3.1 *E. coli* (tentative cut off values recommended by EFSA)

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Amoxicillin cl., AUG	8
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Cefpodoxime, POP	1*
Ceftazidime, CAZ	0.5
Ceftiofur, XNL	1
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.032
Florfenicol, FFN	16
Gentamicin, GEN	2
Nalidixic acid, NAL	16
Streptomycin, STR	16
Sulfonamides, SMX	256**
Tetracycline, TET	8
Trimethoprim, TMP	2
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	0,5

* Tentative
** CLSI

It is optional to continue with the following tests regarding ESBL production:

All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) could be confirmed by confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

3.3.2 **Enterococci** (tentative cut off values recommended by EFSA)

Antimicrobials for enterococci	MIC (µg/mL) **R is >** *E. faecium*	MIC (µg/mL) **R is >** *E. faecalis*
Ampicillin, AMP	4	4
Avilamycin, AVI	16	8
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Daptomycin, DAP	4	4
Erythromycin, ERY	4	4
Florfenicol, FFN	8	8
Gentamicin, GEN	32	32
Linezolid. LZD	4	4
Streptomycin (high level), STR	2048	2048
Quinpristin-dalfopristin (Synacid), SYN	4	32
Tetracycline, TET	2	2
Tigecycline, TGC	0,25	0,25
Vancomycin, VAN	4	4

3.3.3 **Staphylococci** (tentative cut off values recommended by EFSA)

Antimicrobials for *S. aureus*	MIC (µg/mL) **R is >**
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	1
Penicillin, PEN	0,25
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	4

Some of the strains may be MRSA positive. It is optional to continue with tests regarding MRSA, and also the strains may be tested by any method that you prefer. The result that you are asked to upload is ‘positive’ or ‘negative’.

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the enclosed test form. Please enter your results into the interactive web database. Below 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. 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 August 20th, 2007.

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. Finally, a summary report with all results will be performed and made available.

For participants that have received additional strains as a retest for the 2006 EQAS: Please send us the results by the enclosed document(s) 'Retest EQAS 2006, *Salmonella*' and/or 'Retest EQAS 2006, *Campylobacter*'.

If you have any questions, please do not hesitate to contact me:

Rene Hendriksen

The National Food Institute

Technical University of Denmark

27 Bülowsvej, DK-1790 Copenhagen V

Denmark

Tel: +45 7234 6288

Fax: +45 7234 6001

E-mail: rsh@food.dtu.dk

5 HOW TO ENTER RESULTS 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.

Enter the EU CRL-AR EQAS 2007 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 EQAS 2006. If you have problems with the login please contact us.

Click on either “enterococci tests”, “staphylococci tests” or “*E. coli* tests”, depending on your results. The below description is aimed at enterococci entry but is exactly the same as for *E. coli*, and staphylococci entry.

Click on "Start of Data Entry - Methods and Breakpoints for enterococci”

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 enterococci and the brand of discs, tablets, MIC panels etc.

Fill the breakpoints that are routinely used at your laboratory 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.

Click on "save and go to next page”

In the data entry pages for each enterococci, staphylococci and *E. coli* strain, you enter the read value and the interpretation as R or S.

Click on "save and go to next page"

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

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.

Click on "Save and go to next page"

To approve your input and to see and print the evaluated results, please go through the pages and make corrections if necessary. Remember to save a page if you make any corrections.

Approve input pages. 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.

See evaluation report again. 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".

6 TEST FORMS

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

Comments:

National Food Institute

TEST FORM

Survey for routinely applied breakpoints for antimicrobial susceptibility testing of enterococci

Antimicrobial	Interpretation, Zonediam (mm) or MIC-value (µg/ml)				
	<, ≤	Sensitive	Intermediate	>, ≥	Resistant
Ampicillin AMP					
Avilamycin, AVI					
Chloramphenicol, CHL					
Ciprofloxacin, CIP					
Daptocymin, DAP					
Erythromycin, ERY					
Florphenicol, FFN					
Gentamicin, GEN					
Linezolid, LZD					
Streptomycin, STR					
Synacid, SYN					
Tetracycline, TET					
Tigecycline, TGC					
Vancomycin, VAN					

Regarding method used for antimicrobial susceptibility testing of enterococci in this EQAS:

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

National Food Institute

TEST FORM

Survey for routinely applied breakpoints for antimicrobial susceptibility testing of staphylococci

Antimicrobial	Interpretation, Zonediam (mm) or MIC-value (µg/ml)				
	<, ≤	Sensitive	Intermediate	>, ≥	Resistant
Chloramphenicol, CHL					
Ciprofloxacin, CIP					
Erythromycin, ERY					
Florfenicol, FFN					
Gentamicin, GEN					
Penicillin, PEN					
Streptomycin, STR					
Suphonamides, SMX					
Tetracycline, TET					
Trimethoprim, TMP					

Regarding method used for antimicrobial susceptibility testing of staphylococci in this EQAS:

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

National Food Institute

TEST FORM

Survey for routinely applied breakpoints for antimicrobial susceptibility testing of *E. coli*

Antimicrobial	Interpretation, Zonediam (mm) or MIC-value (µg/ml)				
	<, ≤	Sensitive	Intermediate	>, ≥	Resistant
Amoxicillin cl., AUG					
Ampicillin, AMP					
Cefotaxime, CTX					
Cefpodoxime, POD					
Ceftazidime, CAZ					
Ceftiofur, XNL					
Chloramphenicol, CHL					
Ciprofloxacin CIP					
Florphenicol, FFN					
Gentamicin, GEN					
Nalidixic acid, NAL					
Streptomycin, STR					
Sulphonamides, SMX					
Tetracycline, TET					
Trimethoprim, TMP					
TMP+SMX, SXT					

Regarding method used for antimicrobial susceptibility testing of *E. coli* in this EQAS:

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.1	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.2	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.3	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.4	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.5	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.6	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.7	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Enterococci CRL ENT. 1.8	Ampicillin AMP			
	Avilamycin, AVI			
	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Daptocymin, DAP			
	Erythromycin, ERY			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Linezolid, LZD			
	Streptomycin, STR			
	Synacid, SYN			
	Tetracycline, TET			
	Tigecycline, TGC			
	Vancomycin, VAN			

National Food Institute

TEST FORM

Susceptibility testing of *Ent. faecalis* ATCC 29212 ref. strain

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
E. faecalis ATCC 29212	Ampicillin, AMP	
	Avilamycin, AVI	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Daptocymin, DAP	
	Erythromycin, ERY	
	Florphenicol, FFN	
	Gentamicin, GEN	
	Linezolid, LZD	
	Streptomycin, STR	
	Synacid, SYN	
	Tetracycline, TET	
	Tigecycline, TGC	
	Vancomycin, VAN	

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.1	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.2	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.3	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.4	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.5	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.6	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.7	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
Staphylococci CRL ST 1.8	Chloramphenicol, CHL			
	Ciprofloxacin, CIP			
	Erythromycin, ERY			
	Florfenicol, FFN			
	Gentamicin, GEN			
	Penicillin, PEN			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			

Optional test regarding MRSA	☐ Positive ☐ Negative

National Food Institute

TEST FORM

Susceptibility testing of *S. aureus* reference strain ATCC 29213 (MIC) / 25923 (disk diffusion)

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
Please mark the tested strain ☐ *S. aureus* ATCC 29213 ☐ *S. aureus* ATCC 25923	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Erythromycin, ERY	
	Florphenicol, FFN	
	Gentamicin, GEN	
	Penicillin, PEN	
	Streptomycin, STR	
	Sulphonamides, SMX	
	Tetracycline, TET	
	Trimethoprim, TMP	

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.1	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.2	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.3	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.4	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.5	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.6	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.7	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Strain	Antimicrobial	Interpretation		
		≤ >	Zonediam (mm) or MIC-value (µg/ml)	S / R
E. coli CRL EC 1.8	Amoxicillin cl., AUG			
	Ampicillin, AMP			
	Cefotaxime, CTX			
	Cefpodoxime, POD			
	Ceftazidime, CAZ			
	Ceftiofur, XNL			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Florphenicol, FFN			
	Gentamicin, GEN			
	Nalidixic acid, NAL			
	Streptomycin, STR			
	Sulphonamides, SMX			
	Tetracycline, TET			
	Trimethoprim, TMP			
	TMP+SMX, SXT			

Optional tests regarding ESBL production: All strains classified reduced susceptibility against CTX or CAZ (MIC > 0.25 and MIC > 0.50 respectively) or resistance against XNL (MIC > 1) are relevant to include for confirmatory tests for ESBL production.

The confirmatory tests for ESBL (CTX, CAZ) include as well tests for AmpC detection (FOX) and Metallo beta lactamase (IMI). Some of them consist of a susceptibility test with a pure antibiotic vs. a test with the same antibiotic combined with clavulanic acid or EDTA. If there is a 3 dilution steps difference in at least one of the 2 cases (mic ratio >= 8, E-test 3 dilution steps) or an increase in zone diameter >= 5 mm, the test is confirmed ESBL positive according to CLSI M100 Table 2A (enterobacteria). If the test shows signs of synergy it is an indication of the presence of ESBL.

	MIC, value or ratio		**Disks, zone diameter or increase**
CTX/CL : CTX mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or synergy ☐ Incr.< 5 mm
CAZ/CL : CAZ mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8	Incr. in zone diam	☐ Incr. >= 5 mm or 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	Comments:	
IMI/E : IMI mic ratio	☐ MIC ratio >= 8 or synergy ☐ MIC ratio < 8		

National Food Institute

TEST FORM

Susceptibility testing of *E. coli* reference strain ATCC 25922

Strain	Antimicrobial	Zonediameter (mm) or MIC-value (µg/ml)
E. coli ATCC 25922	Amoxicillin, AMX	
	Amoxicillin cl., AUG	
	Ampicillin, AMP	
	Cefotaxime, CTX	
	Cefpodoxime, POD	
	Ceftazidime, CAZ	
	Ceftiofur, XNL	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Florphenicol, FFN	
	Gentamicin, GEN	
	Nalidixic Acid, NAL	
	Streptomycin, STR	
	Sulphonamides, SMX	
	Tetracycline, TET	
	Trimethoprim, TMP	
	Trimethoprim + Sulphonamides, SXT	
	Cefoxitin, FOX	
	Imipenem, IMI	

INSTRUCTIONS FOR OPENING AND REVIVING FREEZE-DRIED CULTURES

Manual for *E. coli* CCM 3954/ATCC 25922, *E. faecalis* CCM 4224/ATCC 29212, *S. aureus* CCM 3953/ATCC 25923 (disk diffusion) and *S. aureus* CCM 4223/ATCC 29213 (MIC determination)

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

1 HANDLING THE AMPOULES

Freeze-dried 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!

2 SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

2.1 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.

2.2 References

M100-S17, January 2007 (Performance Standards for Antimicrobial Susceptibility Testing)

M07-A6, January 2003 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved Standard)

2.3 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

2.4 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

2.5 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.

2.6 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:

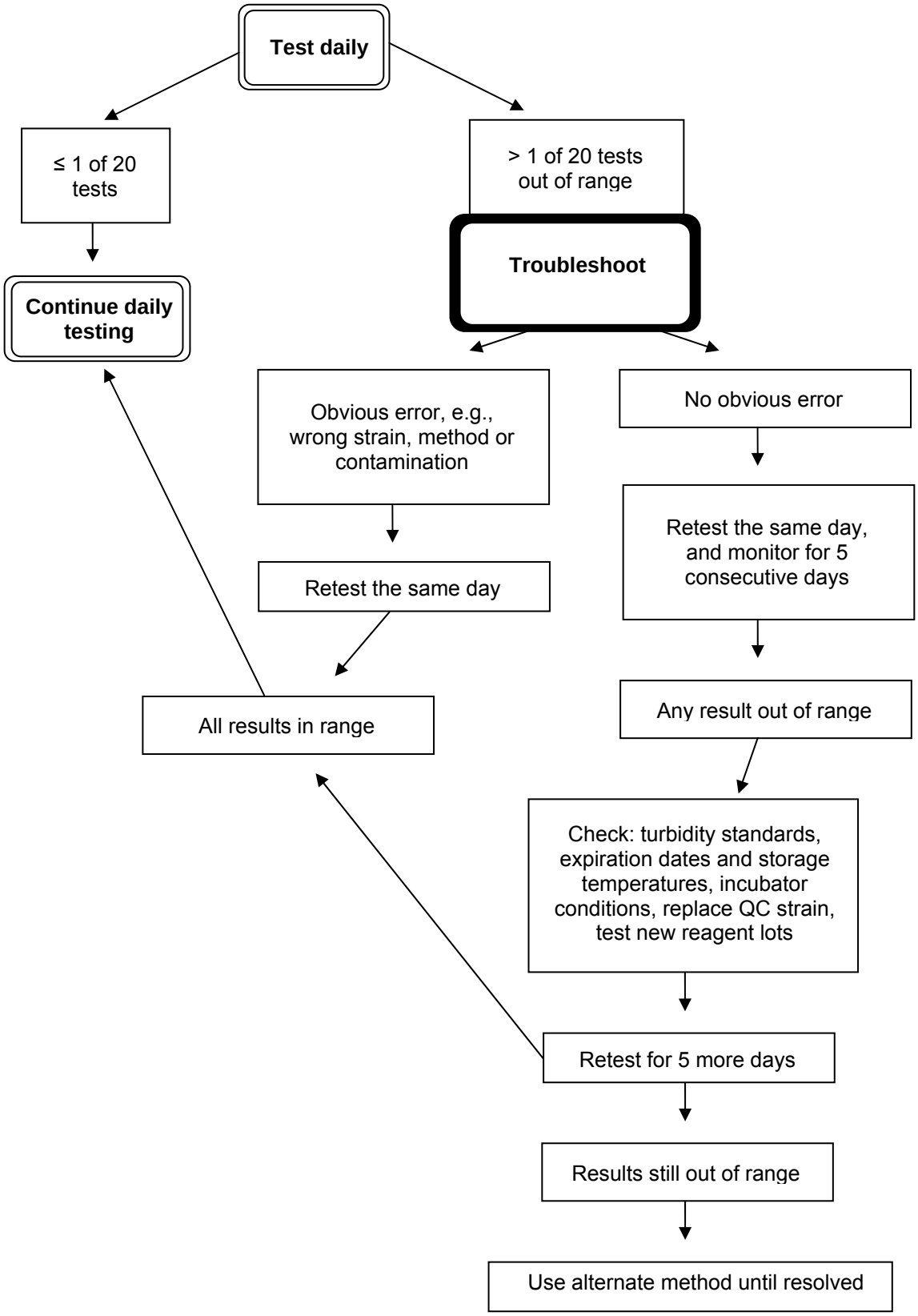
- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

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.

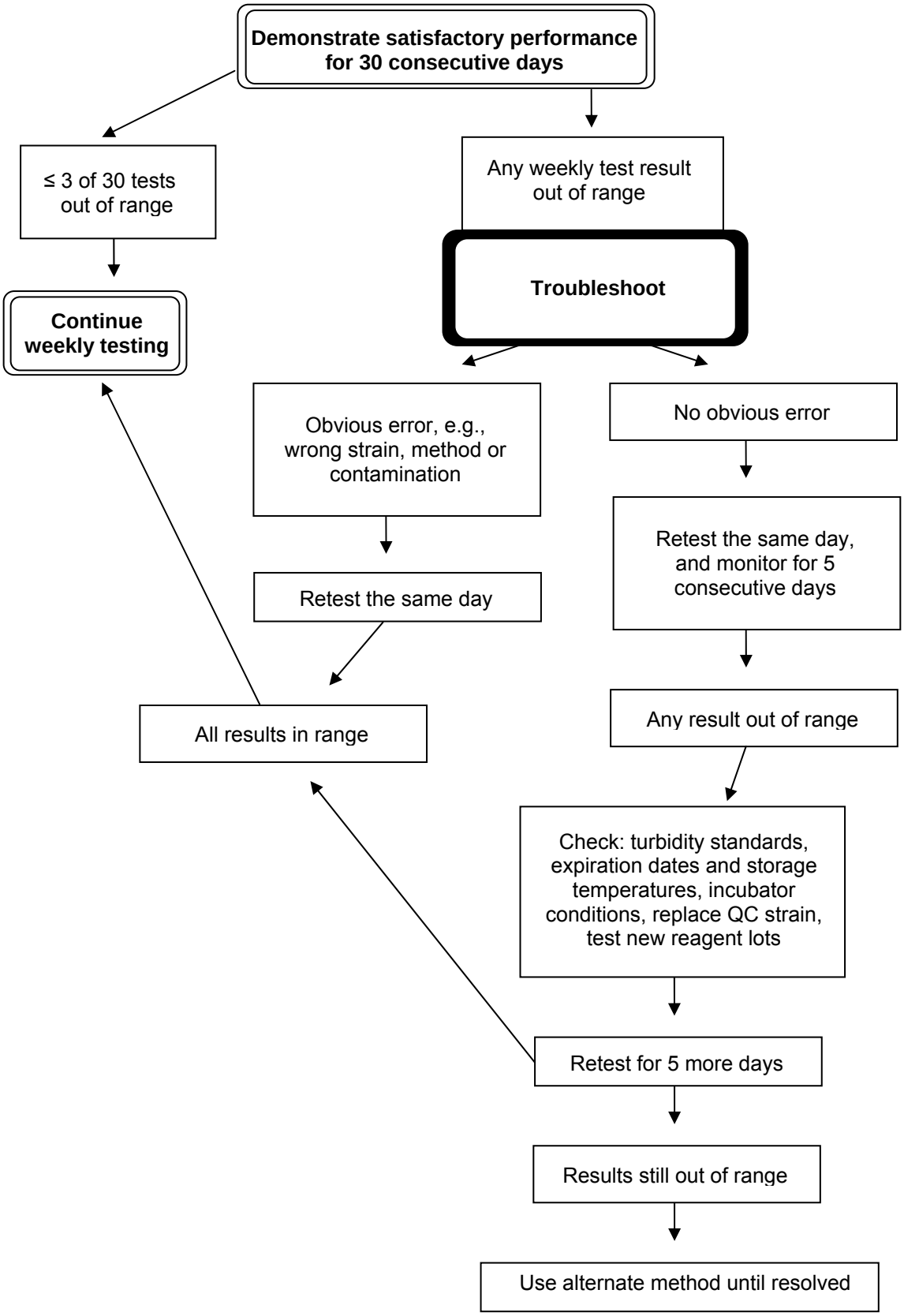
Repeat the 30 days validation before resuming weekly testing.

3 DAILY MIC QC CHART



Modified from CLSI M7-A6, page 35

4 WEEKLY MIC QC CHART



Modified from CLSI M7-A6, page 36

App. 4c
EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2007

National Food Institute

EVALUATION FORM

As means of improving the quality and usefulness of the EQAS
we kindly ask you to take a moment to complete this evaluation form

---

Name:

Country:

---

1. Information received during the CRL AR EQAS 2007 and how the EQAS was performed:

	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general	☐	☐	☐	☐	☐
The EQAS protocol and test forms	☐	☐	☐	☐	☐
The distribution of the samples	☐	☐	☐	☐	☐
What is your overall impression of the interactive web database	☐	☐	☐	☐	☐
How did participation in this EQAS meet your expectations	☐	☐	☐	☐	☐

Comments, suggestions:

2. Did you enter your results in the interactive web database?

☐ yes ☐ no

If not, please specify why:

3. Did you meet limitations or problems when entering data into the interactive web database?

☐ yes ☐ no

If yes, please specify:

4. General comments or suggestions for the EQAS (procedures, species, number of strains, antimicrobials etc.):

App. 4d
EU Community Reference Laboratory for Antimicrobial Resistance

Questionnaire
CRL EQAS EC/Ent/Staph 2007

As means of having updated information on your laboratory's work with *E. coli*, enterococci and staphylococci, we ask you to please fill in the information listed below.

Considering the antimicrobials we ask for information on your *routine diagnostic methods* in you laboratory as well as the test ranges.

Please send the questionnaire back by email to Michael Krause (mik@food.dtu.dk).

Annual isolates and susceptibility tests

How many *E. coli* isolates does your laboratory annual isolate:

How many enterococci isolates does your laboratory annual isolate:

How many staphylococci isolates does your laboratory annual isolate:

How many *E. coli* isolates does your laboratory annual susceptibility test:

How many enterococci isolates does your laboratory annual susceptibility test:

How many staphylococci isolates does your laboratory annual susceptibility test:

Please list information on antimicrobials as described on the following pages.

Comments or additional information:

App. 4d

EU Community Reference Laboratory for Antimicrobial Resistance

Routine diagnostic method used for AST of *E. coli*

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

	Antimicrobial	Disk content	Test-range for MIC

App. 4d

EU Community Reference Laboratory for Antimicrobial Resistance

Routine diagnostic method used for AST of enterococci

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

	Antimicrobial	Disk content	Test-range for MIC

App. 4d

EU Community Reference Laboratory for Antimicrobial Resistance

Routine diagnostic method used for AST of staphylococci

☐ MIC – Microbroth dilution
☐ MIC – Macro dilution tubes
☐ MIC – Agar dilution
☐ E-test
☐ Disc diffusion
☐ Tablets – Neo Sensitabs, Rosco
Brand**:**
Incubation conditions: °C/ h

	Antimicrobial	Disk content	Test-range for MIC

Appendix 5

Breakpoints used by the participants for disk diffusion

DD enterococci break points							
Antibiotic	**Lab No**	**S val**	**R val**	**Antibiotic**	**Lab No**	**S val**	**R val**
Ampicillin, AMP	15	>=19	<14	Gentamicin, GEN	5	>=10	<=6
	5	>=17	<=16		9	>=10	<=6
	9	>=17	<=16		21	>=10	<=6
	19	>=17	<=16		26	>=10	<=6
	21	>=17	<=16		15	>=17	<11
	22	>=17	<=16		23	>=15	<=12
	23	>=17	<=16		29	>=15	<=12
	26	>=17	<=16		14		<17
	29	>=17	<=16		22	>=23	<=18
	14		<19		19	>=10	
	20		<=19	Linezolid, LZD	5	>=23	<=20
Chloram-phenicol, CHL	5	>=18	<=12		21	>=23	<=20
	19	>=18	<=12		20		<=21
	20		<=12		14		<24
	21	>=18	<=12	Streptomycin, STR	5	>=10	<=6
	22	>=18	<=12		9	>=10	<=6
	23	>=18	<=12		21	>=10	<=6
	26	>=18	<=12		20		<=10
	29	>=18	<=12		22	>=15	<=11
	14		<19		23	>=15	<=11
	9	>=18	<13		29	>=15	<=11
Ciprofloxacin, CIP	15	>=22	<14		15	>=14	<12
	5	>=21	<=15		19	>=10	
	9	>=21	<=15	Synacid, SYN	20		<=15
	19	>=21	<=15		21	>=19	<=15
	20		<=15	Tetracycline, TET	5	>=19	<=14
	21	>=21	<=15		9	>=19	<=14
	22	>=21	<=15		19	>=19	<=14
	23	>=21	<=15		21	>=19	<=14
	26	>=21	<=15		22	>=19	<=14
	29	>=21	<=15		23	>=19	<=14
	14		<19		26	>=19	<=14
Erythromycin, ERY	5	>=23	<=13		29	>=19	<=14
	9	>=23	<=13		15	>=19	<17
	19	>=23	<=13		14		<19
	21	>=23	<=13		20		<=21
	22	>=23	<=13	Vancomycin, VAN	5	>=17	<=14
	23	>=23	<=13		19	>=17	<=14
	26	>=23	<=13		21	>=17	<=14
	29	>=23	<=13		23	>=17	<=14
	20		<=16		26	>=17	<=14
	14		<=17		29	>=17	<=14
	15	>=22	<=17		14		<17
Florfenicol, FFN	9	>=18	<=12		15	>=17	<17
	23	>=18	<=12		20		<=17
	29	>=18	<=12				
	19	>=19	<=14				
	26	>=19	<=14				
	15	>=19	<15				
	20		<=16				
	22	>=20	<=16				
	14		<19				

Appendix 5

Breakpoints used by the participants for disk diffusion

DD staphylococci p. 1							
Antibiotic	**Lab No**	**S**	**R**	**Antibiotic**	**Lab No**	**S**	**R**
Chloram-	2	>=18	<=12	**Florfenicol,**	9	>=18	<=12
phenicol, CHL	5	>=18	<=12	**FFN**	23	>=18	<=12
	9	>=18	<=12		34	>13	<=13
	18	>=18	<=12		18	>=19	<=14
	19	>=18	<=12		19	>=19	<=14
	21	>=18	<=12		29	>=19	<=14
	22	>=18	<=12		30	>=19	<=14
	23	>=18	<=12		15	>=19	<15
	28	>=18	<=12		20		<=16
	29	>=18	<=12		22	>=20	<=16
	30	>=18	<=12		14		<19
	34	>14	<=14	**Gentamicin,**	2	>=15	<=12
	20		<=15	**GEN**	5	>=15	<=12
	14		<19		9	>=15	<=12
Ciprofloxacin, CIP	2	>=21	<=15		18	>=15	<=12
	5	>=21	<=15		19	>=15	<=12
	9	>=21	<=15		21	>=15	<=12
	18	>=21	<=15		22	>=15	<=12
	19	>=21	<=15		23	>=15	<=12
	21	>=21	<=15		28	>=15	<=12
	22	>=21	<=15		29	>=15	<=12
	23	>=21	<=15		30	>=15	<=12
	28	>=21	<=15		34	>19	<=19
	29	>=21	<=15		13	>=20	<20
	30	>=21	<=15		14		<20
	15	>=21	<17		15	>=20	<20
	34	>17	<=17		20		<20
	13	>=22	<19	**Penicillin, PEN**	34	>24	<=24
	20		<=21		20		<=26
	14		<22		2	>=29	<=28
Erythromycin,	2	>=23	<=13		5	>=29	<=28
ERY	5	>=23	<=13		9	>=29	<=28
	9	>=23	<=13		19	>=29	<=28
	18	>=23	<=13		21	>=29	<=28
	19	>=23	<=13		22	>=29	<=28
	21	>=23	<=13		23	>=29	<=28
	22	>=23	<=13		28	>=29	<=28
	28	>=23	<=13		29	>=29	<=28
	29	>=23	<=13		30	>=29	<=28
	30	>=23	<=13		13	>=29	<=28
	34	>13	<=13		14		<29
	23	>=21	<=16		15	>=29	<29
	13	>=22	<17				
	15	>=22	<17				
	20		21				
	14		<22				

Appendix 5

Breakpoints used by the participants for disk diffusion

DD staphylococci p. 2							
Antibiotic	**Lab No**	**S**	**R**				
Streptomycin, STR	9	>=15	<=11				
	18	>=15	<=11				
	19	>=15	<=11				
	22	>=15	<=11				
	23	>=15	<=11				
	29	>=15	<=11				
	13	>=15	<13				
	14		<13				
	20		<=13				
	28	>=8	<16				
Suphonamides, SMX	9	>=17	<=12				
	13	>=17	<=12				
	18	>=17	<=12				
	19	>=17	<=12				
	21	>=17	<=12				
	22	>=17	<=12				
	23	>=17	<=12				
	28	>=17	<=12				
	29	>=17	<=12				
	30	>=17	<=12				
	34	>13	<=13				
	20		<=16				
	14		<17				
Tetracycline, TET	34	>13	<=13				
	2	>=19	<=14				
	5	>=19	<=14				
	9	>=19	<=14				
	18	>=19	<=14				
	19	>=19	<=14				
	21	>=19	<=14				
	22	>=19	<=14				
	23	>=19	<=14				
	28	>=19	<=14				
	29	>=19	<=14				
	30	>=19	<=14				
	13	>=19	<17				
	15	>=19	<17				
	14		<19				
	20		<=24				
Trimethoprim, TMP	2	>=16	<=10				
	9	>=16	<=10				
	18	>=16	<=10				
	19	>=16	<=10				
	21	>=16	<=10				
	22	>=16	<=10				
	23	>=16	<=10				
	28	>=16	<=10				
	30	>=16	<=10				
	14		<16				
	20		<=16				

Appendix 5

Breakpoints used by the participants for disk diffusion

DD E. coli p. 1							
Antibiotic	**Lab #**	**S**	**R**	**Antibiotic**	**Lab #**	**S**	**R**
Amoxicillin+cl, AUG	**5**	>=18	<=13	Ceftazidime, CAZ	**5**	>=18	<=14
	9	>=18	<=13		**19**	>=18	<=14
	18	>=18	<=13		**21**	>=18	<=14
	19	>=18	<=13		**23**	>=18	<=14
	21	>=18	<=13		**28**	>=18	<=14
	23	>=18	<=13		**30**	>=18	<=14
	28	>=18	<=13		**15**	>=21	<15
	29	>=18	<=13		**9**	>=21	<=17
	30	>=18	<=13		**14**	=	<21
	34	>13	<=13		**34**	>=21	<=21
	15	>=21	<14	Ceftiofur, XNL	**34**	>13	<=13
	20		<=18		**18**	>=18	<=14
	22	>=18	<=19		**9**	>=21	<=17
	14	=	<21		**19**	>=21	<=17
Ampicillin, AMP	**5**	>=17	<=13		**15**	>=21	<18
	9	>=17	<=13		**30**	>=23	<=19
	18	>=17	<=13		**14**	=	<21
	19	>=17	<=13		**20**	=	<=26
	21	>=17	<=13	Chloram-phenicol, CHL	**5**	>=18	<=12
	22	>=17	<=13		**9**	>=18	<=12
	23	>=17	<=13		**18**	>=18	<=12
	28	>=17	<=13		**19**	>=18	<=12
	29	>=17	<=13		**21**	>=18	<=12
	30	>=17	<=13		**22**	>=18	<=12
	34	>13	<=13		**23**	>=18	<=12
	15	>=21	<14		**28**	>=18	<=12
	20	=	<=17		**29**	>=18	<=12
	14	=	<19		**30**	>=18	<=12
Cefotaxime, CTX	**5**	>=23	<=14		**34**	>13	<=13
	9	>=23	<=14		**20**	=	<=16
	18	>=23	<=14		**14**	=	<19
	19	>=23	<=14		**15**	>=23	<19
	21	>=23	<=14	Ciprofloxacin, CIP	**34**	>13	<=13
	22	>=23	<=14		**5**	>=21	<=15
	28	>=23	<=14		**9**	>=21	<=15
	29	>=23	<=14		**18**	>=21	<=15
	30	>=23	<=14		**19**	>=21	<=15
	15	>=21	<15		**21**	>=21	<=15
	14	=	<21		**22**	>=21	<=15
	20	=	<=27		**23**	>=21	<=15
	34	>29	<=29		**28**	>=21	<=15
Cefpodoxime, POD	**9**	>=18	<=14		**29**	>=21	<=15
	19	>=21	<=17		**30**	>=21	<=15
	30	>=21	<=17		**15**	>=22	<17
	34	>19	<=19		**14**	=	<25
	20	=	<=23		**20**	=	<=29

Appendix 5

Breakpoints used by the participants for disk diffusion

DD E. coli p. 2							
Antibiotic	**Lab #**	**S**	**R**	**Antibiotic**	**Lab #**	**S**	**R**
Gentamicin, GEN	**5**	>=15	<=12	Sulphonamides, SMX	**5**	>=17	<=12
	9	>=15	<=12		**9**	>=17	<=12
	18	>=15	<=12		**15**	>=17	<=12
	19	>=15	<=12		**18**	>=17	<=12
	21	>=15	<=12		**19**	>=17	<=12
	22	>=15	<=12		**21**	>=17	<=12
	23	>=15	<=12		**22**	>=17	<=12
	28	>=15	<=12		**23**	>=17	<=12
	29	>=15	<=12		**28**	>=17	<=12
	30	>=15	<=12		**29**	>=17	<=12
	15	>=18	<16		**30**	>=17	<=12
	20	=	<=17		**20**	=	<=13
	14	=	<18		**34**	>13	<=13
	34	>19	<=19	Tetracycline,TET	19	>=15	<=11
Florphenicol, FFN	**23**	>=18	<=12		21	>=15	<=11
	29	>=18	<=12		20	=	<=11
	34	>13	<=13		34	>13	<=13
	9	>=19	<=14		5	>=19	<=14
	18	>=19	<=14		9	>=19	<=14
	19	>=19	<=14		18	>=19	<=14
	20	=	<=14		22	>=19	<=14
	30	>=19	<=14		23	>=19	<=14
	15	>=19	<=16		28	>=19	<=14
	22	>=20	<=16		29	>=19	<=14
	14	=	<19		30	>=19	<=14
Nalidixic acid, NAL	**5**	>=19	<=13		14	=	<17
	9	>=19	<=13		15	>=19	<17
	18	>=19	<=13	TMP+SMX, SXT	5	>=16	<=10
	19	>=19	<=13		9	>=16	<=10
	21	>=19	<=13		18	>=16	<=10
	22	>=19	<=13		19	>=16	<=10
	23	>=19	<=13		21	>=16	<=10
	28	>=19	<=13		22	>=16	<=10
	29	>=19	<=13		23	>=16	<=10
	30	>=19	<=13		28	>=16	<=10
	34	>13	<=13		29	>=16	<=10
	14	=	<=15		30	>=16	<=10
	15	>=20	<=15		34	>13	<=13
	20	=	<=15		14	=	<16
Streptomycin, STR	**9**	>=15	<=11		20	=	<=22
	18	>=15	<=11	Trimethoprim, TMP	5	>=16	<=10
	19	>=15	<=11		9	>=16	<=10
	21	>=15	<=11		18	>=16	<=10
	22	>=15	<=11		19	>=16	<=10
	23	>=15	<=11		21	>=16	<=10
	28	>=15	<=11		22	>=16	<=10
	29	>=15	<=11		23	>=16	<=10
	30	>=15	<=11		28	>=16	<=10
	14	=	<=13		30	>=16	<=10
	15	>=15	<=13		15	>=16	<12
	20	=	<=13		34	>13	<=13
	34	>12	<=13		20	=	<=20

Formateret: Punktopstilling

Appendix 6
Tentative cut off values recommended by EFSA

Antimicrobials for enterococci	MIC (µg/mL) **R is >**	MIC (µg/mL) **R is >**
Ampicillin, AMP	4	4
Avilamycin, AVI	16	8
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Daptomycin, DAP	4	4
Erythromycin, ERY	4	4
Florfenicol, FFN	8	8
Gentamicin, GEN	32	32
Linezolid. LZD	4	4
Streptomycin (high level), STR	2048	2048
Quinpristin-dalfopristin (Synacid), SYN	4	32
Tetracycline, TET	2	2
Tigecycline, TGC	0,25	0,25
Vancomycin, VAN	4	4

Antimicrobials for *S. aureus*	MIC (µg/mL) **R is >**
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	1
Penicillin, PEN	0,25
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	4

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Amoxicillin cl., AUG	8
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Cefpodoxime, POP	1*
Ceftazidime, CAZ	0.5
Ceftiofur, XNL	1
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.032
Florfenicol, FFN	16
Gentamicin, GEN	2
Nalidixic acid, NAL	16
Streptomycin, STR	16
Sulfonamides, SMX	256**
Tetracycline, TET	8
Trimethoprim, TMP	2
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	0,5

* Tentative ** CLSI

Appendix 7 QC range for reference strains

Antimicrobial	QC ranges for *E. faecalis* 29212 MIC determination.
Ampicillin, AMP	.5 – 2
Avilamycin, AVI	.5 – 4
Chloramphenicol, CHL	4-16
Ciprofloxacin, CIP	.25 – 2
Daptomycin, DAP	1-8
Erythromycin, ERY	1-4
Florfenicol, FFN	2-8
Gentamicin, GEN	4-16
Linezolid, LZD	1-4
Synacid, SYN	2-8
Tetracycline, TET	8-32
Tigecycline, TGC	.03 - .12
Vancomycin, VAN	1-4

Antimicrobial	QC ranges for *S. aureus* 25923 by disk diffusion
Chloramphenicol, CHL	16 - 26
Ciprofloxacin, CIP	22 - 30
Erythromycin, ERY	22 - 30
Gentamicin, GEN	19 - 27
Penicillin, PEN	26 - 37
Streptomycin, STR	14 - 22
Suphonamides, SMX	24 - 30
Tetracycline, TET	24 - 34
Trimethoprim, TMP	19 - 26

Antimicrobial	QC range for *S. aureus* 25923 by E-test
Chloramphenicol, CHL	2-8
Ciprofloxacin, CIP	.125 - .5
Erythromycin, ERY	.125 - .5
Suphonamides, SMX	8-32
Tetracycline, TET	.125 - 1
Trimethoprim, TMP	.5 - 2

.

Antimicrobial	QC ranges for *S. aureus* 25913 by MIC determination
Chloramphenicol, CHL	2-8
Ciprofloxacin, CIP	.12 - .5
Erythromycin, ERY	.25 - 1
Florfenicol, FFN	2-8
Gentamicin, GEN	.12 - 1
Penicillin, PEN	.25 - 2
Suphonamides, SMX	32 - 128
Tetracycline, TET	.12 - 1
Trimethoprim, TMP	1-4

Antibiotic	QC range for *E. coli* ATCC 25922 by disk diffusion
Amoxicillin cl., AUG	18 - 24
Amoxicillin, AMX	0 - 50
Ampicillin, AMP	16 - 22
Cefotaxime, CTX	29 - 35
Cefpodoxime, POD	23 - 28
Ceftazidime, CAZ	25 - 32
Ceftiofur, XNL	26 - 31
Chloramphenicol, CHL	21 - 27
Ciprofloxacin, CIP	30 - 40
Florphenicol, FFN	22 - 28
Gentamicin, GEN	19 - 26
Nalidixic acid, NAL	22 - 28
Streptomycin, STR	0 - 50
Sulphonamides, SMX	15 - 23
Tetracycline, TET	18 - 25
Trimethoprim, TMP	21 - 28

Antimicrobial	QC ranges for *E. coli* ATCC 25922 by E-test
Ampicillin, AMP	2-8
Nalidixic acid, NAL	1-4
Streptomycin, STR	2-8
Sulphonamides, SMX	32 - 128
Tetracycline, TET	.5 - 2
Trimethoprim, TMP	.5 - 2

Appendix 7 QC range for reference strains

Antimicrobial	QC ranges for *E. coli* ATCC 25922 using MIC determination
Amoxicillin cl., AUG	2-8
Amoxicillin, AMX	No range
Ampicillin, AMP	2-8
Cefotaxime, CTX	.03 - .12
Cefpodoxime, POD	.25 - 1
Ceftazidime, CAZ	.06 - .5
Ceftiofur, XNL	.25 - 1
Chloramphenicol, CHL	2-8
Ciprofloxacin, CIP	.004 - .015
Florphenicol, FFN	2-8
Gentamicin, GEN	.25 - 1
Nalidixic acid, NAL	1-4
Streptomycin, STR	4-16
Sulphonamides, SMX	8-32
Tetracycline, TET	.5 - 2
Trimethoprim, TMP	.5 - 2

Appendix 8.: The summarised evaluation form

Participants' evaluation of the CRL EQAS EC/Ent/Staph 2007

The number of participating laboratories in the CRL EQAS EC/Ent/Staph 2007 was 32. Subsequent to submitting their results, the participants were asked to fill in an evaluation form as a means of improving the quality and usefulness of the EQAS. In the following, the information obtained through the 20 completed evaluation forms is collected and commented. It is of great value to have comments from the participants, it helps us to optimise the EQAS. Please find comments from the CRL in *italic* in the following.

1. Information received during the CRL AR EQAS 2007 and how the EQAS was performed:

Opinion Percentage (number of laboratories)	Very poor	Poor	Satisfactory	Good	Very good
Information about the EQAS in general	-	-	10% (2)	37% (7)	53% (10)
The EQAS protocol and test forms	-	-	16% (3)	37% (7)	47% (9)
The distribution of the samples	-	5% (1)	16% (3)	37% (7)	42% (8)
What is your overall impression of the interactive web database	-	-	18% (3)	41% (7)	41% (7)
How did participation in this EQAS meet your expectations	-	-	29% (5)	35% (7)	35% (6)

Comments and proposals from participants:

Samples arrived at ambient temperature without storage conditions on the packaging whereas in the protocol, it was noticed that strains must be kept refrigerated. Without information on the day of shipment and the absence of the person in charge of the trial, strains were stored at room temperature during 4 days. *In future EQAS's the participants will receive a message when we are closing in on the shipment date and also it will be marked on the parcel that it should be refrigerated on arrival.*

It was not possible to get the evaluation of the results. *Evaluation of the results should instantly be available after approving the results, if this should not be the case or if other database problems occur, please do not hesitate to contact the CRL and we will look into the problem.*

Some of the expected values were not correct, but was quickly adjusted. Very good communication and ability to react when questions or problems during the EQAS. *The CRL are always very alert when an EQAS has just been launched and it is very valuable to have incorrect values pointed out.*

The samples had to be collected at the airport custom office 300 kilometers away from the laboratory whereas they should be delivered by door-to-door service. *When shipping the strains we use Fedex as the first choice (door-door delivery), but to a number of countries they do not have a service regarding UN3373. Our second choice has been DSV who deliver door-airport. The airport should of course always be the one nearest to the consignee, which was not the case in the situation mentioned and which has been pointed out to the shipper. The CRL aim to use DSV door-door service as a second choice in the future.*

We did not receive the reference strain for *E. coli*. *All new participants to an EQAS of either* E. coli, *enterococci, staphylococci,* Salmonella *or* Campylobacter *should receive the relevant reference strain. The participants are expected to maintain the reference strain and thus it is only supplied once. Specifically for* E. coli *the reference strain is the same as for* Salmonella, *therefore the reference strain was not part of the shipments to the EC/Ent/Staph EQAS for participants of the Salm/Camp EQAS 2006.*

One E. coli was not cultivable in our laboratory conditions. *Unfortunately, this might occasionally occur. The CRL perform a number of tests on the test material as means of ensuring the best possible test material for the participants to work with.*

2. Did you enter your results in the interactive database?

Opinion Percentage (number of laboratories)	
Yes	89% (16)
No	11% (2)

Comments and expectations and suggestions from participants:

Last year I had problems with the final report. *The database is an important tool in terms of evaluation of the obtained results. You are very welcome to contact the CRL if you experience technical problems with uploading and approving the results in the database.*

There was no username and password together with the strains. *In future EQAS's the username and password will follow the strains in the parcel. The participants' username and password does not change from year to year.*

3. Did you meet limitations or problems when entering data into the interactive web database?

Opinion Percentage (number of laboratories)	
Yes	41% (7)
No	59% (10)

Intermediate results could not be entered, and also epidemiological breakpoints were used for definition of "susceptible" or "resistant"; however only MIC values were given for this issue - for some antimicrobial agents we used disc diffusion testing and the CLSI breakpoints - this inevitably results in somewhat different assignment of the categories as CLSI uses "clinical breakpoints". *At the workshop in May in Copenhagen it was agreed that the CRL EQAS's will use the breakpoints EUCAST have recommended for the EFSA monitoring. These are epidemiological cut-off values that only categorize isolates as either sensitive or resistant which is the reason why it is not possible to categorize a strain as 'intermediate'. For laboratories which use disc diffusion it is relevant to keep in mind that it is results from routine methods that are interesting for this EQAS which also counts for breakpoints used for interpretation of disk diffusion diameters (could be eg. CLSI breakpoints or breakpoints suggested by the supplier). Therefore, our suggestion is that when interpreting disc diffusion results you use a relevant interpretation guideline, and – if no such one exists – leave the result out from the database.*

As we are using CLSI standard, we did not use some of the antibiotic substances indicated in the forms. *With regard to the antimicrobials we also follow the EUCAST recommendations, as decided at the workshop in May 2007. In terms of the EQAS it is not a problem if a participant does not report results for all the listed antimicrobials.*

4. General comments or suggestions for the EQAS (procedures, species, number of strains, antimicrobials etc.):

I miss a field for entering the final conclusion on the ESBL confirmatory testing, fx ESBL-production yes/no. *This is added to the test form and in the database.*

SMX QC range does not exist, the CLSI only provide QC range for sulfisoxazole. *This has been corrected in the database.*

Would it be interesting to add an open field for results of not expected antimicrobials? And how about including *Pseudomonas* strains for MBLS and ESBL detection? Also, the selection of antimicrobials to test could be optimised (daptomycin, linezolid?) In the (distant) future, clinically (veterinary) relevant species would be great. Also, the list of mandatory antimicrobials could be established by a multinational experts panel at the European level, both for human and veterinary purposes. *In general, the EFSA recommendations regarding microorganisms and antimicrobials*

will be followed. At the conference and the workshop in June 2008 the selection of microorganisms and antimicrobials can be discussed.

For some antimicrobials we should provide much more details on which exact molecule we used, for instance the sulfamides family. Is everyone testing exactly the same molecule? Isn't it a source of tiny variation from one lab to another? *With regard to the breakpoints in general the CLSI guidelines are not specific as to which specific sulfonamide is used. Regarding QC range, though, the CLSI guidelines specifically mention sulfisoxazole.*

Send an e-mail few days before or the day of strains shipment to allow to leave the time of participants to organize the reception of the samples. *This will be done for future EQAS's.*

One participant states that it would be nice to see the results from the CRL from one or more independent tests of the test strains, making it clear, what was the basis for the evaluation is. Also, it is stated that it should be shown which breakpoints were used for the evaluation. *Tthe breakpoints used for evaluation are presented in the protocol. The basis for the categorization of the test strains are MIC tests performed by the CRL and verified by FDA. These results are presented in the report as expected values.*

Because of our country's new provision system, we would like to know at least one year earlier, if possible, any changes on antimicrobials or methodology. *Any major changes to the EQAS will be decided in the network at the annual workshop. In general, the antimicrobials and the methodology recommended by EUCAST/EFSA will be the basis for the CRL EQAS's.*

Strains should be sent in September. *With regard to shipment of strains we need to hold on to the plan. If the test period is a holiday period this will be taken into consideration when planning the deadline – there will always be a period of at least 1½ months from shipment to deadline.*

While entering the results for enterococci, staphylococci and *E. coli*, we noticed that it is necessary to enter breakpoints data every time and it is quite time-consuming. I was wondering if it is possible for you to save the data entered by each lab in the database so that only when there are changes in breakpoints used, any given lab can go to the page and modify them. *This suggestion has been presented to the systems developer and is effectuated from the following EQAS.*

We should like to receive reference strains, including those for special tests together with their certificate. *Certificates for the reference strains sent in connection to a CRL-EQAS are available for download from the CRL-website (www.crl-ar.eu).*

One participant states that number of strains is fine, whereas another would like it to be only 6 strains per species. *Eight strains of each species gives a number of tests that can be evaluated with some certainty with regard to determining the level of performance of the laboratory. This will be the number of strains of each species that we will be using in the future.*

There should be the possibility to add data of two different methods of testing the samples at the same time. *This is actually possible. If it is the case that you supplement one method with another, and thus would like to mix the methods used when you upload the data – this is no problem. The database evaluates on the interpretations and therefore it does not make a difference whether the obtained result is given as a zone diameter or a MIC-value. If you choose to mix the methods like this, please note that the method you have used for the QC-strain is the method you should mark on the first page! In case of using two different methods paralel on all antimicrobials it is possible for us to provide you with an extra username and password and thus you have the opportunity to upload two sets of results. If you choose to get an extra username and password please note that this extra set of results will not be evaluated in the report – only one set of results from each NRL will be evaluated in the report.*

You are collecting information on the zone diameter obtained in disc diffusion tests for the ATCC strains. We would of course not expect the diameter obtained to be the same with different disc diffusion methods (eg CLSI versus BSAC) even if the same concemtration disc is used, because the density of the inoculum is much greater with CLSI, resulting in a smaller zone. However, the end result (S, I or R) may be correct for all methods if the zone diameter breakpoints appropriate for each method (and different for each method) are used to interpret the results. The zone sizes obtained from the different methods should not be compared; only zone sizes for participating labs using the same method (eg CLSI or BSAC) can be validly compared.

This point is illustrated in the attached table which show the zone sizes we obtained from ATCC 25922 using the BSAC method and the target ranges for this isolate as quoted by BSAC. In the EQAS exercise some of our zone sizes fall outside of the CLSI targets for ATCC 25922 and were scored accordingly but when compared to available BSAC targets all zones were within target.

It is correct that the use of other guidelines than CLSI regarding QC strains gives deviations in the database. Since the focus of the EQAS is to harmonise the work with AST we need work with the guidelines agreed upon in the network.

Additional comments from the CRL

It is very useful for us to have had all these comments. Thank you very much for taking your time to write them down. In general, we welcome any comments or enquiries that you may have. You are welcome to write us an email and we will make an effort to get back to you a.s.a.p. with an answer or some relevant advice.

Appendix 9 The summarised questionnaire

Antimicrobials and ranges used in the daily routine by participants for AST of enterococci using MIC

Antimicrobial	Laboratory #						
	2	**9**	**12**	**16**	**25**	**28**	**33**
Ampicillin AMP		0.5-64	0.25-32	0.25-128	1-128	0.5-16	0.25-32
Avilamycin AVI	1-32			0.06-64			
Bacitracin			1-128		2-256		1U-128U
Chloramphenicol CHL	2-64		0.5-64	2-64	2-64	0.5-256	0.5-64
Ciprofloxacin CIP					0.5-64	0.5-256	
Erythromycin ERY	1-32		0.5-64	0.03-128	1-128	0.5-256	0.5-64
Flavomycin					4-512		
Flophenicol FFN	2-32						
Gentamicin GEN			2-256	0.5-512	128-1024	500 (high)	2-256
Kanamycin			16-2048				16-2048
Levofloxacin						0.5-256	
Linezolid LZD			0.5-16		0.25-32	0.016-256	0.5-16
Narasin			0.12-16				0.12-16
Norfloxacin						1-256	
Penicillin PEN						0.5-16	
Pristinamycin				0.06-64			
Quinupristin/Dalfopristin		0.125-16				0.02-32	
Salinomycin					0.5-64		
Streptomycin STR	128-2048		8-1024	4-2048	512-2048	2000 (high)	8-1024
Synacid SYN	0.5-32				0.5-32		
Teicoplanin		0.25-32					
Tetracycline TET	1-32	0.25-32	0.5-64	0.06-128	0.5-64	0.5-256	0.5-64
TMP+SMX .SXT							
Vancomycin VAN	1-32	0.25-32	1-128	0.12-128	0.5-64	0.5-256	1-128
Virginiamycin			0.5-64				0.5-64

Antimicrobials and ranges used in the daily routine by participants for AST of enterococci using disk

Antimicrobial	Laboratory #					
	2	**5**	**9**	**19**	**28**	**29**
Ampicillin AMP	10	10		10	10	
Chloramphenicol CHL		30			30	30
Ciprofloxacin CIP	5	5			5	5
Erythromycin ERY		15		15	15	15
Flophenicol FFN						30
Gentamicin GEN	10/120	120	120	120	120	10
Levofloxacin					5	
Linezolid LZD	30	30			30	
Norfloxacin					15	
Penicillin PEN					10	10IU
Quinupristin/Dalfopristin					15	
Streptomycin STR		300	300	300	300	10
Sulphonamides SMX						200
Tetracycline TET		30		30	30	30
TMP+SMX .SXT						1.25/23.75
Vancomycin VAN		30		30	30	

Appendix 9 The summarised questionnaire

Antimicrobials and disk content used in the daily routine by participants for AST of staphylococci using disk

Antimicrobial	**Laboratories #**					
	2	**5**	**9**	**19**	**28**	**29**
Ampicillin AMP						10
Ampicillin AMP cl. AUG					20/10	
Cefoxitin FOX		30	30	30	30 (screen VITEK)	
Cephalotin CEP					30	
Cetiofur XNL				30		
Chloramphenicol CHL	30	30			30	30
Ciprofloxacin CIP	5	5			5	4
Clindamycin		2			2	
Doxicyclin				30		
Erythromycin ERY	15			16	15	15
Flophenicol FFN				30		30
Fucidic Acid		10				
Gentamicin GEN	10	10		10	10	10
Kanamycin KAN					30	
Linezolid LZD		30			30	
Lincomycin				2		
Mupirocin		5				
Neomycin NEO				30		
Novobiocin				30		
Oxacillin OXA	1	1		1		
Quinopristin/Dalfopristin		15			15	
Penicillin PEN	10	10U		10	10U	
Rifampin		5			5	
Streptomycin STR						10
Teicoplanin		30				
Telithromycin					15	
Tetracycline TET	30	30		30	30	30
Tobramycin						
Trimethoprim TMP	5				25	
Tilmicosin				15		
TMP+SMX .SXT		1,25/23.75				
Tobramycin				10		
Vancomycin VAN	30			30	30	30

Appendix 9 The summarised questionnaire

Antimicrobials and range used in the daily routine by participants for AST of staphylococci using MIC

Antimicrobial/Laboratories#					
	9	**12**	**25**	**28**	**33**
Augmentin			0.12/0.06-16/8		
Ampicillin AMP cl. AUG				2/1-32/16	
Avilamycin AVI		1-64			
Cefoxitin FOX		0.12-16		6 (Screen VITEK)	
Cephalotin CEP		0.06-8	0.06-4		0.06-8
Chloramphenicol CHL	0.25-30				0.5-64
Ciprofloxacin CIP	0.125-16	0.06-4		0.5-8	0.06-4
Clindamycin	0.032-4	0.25-32	0.06-8	0.25-8	0.25-32
Doxicyclin					
Erythromycin ERY	0.032-4	0.25-32	0.12-16	0.25-8	0.25-32
Fucidic Acid	0.032-4	0.06-8			0.06-8
Gentamicin GEN	0.125-16	0.5-64		0.5-16	0.5-64
Kanamycin KAN		0.25-32	0.5-64	8-32	0.25-32
Linezolid LZD	0.125-16			0.5-8	
Mupirocin	0.125-16				
Neomycin NEO			0.12-16		
Oxacillin OXA	0.125-16	0.12-16	0.06-8		0.12-16
Quinopristin/Dalfopristin				0.25-16	
Penicillin PEN		0.03-4	0.06-8	0.03-0.5	0.03-4
Pirlymycin			0.12-16		
Rifampin	0.032-4			0.5-32	
Streptomycin STR		2-256	0.5-64		
Sulphonamides SMX					
Synacid SYN					
Teicoplanin	0.25-30				
Telithromycin					
Tetracycline TET		0.5-64	0.12-16	1-16	0.5-64
Tobramycin	0.125-16			4-16	
Trimethoprim TMP		0.5-32			0.5-32
TMP+SMX .SXT	0.125/2.38-2/76		0.12/2.38-16/304	10 (0.5/9.5)-320	
Vancomycin VAN	0.125-16	1-128			
Virginiamycin		0.25-32		1-32	

Appendix 9 The summarised questionnaire

Antimicrobials and disk content used in the daily routine by participants for AST of *E. coli* using disk

Antimicrobial	**Laboratory #**						
	2	**5**	**9**	**18**	**19**	**28**	**29**
Amikacin		30				30	
Amoxicillin cl. AUG		20/10	30	20/10	20/10	20/10	20/10
Ampicillin AMP				10	10	10	10
Apramycin APR					15		
Aztreonam			30			30	
Carbenicillin		100					
Cefixime						30	
Cefotaxime CTX	30	30	30	30	30	30	30
Cefoxitin FOX		30	30			5	
Cefpodoxime POD	10				10		
Cefuroxime		30				30	
Ceftazidime CAZ	30	30	30		30	30	
Cephalotin CEP		30	30			30	
Cetiofur XNL				30			
Chloramphenicol CHL		30		30	20	30	30
Ciprofloxacin CIP		5		5		5	5
Colistin				10			
Doxycyclin					30		
Enrofloxacin				5	5		
Flophenicol FFN				30	30		30
Flumequin					30		
Gentamicin GEN		10		10	10	10	10
Imipeneme						10	
Kanamycin KAN				30			
Meropenem							
Nalidixic acid NAL		30		30		30	30
Neomycin					30		
Ofloxacin						5	
Oxolinic acid					2		
Piperacillin						100	
Piperacillin-tazobactam						100/10	
Spectinomycin					100		
Streptomycin STR				10	10		15
Sulphonamides SMX				300	300		200
Synacid SYN							
Tetracycline TET		30		30	30	30	30
Ticarcillin						75	
Tobramycin						10	
Trimethoprim TMP				5		5	
TMP+SMX .SXT	25	1.25/23.75			25	1.25/23.75	1.25/23.75

Appendix 9 The summarised questionnaire

Antimicrobials and range used in the daily routine by participants for AST of E. *coli* using MIC

Antimicrobial	**Laboratory s#**						
	2	**9**	**12**	**16**	**25**	**28**	**33**
Amikacin		0.25-32		0.5-16		2-64	
Amoxicillin cl. AUG	2-32	0.25-32		1/0.5-32/16		2/1-32/16	
Ampicillin AMP	1-32	0.25-32	0.5-32	1-512	0.5-64	2-32	0.25-32
Avilamycin AVI							
Aztreonam						1-64	
Cefepime		0.25-32					
Cefixime						0.25-4	
Cefotaxime CTX		0.25-32	0.06-2	0.0015-8	0.12-16	1-64	0.06-2
Cefotoxine FX							
Cefoxitin FOX		0.25-32		0.25-128		4-64	
Cefpodoxime POD							
Ceftazidime CAZ		0.25-32		0.03-16	0.12-16	1-64	
Cefuroxime				0.5-32		1-64	
Cephalotin CEP						2-64	
Cetiofur XNL	0.5-8		0.12-16	0.25-8			0.12-16
Chloramphenicol CHL	2-64		1-128	2-512	2-128		1-128
Ciprofloxacin CIP	0.03-4.0	0.063-8	0.008-1	0.008-8	0.06-16	0.25-4	0.008-1
Colistin		0.25-32		0.03-16			
Flophenicol FFN	2-64		4-32	2-32	1-128		4-32
Gentamicin GEN	1-32	0.25-32	0.5-64	0.25-32	0.25-32	1-6	0.5-64
Imipeneme						0.5-16	
Kanamycin KAN			2-16	0.25-128			2-16
Meropenem		0.25-32					
Nalidixic acid NAL	8-128		1-128	1-256	2-128	2-32	1-128
Neomycin				0.5-64	1-128		
Ofloxacin						0.25-8	
Piperacillin						4-128	
Piperacillin-tazobactam		0.25-32				4/4-128/4	
Streptomycin STR	4-64	0.25-32	2-256	5-512			2-256
Sulphonamides SMX	64-1024		16-2048		8-1024		16-2048
Tetracycline TET	2-32		0.5-64	0.5-256	0.5-64	1-16	0.5-64
Ticarcillin						8-128	
Tigecycline TGC		0.063-8					
Tobramycin		0.25-32		0.25-16		1-16	
Trimethoprim TMP	4-32	0.25-32	0.25-32	0.12-64	0.5-64	0.5-16	0.25-32
TMP+SMX .SXT		0.063/1.19-4/152		1/19-16/304			

Appendix 10 Correct % R-S antimicrobial/strain

Enterococci

Antimicrobial	Strain	Correct	% R	%S
Ampicillin, AMP	CRL ENT.1,1	S	40	60
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	20	80
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	12	88
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	4	96
	CRL ENT.1,8	S	4	96
Avilamycin, AVI	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	0	100
	CRL ENT.1,8	R	80	20
Chloramphenicol, CHL	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	5	95
	CRL ENT.1,7	R	96	4
	CRL ENT.1,8	S	14	86
Ciprofloxacin, CIP	CRL ENT.1,1	S	60	40
	CRL ENT.1,2	S	11	89
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	37	63
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	R	95	5
	CRL ENT.1,8	S	21	79
Daptomycin, DAP	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	0	100
	CRL ENT.1,8	S	0	100
Erythromycin, ERY	CRL ENT.1,1	R	100	0
	CRL ENT.1,2	S	4	96
	CRL ENT.1,3	S	27	73
	CRL ENT.1,4	S	19	81
	CRL ENT.1,5	S	32	68
	CRL ENT.1,6	R	100	0
	CRL ENT.1,7	R	100	0
	CRL ENT.1,8	R	96	4

Enterococci

Antimicrobial	Strain	Correct	% R	%S
Florfenicol, FFN	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	8	92
	CRL ENT.1,8	S	8	92
Gentamicin, GEN	CRL ENT.1,1	S	8	92
	CRL ENT.1,2	S	4	96
	CRL ENT.1,3	S	8	92
	CRL ENT.1,4	S	8	92
	CRL ENT.1,5	S	4	96
	CRL ENT.1,6	R	80	20
	CRL ENT.1,7	R	96	4
	CRL ENT.1,8	S	4	96
Linezolid, LZD	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	0	100
	CRL ENT.1,8	S	0	100
Streptomycin, STR	CRL ENT.1,1	R	95	5
	CRL ENT.1,2	S	18	82
	CRL ENT.1,3	R	95	5
	CRL ENT.1,4	S	32	68
	CRL ENT.1,5	S	18	82
	CRL ENT.1,6	R	91	9
	CRL ENT.1,7	R	91	9
	CRL ENT.1,8	R	86	14
Synacid, SYN	CRL ENT.1,1	S	22	78
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	20	80
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	11	89
	CRL ENT.1,6	S	44	56
	CRL ENT.1,7	S	44	56
	CRL ENT.1,8	S	44	56
Tetracycline, TET	CRL ENT.1,1	R	100	0
	CRL ENT.1,2	S	4	96
	CRL ENT.1,3	R	100	0
	CRL ENT.1,4	S	4	96
	CRL ENT.1,5	R	100	0
	CRL ENT.1,6	R	100	0
	CRL ENT.1,7	R	96	4
	CRL ENT.1,8	R	38	62

Enterococci				
Antimicrobial	**Strain**	**Correct**	**% R**	**%S**
Tigecycline, TGC	CRL ENT.1,1	S	0	100
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	0	100
	CRL ENT.1,5	S	0	100
	CRL ENT.1,6	S	0	100
	CRL ENT.1,7	S	0	100
	CRL ENT.1,8	S	0	100
Vancomycin, VAN	CRL ENT.1,1	S	4	96
	CRL ENT.1,2	S	0	100
	CRL ENT.1,3	S	0	100
	CRL ENT.1,4	S	4	96
	CRL ENT.1,5	R	100	0
	CRL ENT.1,6	S	9	91
	CRL ENT.1,7	S	13	88
	CRL ENT.1,8	S	13	87

Appendix 10 Correct % R-S antimicrobial/strain

Staphylococci

Antimicrobial	Strain	Correct	% R	%S
Chloramphenicol, CHL	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	0	100
	CRL ST.1,3	S	4	96
	CRL ST.1,4	S	0	100
	CRL ST.1,5	S	4	96
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	S	0	100
Ciprofloxacin, CIP	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	0	100
	CRL ST.1,3	S	0	100
	CRL ST.1,4	R	97	3
	CRL ST.1,5	S	0	100
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	R	29	71
Erythromycin, ERY	CRL ST.1,1	S	0	100
	CRL ST.1,2	R	97	3
	CRL ST.1,3	R	100	0
	CRL ST.1,4	S	7	93
	CRL ST.1,5	R	100	0
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	S	0	100
Florfenicol, FFN	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	0	100
	CRL ST.1,3	S	0	100
	CRL ST.1,4	S	0	100
	CRL ST.1,5	S	0	100
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	S	0	100
Gentamicin, GEN	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	0	100
	CRL ST.1,3	S	4	96
	CRL ST.1,4	S	0	100
	CRL ST.1,5	S	0	100
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	R	96	4
Penicillin, PEN	CRL ST.1,1	R	69	31
	CRL ST.1,2	R	96	4
	CRL ST.1,3	R	96	4
	CRL ST.1,4	R	100	0
	CRL ST.1,5	R	100	0
	CRL ST.1,6	S	0	100
	CRL ST.1,7	R	100	0
	CRL ST.1,8	R	100	0

Staphylococci				
Antimicrobial	**Strain**	**Correct**	**% R**	**%S**
Streptomycin, STR	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	0	100
	CRL ST.1,3	S	5	95
	CRL ST.1,4	R	95	5
	CRL ST.1,5	R	95	5
	CRL ST.1,6	S	0	100
	CRL ST.1,7	S	0	100
	CRL ST.1,8	R	100	0
Suphonamides, SMX	CRL ST.1,1	R	83	17
	CRL ST.1,2	S	28	72
	CRL ST.1,3	S	18	82
	CRL ST.1,4	S	6	94
	CRL ST.1,5	S	11	89
	CRL ST.1,6	S	6	94
	CRL ST.1,7	S	0	100
	CRL ST.1,8	R	84	16
Tetracycline, TET	CRL ST.1,1	R	17	83
	CRL ST.1,2	R	100	0
	CRL ST.1,3	R	100	0
	CRL ST.1,4	R	100	0
	CRL ST.1,5	S	3	97
	CRL ST.1,6	S	0	100
	CRL ST.1,7	R	100	0
	CRL ST.1,8	R	100	0
Trimethoprim, TMP	CRL ST.1,1	S	0	100
	CRL ST.1,2	S	5	95
	CRL ST.1,3	R	100	0
	CRL ST.1,4	R	100	0
	CRL ST.1,5	R	100	0
	CRL ST.1,6	S	0	100
	CRL ST.1,7	R	100	0
	CRL ST.1,8	S	0	100

Appendix 10 Correct % R-S antimicrobial/strain

E. coli

Antimicrobial	Strain	Correct	% R	%S
Amoxicillin cl., AUG	CRL EC.1,1	S	5	95
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	22	78
	CRL EC.1,7	R	100	0
	CRL EC.1,8	S	5	95
Ampicillin, AMP	CRL EC.1,1	R	100	0
	CRL EC.1,2	S	0	100
	CRL EC.1,3	R	100	0
	CRL EC.1,4	S	12	88
	CRL EC.1,5	S	0	100
	CRL EC.1,6	R	96	4
	CRL EC.1,7	R	100	0
	CRL EC.1,8	R	100	0
Cefotaxime, CTX	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	R	96	4
	CRL EC.1,7	R	91	9
	CRL EC.1,8	S	0	100
Cefpodoxime, POD	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	R	100	0
	CRL EC.1,7	R	100	0
	CRL EC.1,8	S	0	100
Ceftazidime, CAZ	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	6	94
	CRL EC.1,5	S	0	100
	CRL EC.1,6	R	59	41
	CRL EC.1,7	R	100	0
	CRL EC.1,8	S	0	100
Ceftiofur, XNL	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	R	94	6
	CRL EC.1,7	R	88	12
	CRL EC.1,8	S	0	100

E. coli				
Antib	**Strain**	**Correct**	**% R**	**%S**
Chloramphenicol, CHL	CRL EC.1,1	R	100	0
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	4	96
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	R	100	0
	CRL EC.1,8	S	0	100
Ciprofloxacin, CIP	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	7	93
	CRL EC.1,5	S	3	97
	CRL EC.1,6	S	3	97
	CRL EC.1,7	S	3	97
	CRL EC.1,8	R	90	10
Florphenicol, FFN	CRL EC.1,1	R	100	0
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	S	0	100
	CRL EC.1,8	S	0	100
Gentamicin, GEN	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	R	89	11
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	S	0	100
	CRL EC.1,8	S	0	100
Nalidixic acid, NAL	CRL EC.1,1	S	0	100
	CRL EC.1,2	S	0	100
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	0	100
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	S	4	96
	CRL EC.1,8	R	100	0
Streptomycin, STR	CRL EC.1,1	R	100	0
	CRL EC.1,2	R	100	0
	CRL EC.1,3	R	100	0
	CRL EC.1,4	R	92	8
	CRL EC.1,5	S	8	92
	CRL EC.1,6	S	0	100
	CRL EC.1,7	R	100	0
	CRL EC.1,8	R	92	8

E. coli				
Antib	**Strain**	**Correct**	**% R**	**%S**
Sulphonamides, SMX	CRL EC.1,1	R	96	4
	CRL EC.1,2	S	4	96
	CRL EC.1,3	S	4	96
	CRL EC.1,4	S	8	92
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	R	100	0
	CRL EC.1,8	R	100	0
TMP+SMX, SXT	CRL EC.1,1	R	100	0
	CRL EC.1,2	S	45	55
	CRL EC.1,3	S	0	100
	CRL EC.1,4	S	11	89
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	5	95
	CRL EC.1,7	S	0	100
	CRL EC.1,8	R	100	0
Tetracycline, TET	CRL EC.1,1	R	100	0
	CRL EC.1,2	S	0	100
	CRL EC.1,3	R	100	0
	CRL EC.1,4	R	100	0
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	R	100	0
	CRL EC.1,8	R	100	0
Trimethoprim, TMP	CRL EC.1,1	R	100	0
	CRL EC.1,2	R	100	0
	CRL EC.1,3	S	0	100
	CRL EC.1,4	R	100	0
	CRL EC.1,5	S	0	100
	CRL EC.1,6	S	0	100
	CRL EC.1,7	S	4	96
	CRL EC.1,8	R	100	0

Lab	Strain	Antibiotic	Obtained	Expected
1	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
2	CRL ENT.1,6	Gentamicin, GEN	S	R
	CRL ENT.1,6	Streptomycin, STR	S	R
	CRL ENT.1,8	Streptomycin, STR	S	R
	ATCC 29212	Ampicillin, AMP	29	,5-2
	ATCC 29212	Ciprofloxacin, CIP	26	,25-2
	ATCC 29212	Gentamicin, GEN	19	4-16
	ATCC 29212	Linezolid, LZD	28	1-4
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
5	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,2	Streptomycin, STR	R	S
	CRL ENT.1,3	Ampicillin, AMP	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Erythromycin, ERY	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,5	Streptomycin, STR	R	S
	CRL ENT.1,6	Chloramphenicol, CHL	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
9	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,3	Ampicillin, AMP	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Erythromycin, ERY	R	S
	CRL ENT.1,5	Ampicillin, AMP	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,8	Ciprofloxacin, CIP	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
10	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,3	Ampicillin, AMP	R	S
	CRL ENT.1,6	Gentamicin, GEN	S	R
	CRL ENT.1,6	Synacid, SYN	R	S
	CRL ENT.1,7	Synacid, SYN	R	S
	CRL ENT.1,8	Synacid, SYN	R	S
	ATCC 29212	Ampicillin, AMP	4	,5-2
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
11	CRL ENT.1,2	Tetracycline, TET	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
12	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R

Lab	Strain	Antibiotic	Obtained	Expected
14	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,6	Gentamicin, GEN	S	R
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
15	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,6	Gentamicin, GEN	S	R
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
16	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
17	CRL ENT.1,1	Streptomycin, STR	S	R
	CRL ENT.1,3	Streptomycin, STR	S	R
	CRL ENT.1,6	Streptomycin, STR	S	R
	CRL ENT.1,7	Streptomycin, STR	S	R
	CRL ENT.1,8	Streptomycin, STR	S	R
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
19	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Erythromycin, ERY	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,6	Gentamicin, GEN	S	R
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
20	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,7	Vancomycin, VAN	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
	CRL ENT.1,8	Vancomycin, VAN	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
21	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,7	Chloramphenicol, CHL	S	R
	CRL ENT.1,7	Ciprofloxacin, CIP	S	R
	CRL ENT.1,7	Gentamicin, GEN	S	R
	CRL ENT.1,7	Streptomycin, STR	S	R
	CRL ENT.1,7	Tetracycline, TET	S	R
	CRL ENT.1,8	Ciprofloxacin, CIP	R	S
	CRL ENT.1,8	Streptomycin, STR	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
22	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,2	Streptomycin, STR	R	S
	CRL ENT.1,3	Ampicillin, AMP	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,5	Streptomycin, STR	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R

Lab	Strain	Antibiotic	Obtained	Expected
23	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,1	Gentamicin, GEN	R	S
	CRL ENT.1,1	Vancomycin, VAN	R	S
	CRL ENT.1,2	Ciprofloxacin, CIP	R	S
	CRL ENT.1,2	Streptomycin, STR	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,3	Gentamicin, GEN	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Gentamicin, GEN	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,4	Vancomycin, VAN	R	S
	CRL ENT.1,5	Streptomycin, STR	R	S
	CRL ENT.1,6	Vancomycin, VAN	R	S
	CRL ENT.1,7	Vancomycin, VAN	R	S
	CRL ENT.1,8	Ciprofloxacin, CIP	R	S
	CRL ENT.1,8	Vancomycin, VAN	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
24	CRL ENT.1,6	Synacid, SYN	R	S
	CRL ENT.1,7	Synacid, SYN	R	S
	CRL ENT.1,8	Synacid, SYN	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
	ATCC 29212	Ciprofloxacin, CIP	0.05	,25-2
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
26	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,8	Erythromycin, ERY	S	R
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
27	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,4	Tetracycline, TET	R	S
	CRL ENT.1,5	Ampicillin, AMP	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,8	Chloramphenicol, CHL	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
28	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,3	Synacid, SYN	R	S
	CRL ENT.1,4	Erythromycin, ERY	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,6	Synacid, SYN	R	S
	CRL ENT.1,7	Synacid, SYN	R	S
	CRL ENT.1,8	Chloramphenicol, CHL	R	S
	CRL ENT.1,8	Synacid, SYN	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R

Lab	Strain	Antibiotic	Obtained	Expected
29	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,1	Ciprofloxacin, CIP	R	S
	CRL ENT.1,1	Gentamicin, GEN	R	S
	CRL ENT.1,2	Ciprofloxacin, CIP	R	S
	CRL ENT.1,2	Erythromycin, ERY	R	S
	CRL ENT.1,2	Gentamicin, GEN	R	S
	CRL ENT.1,2	Streptomycin, STR	R	S
	CRL ENT.1,3	Ampicillin, AMP	R	S
	CRL ENT.1,3	Erythromycin, ERY	R	S
	CRL ENT.1,3	Gentamicin, GEN	R	S
	CRL ENT.1,4	Ciprofloxacin, CIP	R	S
	CRL ENT.1,4	Gentamicin, GEN	R	S
	CRL ENT.1,4	Streptomycin, STR	R	S
	CRL ENT.1,5	Ampicillin, AMP	R	S
	CRL ENT.1,5	Erythromycin, ERY	R	S
	CRL ENT.1,5	Gentamicin, GEN	R	S
	CRL ENT.1,5	Streptomycin, STR	R	S
	CRL ENT.1,6	Vancomycin, VAN	R	S
	CRL ENT.1,7	Ampicillin, AMP	R	S
	CRL ENT.1,7	Florfenicol, FFN	R	S
	CRL ENT.1,7	Vancomycin, VAN	R	S
	CRL ENT.1,8	Ampicillin, AMP	R	S
	CRL ENT.1,8	Chloramphenicol, CHL	R	S
	CRL ENT.1,8	Ciprofloxacin, CIP	R	S
	CRL ENT.1,8	Florfenicol, FFN	R	S
	CRL ENT.1,8	Gentamicin, GEN	R	S
	CRL ENT.1,8	Vancomycin, VAN	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
33	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
34	CRL ENT.1,1	Synacid, SYN	R	S
	CRL ENT.1,3	Synacid, SYN	R	S
	CRL ENT.1,5	Synacid, SYN	R	S
	CRL ENT.1,6	Synacid, SYN	R	S
	CRL ENT.1,7	Synacid, SYN	R	S
	CRL ENT.1,8	Avilamycin, AVI	S	R
	CRL ENT.1,8	Synacid, SYN	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R
Lab.	**Strain**	**Antibiotic**	**Obtained**	**Expected**
35	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,1	Synacid, SYN	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
36	CRL ENT.1,1	Ampicillin, AMP	R	S
	CRL ENT.1,8	Tetracycline, TET	S	R

Lab	Strain	Antibiotic	Obtained	Expected
1	ATCC 29213	Suphonamides, SMX	8	32-128
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
2007	CRL ST.1,1	Penicillin, PEN	S	R
2	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
4	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,2	Suphonamides, SMX	R	S
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 29213	Ciprofloxacin, CIP	0.75	,125-,5
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
5	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
9	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
10	CRL ST.1,3	Chloramphenicol, CHL	R	S
	CRL ST.1,5	Chloramphenicol, CHL	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
11	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
12	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 29213	Chloramphenicol, CHL	16	2-8
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
13	CRL ST.1,1	Tetracycline, TET	S	R
	ATCC 25923	Suphonamides, SMX	22	24-30
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
14	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25923	Chloramphenicol, CHL	27	16-26
	ATCC 25923	Ciprofloxacin, CIP	31	22-30
	ATCC 25923	Penicillin, PEN	38	26-37
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
15	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,1	Methicillin resistant	Neg	Pos
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25923	Ciprofloxacin, CIP	38	22-30
	ATCC 25923	Gentamicin, GEN	29	19-27
	ATCC 25923	Penicillin, PEN	39	26-37

Lab	Strain	Antibiotic	Obtained	Expected
16	CRL ST.1,1	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
17	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,1	Methicillin resistant	Neg	Pos
	CRL ST.1,2	Erythromycin, ERY	S	R
	CRL ST.1,2	Suphonamides, SMX	R	S
	CRL ST.1,3	Suphonamides, SMX	R	S
	CRL ST.1,4	Erythromycin, ERY	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
18	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,2	Suphonamides, SMX	R	S
	CRL ST.1,3	Suphonamides, SMX	R	S
	CRL ST.1,5	Suphonamides, SMX	R	S
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25923	Suphonamides, SMX	21.6	24-30
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
19	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25923	Suphonamides, SMX	20	24-30
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
20	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
21	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Suphonamides, SMX	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
22	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,2	Suphonamides, SMX	R	S
	CRL ST.1,4	Ciprofloxacin, CIP	S	R
	CRL ST.1,4	Erythromycin, ERY	R	S
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25923	Suphonamides, SMX	22	24-30
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
23	CRL ST.1,1	Tetracycline, TET	S	R
	ATCC 25923	Ciprofloxacin, CIP	21	22-30
	ATCC 25923	Penicillin, PEN	19	26-37
	ATCC 25923	Suphonamides, SMX	22	24-30
	ATCC 25923	Tetracycline, TET	22	24-34
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
24	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,4	Streptomycin, STR	S	R
	CRL ST.1,5	Streptomycin, STR	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	CRL ST.1,8	Gentamicin, GEN	S	R

Lab	Strain	Antibiotic	Obtained	Expected
25	CRL ST.1,1	Tetracycline, TET	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
26	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,2	Penicillin, PEN	S	R
	CRL ST.1,3	Penicillin, PEN	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
28	CRL ST.1,1	Suphonamides, SMX	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	CRL ST.1,8	Suphonamides, SMX	S	R
	ATCC 25923	Gentamicin, GEN	29	19-27
	ATCC 25923	Penicillin, PEN	38	26-37
	ATCC 25923	Trimethoprim, TMP	28	19-26
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
29	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,2	Suphonamides, SMX	R	S
	CRL ST.1,3	Gentamicin, GEN	R	S
	CRL ST.1,3	Streptomycin, STR	R	S
	CRL ST.1,3	Suphonamides, SMX	R	S
	CRL ST.1,4	Suphonamides, SMX	R	S
	CRL ST.1,5	Suphonamides, SMX	R	S
	CRL ST.1,6	Suphonamides, SMX	R	S
	ATCC 25923	Ciprofloxacin, CIP	20	22-30
	ATCC 25923	Erythromycin, ERY	19	22-30
	ATCC 25923	Gentamicin, GEN	17	19-27
	ATCC 25923	Penicillin, PEN	15	26-37
	ATCC 25923	Streptomycin, STR	12	14-22
	ATCC 25923	Suphonamides, SMX	0	24-30
	ATCC 25923	Tetracycline, TET	20	24-34
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
30	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
31	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	ATCC 29213	Ciprofloxacin, CIP	1	,12-,5
	ATCC 29213	Erythromycin, ERY	0.12	,25-1
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
33	CRL ST.1,2	Trimethoprim, TMP	R	S
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
34	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,1	Methicillin resistant	Neg	Pos
	CRL ST.1,8	Suphonamides, SMX	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**

35	CRL ST.1,1	Penicillin, PEN	S	R
	CRL ST.1,1	Suphonamides, SMX	S	R
	CRL ST.1,1	Tetracycline, TET	S	R
	CRL ST.1,5	Tetracycline, TET	R	S
	CRL ST.1,8	Ciprofloxacin, CIP	S	R
	CRL ST.1,8	Suphonamides, SMX	S	R
	CRL ST.1,8	Methicillin resistant	Neg	Pos
	ATCC 29213	Suphonamides, SMX	23	32-128
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
36	CRL ST.1,8	Ciprofloxacin, CIP	S	R

Lab	**Strain**	**Antibiotic**	**Obtained**	**Exspected**
4	ATCC 25922	Tetracycline, TET	6	.5-2
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
5	CRL EC.1,3	Gentamicin, GEN	S	R
	CRL EC.1,4	Ampicillin, AMP	R	S
	CRL EC.1,6	Ceftazidime, CAZ	S	R
	CRL EC.1,8	Ciprofloxacin, CIP	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
9	CRL EC.1,6	Amoxicillin cl., AUG	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
10	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,6	Amoxicillin cl., AUG	R	S
	ATCC 25922	Streptomycin, STR	2	4-16
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
11	CRL EC.1,4	Ciprofloxacin, CIP	R	S
	CRL EC.1,4	Sulphonamides, SMX	R	S
	CRL EC.1,7	Trimethoprim, TMP	R	S
	ATCC 25922	Ciprofloxacin, CIP	1	.004-.015
	ATCC 25922	Sulphonamides, SMX	2048	8-32
Lab	**Antibiotic**	**Obtained**	**Expected**	**Expected**
12	CRL EC.1,2	TMP+SMX, SXT	R	S
	ATCC 25922	Cefotaxime, CTX	0.5	.03-.12
	ATCC 25922	Ciprofloxacin, CIP	0.03	.004-.015
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
14	CRL EC.1,2	TMP+SMX, SXT	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
15	CRL EC.1,6	Ceftazidime, CAZ	S	R
	ATCC 25922	Amoxicillin cl., AUG	25	18-24
	ATCC 25922	Ceftazidime, CAZ	33	25-32
	ATCC 25922	Cefotaxime, CTX	37	29-35
	ATCC 25922	Chloramphenicol, CHL	29	21-27
	ATCC 25922	Sulphonamides, SMX	26	15-23
	ATCC 25922	Tetracycline, TET	26	18-25
	ATCC 25922	Cefoxitin, FOX	31	23-29
	ATCC 25922	Imipenem, IMI	40	26-32
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
16	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,4	Sulphonamides, SMX	R	S
	CRL EC.1,4	TMP+SMX, SXT	R	S
	ATCC 25922	Sulphonamides, SMX	512	8-32
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
17	CRL EC.1,1	Sulphonamides, SMX	S	R
	CRL EC.1,4	Ceftazidime, CAZ	R	S
	CRL EC.1,6	Ceftazidime, CAZ	S	R
	CRL EC.1,7	Ciprofloxacin, CIP	R	S
	CRL EC.1,7	Nalidixic acid, NAL	R	S

Lab	Strain	Antibiotic	Obtained	Expected
18	CRL EC.1,3	Sulphonamides, SMX	R	S
	CRL EC.1,7	Cefotaxime, CTX	S	R
	CRL EC.1,7	Ceftiofur, XNL	S	R
	ATCC 25922	Ceftiofur, XNL	25	26-31
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
19	CRL EC.1,4	Ampicillin, AMP	R	S
	CRL EC.1,6	Amoxicillin cl., AUG	R	S
	ATCC 25922	Ceftazidime, CAZ	23	25-32
	ATCC 25922	Cefotaxime, CTX	27	29-35
	ATCC 25922	Florphenicol, FFN	33	22-28
	ATCC 25922	TMP+SMX, SXT	20	23-29
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
20	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,7	Cefoxitin, zone dia.	> 14 mm	<14\ AmpC
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
21	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,4	Streptomycin, STR	S	R
	CRL EC.1,6	Ceftazidime, CAZ	S	R
	CRL EC.1,8	Streptomycin, STR	S	R
	ATCC 25922	Florphenicol, FFN	29	22-28
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
22	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,3	Gentamicin, GEN	S	R
	CRL EC.1,7	Cefotaxime, CTX	S	R
	CRL EC.1,8	Ciprofloxacin, CIP	S	R
Lab	**Antibiotic**	**Obtained**	**Expected**	**Importance *)**
23	CRL EC.1,6	Ceftazidime, CAZ	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
24	CRL EC.1,1	Amoxicillin cl., AUG	R	S
	CRL EC.1,3	Gentamicin, GEN	S	R
	CRL EC.1,6	Amoxicillin cl., AUG	R	S
	CRL EC.1,7	CTX/CL:CTX mic ratio	ESBL	non-ESBL
	CRL EC.1,8	Amoxicillin cl., AUG	R	S
	CRL EC.1,8	Ciprofloxacin, CIP	S	R
	ATCC 25922	Cefotaxime, CTX	0.012	.03-.12
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
27	CRL EC.1,4	Ciprofloxacin, CIP	R	S
	CRL EC.1,5	Ciprofloxacin, CIP	R	S
	CRL EC.1,6	Ciprofloxacin, CIP	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
28	CRL EC.1,6	Ceftazidime, CAZ	S	R
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
29	CRL EC.1,2	Sulphonamides, SMX	R	S
	CRL EC.1,2	TMP+SMX, SXT	R	S
	CRL EC.1,4	TMP+SMX, SXT	R	S
	CRL EC.1,5	Streptomycin, STR	R	S
	CRL EC.1,6	TMP+SMX, SXT	R	S
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
30	CRL EC.1,2	TMP+SMX, SXT	R	S

Lab	Antibiotic	Obtained	Expected	Importance *)
32	CRL EC.1,4	Chloramphenicol, CHL	R	S
	CRL EC.1,5	Streptomycin, STR	R	S
	ATCC 25922	Cefotaxime, CTX	0.25	.03-.12
	ATCC 25922	Chloramphenicol, CHL	16	2-8
	ATCC 25922	Florphenicol, FFN	16	2-8
	ATCC 25922	Gentamicin, GEN	2	.25-1
	ATCC 25922	Nalidixic acid, NAL	16	1-4
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
33	ATCC 25922	Ciprofloxacin, CIP	0.03	.004-.015
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
34	CRL EC.1,4	Streptomycin, STR	S	R
	CRL EC.1,6	Ceftazidime, CAZ	S	R
	CRL EC.1,7	Ceftiofur, XNL	S	R
	CRL EC.1,8	Streptomycin, STR	S	R
	ATCC 25922	Cefpodoxime, POD	30	23-28
	ATCC 25922	Ceftiofur, XNL	34	26-31
	ATCC 25922	Ceftazidime, CAZ	33	25-32
	ATCC 25922	Cefotaxime, CTX	38	29-35
	ATCC 25922	Chloramphenicol, CHL	20	21-27
	ATCC 25922	Nalidixic acid, NAL	29	22-28
	ATCC 25922	Sulphonamides, SMX	25	15-23
	ATCC 25922	Tetracycline, TET	26	18-25
	ATCC 25922	TMP+SMX, SXT	31	23-29
	ATCC 25922	Imipenem, IMI	37	26-32
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
35	CRL EC.1,4	Ampicillin, AMP	R	S
	ATCC 25922	Amoxicillin cl., AUG	22	2-8
	ATCC 25922	Ceftazidime, CAZ	31	.06-.5
	ATCC 25922	Trimethoprim, TMP	26	.5-2
	ATCC 25922	Cefoxitin, FOX	27	2-8
	ATCC 25922	Imipenem, IMI	31	.06-.25
Lab	**Strain**	**Antibiotic**	**Obtained**	**Expected**
36	CRL EC.1,6	Ampicillin, AMP	S	R
	CRL EC.1,6	Cefotaxime, CTX	S	R
	CRL EC.1,6	Ceftiofur, XNL	S	R
	ATCC 25922	Ciprofloxacin, CIP	0.03	.004-.015

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