The 4th CRL-AR Profiency Testing enterococci, staphylococci and *E. coli* 2008

DTU Food
National Food Institute

Community Reference Laboratory – Antimicrobial Resistance

THE 4TH CRL-AR PROFICIENCY TESTING ENTEROCOCCI, STAPHYLOCOCCI AND *E. COLI* - 2008

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1. INTRODUCTION

This report summarises the results of the proficiency trial in antimicrobial susceptibility testing (AST) also known as External Quality Assurance System (EQAS 2008) concerning *Escherichia coli*, enterococci and staphylococci. The National Food Institute (DTU Food) was appointed as Community Reference Laboratory on Antimicrobial Resistance (CRL-AR) by the European Commission (EC) in 2006. Since then, this has been the 4th EQAS trial carried out within the CRL-AR network. The objective was to monitor the quality of the antimicrobial susceptibility data produced by the National Reference Laboratories (NRL) and identify areas of interest and/or laboratories, which may need guidance or assistance to produce reliable susceptibility data.

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

The technical advisory group for the CRL EQAS scheme consists of competent representatives from all NRL's, who meet once a year at the CRL- workshop. During the passed CRL-AR Workshop (2008), the network agreed upon the following decisions for EQAS 2008:

1. The accepted deviation for each laboratory was set up at 7%.
2. Results should be further analysed (possibly ignored) if only 75% are correct (test strain/antimicrobial combination).

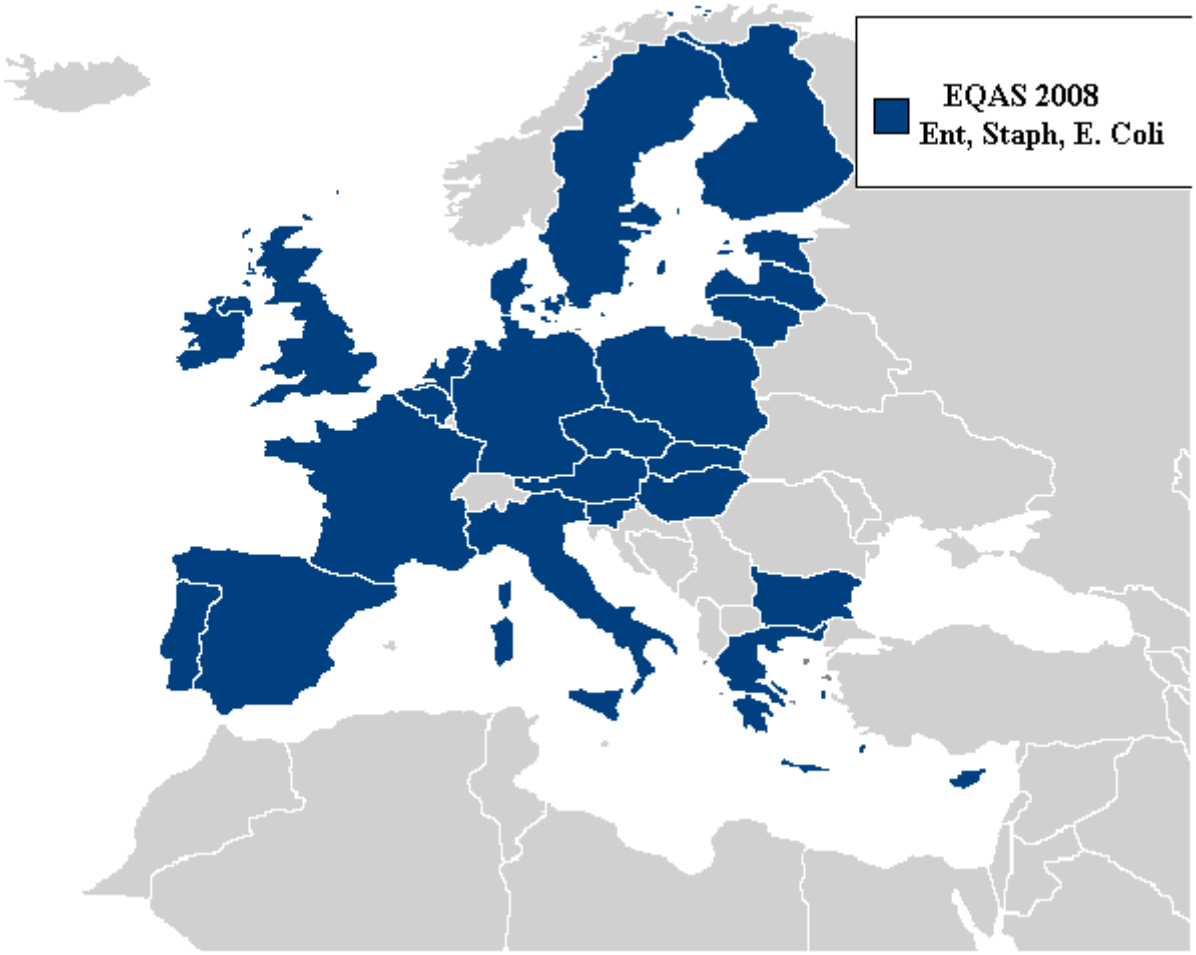
2. MATERIALS AND METHODS

2.1 Participants in EQAS 2008

In April 2008, a pre-notification to announce the EQAS 2008 on susceptibility testing for enterococci, staphylococci and *E. coli* was distributed by e-mail to the 32 European NRLs designated by the member states (App. 1). Three additional laboratories from Spain, Romania and Norway were enrolled by the CRL-AR to make up a total of 35 participating laboratories, although results from these three laboratories were not included in the evaluation. They represented all EU countries except for Luxembourg (App. 2). One of the three NRLs from Spain and the NRL from Romania declined to participate. The NRL from the United Kingdom that received the samples from Malta did not report back any results, therefore out of 35 participating laboratories, a total of 29 submitted results (Figure 1). Of those, 23, 28 and 27 laboratories analysed the enterococci,

staphylococci and the *E. coli* strains, respectively. A minor decrease in participation was observed when compared to EQAS 2007, when out of 34 participating laboratories, 26, 31 and 30 submitted results for enterococci, staphylococci and *E. coli*, respectively.

Figure 1. European map illustrating the participating countries in the EQAS trial 2008.



2.2 Strains

Eight strains of enterococci, staphylococci and *E. coli*, respectively were selected among the DTU Food strain collection. The selection of strains was based on antimicrobial resistance profile. Antimicrobial susceptibility testing on the strains was performed at DTU Food and the MIC values obtained were used as reference for the EQAS trial (App. 3). However, prior to distribution of the strains, the results were verified by the United States Food and Drug Administration (FDA), Centre for Veterinary Medicine. The strains were inoculated in agar stab cultures and subsequently sent to the participating laboratories.

New participating laboratories were provided with the following reference strains, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Furthermore, they were requested to save and maintain the ATCC reference strains for quality assurance purposes and future EQAS trials.

2.3 Antimicrobials

The panel of antimicrobials used for AST is listed in Table 1.

AST guidelines were set 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 determination at the CRL-AR was performed using the Sensititre system from Trek diagnostics Ltd. The MIC results were interpreted using the cut off values set by EUCAST (www.eucast.org), recommended by EFSA and described in the protocol (App. 4). E-test from AB-Biodisk was the method selected for ESBL analysis of the *E. coli* strains. Furthermore, result values of antimicrobials used for ESBL detection were interpreted according to the recommendations from CLSI.

During the previous years (2007-2008), NRL participants at the CRL-AR workshop in Copenhagen have agreed upon harmonising AST analyses by MIC determination using the antimicrobial panel and cut-off values recommended by EFSA.

Table 1. Panel of antimicrobials used for susceptibility testing in each of the organisms examined in the EQAS 2008.

Enterococci trial	Staphylococci trial*	*E. coli* trial
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†
Quinupristin-dalfopristin†		Ciprofloxacin†
Tetracycline†		Florfenicol
Tigecycline		Gentamicin†
Vancomycin†		Imipenem
		Imipenem-EDTA
		Nalidixic acid†
		Streptomycin†
		Sulphonamides†
		Tetracycline†
		Trimethoprim†
		Trimethoprim-sulphonamides

†Antimicrobials recommended by EFSA for monitoring European antimicrobial resistance.
*No specific recommendations have been suggested by EFSA for monitoring resistance in staphylococci.

2.4 Distribution

The protocols and other relevant material were made available to all participants from the CRL-AR website (http://crl-ar.eu). Cultures were dispatched in double pack containers (class UN 6.2) to the participating laboratories according to the International Air Transport Association (IATA) regulations as dangerous goods UN3373, category B.

2.5 Procedure

Upon arrival of the parcel and prior to performing the antimicrobial susceptibility test, the laboratories were instructed to place the tubes in a refrigerator and subculture the strains in accordance with the protocol. The cut off values for the MIC determination were also listed in this protocol (App. 4, tables 3.3.1; 3.3.2 and 3.3.3). Participants using disk diffusion method were advised to interpret the results according to their individual breakpoints (App. 5). In both cases the results were categorized as resistant or sensitive. Intermediate resistance was not accepted as a result. In addition, the laboratories also entered the zone diameter in millimeters or MIC value of the reference strains. The results were individually compared to the quality control ranges according to the CLSI documents M31-A2 (2002) / M100-S18 (2007), Trek Diagnostic Sensititre System or AB-Biodisk E-tests (App. 6).

All participating laboratories were advised to enter the results into an electronic record sheet at the CRL-AR web based database through a secured individual login and password. Alternatively, they were allowed to send the record sheet from the enclosed protocol by fax or email to CRL-AR. The website was opened for data entry until the 15th of September 2008.

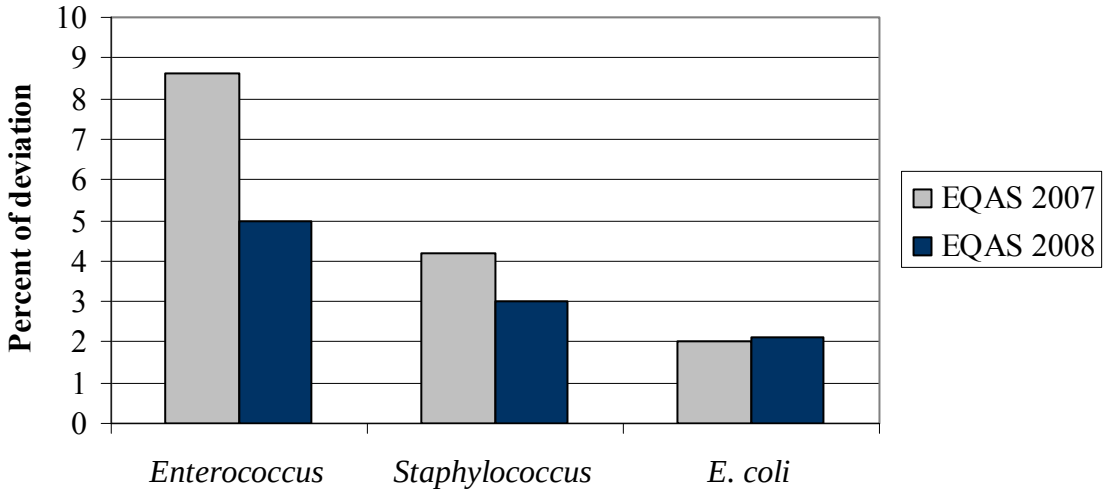
After submitting the data to the secured web site, the laboratories were instructed to retrieve an instantly generated individual report evaluating the submitted results where all deviations from the expected interpretations were reported. In addition and with the aim to improve future EQAS trials, participants were encouraged to fill in an evaluation report generated from the CRL-AR database (App. 10).

3. RESULTS

3.1 EQAS 2008 versus EQAS 2007

The percentages of deviation obtained for the enterococci and staphylococci trials in EQAS 2008 have decreased from 8.6% to 5% and from 4.2% to 3%, respectively when compared to 2007. On the other hand, the deviation results for the *E. coli* strains have registered a minor increased in EQAS 2008. Compared to the EQAS 2007, 95% of the enterococci strains, 96.9% of the staphylococci and 97.9% of the *E. coli* were interpreted correctly instead of the 91.4%, 95.8% and 98% from 2007 (Figure 2).

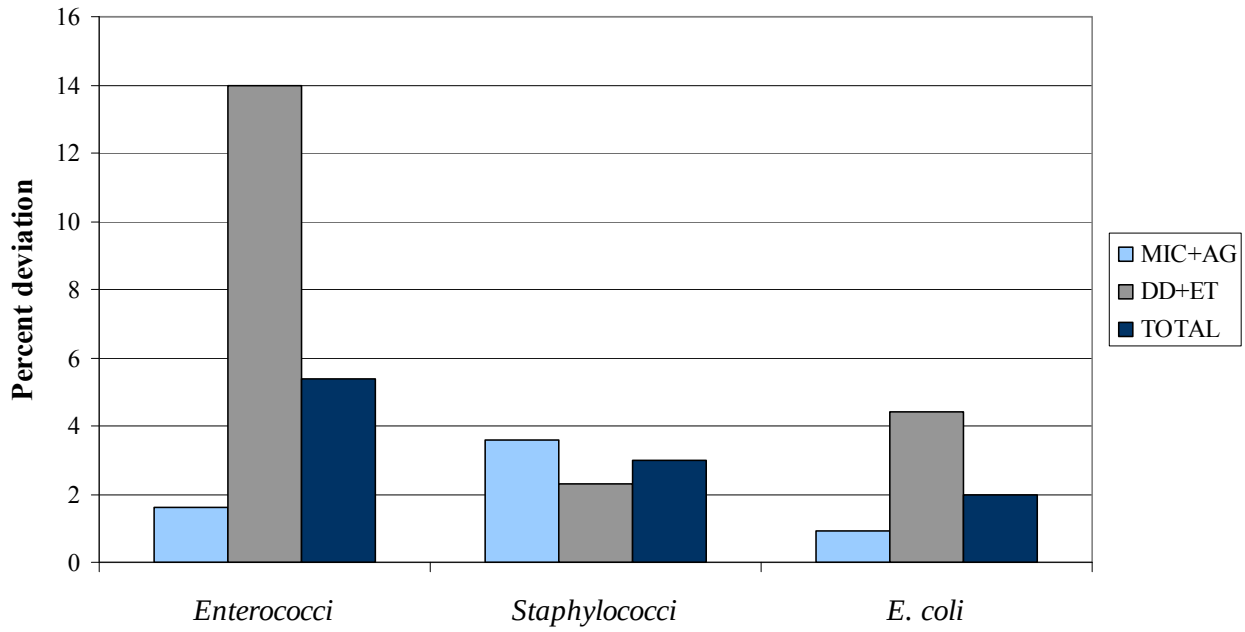
Figure 2. Comparison of results between EQAS 2007 and EQAS 2008 illustrating the deviation levels for the different species tested.



3.2 Deviations by strain

For analysis of data, agar dilution (AG) methods and MIC determination have been evaluated together since the obtained value is a concentration of which the antimicrobial inhibits the growth of the organism. E-test has been considered a disk diffusion (DD) method, since the antimicrobial would diffuse in the agar in the same way as a disk does. Generally, analysing the deviation results for the individual species obtained in the EQAS 2008, enterococci presented the highest deviation with respect to the other two species, mainly caused by the laboratories performing disk diffusion for AST. This method caused 16% deviation by comparison with the 1.4% caused by participants using MIC. Similar results were observed for the *E. coli* trial with values of 4.4% for disk diffusion when compared to the 0.9% for MIC. However, for the staphylococci trial it appeared that laboratories performing disk diffusion and E-test produced lower number of errors than those using MIC (Figure 3).

Figure 3. Percentage of deviations for the different strains comparing the different methods used for AST.



As shown in Table 2, the percentage of correct results per strains ranged from 92.4% to 99.8% depending of strain, with the best results obtained for *E. coli* trial, in which none of the strains obtained values below 93%. On the contrary, the enterococci trial was less successful with two strains out of eight exhibiting deviations higher than the 7% acceptance limit.

Table 2. The number of AST performed and the percentage of correct results for each strain.

Test strain	AST in total	% correct	Test strain	AST in total	% correct	Test strain	AST in total	% correct
ENT.2,1	213	**92.5%**	ST.2,1	224	99.1%	EC.2,1	386	98.7%
ENT.2,2	206	94.6%	ST.2,2	254	99.2%	EC.2,2	326	99.7%
ENT.2,3	215	96.7%	ST.2,3	255	**92.6%**	EC.2,3	353	96.9%
ENT.2,4	157	98.0%	ST.2,4	254	96.9%	EC.2,4	387	98.2%
ENT.2,5	206	95.1%	ST.2,5	254	96.5%	EC.2,5	374	95.7%
ENT.2,6	214	95.8%	ST.2,6	225	97.8%	EC.2,6	355	99.8%
ENT.2,7	203	93.1%	ST.2,7	252	96.0%	EC.2,7	355	99.4%
ENT.2,8	212	**92.4%**	ST.2,8	235	98.3%	EC.2,8	381	95.8%

The following sections describe in detail the deviations obtained for each one of the three EQAS trials carried out in 2008 depending on strain, antimicrobial and laboratory. It also analyses the results obtained for the quality control reference strains.

3.2.1 Enterococci trial

As agreed in previous CRL meetings, when the percentage of correct results was lower than 75% the data was subtracted from the analysis. In this case, the percentage of correct results for the combinations of strains ENT.2,2 with synacid, ENT.2,4 with ampicillin, ciprofloxacin, streptomycin, ENT.2,7 with daptomycin and synacid and ENT.2,8 with daptomycin were below the 75% and therefore they were not included in the evaluation (Table 3). However, to see the total percentage of positive results for each strain and antimicrobial tested refer to Appendix 7a.

Table 3. Enterococci strain and antimicrobial combination omitted from the EQAS evaluation.

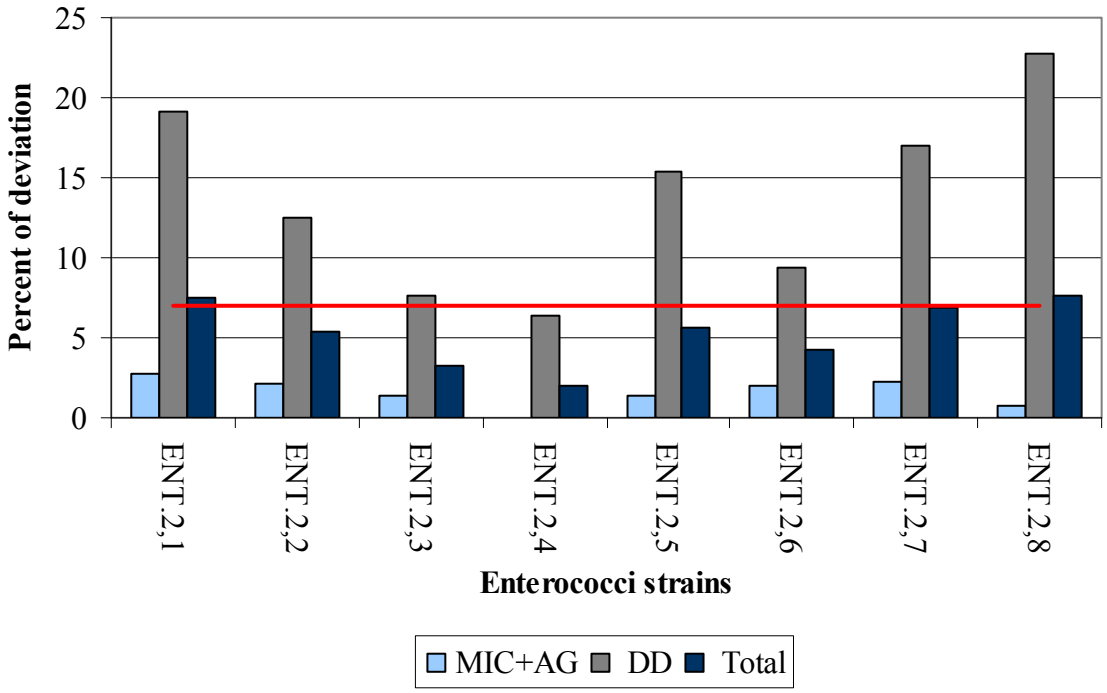
Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off Value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
ENT.2,2	Synacid	S	**63%**	16	32	2/7	1/1
ENT.2,4	Ampicillin	S	**45%**	4	4	9/15	3/7
ENT.2,4	Ciprofloxacin	S	**67%**	4	4	1/4	4/5
ENT.2,4	Streptomycin	R	**25%**	256	128	13/14	2/6
ENT.2,7	Daptomycin	S	**67%**	4	4	1/3	0
ENT.2,7	Synacid	S	**44%**	1	1	5/8	0/1
ENT.2,8	Daptomycin	S	**33%**	4	4	2/3	0

[1]MIC/n= number of laboratories that produced incorrect results by MIC determination / total number of laboratories performing MIC for AST in that specific strain.
[2]DD/n= number of laboratories that produced incorrect results by disk diffusion (DD) / total number of laboratories performing DD for AST in that specific strain

As illustrated in Figure 4, two strains presented deviations exceeding the acceptance level of 7%, those were ENT.2,1, and ENT.2,8 with values of 7.5%, and 7.6% respectively. Out of 23 laboratories participating in the enterococci trial, 16 used MIC determination and seven used disk diffusion. When comparing the different methods for AST used in each of the strains, the major deviations were observed in participating laboratories using disk diffusion. For all strains the deviation percentage is more than three times as high when performed by disk diffusion by

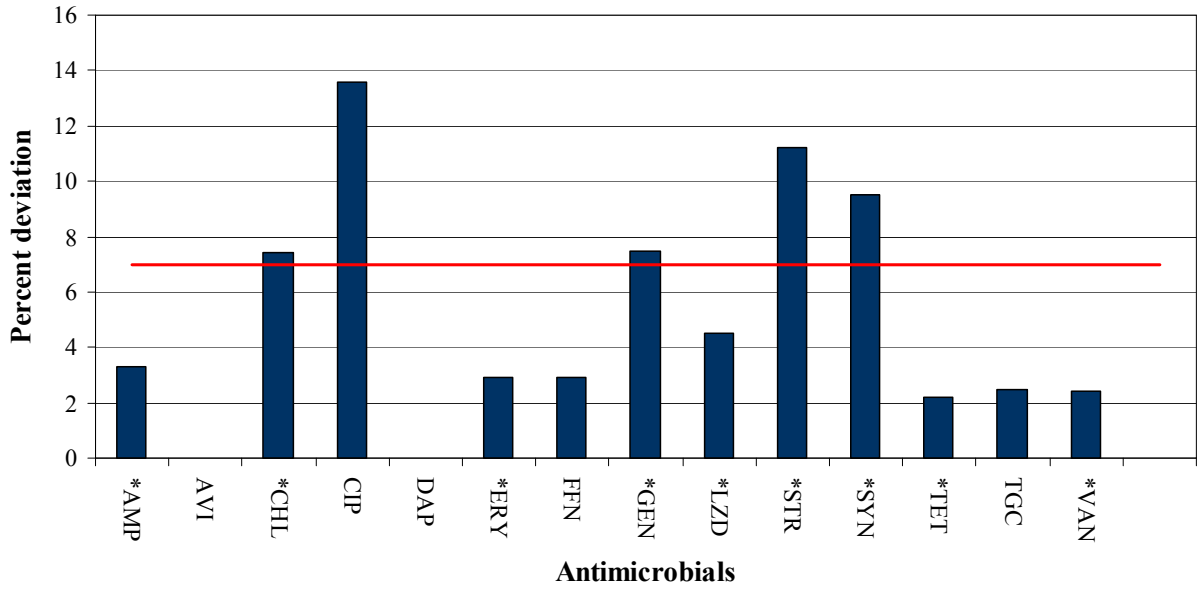
comparison to MIC and agar dilution (AG). Furthermore, significance differences were observed when comparing the two methods ($p < 0.01$)

Figure 4. Summary of the deviations obtained per strain according to the method used for AST by all participants.



Analysis of results per antimicrobial tested as presented in Figure 5, showed deviations above the 7% limit for five different antimicrobials with values as high as of 13.6% for ciprofloxacin, 11.2% for streptomycin, 9.5% for synacid, 7.5% for gentamicin and 7.0% for chloramphenicol. These last four compounds belonged to the panel of antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU.

Figure 5. Deviations in enterococcal strains per antimicrobial tested.



*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU.

3.2.2 Staphylococci trial

For the staphylococci strains, three combinations of strain/antimicrobial produced more than 25% incorrect results and subsequently were extracted from the evaluation. These combinations were ST.2,1 with ciprofloxacin, ST.2,6 with tetracycline and ST.2,8 with streptomycin (Table 4).

Table 4. Staphylococci strain and antimicrobial combination omitted from the EQAS evaluation.

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
ST.2,1	Ciprofloxacin	R	**38%**	2	1	9/17	9/11
ST.2,6	Tetracycline	R	**50%**	4	1	3/17	11/11
ST.2,8	Streptomycin	S	**36%**	16	32	6/12	8/10

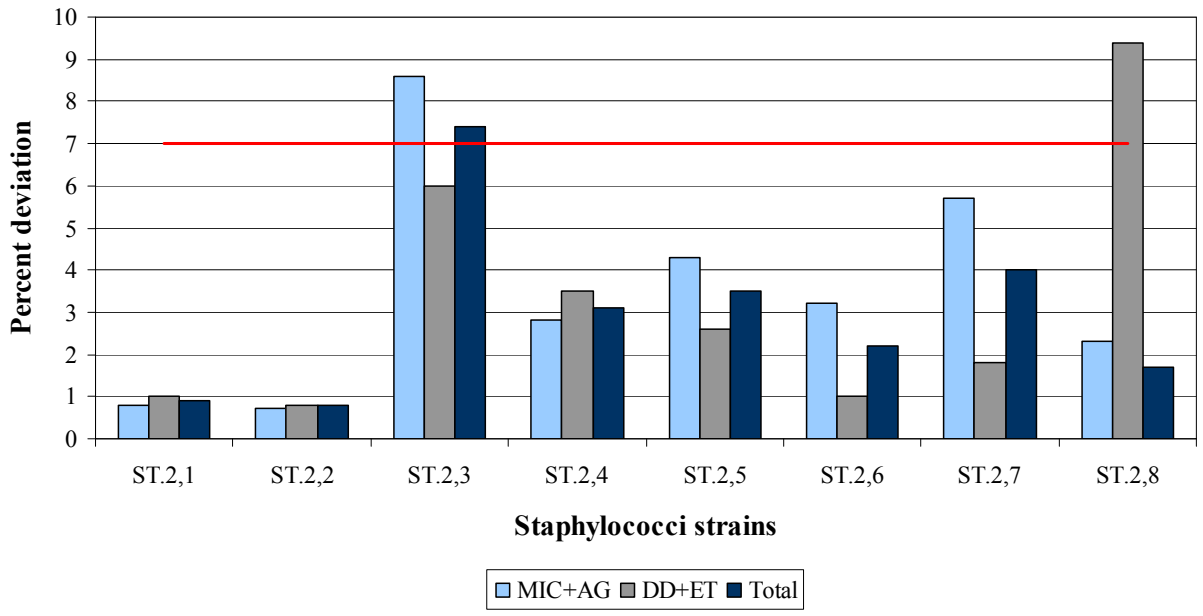
[1]MIC/n= number of laboratories that produced incorrect results by MIC determination / total number of laboratories performing MIC for AST in that specific strain.
[2]DD/n= number of laboratories that produced incorrect results by DD / total number of laboratories performing DD for AST in that specific strain

The results of the staphylococci trial were more positive than those for enterococci, with only one strain, ST.2,2 deviating more than the 7% limit (Figure 6). For the 28 laboratories involved in the

staphylococci trial, 16 used MIC determination, 11 disk diffusion and one E-test. Contrarily to the observations in the enterococci trial where higher deviations in all strains were observed when using disk diffusion methods, for staphylococci this does not appear to be the case. Furthermore not significance difference was observed when comparing the two different methods for AST ($p = 0.11$).

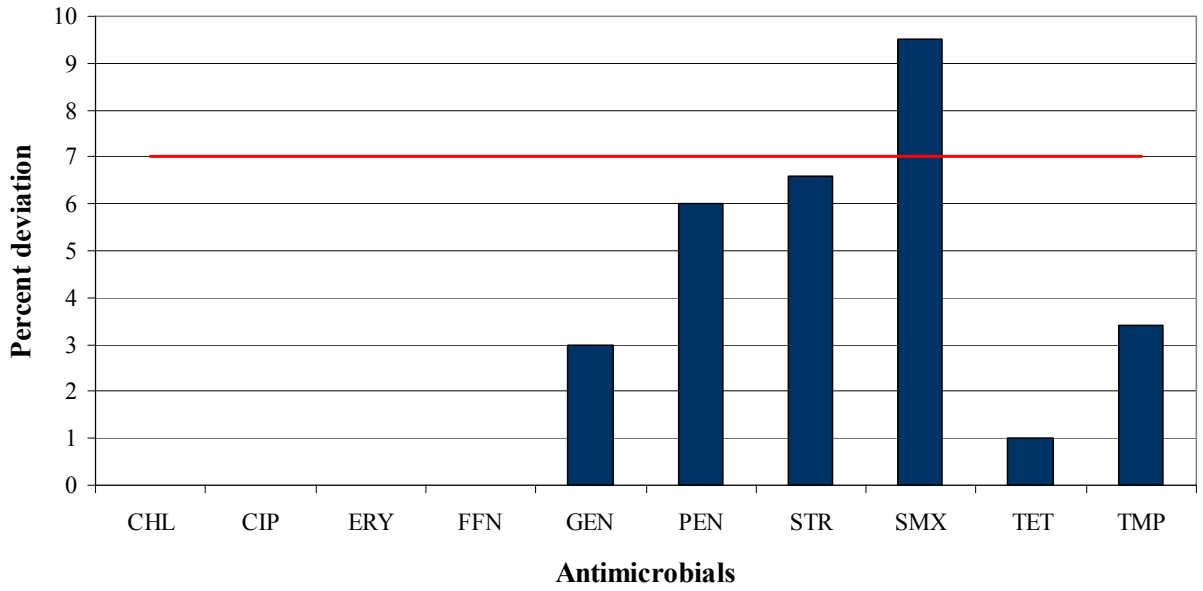
Except for strain ST.2,8 where the deviation value of the disk diffusion test (9.4%) was over four times higher than the deviations value of the MIC test (2.3%), strains such as ST.2,3, ST.2,5, ST.2,6 and ST.2,7 produced better results by using disk diffusion or E-test rather than MIC determination or agar dilution. Furthermore, results obtained by E-test were 100% correct for all strains although it was only one laboratory performing this method. For the rest of strains (ST.2,1, ST.2,2, ST2,4) similar values were obtained independently of the method used for antimicrobial susceptibility testing.

Figure 6. Summary of the deviations obtained per strain according to the method used for AST by all participants. Results produced by MIC and agar dilution have been evaluated together whereas disk diffusion has been evaluated together with E-test.



When analyzing the deviations in the staphylococci proficiency trial with respect to the antimicrobial tested (Figure 7), only sulphonamide exceeded the 7% limit with a value equivalent to 9.5%. To see in more detail all the results generated in the staphylococci trial with respect to each one of the antimicrobials tested, please refer to appendix 7b.

Figure 7. Deviations in staphylococcal strains per antimicrobial tested.



Methicillin resistant strains.

Among the eight staphylococcal strains selected for the trial, ST.2,1, ST.2,3 and ST.2,4 were methicillin resistant according to the expected results. Out of 28 laboratories taking part in the staphylococci EQAS trial, 27 NRLs agreed to test specifically for methicillin resistance genes by *mecA* PCR. In total, 10 participants did not obtain results in accordance with the expected results. Eight of them reported one of the *mecA* positive strains (ST.2,3) as negative, causing a deviation of 7% when analyzing the strain ST.2,3 against methicillin resistance. In addition, one laboratory reported two of the *mecA* positive strains as negative and two of the negative strains as positive. One more NRL reported one strain positive for methicillin that was negative.

3.2.3 *E. coli* trial

Regarding the analysis of the *E. coli* data, two combinations of strain/antimicrobial were subtracted from the evaluation for producing a low percentage of positive results. These combinations were EC.2,2 with streptomycin and EC.2,5 with amoxicillin/clavulanic acid (Table 5).

Table 5. *E. coli* strain and antimicrobial combination omitted from the EQAS evaluation.

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
EC.2,2	Streptomycin	S	12%	16	16	16/19	7/8
EC.2,5	Amoxicillin + clavulanic ac	S	50%	8	8	1/2	4/8

[1]MIC/n= number of laboratories that produced incorrect results by MIC determination / total number of laboratories performing MIC for AST in that specific strain.
[2]DD/n= number of laboratories that produced incorrect results by DD / total number of laboratories performing DD for AST in that specific strain

All of the deviation values in terms of total deviation were below the 7% acceptance limit (Figure 8). The *E. coli* trial was performed in 27 laboratories of which 19 used MIC determination and eight used disk diffusion. Thus, when analyzing the results based on the different methods used for AST, the values achieved using disk diffusion were over four times higher than when using MIC, obtaining deviations of 8%, 10.6% and 10.4% for the strains EC.2,3, EC.2,5 and EC.2,8, respectively. Furthermore, significance difference wertr observed when comparing the two methods for AST ($p < 0.01$). For more details in all deviations per antimicrobial refer to Appendix 7c.

As illustrated in Figure 9, the highest deviations per antimicrobial in the *E. coli* trial were obtained for amoxicillin+clavulanic acid (AUG) and ciprofloxacin with values equal to 9.2% and 7.8%, respectively.

Figure 8. Summary of the deviations obtained per strain according to the method used for AST by all participants.

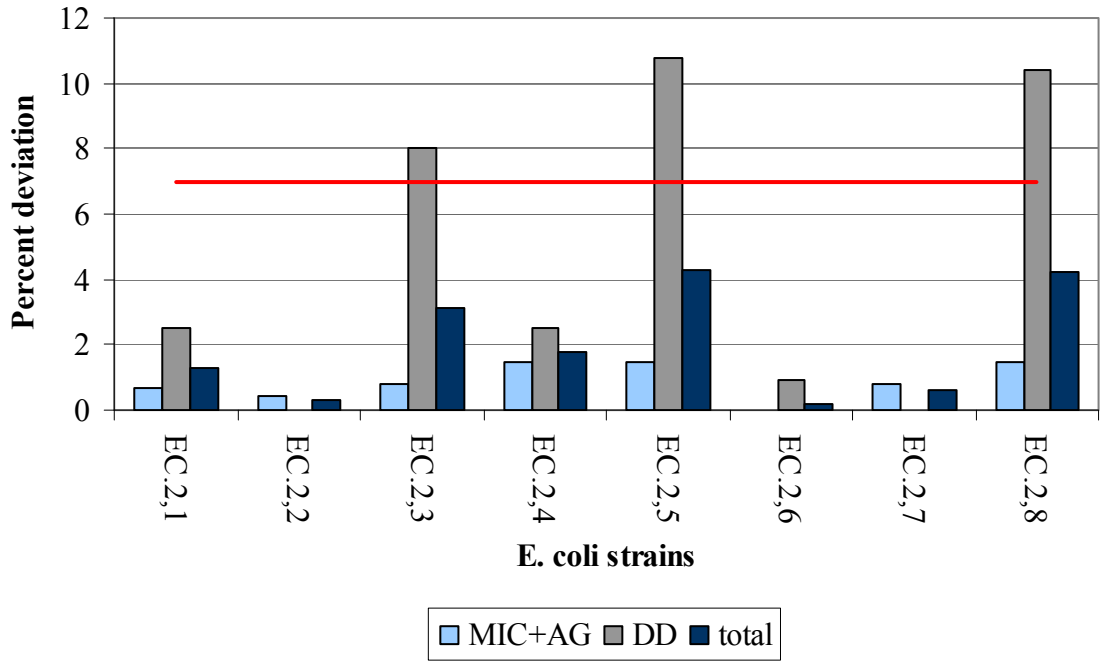
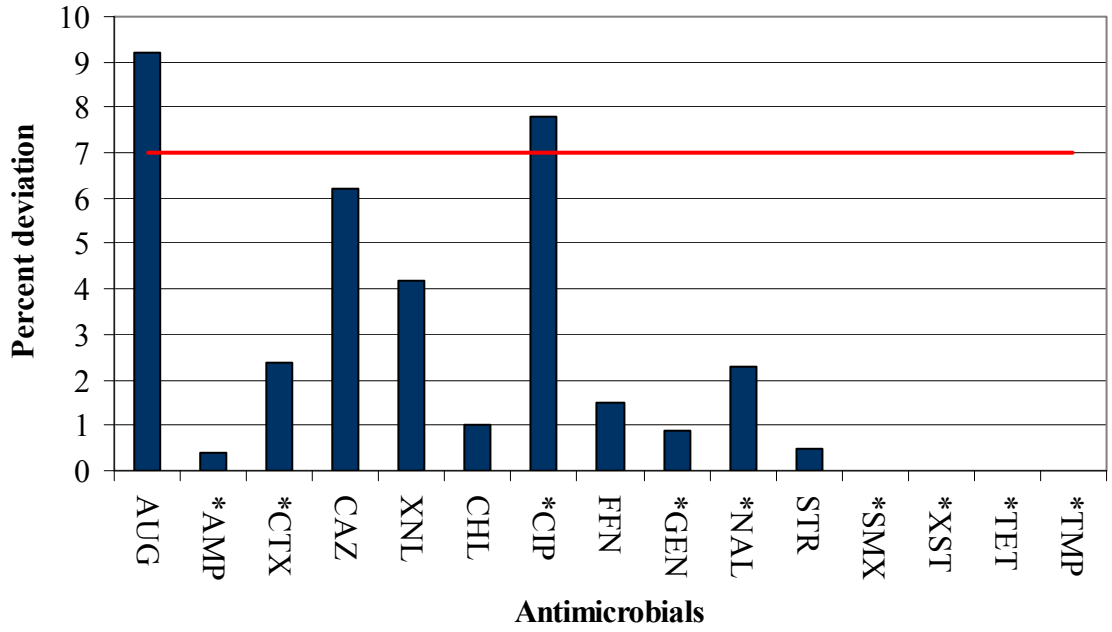


Figure 9. Deviations in *E. coli* strains per antimicrobial tested.



*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU.

Ciprofloxacin is one of the compounds recommended by EFSA for monitoring antimicrobial resistance. Furthermore, three of the eight *E. coli* strains from the panel deviated when tested

against ciprofloxacin. They were EC.2,3, EC.2,5 and EC.2,8 (Table 6). Laboratories performing MIC determination produced higher number of correct results when compared with the disk diffusion method which ended up causing 94% of the deviation.

Table 6. *E. coli* strains with deviations in ciprofloxacin antimicrobial higher than 7%.

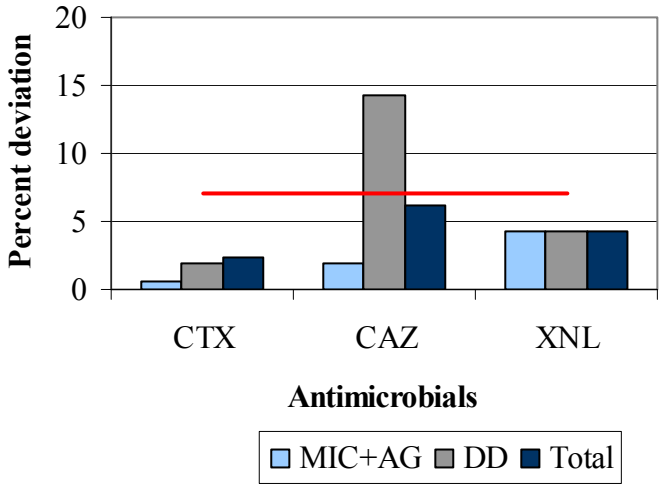
Strain	Mutation /Gene	Antimicrobial	Correct R/S	Correct results (%)	Expected MIC	Cut off value (R>)	Deviations MIC/n[1]	Deviations DD/n[2]
EC.2,3	GyrA	Ciprofloxacin	R	**72%**	0.06	0.032	1/18	6/7
EC.2,5	QnrS1	Ciprofloxacin	R	**85%**	0.5	0.032	0/19	4/7
EC.2,8	QnrA	Ciprofloxacin	R	**80%**	0.12	0.032	0/19	5/6

[1]MIC/n= number of laboratories that produced incorrect results by MIC determination / total number of laboratories performing MIC for AST in that specific strain.
[2]DD/n= number of laboratories that produced incorrect results by DD / total number of laboratories performing DD for AST in that specific strain

Extended spectrum betalactamase (ESBL) producing strains

With regard to the panel of cephalosporins selected to identify possible ESBL producing strains, three antimicrobials, cefotaxime (CTX), ceftazidime (CAZ) and ceftiofur (XNL) were further analysed in terms of deviations caused by AST (Figure 10). The highest deviation values were observed when using disk diffusion for AST. For the ceftazidime (CAZ) and cefotazime (CTX) the deviations were caused by two participants (#23 and #40) using disk diffusion for AST. For ceftiofur (XNL), two laboratories performing MIC determination together with two laboratories performing disk diffusion failed to identify the strain EC.2,8 as ESBL producing.

Figure 10. Further analysis of the deviations obtained for the antimicrobials used for ESBL detection in correspondence with the method used for AST.



The laboratories were requested to test for ESBL producing *E. coli* strains according to the clinical guidelines described by CLSI. The CRL-AR specified that an ESBL producing strain should be interpreted as resistant to **all** cephalosporins if it is resistant to **one** cephalosporin regardless of the value detected from the results.

Four out of the eight *E. coli* strains selected for the EQAS 2008 were ESBL producing. The EC.2,1 strain was CTX M-1, EC.2,4 was TEM-52b, EC.2,5 was CTX M-14 and finally EC.2,8 was a SHV-12. Out of 25 laboratories that performed the analysis for detection of ESBL producing strains, 23 identified the strains correctly. However, one laboratory (#1) failed to detect EC.2,5 and EC.2,8 as ESBL producing strains. Regarding strain EC.2,5, the difference in diameter zones obtained in the two confirmatory tests (CAZ/CL:CAZ and CTX/CL:CTX) were both <5 mm and the tests were consequently interpreted as negative. On the other hand, for strain EC.2,8 the difference in diameter zones in the confirmatory tests (CAZ/CL:CAZ) was ≥ 5mm but the laboratory failed to interpret the result as positive. In addition, another laboratory (#6) did not detect EC.2,4 as ESBL producing *E. coli* as differences in zones obtained in the two confirmatory tests were both < 5mm, therefore the test was interpreted as negative for ESBL production.

3.3 Deviations by laboratory

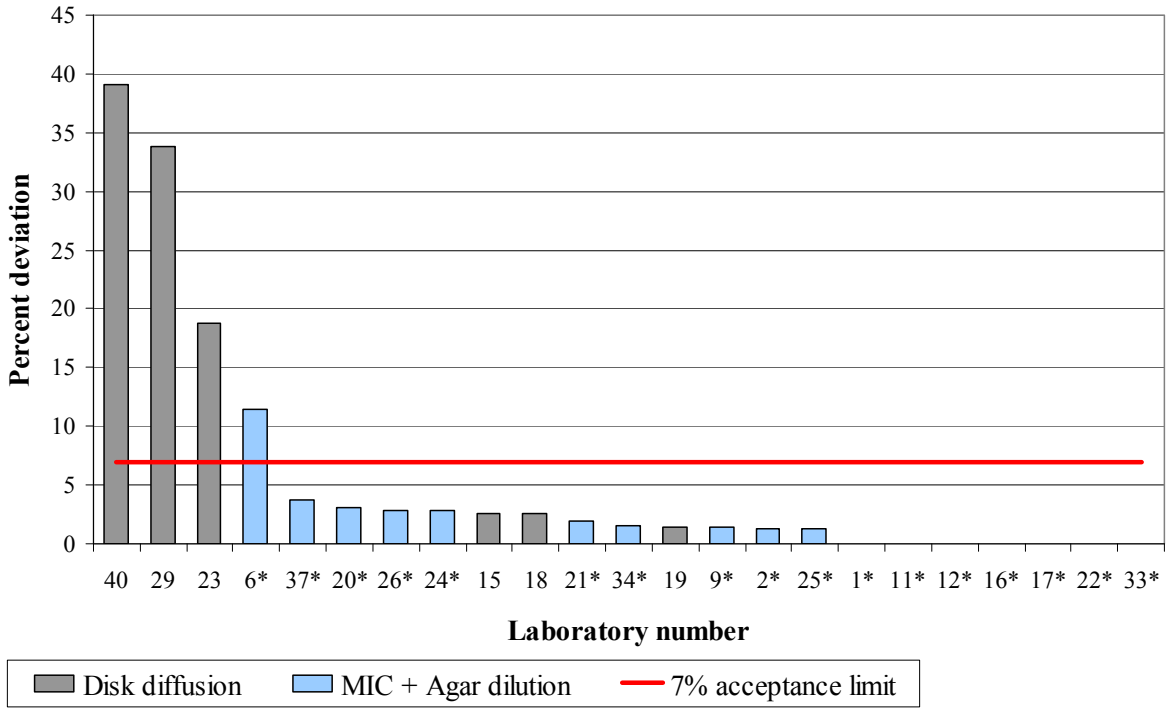
3.3.1 Enterococci trial

Out of the 23 participating laboratories, four obtained deviations greater than the previously agreed 7% acceptance limit (Figure 11). The percentage of deviations differed widely between the

laboratories with a maximum of 39% for laboratory #40 followed by laboratories #29, #23 and #6 with deviations of 33.8%, 18.8% 11.5% respectively. Three of these laboratories performed disk diffusion test whereas the last one (#6) performed MIC determination. In laboratory #6 deviations appeared to be caused mainly by three antimicrobials, gentamicin, streptomycin and tetracycline. For laboratory #23 and #29 gentamicin, streptomycin and ciprofloxacin as well as florphenicol for laboratory #29 appeared to have caused the deviations.

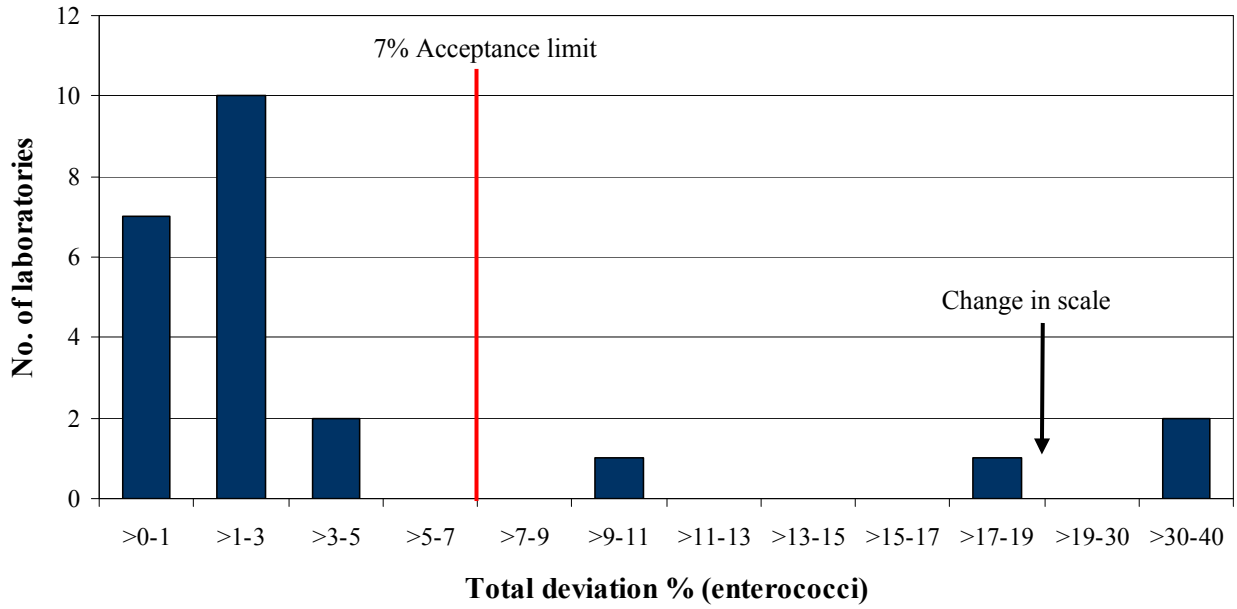
Interestingly, all the participants (seven) that produced 100% positive results, performed MIC determination rather than disk diffusion. Appendix 8a summarises all deviations by laboratory.

Figure 11. Individual deviations per laboratory in percentage of their total number of enterococci tests. The laboratories were ranked by decreasing percentage of deviations.



*Laboratories performing MIC for AST

Figure 12. The number of laboratories listed in intervals of percent of total deviations. The first line marks the acceptance limit set by the CRL at 7%.

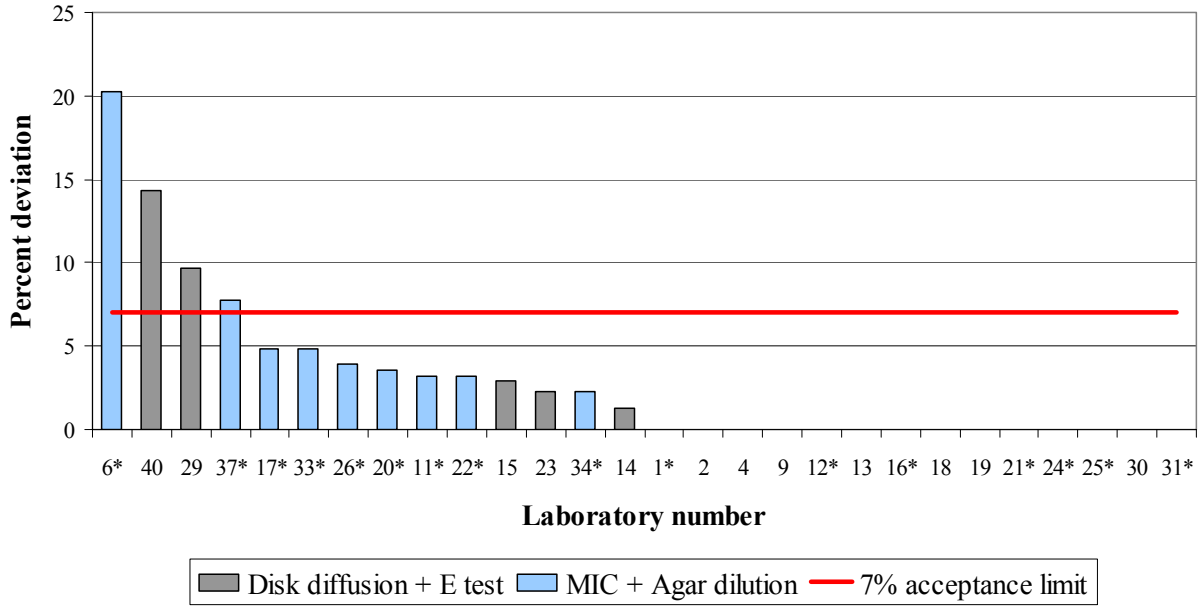


As shown in Figure 12, a total of 19 laboratories (N=23) achieved the acceptance level of performance lower than 7%. Three out of the four laboratories that clustered outside the 7% acceptance limit were identified as outliers for the enterorocci EQAS trial.

3.3.2 Staphylococci trial

In this EQAS 2008 as well as the EQAS 2007, four laboratories exceeded the 7% acceptance limit of deviation in the staphylococci trial. The percentage of deviation per individual laboratory was higher in this staphylococci trial when compared to the previous year, with values equivalent to 20.3%, 14.3%, 9.7% and 7.8% for laboratories #6, #40, #29 and #37, respectively (Figure 13). For laboratory #6 deviations were caused mainly by the antimicrobials gentamicin, sulfamethoxazole and streptomycin. Participant #40 also had problems with sulfamethoxazole and failed to confirm methicillin resistance by PCR in two occasions whereas in another two strains they reported false positive results. Laboratory #29 obtained the deviations in the streptomycin antimicrobial and #37 in sulfamethoxazole.

Figure 13. Individual deviations per laboratory in percentage of their total number of staphylococci tests. The laboratories were ranked by decreasing percentage of deviations.

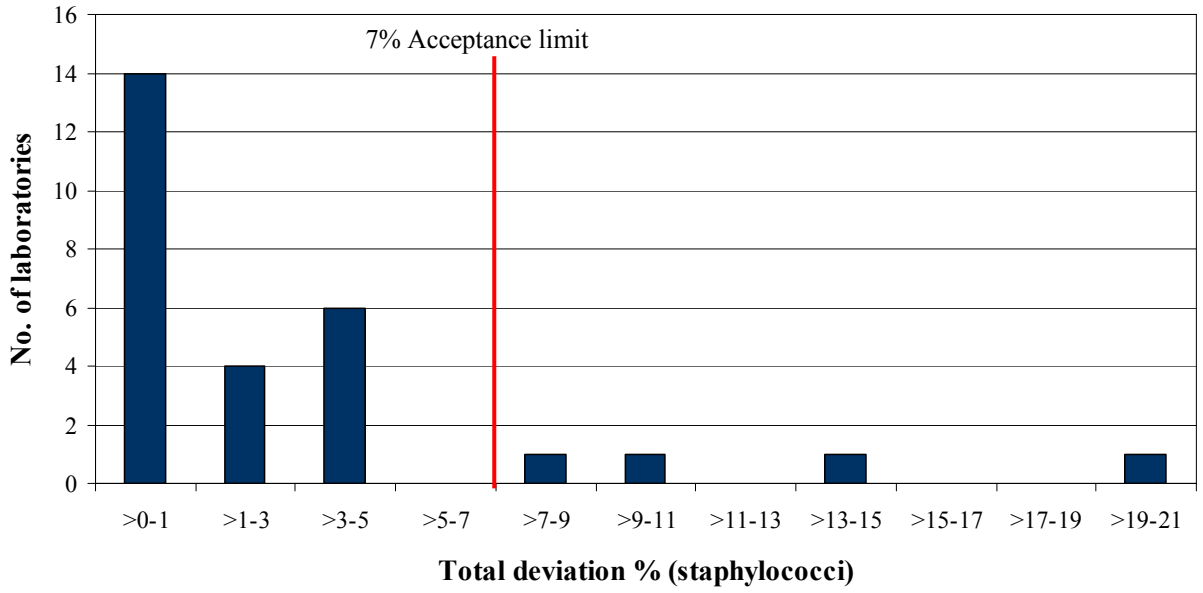


*Laboratories performing MIC for AST

In total, 14 laboratories out of the 28 taking part on the staphylococci trial obtained 100% correct results. Of those, seven performed MIC determination and seven performed disk diffusion for AST (Appendix 8b shows in detail the deviations per laboratory). Interestingly, nine of the 16 laboratories performing MIC determination exhibited deviations between 2.3% and 20.3% whereas five out of the 12 laboratories performing disk diffusion exhibited deviations with lower values, ranging between 1.3% and 14.3%. One cause of deviations at the individual level was the identification of ST.2,3 as a non-methicillin resistant strain.

When clustering the laboratories in intervals of deviation as illustrated in Figure 14, out of the four participants obtaining deviations higher than the 7%, mainly two participants showed high level of deviations and were identified as outliers. On the contrary, the majority of the laboratories obtained deviations in the lowest interval between 0% and 1%.

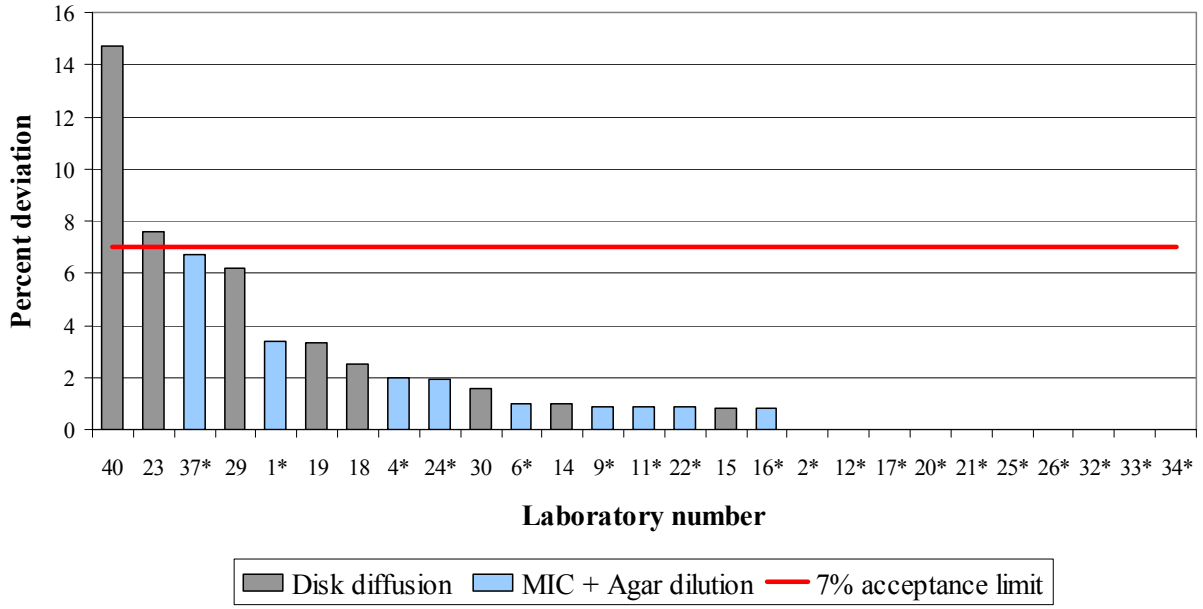
Figure 14. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the 7% acceptance limit set by the CRL.



3.3.3 *E. coli* trial

In general, the deviations per laboratory in the *E. coli* trial were higher than those from the EQAS 2007 where all laboratories obtained results below the 7% acceptance limit. As illustrated in Figure 15, out of the 27 participating laboratories, two obtained deviations higher than the 7% recommended. Both participants used disk diffusion method for AST. For laboratory #40, identified as an outlier in this trial, the 14.7% deviation was caused by the two cephalosporins (cefotaxime, ceftazidime) used to identify possible ESBL producing strains, and also gentamicin and ciprofloxacin. Laboratory #23 obtained a 7.3% deviation, just above the limit which was mainly caused by the results obtained for ceftazidime and ciprofloxacin. All of the ten laboratories that presented 100% correct results performed MIC determination for AST instead of disk diffusion. To see the deviations for each individual laboratory refer to Appendix 8c.

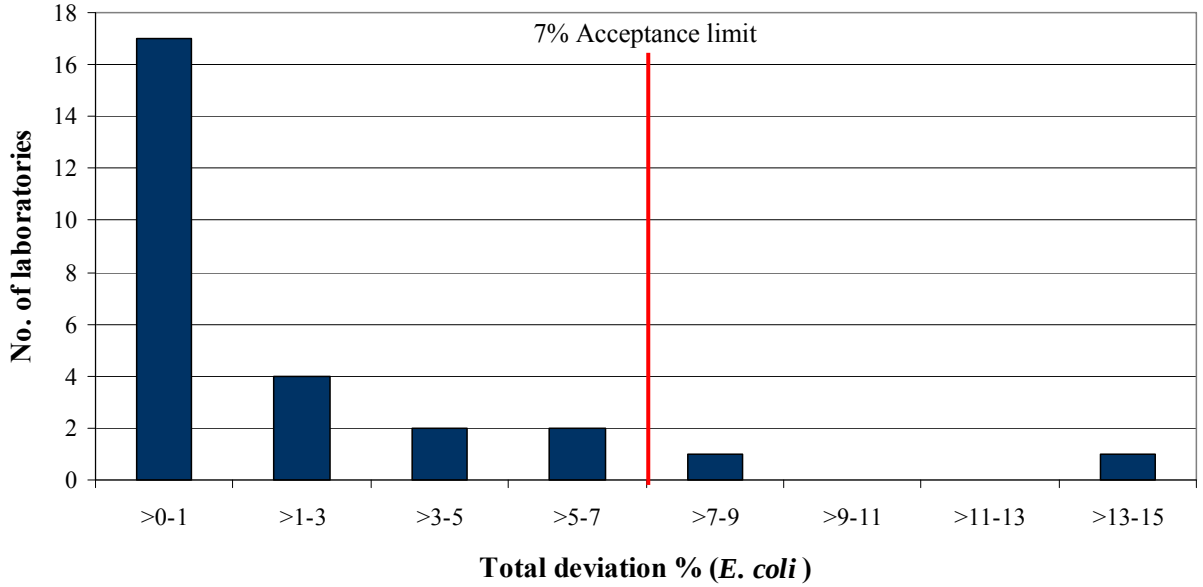
Figure 15. Individual deviations per laboratory in percentage of their total number of *E. coli* tests. The laboratories were ranked by decreasing percentage of deviations.



*Laboratories performing MIC for AST

As illustrated in Figure 16 the majority of the laboratories obtained deviations in the interval between 0% and 1%. Only laboratory #40 was clustered alone and identified as an outlier.

Figure 16. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the acceptance limit set by the CRL.



Appendix 8 is a summary of all the deviations obtained per participating laboratory. Thus, in total, four laboratories (#40, #29, #23 and #6) were identified as outliers in more than one of the three test carried out. Of them, only participant #6 used MIC for AST. These four laboratories will be contacted by the CRL-AR in an attempt to identify possible deficiencies in the procedures.

3.4 Deviations by reference strains

We could not find any comments on the database of the different methods used for AST differing from CLSI, therefore results for disk diffusion on the reference strains have been evaluated following CLSI guidelines.

3.4.1 Enterococci

The 15 participating laboratories that carried out MIC determination in the reference strain *E. faecalis* ATCC 29212 obtained 100% of correct results (Table 7). This is a total of 139 correct tests performed in this strain.

Table 7. Deviations obtained for the reference strain *E. faecalis* ATCC 29212 by MIC determination

***E. faecalis* ATCC 29212**				
Antimicrobial	**MIC deviations /Total no. of test**	**QC range MIC**	**Min value**	**Max value**
Ampicillin	**0**/15	0.5 - 2	0.5	2
Avilamycin	**0**/3	0.5 - 4	01	4
Chloramphenicol	**0**/15	4 - 16	4	8
Ciprofloxacin	**0**/9	0.25 - 2	0.5	1
Daptomycin	**0**/3	1 - 8	1	2
Erythromycin	**0**/14	1 - 4	1	4
Florfenicol	**0**/6	2 - 8	2	4
Gentamicin	**0**/14	4 - 16	8	≤128
Linezolid	**0**/10	1 - 4	1	2
Synacid	**0**/7	2 - 8	4	8
Tetracycline	**0**/15	8 - 32	16	32
Tigecycline	**0**/3	0.03 – 0.12	0.06	0.12
Vancomycin	**0**/13	1 - 4	1	4

As CLSI has not published a QC range for *E. faecalis* 29212 using disk diffusion, the six laboratories performing this method for AST could not be evaluated.

The unique participant using agar dilution for susceptibility testing reported the wrong outcome for vancomycin antimicrobial introducing a millimetre zone as a value for this antimicrobial. It appeared that this participant has combined the use of agar dilution with disk diffusion; therefore results from this laboratory have not been included in Table 7.

3.4.2 Staphylococci

A total of 11 laboratories performed disk diffusion in the reference strain *S. aureus* ATCC 25923. Table 8 shows the results and the deviations obtained by antimicrobial. Six antimicrobials produced deviations higher than the 7% acceptance limit, being the highest value of 28.6% for sulfisoxazole (FIS) followed by erythromycin (18.2%), trimethoprim (12.5%), streptomycin (11.1%) and gentamicin together with penicillin (9.1%).

The total number of tests performed with this reference strain was 96, of which eight were out of range, producing a deviation of 8.3%.

Table 8. Deviations obtained for the reference strain *S. aureus* ATCC 25923 by disk diffusion.

Antimicrobial	QC range	Deviation/Total no. of test	Min value	Max value
Chloramphenicol	16 - 26	0/9	18	24
Ciprofloxacin	22 - 30	0/11	22	30
Erythromycin	22 - 30	**2/11 (18.2%)**	20	31
Gentamicin	19 - 27	**1/11 (9.1%)**	19	29
Penicillin	26 - 37	**1/11 (9.1%)**	30	40
Streptomycin	14 - 22	**1/9 (11.1%)**	14	31
Sulfisoxazole	24 - 30	**2/7 (28.6%)**	6	26
Tetracycline	24 - 34	0/11	24	30
Trimethoprim	19 - 26	**1/8 (12.5%)**	16	24

The 13 laboratories that tested the reference strain *S. aureus* ATCC 25913 using MIC did not produce any deviation. This means a total of 104 correct tests performed in this strain. However, the unique laboratory which chose to perform agar dilution method for AST in *S. aureus* ATCC 25913 produced two incorrect results in nine of the tests performed. They reported MIC values of 256 mg/L for sulfisoxazole and 0.125 mg/L for penicillin instead of the expected ≤128 mg/L and ≥0.25 mg/L, respectively causing a deviation of 1.0% (Table 9).

One laboratory performed E-test in the reference strain *S. aureus* ATCC 29213. The obtained results were 100% correct but they have not been included in any of the tables.

Table 9. Range of obtained values for *S. aureus* ATCC 25913 by MIC determination.

Antimicrobial	QC range	Deviation/Total no. of test	Min value	Max value
Chloramphenicol	2 - 8	0/14	4	8
Ciprofloxacin	0.12 – 0.5	0/13	0.12	≤1
Erythromycin	0.25 - 1	0/13	≤0.25	0.5
Florfenicol	2 - 8	0/9	2	4
Gentamicin	0.12 - 1	0/12	≤0.25	0.5
Penicillin	0.25 - 2	**1/13 (7.8%)**	0.125	1
Streptomycin	0 - 258	0/9	≤2	≤1000
Sulfisoxazole	32 - 128	**1/6 (16.6%)**	32	256
Tetracycline	0.12 - 1	0/14	0.5	4
Trimethoprim	1 - 4	0/11	1	2

3.4.3 *E. coli*

Seven laboratories carried out disk diffusion on the reference strain *E. coli* ATCC 25922. The total number of test performed in this strain was 106 of which 13 were incorrect. Furthermore, seven out of the 13 incorrect results were caused by only one participant. Thus, the resulting deviation in EQAS 2008 for this strain was 12.3%. A small increase was observed when compared to EQAS 2007 when the average deviation was 11.1%. A total of eight antimicrobials deviated more than 7%

(Table 10). The highest percentages of deviations were caused by sulfisoxazole followed by cefotaxime together with ceftazidime.

Table 10. Range of obtained values for the reference strain *E. coli* ATCC 25922 by disk diffusion.

Antimicrobial	QC range	Deviation/Total no of test (%)	Min value	Max value
Amoxicillin+clavulanic ac	18 – 24	**1/6 (16.7%)**	20	25
Amoxicillin		0/4	16	24
Ampicillin	16 – 22	**1/7 (14.3%)**	16	24
Cefotaxime	29 – 35	**2/6 (33.3%)**	27	37
Cefoxitin		0/4	25	29
Ceftazidime	25 – 32	**2/6 (33.3%)**	24	33
Ceftiofur	26 – 31	**1/6 (16.7%)**	22	30
Chloramphenicol	21 – 27	0/7	22	27
Ciprofloxacin	30 – 40	0/7	30	40
Florphenicol	22 – 28	0/7	23	27
Gentamicin	19 – 26	0/7	20	26
Imipenem		0/3	27	31
Nalidixic acid	22 – 28	**1/7 (14.3%)**	21	28
Streptomycin	0 – 50	0/5	14	20
Sulfisoxazole	15 – 23	**3/6 (50.0%)**	6	26
Tetracycline	18 – 25	0/7	22	25
TMP+SMX		0/6	22	29
Trimethoprim	21 – 28	**1/5 (16.7%)**	20	27

Finally, 18 laboratories tested the reference strain using MIC determination. They performed a total of 231 tests of which eight were incorrect causing an average deviation of 3.5%. The deviations in this strain were caused by cefoxitin, ciprofloxacin, streptomycin, ampicillin, cefotaxime and sulfisoxazole (Table 11).

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

Antimicrobial	QC range	Deviation/Total no of test (%)	Min value	Max value
Amoxicillin+clavulanic ac	2 – 8	0/4	4	8
Ampicillin	2 – 8	**1/18 (5.5%)**	1	8
Cefotaxime	0.03 – 0.12	**1/19 (5.3%)**	≤0.06	0.25
Cefoxitin		**1/3 (33.3%)**	4	26
Ceftazidime	0.06 – 0.5	0/13	≤0.25	0.25
Ceftiofur	0.25 – 1	0/5	≤0.25	0.5
Chloramphenicol	2 – 8	0/18	4	8
Ciprofloxacin	0.004 – 0.016	**3/18 (16.6%)**	≤0.08	0.03
Florphenicol	2 – 8	0/18	2	8
Gentamicin	0.25 – 1	0/19	≤0.25	0.5
Nalidixic acid	1 – 4	0/19	1	4
Streptomycin	4 – 16	**1/18 (5.5%)**	2	8
Sulfisoxazole	8 – 32	**1/17(5.9%)**	8	64
Tetracycline	0.5 – 2	0/19	1	2
TMP+SMX		0/3	<0.12	1
Trimethoprim	0.5 – 2	0/19	≤0.5	2

4. DISCUSSION

4.1 Enterococci trial

As established in previous meetings, when the percentage of positive results was lower than 75% the combination of strain/antimicrobial was omitted from the evaluation. For the enterococci trial, this resulted in the subtraction of seven combinations enterococci/antimicrobial. With the exception of streptomycin, the obtained MIC by the participants, the expected MIC and the cut off values to categorise the strains as resistant/sensitive were within one fold dilution difference. Therefore, it appeared that the strains had low susceptibility to these antimicrobials. In addition for the daptomycin, only three participants tested for it, therefore the reporting of one error would immediately be interpreted as a 33% deviation in the final outcome. Out of 14 laboratories performing MIC determination against streptomycin in strain ENT.2,4, 13 reported incorrect results. With the exception of one laboratory that obtained an MIC of 512 mg/L and recorded the strain as sensitive, the majority of laboratories obtained values between 64 mg/L and 128 mg/L for this antimicrobial. Furthermore, this antimicrobial appeared to be the cause of 11% deviation in the enterococci trial when tested against the other seven strains. The discrepancy in results appears to be caused by the methodology of the laboratory performing the test rather than by the stability of the antimicrobial, since streptomycin appears to be very stable [1, 2].

Five antimicrobials have deviated in this EQAS 2008 by comparison to the seven that deviated in EQAS 2007. As well as for streptomycin, deviations higher than 7% were also recorded for ciprofloxacin, gentamicin, chloramphenicol and synacid. All of them except for ciprofloxacin are antimicrobials recommended by EFSA for monitoring antimicrobial resistance. These deviations were mainly caused by laboratories performing disk diffusion for AST. Moreover, deviations in these antimicrobials were mainly caused by the four laboratories identified as outliers in this enterococci trial (#40, #29, #23 and #6). Results obtained by MIC determination were better than those by disk diffusion; furthermore all laboratories with 100% correct results performed MIC determination. The number of laboratories deviating more than the 7% acceptance limit has also decreased, from 14 registered in 2007 to four in 2008, with the majority of participants clustered in the deviation interval between 0% and 3%.

The analysis of the reference strains was used as an internal quality control system to monitor the excellence of the laboratories procedures. MIC determination for *E. faecalis* ATCC 29212 revealed

no deviation in the EQAS 2008 whereas in the EQAS 2007, 1.8% of the tests for this reference strain differed from the expected results. The one laboratory performing agar dilution recorded the incorrect outcome for vancomycin. It appeared that this participant has combined the use of agar dilution with disk diffusion and has reported a millimetre zone diameter instead of a concentration range.

Over all, the percentage of correct results in the susceptibility test for enterococci was 95% by comparison to 91.4% from 2007, results that have demonstrated a great improvement for all the participating laboratories. However, there are still areas that need attention since four out of nine antimicrobials recommended by EFSA failed to produce 100% of correct results and still three laboratories were identified as outliers. Since they were all using disk diffusion for AST it appeared that the main cause for obtaining incorrect results could be the methodology. When performing disk diffusion it is important to consider many factors that could have an influence in the results, such as temperature, age and concentration of the antimicrobial disks, volume and pH of the agar media in the petri dish and the turbidity and density of the inoculum. However, these laboratories will be advised to participate in a workshop to improve their procedures.

4.2 Staphylococci trial

For the staphylococci trial three combinations strain/antimicrobial were omitted from the dataset; streptomycin, ciprofloxacin and tetracycline. However, with the exception of strain ST.2,6, tetracycline obtained a low deviation value (1%) when tested against the rest of the strains, as well as against the reference strains. This antimicrobial is known to be pH dependent, and that may explain the deviations on the results. On the other hand, for streptomycin and ciprofloxacin, the expected results and the cut off values to categorise the strains as susceptible/resistant were within one fold dilution. Thus, producing results within the correct range (± one fold dilution) could conclude in the wrong outcome. The difference in the obtained results appeared to be mainly caused by the methodology used for AST, since the majority of participants using disk diffusion reported results deviating from the expected results for these three combinations staphylococci/ antimicrobial.

One antimicrobial, sulfamethoxazole exhibited deviations higher than 7%. This last antimicrobial has a bacteriostatic effect in the microorganisms and takes special reading technique; therefore the interpretation of results can be uncertain for both, MIC determination and disk diffusion. Still, the

deviation was mainly caused by three laboratories (#40, #37 and #6). Overall, two laboratories were identified as outliers for the staphylococci trial by comparison to the seven from EQAS 2007. This year, half of the participants were clustered in the 0% to 1% deviation interval which is a very positive outcome.

MIC results for the quality control strain *S. aureus* ATCC 25913 were 100% within range, whereas in 2007 this percentage was 94.1%. Similar results were observed for the disk diffusion test carried out in *S. aureus* ATCC 25923 were the average deviation has reduced from 18.3% in the EQAS 2007 to 8.3% in 2008. Although this value is still high, we can conclude that results have improved when compared to the previous year.

Methicillin resistant strains

In the staphylococci trial only strain ST.2,3 resulted in a deviation higher than the 7% accorded limit. This strain was methicillin resistant but exhibited an MIC to penicillin of 0.12 mg/L. The major cause of the deviations for this strain was failing to detect the *mecA* gene or the production of PBP 2a (the *mecA* gene product) by the participants. Furthermore, according to the CLSI recommendations (M100-S18, table 2C), if a strain is identified as **methicillin resistant staphylococci**, should also be reported as resistant to **all β-lactam** antibiotics, including penicillin. Therefore, laboratories failing to identify resistance to methicillin also failed to interpret the value of the penicillin test as resistant, causing an additional deviation of 6% for this antimicrobial.

As well as ST.2,3, strain ST.2,1 and ST.2,4 were also methicillin resistant. In total, eight out of 27 laboratories taking part on the methicillin resistance identification did not obtain correct results in one or more tests, which is 30% of the laboratories by comparison to the 17% (4/23) that failed in the EQAS 2007. Considering the severity of the MRSA problem, the outcome of this test was not as successful as expected. Consequently, the detection of MRSA will be a key issue on the forthcoming year. The NRLs should consider harmonising protocols regarding the identification of *mecA* gene. For comparable purposes and easy interpretation of errors, the use of the same solid validated method for identification of *mecA* would be a good improvement. Suggested protocols are available on the CRL-AR website (http://www.crl-ar.eu/data/images/meca-pcr_protocol).

4.3 *E. coli* trial

For this trial only two combinations strain/antimicrobial were subtracted from the dataset, EC.2,2/streptomycin and EC.2,5/amoxicillin+clavulanic acid. In both cases, the MIC values obtained by the participants were higher than the cut off value or the expected value; these strains may exhibit low susceptibility against these antimicrobials.

Although a minor increase of 0.1% has been observed in the average deviation for the *E. coli* trial from 2% from EQAS 2007 to 2.1% this year, none of the *E. coli* strains deviated higher than the 7% acceptance limit. The major percentages of error were observed for two antimicrobials, they were amoxicillin+clavulanic acid (AUG) and ciprofloxacin.

Strains EC.2,3, EC.2,5 and EC.2,8 that exhibited low level of resistance to ciprofloxacin were reported as susceptible by most of the participants performing disk diffusion for AST. It appears to be a discrepancy on the cut off values recommended by EUCAST (> 0.032 mg/L) and those recommended by CLSI (≥ 4 mg/L) for the MIC interpretation of ciprofloxacin. EUCAST values are much lower than CLSI which also reports intermediate resistance (2 mg/L). Furthermore, performing disk diffusion and following CLSI guidelines would probably reflect this discrepancy when reading the zone diameters. In addition, there is also a range of zone diameter between 16-20 mm categorised as intermediate resistance which may have confused some of the participants. Identification of low level ciprofloxacin resistance is an important issue, especially nowadays as the occurrence of this low resistance is increasing and the use of ciprofloxacin may induce the emergence of more resistant strains by additional mutations. Harmonization of methodology would prevent deviations to occur since participants performing MIC had not reported incorrect results. On the other hand, the deviations obtained for ciprofloxacin in the reference strain *E. coli* ATCC 25922 were higher for participants using MIC than those using disk diffusion, contradiction that cannot be easily explained.

Regarding the reference strain *E. coli* ATCC 25922, the percentage of positive results for all test performed has increased from 90% in EQAS 2007 to 96.8% this year, which is a very positive outcome. However, there are still differences in the methodology and the major part of the deviations were caused by laboratories performing disk diffusion against cephalosporins which

appeared to be the same problem encountered last year. Participants using disk diffusion should take into consideration all factors that can have a negative influence in the results.

The deviations obtained in antimicrobial AUG (five out of seven errors) was mainly produced by laboratory #37 performing agar dilution for AST. This laboratory reported to mix the compounds ampicillin and clavulanic acid themselves to prepare the different agar dilutions. Errors in the procedure would definitely result in the incorrect concentration of the antimicrobials on the media. Laboratories #23 and #40 performing disk diffusion were the main cause of the deviation obtained for ceftazidime. These last two participants were also identified as outliers for the *E. coli* test. However, the majority of the participants (63%) clustered in the interval of deviation between 0% and 1%, and 10 of the 27 taking part in the *E. coli* trial obtained 100% correct results, which is a very positive outcome when compared to 2007 when only six participants achieved 100% correct results.

Extended spectrum betalactamase (ESBL)

Four out of the *E. coli* strains were ESBL producing however, none of the eight strains produced AmpC or MBL, which was correctly confirmed by all laboratories. On the contrary, two out of 25 laboratories that uploaded results for ESBL detection, failed to identify ESBL producing organisms in one or two strains. For laboratory #1 it appeared that in one of the cases an error was produced on the interpretation of results, since one of the double disk confirmatory tests (CAZ/CL:CAZ) was positive for ESBL production, but the result was uploaded as negative. In the other occasions for participants #1 as well as #6, the difference in diameter zones for the two tests (CAZ/CL:CAZ and CTX/CL:CTX) were smaller than expected and the deviations were more likely caused by a methodological error. Overall, the use of the combination disk confirmatory test appeared to be a successful test for identifying ESBL producing strains when it is correctly performed. Giving the threat that these organisms may pose to human health, the detection of ESBL producing *E. coli* has a priority for the CRL. The laboratories that failed to obtain the correct results for this test will be contacted and invited to a training course to refresh and improve their methodology. They will be also encouraged to retake the test to ensure the implementation of a better detection system.

5. CONCLUSIONS

One of the aims of the CRL-AR through the EQAS is to work towards all laboratories performing the susceptibility test with a deviation margin below 7%, and thus generate correct and reliable data for the monitoring programmes implemented by the European Commission.

From EQAS 2008, we can conclude that the overall performance of the participants has improved in the enterococci and staphylococci trial when comparing to 2007, although the testing of enterococci needs attention regarding the antimicrobials recommended by EFSA. For the *E. coli* trial the percentage of deviations has suffered a small increase (0.1%) when compared to EQAS 2007. ESBL producing *E. coli* are still considered a priority area for the CRL-AR.

As in previous years, the main cause of deviations was strains with expected MIC values close to the cut off values to define them as resistant. Also laboratories performing disk diffusion appeared to produce lower number of correct results, which probably could be improved if the different laboratories would harmonise methodology or at least agree upon using the same zone diameters to define breakpoints. However for all species tested, the number of laboratories clustered in the deviation interval between 0% and 1% as well as the number of laboratories performing all tests 100% correctly has increased considerably, which is a great success.

Out of the 29 laboratories participating in the EQAS proficiency test, four laboratories have been categorised as outliers. The four of them have obtained deviations above the 7% acceptance limit in two or more tests. They were also the main causes of deviations per strain and antimicrobials. Therefore, future work will be done to assess the reasons for these deviations as well as to provide guidelines to improve their methodology. These participants will be invited to take part in a comprehensive training course organised by the CRL-AR in February 2009. Subsequently, they will be encouraged to perform a re-test trial in the relevant strains and surely they will achieve a better outcome in the forthcoming EQAS 2009.

Still there are areas which need attention. One of them is the MRSA identification which appeared to be a cause of major deviations for the staphylococci trial. Harmonisation of methodology in terms of protocols to improve these results could be relevant. To discuss all these issues, representatives from all Member States have been encouraged to participate in an MRSA workshop

organised by the CRL-AR in April 2009. This workshop will facilitate the harmonization of methodology and the identification of areas that may require improvement for each particular laboratory. Furthermore, the CRL-AR is working on the implementation of a novel MRSA ring trial in summer 2009 where all NRLs will be invited to participate.

To finalise, the CRL-AR will take into consideration all the suggestions received from the NRLs to improve and ensure a good quality of work which certainly will reflect in the forthcoming EQAS 2009.

6. REFERENCE LIST

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Appendix 1

National Food Institute DTU

Technical University of Denmark

CRL-AR EQAS pre-notification

EQAS 2008 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, new participants will be offered the following QC strains: *E. coli* ATCC 25922 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC).

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 EQAS coordinator with documents or other information that can ease the parcel's way through customs (eg. specific text that should be written on the invoice). As means of avoiding passing the deadline we ask you to send us this information already at this stage. For your information, the contents of the parcel are "Biological Substance Category B": Eight *E. coli*, eight staphylococci, eight enterococci and for new participants also the QC strains mentioned above. The strains are expected to arrive at your laboratory in June 2008.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped in June 2008. The protocol will be provided electronically.

Returning of results: Results must be returned to the National Food Institute, by September 1st, 2008. 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 2008.

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

EU Community Reference Laboratory, Antimicrobial Resistance, Bülowsvej 27, DK-1790, Copenhagen V, Denmark
Ph: +45 7234 6288, Fax: +45 7234 6001, e-mail: rsh@food.dtu.dk

Participant List

Ent	Stap	*E.coli*	Institute	Country
X	X	X	Austrian Agency for Health and Food Safety	Austria
	X	X	Institute of Public Health	Belgium
X	X	X	NRL AR on food, National Diagnostic and Research Veterinary Institute	Bulgaria
X	X	X	Veterinary Services	Cyprus
X	X	X	State Veterinary Institute Prague	Czech Republic
X	X	X	The National Food Institute	Denmark
X	X	X	Estonian Veterinary and Food Laboratory	Estonia
X	X	X	Finnish Food Safety Authority EVIRA	Finland
	X		AFSSA LERQAP	France
	X	X	AFSSA Ploufragan - LERAP	France
X	X	X	AFSSA Lyon	France
X	X	X	AFSSA Fougères LERMVD	France
X	X	X	Federal Institute for Risk Assessment	Germany
X	X	X	Veterinary Laboratory of Chalkis	Greece
X	X	X	Central Agricultural Office, Veterinary Diagnostical Directorate	Hungary
X	X	X	Central Veterinary Research Laboratory	Ireland
X	X	X	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
X	X	X	National Diagnostic Centre of Food and Veterinary Service	Latvia
X	X	X	National Veterinary Laboratory	Lithuania
			*Centre for Infections Health Protection Agency	Malta/UK
X	X	X	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
			Veterinærinstituttet	Norway
X	X	X	National Veterinary Research Institute	Poland
X	X	X	Laboratorio National de Investigacáo Veterinaria	Portugal
			*Institute for Hygiene and Veterinary Public Health	Romania
			National Institute of Research-Development for Microbiology and Immunology “Cantacuzino”	Romania
X	X	X	State Veterinary and Food Institute (SVFI)	Slovakia
	X	X	National Veterinary Institute	Slovenia
	X		Laboratorio Central de Sanidad Animal de Santa Fe	Spain
		X	Laboratorio Central de Sanidad Animal de Algete	Spain
			*C N de Alimentacion. Agencia Espanola de Seguridad Alimentria y	Spain
			Complutense University of Madrid	Spain
X	X	X	National Veterinary Institute, SVA	Sweden
X	X	X	The Veterinary Laboratory Agency	United Kingdom

Designated NRL by the compentent authority of the member state
Laboratories enroled by the CRL
Not a Member State of the EU
* The laboratory decline to participate or did not submit results

Enterococci test strains and reference values (MIC)

Strain	Species	AMP	AVI	CHL	CIP	DAP	ERY	FFN	GEN	KAN	LZD	STR	SYN	TET	TGC	VAN
ENT-2.1	*E. faecium*	4	≤2	4	0.50	1.0	4.0	≤4	≤128	256	2	≤128	4	>32	0.06	>32
ENT-.2.2	*E. faecalis*	≤2	≤2	8	0.50	1.0	>32.0	≤4	2048	>2048	2	>2048	16	>32	0.12	≤2
ENT- 2.3	*E. faecium*	≤2	>16	≤2	1.0	1.0	≤0.5	≤4	≤128	256	≤1	≤128	≤0.5	>32	0.12	>32
ENT-2.4	*E. faecium*	4.0	>16	4	4	4.0	>32	≤4	≤128	512	2	256	16.0	>32	0.06	≤2
ENT-2.5	*E. faecium*	≤2	>16	16	2.0	2.0	>32	≤4	≤128	>2048	≤1	2048	1.0	>32	0.12	≤2
ENT-2.6	*E. faecium*	4	>16	4	4.0	4.0	>32	≤4	≤128	>2048	≤1	>2048	16.0	>32	0.12	≤2
ENT-2.7	*E. faecium*	≤2	≤2	4	1.0	4.0	≤0.5	≤4	≤128	512	2	≤128	1	≤1	0.03	≤2
ENT- 2.8	*E. faecium*	≤2	≤2	16	1.0	4.0	>32.0	≤4	≤128	>2048	2	≤128	2.0	>32	0.12	≤2

Strain	Species	AMP	AVI	CHL	CIP	DAP	ERY	FFN	GEN	KAN	LZD	STR	SYN	TET	TGC	VAN
ENT-2.1	*E. faecium*	S	S	S	S	S	S	S	S	S	S	S	R	R	S	R
ENT-.2.2	*E. faecalis*	S	S	S	S	S	R	S	R	R	S	R	S	R	S	S
ENT- 2.3	*E. faecium*	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R
ENT-2.4	*E. faecium*	S	R	S	S	S	R	S	S	S	S	R	R	R	S	S
ENT-2.5	*E. faecium*	S	R	S	S	S	R	S	S	R	S	R	S	R	S	S
ENT-2.6	*E. faecium*	S	R	S	S	S	R	S	S	R	S	R	R	R	S	S
ENT-2.7	*E. faecium*	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
ENT- 2.8	*E. faecium*	S	S	S	S	S	R	S	S	R	S	S	R	R	S	S

Resistant

Staphylococci test strains and reference values (MIC)

Strain		CHL	CIP	ERY	FFN	GEN	MRS	PEN	STR	SMX	TET	TMP	SXT
CRL: ST-2.1	*S. aureus*	4	2.0	0.25	2	16	+	>16	>128	256	32	≤1	≤0.25
CRL: ST-2.2	*S. aureus*	8	0.5	0.25	4	0.25	-	≤0.06	≤2	16	≤0.5	≤1	≤0.25
CRL: ST-2.3	*S. pisciferm*	8	0.25	>16	4	0.125	+	0,12	>128	32	32	>32	0.5
CRL: ST-2.4	*S. aureus*	16	0.5	>16	8	0.25	+	8	4	32	>32	>32	≤0.25
CRL: ST-2.5	*S. aureus*	8	1.0	0.25	4	0.5	-	≤0.06	32	16	1.0	≤1	≤0.25
CRL: ST-2.6	*S. epidermis*	4	≤0.12	0.25	2	16	-	0.5	≤2	256	4.0	>32	8.0
CRL: ST-2.7	*S. pseudointermedius*	8	≤0.12	>16	8	0.125	-	16	≤2	≤8	>32	≤1	≤0.25
CRL: ST-2.8	*S. pasteuri*	8	0.5	0.5	4	0.125	-	2.0	16	≤8	>32	>32	≤0.25

Strain		CHL	CIP	ERY	FFN	GEN	MRS	PEN	STR	SMX	TET	TMP	SXT
CRL: ST-2.1	*S. aureus*	S	R	S	S	R	+	R	R	R	R	S	S
CRL: ST-2.2	*S. aureus*	S	S	S	S	S	-	S	S	S	S	S	S
CRL: ST-2.3	*S. pisciferm*	S	S	R	S	S	+	R*	R	S	R	R	S
CRL: ST-2.4	*S. aureus*	S	S	R	S	S	+	R	S	S	R	R	S
CRL: ST-2.5	*S. aureus*	S	S	S	S	S	-	S	R	S	S	S	S
CRL: ST-2.6	*S. epidermis*	S	S	S	S	R	-	R	S	R	R	R	R
CRL: ST-2.7	*S. pseudointermedius*	S	S	R	S	S	-	R	S	S	R	S	S
CRL: ST-2.8	*S. pasteuri*	S	S	S	S	S	-	R	S	S	R	R	S

R*= MRS positive strain

Resistant

E. coli **test strains and reference values (MIC)**

Strain	AMP	AUG	CAZ	CAZ/CLV	CHL	CIP	CTX	CTX/CLV	ESBL gene	FFN	FOX	GEN	IP/IPE	NAL	SMX	STR	SXT	TET	TMP	XNL
EC-2.1	>32	8	8	0.125	4	<0.015	>16	0.064	**Positive**	4	4	≤0.5	MIC ratio <8	≤4	≤64	≤8	0.032	≤2	≤1	>8
EC-2.2	>32	8	0.25	0.125	≤2	>4.0	≤0.12	0.032	Negative	≤2	4	≤0.5	MIC ratio <8	>64	>1024	16	0,125	>32	≤1	≤0.5
EC-2.3	>32	4	0.064	0.064	32	0.06	≤0.12	0.032	Negative	8	4	16.0	MIC ratio <8	32	>1024	64	>32	>32	>32	0.5
EC-2.4	>32	8	16	0.125	16	>4.0	>4.0	0.125	**Positive**	16	16	0.5	MIC ratio <8	>64	1024	≤8	>32	4	>32	>8.0
EC-2.5	>32	8	1.0	0.125	4	0.05	>16	0.032	**Positive**	4	4	>16.0	MIC ratio <8	8	>1024	>128	>32	>32	>32	>8.0
EC-2.6	4	4	0.5	0.25	≤2	0.015	0.12	0.125	Negative	≤2	8	≤0.5	MIC ratio <8	≤4	≤64	≤8	0.125	≤2	≤1	0.5
EC-2.7	>32	4	0.125	<0.064	4	<0.015	≤0.12	0.032	Negative	≤2	2	>16.0	MIC ratio <8	≤4	>1024	128	0.125	>32	≤1	≤0.5
EC-2.8	>32	4	8	<0.064	>64	0.12	0.5	0.016	**Positive**	4	4	>16.0	MIC ratio <8	≤4	>1024	256	>32	≤2	>32	≤0.5

Strain	AMP	AUG	CAZ	CAZ/CLV	CHL	CIP	CTX	CTX/CLV	ESBL gene	FFN	FOX	GEN	IP/IPE	NAL	SMX	STR	SXT	TET	TMP	XNL
EC-2.1	R	S	R	Synergy*	S	S	R	Synergy*	**CTX M-1**	S	none ampC	S	none MBL	S	S	S	S	S	S	R
EC-2.2	R	S	S		S	R	S		none ESBL	S	none ampC	S	none MBL	R	R	S	S	R	S	S
EC-2.3	R	S	S		R	R	S		none ESBL	S	none ampC	R	none MBL	R	R	R	R	R	R	S
EC-2.4	R	S	R	Synergy*	S	R	R	Synergy*	**TEM-52b**	S	none ampC	S	none MBL	R	R	S	R	S	R	R
EC-2.5	R	S	R	Synergy*	S	R	R	Synergy*	**CTX M-14**	S	none ampC	R	none MBL	S	R	R	R	R	R	R
EC-2.6	S	S	S		S	S	S		none ESBL	S	none ampC	S	none MBL	S	S	S	S	S	S	S
EC-2.7	R	S	S		S	S	S		none ESBL	S	none ampC	R	none MBL	S	R	R	S	R	S	S
EC-2.8	R	S	R	Synergy*	R	R	R	Synergy*	**SHV-12**	S	none ampC	R	none MBL	S	R	R	R	S	R	R

ESBL genes

Resistant

*Synergy when CAZ/CLV and CTX/CLV ≥ 8

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 2008 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 (CCM 3954), *E. faecalis* ATCC 29212 (CCM 4224), *S. aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC).

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

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 to EFSA by different laboratories on *E. coli*, enterococci and staphylococci and to harmonise the breakpoints used within the EU.

3 OUTLINE OF THE EQAS 2008

3.1 Shipping, receipt and storage of strains

In June 2008 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. Reference strains will be included for participants who have not previously received these. All strains are non-toxin producing human pathogens Class II. There might be ESBL-producing strains among the selected material. The reference strains are shipped lyophilised, and the test strains are stab cultures. On arrival, the stab cultures must be subcultured, and all cultures should be kept refrigerated until testing. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please see the document 'instructions for opening and reviving lyophilised cultures' for additional information.

a) Open the ampoule. Take some of the material and dissolve it in 0.5 ml appropriate broth. Leave it for 10 minutes. Inoculate the solution on a non selective agar plate using either a 10 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 10 µl loop or a cotton swab on none selective agar and incubate.

If you do not succeed with a) or b), shake the vial/ampoule and empty it directly onto a non-selective agar plate. Add 100 µl saline 0.9% to the plate, and spread the culture properly with a triangle or 'hockey stick'. Incubate as mentioned above.

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the method used in the laboratory when performing monitoring for EFSA. For MIC, the cut off values listed in tables 3.3.1; 3.3.2 and 3.3.3 should be used. The epidemiological cut-off values allow two categories of characterisation – resistant or sensitive. Participants using disk diffusion are recommended to interpret the results according to their individual breakpoints, categorising them into the terms resistant and sensitive. A categorization as intermediary is not accepted. 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).

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

3.3.1 *E. coli*

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
Amoxicillin cl., AUG	8
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
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

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

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

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

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

Also, when testing cephalosporins, please note that when an isolate is found resistant to one cephalosporin, the isolate is regarded resistant to all cephalosporins.

3.3.2 Enterococci

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, STR	128	512
Quinpristin-dalfopristin (Synacid), SYN	1	32
Tetracycline, TET	2	2
Tigecycline, TGC	0.25	0.25
Vancomycin, VAN	4	4

Please find information on the test form below showing which test strains are *E. faecium* and *E. faecalis* respectively.

3.3.3 Staphylococci

Antimicrobials for *S. aureus*	MIC (µg/mL) **R is >**
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	2
Penicillin, PEN	0.125
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	4

Some of the strains may be methicillin resistant. Testing the staphylococci also include tests regarding methicillin resistance. The strains may be tested by any method that you prefer. The result must be uploaded as 'positive' or 'negative'. According to the CLSI recommendations (M100-S18, table 2C), all MRS should be regarded resistant for all β-lactam antibiotics.

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the enclosed test form. Please enter your results into the interactive web database. Please read the detailed description below before entering the web database. 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 September 1st 2008.

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

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

For participants that have received additional strains as a retest for the 2007 Salm/Camp EQAS: Please send us the results by the document(s) 'Retest EQAS 2007, *Salmonella*' and/or 'Retest EQAS 2007, *Campylobacter*'.

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

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5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

Please read this passage before entering the web page. Before you go ahead, you need your test form by your side together with your breakpoint values.

You are able to browse back and forth by using the forward and back keys or click on the CRL logo.

You enter the EU CRL-AR EQAS 2008 start web page (http://thor.dfvf.dk/crl) then write your username and password in low cases and press enter. Your username and password is the same as in the previous EQAS's arranged by The National Food Institute. If you have problems with the login please contact us.

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

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

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

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

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

Click on "save and go to next page"

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

If relevant for the microorganism, you also have the option to type in results for the ESBL tests.

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

Click on "save and go to next page"

When uploading data on the reference strains please enter the zonediameters in mm or MIC values in µg/ml. Remember to use the operator keys to show e.g. equal to, etc.

Click on "save and go to next page"

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

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

Please fill in the evaluation form.

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

See the evaluated results. You can print each page. *You may have to choose a smaller text* size to print the whole screen on one piece of paper. In the Internet Explorer (or the Internet program you may have), you click on "view", "text size" and e.g. "smallest".

Breakpoints used in daily routine (disk diffusion) - Enterococci

Antimicrobial	Lab. no.	R≤ mm	S≥ mm
Ampicillin, AMP	15	14	19
	18	16	17
	19	16	17
	23	16	17
	26	16	17
	29	16	17
	40	16	17
Chloramphenicol, CHL	15	19	23
	18	12	18
	19	12	18
	23	12	18
	26	12	18
	29	17	21
	40	12	18
Ciprofloxacin, CIP	18	15	21
	19	15	21
	23	15	21
	26	15	21
	29	16	23
	40	15	21
Erythromycin, ERY	15	17	22
	18	13	23
	19	13	23
	23	13	23
	26	13	23
	29	15	21
	40	13	23
Florfenicol, FFN	15	15	19
	18	12	18
	19	14	19
	23	12	18
	26	14	19
	29	17	21
Gentamicin, GEN	15	11	17
	18	12	15
	19		10
	23	12	15
	29	12	15
	40	12	15

Antimicrobial	Lab. no.	R≤ mm	S≥ mm
Linezolid, LZD	15	24	24
	18	20	23
	26		19
	40	20	23
Streptomycin, STR	15	12	14
	18	11	12
	19		10
	23	11	15
	29	11	15
	40	11	15
Synacid, SYN	26	15	19
Tetracycline, TET	15	17	19
	18	14	19
	19	14	19
	23	14	19
	26	14	19
	29	18	23
	40	14	16
Tigecycline, TGC	15	22	22
	26		21
Vancomycin, VAN	15	17	17
	18	14	17
	19	14	17
	23	14	17
	26	14	17
	29	14	17
	40	14	17

Breakpoints used in daily routine (disk diffusion) - Staphylococci

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Chloramphenicol, CHL	2	12	18
	9	12	18
	14	18	23
	15	19	22
	18	12	18
	19	12	18
	23	12	18
	28	12	18
	29	12	18
	30	12	18
	40	12	18
Ciprofloxacin, CIP	2	15	21
	9	15	21
	13	15	21
	14	18	22
	15	19	22
	18	15	21
	19	15	21
	23	15	21
	28	15	21
	29	16	23
	30	15	21
	40	15	21
Erythromycin, ERY	2	13	23
	9	13	23
	13	16	22
	14	16	22
	15	17	22
	18	13	23
	19	13	23
	23	16	21
	28	13	23
	29	13	23
	30	13	23
	40	13	23

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Florfenicol, FFN	9	12	18
	14	18	22
	15	14	19
	18	12	18
	19	14	19
	23	12	18
	29	12	18
	30	14	19
Gentamicin, GEN	2	12	15
	9	12	15
	13	12	15
	14	19	
	15	20	20
	18	12	15
	19	12	15
	23	12	15
	28	12	15
	29	12	16
	30	12	15
	40	12	15
Penicillin, PEN	2	28	29
	9	28	29
	13	28	29
	14	28	
	15	29	29
	18	28	29
	19	28	29
	23	28	29
	28	28	29
	29	28	29
	30	28	29
	40	28	29
Streptomycin, STR	9	11	15
	13	12	15
	14	12	
	15	13	15
	18	11	12
	19	11	15
	23	11	15
	28	16	8
	29	12	16
	40	11	15

Antimicrobial	**Lab. no.**	**R ≤ mm**	**S ≥ mm**
Sulfamethoxazole, SMX	2	12	17
	9	12	17
	13	12	17
	14	11	17
	18	12	17
	19	12	17
	23	12	17
	28	12	17
	29	12	17
	30	12	17
	40	12	17
Tetracycline, TET	2	14	19
	9	14	19
	13	14	19
	14	16	19
	15	17	19
	18	14	19
	19	14	19
	23	14	19
	28	14	19
	29	14	19
	30	14	19
	40	14	19
Trimethoprim, TMP	2	10	16
	9	10	16
	14	11	16
	18	10	16
	19	10	16
	23	10	16
	28	10	16
	30	10	16
	40	10	16

Breakpoints used in daily routine (disk diffusion) - *E. coli*

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Amoxicillin+cl, AUG	15	13	21
	18	13	18
	19	13	18
	23	13	18
	28	13	18
	29	13	18
	30	13	18
Ampicillin, AMP	14	18	
	15	13	21
	18	13	17
	19	13	17
	23	13	17
	28	13	17
	29	13	17
	30	13	17
	40	16	17
Cefotaxime, CTX	14	20	
	15	22	26
	18	27	
	19	14	23
	23	14	23
	28	14	23
	29	14	23
	30	14	23
	40	14	23
Ceftazidime, CAZ	14	20	
	15	18	26
	18	22	
	19	14	18
	23	14	18
	28	14	18
	29	14	18
	30	14	18
	40	14	18
Ceftiofur, XNL	14	20	
	15	17	21
	19	17	21
	23	17	18
	29	17	21
	30	19	23

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Chloramphenicol, CHL	14	18	
	15	18	22
	18	12	18
	19	12	18
	23	12	18
	28	12	18
	29	12	18
	30	12	18
	40	12	18
Ciprofloxacin, CIP	14	24	
	18	15	21
	19	15	21
	23	15	21
	28	15	21
	29	15	21
	30	15	21
	40	15	21
Florphenicol, FFN	14	18	
	15	14	19
	18	12	18
	19	14	19
	23	12	18
	29	14	19
	30	14	19
Gentamicin, GEN	14	17	
	15	15	18
	18	12	15
	19	12	15
	23	12	15
	28	12	15
	29	12	15
	30	12	15
	40	12	18
Nalidixic acid, NAL	14	14	
	15	14	20
	18	13	19
	19	13	19
	23	13	19
	28	13	19
	29	13	19
	30	13	19
	40	13	19

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Streptomycin, STR	14	12	
	15	12	15
	18	11	15
	19	11	15
	23	11	15
	28	11	15
	29	11	15
	30	11	15
	40	11	15
Sulfamethoxazole, SMX	15	11	17
	18	12	17
	19	12	17
	23	12	17
	28	12	17
	29	12	17
	30	12	17
	40	12	17
TMP+SMX, SXT	14	15	
	18	10	16
	19	10	16
	23	10	16
	28	10	16
	29	10	16
	30	10	16
	40	10	16
Tetracycline,TET	14	16	
	15	16	19
	18	11	15
	19	11	15
	23	14	19
	28	14	19
	29	14	19
	30	14	19
	40	11	15
Trimethoprim, TMP	15	11	16
	18	10	16
	19	10	16
	23	10	16
	28	10	16
	30	10	16
	40	10	16

Quality control ranges for the control strains

E. faecalis 29212	
Antimicrobial	**MIC**
Ampicillin, AMP	0.5 - 2
Avilamycin, AVI	0.5 - 4
Chloramphenicol, CHL	4 - 16
Ciprofloxacin, CIP	0.25 - 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	0.03 - 0.12
Vancomycin, VAN	1 - 4

	S. aureus ATCC 25923	*S. aureus* ATCC 29213	
Antimicrobial	**Disk diffusion**	**E-test**	**MIC**
Chloramphenicol, CHL	16 - 26	2 - 8	2 - 8
Ciprofloxacin, CIP	22 - 30	0.125 - 0.5	0.12 - 0.5
Erythromycin, ERY	22 - 30	0.125 - 0.5	0.25 - 1
Florfenicol, FFN	None	None	2 - 8
Gentamicin, GEN	19 - 27	None	0.12 - 1
Penicillin, PEN	26 - 37	None	0.25 - 2
Streptomycin, STR	14 - 22	None	None
Suphonamides, SMX	24 - 30	8 - 32	32 - 128
Tetracycline, TET	24 - 34	0.125 - 1	0.12 - 1
Trimethoprim, TMP	19 - 26	0.5 - 2	1-4

E-test ranges are according to AB-Biodisk

E. coli ATCC 25922		
Antimicrobial	**Disk difusion**	**MIC**
Amoxicillin cl., AUG	18 - 24	2 - 8
Amoxicillin, AMX	0 - 50	None
Ampicillin, AMP	16 - 22	2 - 8
Cefotaxime, CTX	29 - 35	0.03 - 0.12
Cefpodoxime, POD	23 - 28	0.25 - 1
Ceftazidime, CAZ	25 - 32	0.06 - 0.5
Ceftiofur, XNL	26 - 31	0.25 - 1
Chloramphenicol, CHL	21 - 27	2 - 8
Ciprofloxacin, CIP	30 - 40	0.004 - 0.015
Florphenicol, FFN	22 - 28	2 - 8
Gentamicin, GEN	19 - 26	0.25 - 1
Nalidixic acid, NAL	22 - 28	1 - 4
Streptomycin, STR	0 - 50	4 - 16
Sulphonamides, SMX	15 - 23	8 - 32
Tetracycline, TET	18 - 25	0.5 - 2
Trimethoprim, TMP	21 - 28	0.5 - 2

MIC ranges and disc diffusion ranges are according to CLSI M100-S18 with one exception: The MIC range for streptomycin is according to Sensititre. Additionally, the range for ciprofloxacin is extended to include 0.016 as well.

Deviations per laboratory for the enterococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	CRL ENT.2,4	Streptomycin	S	≤64	R	256	MIC
2	CRL ENT.2,4	Ampicillin	R	8	S	4.0	MIC
2	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
2	CRL ENT.2,5	Synacid	R	2	S	1.0	MIC
2	CRL ENT.2,7	Synacid	R	2	S	1	MIC
6	CRL ENT.2,2	Gentamicin	S	1	R	2048	MIC
6	CRL ENT.2,3	Gentamicin	R	>32	S	≤128	MIC
6	CRL ENT.2,3	Streptomycin	R	>128	S	≤128	MIC
6	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
6	CRL ENT.2,7	Streptomycin	R	>128	S	≤128	MIC
6	CRL ENT.2,7	Tetracycline	R	64	S	≤1	MIC
6	CRL ENT.2,8	Tetracycline	S	2	R	>32	MIC
9	CRL ENT.2,4	Streptomycin	S	64	R	256	MIC
9	CRL ENT.2,6	Ciprofloxacin	R	8	S	4.0	MIC
11	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
12	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
15	CRL ENT.2,4	Streptomycin	S	19	R	256	DD
15	CRL ENT.2,5	Chloramphenicol	R	13	S	16	DD
15	CRL ENT.2,8	Chloramphenicol	R	13	S	16	DD
16	CRL ENT.2,4	Streptomycin	S	64	R	256	MIC
17	CRL ENT.2,4	Streptomycin	S	64	R	256	MIC
18	CRL ENT.2,3	Streptomycin	R	6	S	≤128	DD
18	CRL ENT.2,8	Streptomycin	R	6	S	≤128	DD
19	CRL ENT.2,1	Erythromycin	R	13	S	4.0	DD
19	CRL ENT.2,4	Streptomycin	S	18	R	256	DD
20	CRL ENT.2,1	Gentamicin	R	>1024	S	≤128	MIC
20	CRL ENT.2,1	Streptomycin	R	>2048	S	≤128	MIC
20	CRL ENT.2,2	Tigecycline	R	0.5	S	0.12	MIC
20	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
21	CRL ENT.2,6	Ampicillin	R	8	S	4	MIC
23	CRL ENT.2,1	Ampicillin	R	15	S	4	DD
23	CRL ENT.2,1	Erythromycin	R	13	S	4.0	DD
23	CRL ENT.2,1	Streptomycin	R	6	S	≤128	DD
23	CRL ENT.2,3	Streptomycin	R	6	S	≤128	DD
23	CRL ENT.2,4	Gentamicin	R	9	S	≤128	DD
23	CRL ENT.2,5	Chloramphenicol	R	15	S	16	DD
23	CRL ENT.2,5	Ciprofloxacin	R	15	S	2.0	DD
23	CRL ENT.2,6	Ciprofloxacin	R	15	S	4.0	DD
23	CRL ENT.2,6	Gentamicin	R	12	S	≤128	DD
23	CRL ENT.2,7	Streptomycin	R	6	S	≤128	DD
23	CRL ENT.2,8	Chloramphenicol	R	12	S	16	DD
23	CRL ENT.2,8	Gentamicin	R	12	S	≤128	DD
23	CRL ENT.2,8	Streptomycin	R	6	S	≤128	DD

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
24	CRL ENT.2,1	Synacid	S	4	R	4	MIC
24	CRL ENT.2,6	Ampicillin	R	8	S	4	MIC
25	CRL ENT.2,4	Streptomycin	S	≤ 512	R	256	MIC
25	CRL ENT.2,5	Synacid	R	2	S	1.0	MIC
26	CRL ENT.2,1	Synacid	S	22	R	4	DD
26	CRL ENT.2,8	Synacid	S	21	R	2.0	DD
29	CRL ENT.2,1	Erythromycin	R	16	S	4.0	DD
29	CRL ENT.2,1	Streptomycin	R	8	S	≤128	DD
29	CRL ENT.2,2	Chloramphenicol	R	20	S	8	DD
29	CRL ENT.2,2	Ciprofloxacin	R	21.5	S	0.50	DD
29	CRL ENT.2,2	Florfenicol	R	20	S	≤4	DD
29	CRL ENT.2,2	Vancomycin	R	16	S	≤2	DD
29	CRL ENT.2,3	Ciprofloxacin	R	21	S	1.0	DD
29	CRL ENT.2,3	Streptomycin	R	0	S	≤128	DD
29	CRL ENT.2,4	Ciprofloxacin	R	17	S	4	DD
29	CRL ENT.2,4	Gentamicin	R	9.5	S	≤128	DD
29	CRL ENT.2,5	Chloramphenicol	R	16.5	S	16	DD
29	CRL ENT.2,5	Ciprofloxacin	R	17.5	S	2.0	DD
29	CRL ENT.2,5	Florfenicol	R	16.5	S	≤4	DD
29	CRL ENT.2,5	Gentamicin	R	14.5	S	≤128	DD
29	CRL ENT.2,6	Ciprofloxacin	R	16.5	S	4.0	DD
29	CRL ENT.2,6	Gentamicin	R	12.5	S	≤128	DD
29	CRL ENT.2,7	Ciprofloxacin	R	20.5	S	1.0	DD
29	CRL ENT.2,7	Gentamicin	R	12	S	≤128	DD
29	CRL ENT.2,7	Streptomycin	R	0	S	≤128	DD
29	CRL ENT.2,8	Chloramphenicol	R	16	S	16	DD
29	CRL ENT.2,8	Ciprofloxacin	R	18.5	S	1.0	DD
29	CRL ENT.2,8	Florfenicol	R	16	S	≤4	DD
29	CRL ENT.2,8	Gentamicin	R	13.5	S	≤128	DD
29	CRL ENT.2,8	Streptomycin	R	0	S	≤128	DD
33	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
34	CRL ENT.2,1	Ampicillin	R	8	S	4	MIC
34	CRL ENT.2,4	Streptomycin	S	128	R	256	MIC
37	CRL ENT.2,2	Vancomycin	R	18	S	≤2	AGA
37	CRL ENT.2,4	Streptomycin	S	64	R	256	AGA
37	CRL ENT.2,7	Tetracycline	R	16	S	≤1	AGA
40	CRL ENT.2,1	Ampicillin	R	12	S	4	DD
40	CRL ENT.2,1	Chloramphenicol	R	15	S	4	DD
40	CRL ENT.2,1	Ciprofloxacin	R	11	S	0.50	DD
40	CRL ENT.2,1	Erythromycin	R	19	S	4.0	DD
40	CRL ENT.2,1	Linezolid	R	12	S	2	DD
40	CRL ENT.2,2	Chloramphenicol	R	0	S	8	DD
40	CRL ENT.2,2	Ciprofloxacin	R	17	S	0.50	DD
40	CRL ENT.2,2	Linezolid	R	14	S	2	DD
40	CRL ENT.2,2	Vancomycin	R	15	S	≤2	DD
40	CRL ENT.2,3	Streptomycin	R	12	S	≤128	DD
40	CRL ENT.2,4	Linezolid	R	16	S	2	DD

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
40	CRL ENT.2,5	Chloramphenicol	R	13	S	16	DD
40	CRL ENT.2,5	Ciprofloxacin	R	14	S	2.0	DD
40	CRL ENT.2,5	Vancomycin	R	19	S	≤2	DD
40	CRL ENT.2,6	Chloramphenicol	R	17	S	4	DD
40	CRL ENT.2,6	Ciprofloxacin	R	14	S	4.0	DD
40	CRL ENT.2,7	Chloramphenicol	R	14	S	4	DD
40	CRL ENT.2,7	Ciprofloxacin	R	11	S	1.0	DD
40	CRL ENT.2,7	Erythromycin	R	19	S	≤0.5	DD
40	CRL ENT.2,7	Gentamicin	R	13	S	≤128	DD
40	CRL ENT.2,7	Linezolid	R	15	S	2	DD
40	CRL ENT.2,7	Streptomycin	R	0	S	≤128	DD
40	CRL ENT.2,7	Tetracycline	R	0	S	≤128	DD
40	CRL ENT.2,8	Chloramphenicol	R	12	S	16	DD
40	CRL ENT.2,8	Ciprofloxacin	R	18	S	1.0	DD
40	CRL ENT.2,8	Linezolid	R	21	S	2	DD
40	CRL ENT.2,8	Streptomycin	R	12	S	≤128	DD

Deviations per laboratory for the staphylococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
6	CRL ST.2,3	Gentamicin	R	16	S	0.125	MIC
6	CRL ST.2,3	Methicillin	Neg		Pos		MIC
6	CRL ST.2,3	Sulfamethoxazole	R	256	S	32	MIC
6	CRL ST.2,4	Gentamicin	R	16	S	0.25	MIC
6	CRL ST.2,4	Streptomycin	R	>128	S	4	MIC
6	CRL ST.2,5	Gentamicin	R	4	S	0.5	MIC
6	CRL ST.2,5	Sulfamethoxazole	R	512	S	16	MIC
6	CRL ST.2,5	Tetracycline	R	16	S	1	MIC
6	CRL ST.2,5	Trimethoprim	R	>32	S	≤1	MIC
6	CRL ST.2,6	Streptomycin	R	>128	S	≤2	MIC
6	CRL ST.2,7	Gentamicin	R	4	S	0.125	MIC
6	CRL ST.2,7	Streptomycin	R	32	S	≤2	MIC
6	CRL ST.2,7	Sulfamethoxazole	R	>1024	S	≤8	MIC
6	CRL ST.2,8	Gentamicin	R	4	S	0.125	MIC
6	CRL ST.2,8	Streptomycin	R	128	S	16	MIC
11	CRL ST.2,1	Trimethoprim	R	2	S	≤1	MIC
11	CRL ST.2,8	Methicillin resistant	Pos		Neg		MIC
14	CRL ST.2,3	Penicillin	S	33	R	0.12	MIC
15	CRL ST.2,3	Streptomycin	S	18	R	>128	DD
15	CRL ST.2,5	Streptomycin	S	21	R	32	DD
15	ATCC 25923	Erythromycin	31		22-30		DD
15	ATCC 25923	Gentamicin	29		19-27		DD
15	ATCC 25923	Penicillin	40		26-37		DD
15	ATCC 25923	Streptomycin	31		14-22		DD
17	CRL ST.2,3	Methicillin	Neg		Pos		MIC
17	CRL ST.2,6	Penicillin	S	≤0.5	R	0.5	MIC
17	CRL ST.2,7	Penicillin	≤0.5	R	16		MIC
17	CRL ST.2,8	Penicillin	S	≤0.5	R	2	MIC
18	ATCC 25923	Sulfisoxazole	23		24-30		
20	CRL ST.2,3	Methicillin	Neg		Pos		MIC
20	CRL ST.2,4	Sulfamethoxazole	R	256	S	32	MIC
20	CRL ST.2,5	Sulfamethoxazole	R	>1024	S	16	MIC
22	CRL ST.2,3	Penicillin	S	0.12	R	0.12	MIC
22	CRL ST.2,6	Penicillin	S	0.12	R	0.5	MIC
23	CRL ST.2,3	Penicillin	S	29	R	0.12	MIC
23	CRL ST.2,3	Methicillin	Neg		Pos		MIC
26	CRL ST.2,3	Penicillin	S	≤0.06	R	0.12	MIC
26	CRL ST.2,3	Methicillin	Neg		Pos		MIC
26	CRL ST.2,6	Penicillin	S	≤0.06	R	0.5	MIC
29	CRL ST.2,2	Streptomycin	R	14.5	S	≤2	DD
29	CRL ST.2,3	Methicillin	Neg		Pos		DD
29	CRL ST.2,4	Streptomycin	R	14	S	4	DD
29	CRL ST.2,6	Streptomycin	R	15.5	S	≤2	DD
33	CRL ST.2,3	Penicillin	S	0.12	R	0.12	MIC
33	CRL ST.2,3	Methicillin	Neg		Pos		MIC
33	CRL ST.2,7	Trimethoprim	R	8	S	≤1	MIC

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
34	CRL ST.2,3	Sulfamethoxazole	R	256	S	32	MIC
34	CRL ST.2,7	Trimethoprim	R	8	S	≤1	MIC
37	CRL ST.2,2	Tetracycline	R	4	S	≤0.5	AGA
37	CRL ST.2,3	Sulfamethoxazole	R	256	S	32	AGA
37	CRL ST.2,4	Sulfamethoxazole	R	256	S	32	AGA
37	CRL ST.2,5	Sulfamethoxazole	R	256	S	16	AGA
37	CRL ST.2,7	Sulfamethoxazole	R	256	S	≤8	AGA
37	CRL ST.2,7	Trimethoprim	R	256	S	≤1	AGA
37	CRL ST.2,8	Streptomycin	R	32	S	16	AGA
37	ATCC 29213	Penicillin	0.125		0.25-2		AGA
37	ATCC 29213	Sulfisoxazole	256		32-128		AGA
40	CRL ST.2,1	Ciprofloxacin	S	21	R	2	DD
40	CRL ST.2,1	Gentamicin	S	13	R	16	DD
40	CRL ST.2,3	Methicillin	Neg		Pos		DD
40	CRL ST.2,3	Penicillin	S	29	R	0.12	DD
40	CRL ST.2,4	Methicillin	Neg		Pos		DD
40	CRL ST.2,4	Sulfamethoxazole	R	11	S	32	DD
40	CRL ST.2,4	Trimethoprim	S	13	R	>32	DD
40	CRL ST.2,5	Sulfamethoxazole	R	6	S	16	DD
40	CRL ST.2,5	Streptomycin	S	12	R	32	DD
40	CRL ST.2,6	Tetracycline	S	17	R	4	DD
40	CRL ST.2,7	Methicillin	Pos		Neg		DD
40	CRL ST.2,7	Sulfamethoxazole	R	6	S	≤8	DD
40	CRL ST.2,8	Methicillin	Pos		Neg		DD
40	ATCC 25923	Erythromycin	20		22-30		DD
40	ATCC 25923	Sulfisoxazole	6		24-30		DD
40	ATCC 25923	Trimethoprim	16		19-26		DD

Deviations per laboratory for the *E. coli* strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
1	CRL EC.2,1	Amoxicillin cl	R	16	S	8	MIC
1	CRL EC.2,5	Confirmed ESBL	No		Yes		MIC
1	CRL EC.2,8	Ceftiofur	S	≤0.5	R	<0.5	MIC
1	CRL EC.2,8	Confirmed ESBL	No		Yes		MIC
4	CRL EC.2,2	Streptomycin	R	32	S	16	MIC
4	CRL EC.2,5	Ceftazidime	S	0.5	R	1.0	MIC
4	CRL EC.2,7	Cefotaxime	R	0.5	S	≤0.12	MIC
6	CRL EC.2,4	Confirmed ESBL	No		Yes		MIC
9	CRL EC.2,5	Nalidixic acid	R	32	S	8	MIC
11	CRL EC.2,4	Streptomycin	R	64	S	16	MIC
11	ATCC 25922	Ciprofloxacin	0.03		0.004-0.016		MIC
12	ATCC 25922	Ciprofloxacin	0.03		0.004-0.016		MIC
14	CRL EC.2,3	Ciprofloxacin	S	27	R	0.06	DD
14	ATCC 25922	Ampicillin	24		16-22		DD
14	ATCC 25922	Amoxicillin cl	25		18-24		DD
14	ATCC 25922	Cefotaxime	37		29-35		DD
15	CRL EC.2,2	Streptomycin	R	9	S	16	DD
15	CRL EC.2,5	Nalidixic acid	R	13	S	8	DD
16	CRL EC.2,2	Streptomycin	R	64	S	16	MIC
16	CRL EC.2,5	Amoxicillin cl	R	16	S	8	MIC
16	CRL EC.2,8	Ceftiofur	S	1	R	<0.5	MIC
17	CRL EC.2,2	Streptomycin	R	32	S	16	DD
18	CRL EC.2,2	Streptomycin	R	9	S	16	DD
18	CRL EC.2,3	Ciprofloxacin	S	27	R	0.06	DD
18	CRL EC.2,5	Ciprofloxacin	S	21	R	0.5	DD
18	CRL EC.2,8	Ciprofloxacin	S	26	R	0.12	DD
19	CRL EC.2,2	Streptomycin	R	11	S	16	DD
19	CRL EC.2,3	Ciprofloxacin	S	27	R	0.06	DD
19	CRL EC.2,5	Amoxicillin cl	R	14	S	8	DD
19	CRL EC.2,5	Ciprofloxacin	S	22	R	0.5	DD
19	CRL EC.2,5	Nalidixic acid	R	17	S	8	DD
19	CRL EC.2,8	Ciprofloxacin	S	27	R	0.12	DD
19	ATCC 25922	Sulfisoxazole	26		15-23		DD
20	CRL EC.2,2	Streptomycin	R	32	S	16	MIC
21	ATCC 25922	Ampicillin	1		2-8		MIC
21	ATCC 25922	Cefotaxime	0.25		0.03-0.12		MIC
21	ATCC 25922	Streptomycin	2		4-16		MIC
22	CRL EC.2,2	Streptomycin	R	32	S	16	MIC
22	CRL EC.2,5	Ceftazidime	S	0.5	R	1	MIC

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
23	CRL EC.2,1	Ceftazidime	S	23	R	8	DD
23	CRL EC.2,2	Streptomycin	R	11	S	16	DD
23	CRL EC.2,3	Ciprofloxacin	S	27	R	0.06	DD
23	CRL EC.2,4	Ceftazidime	S	18	R	16	DD
23	CRL EC.2,5	Ceftazidime	S	26	R	1.0	DD
23	CRL EC.2,5	Ciprofloxacin	S	21	R	0.5	DD
23	CRL EC.2,8	Cefotaxime	S	23	R	0.5	DD
23	CRL EC.2,8	Ceftazidime	S	20	R	8	DD
23	CRL EC.2,8	Ceftiofur	S	19	R	<0.5	DD
23	CRL EC.2,8	Ciprofloxacin	S	25	R	0.12	DD
23	ATCC 25922	Ceftazidime	33		25-32		DD
23	ATCC 25922	Sulfisoxazole	24		15-23		DD
24	CRL EC.2,2	Streptomycin	R	32	S	16	MIC
24	CRL EC.2,4	Chloramphenicol	R	32	S	16	MIC
24	CRL EC.2,4	Florphenicol	R	32	S	16	MIC
29	CRL EC.2,2	Streptomycin	R	7	S	16	DD
29	CRL EC.2,3	Ciprofloxacin	S	27.5	R	0.06	DD
29	CRL EC.2,3	Florphenicol	R	10	S	8	DD
29	CRL EC.2,5	Nalidixic acid	R	15,5	S	8	DD
29	CRL EC.2,8	Ceftiofur	S	23	R	<0.5	DD
29	CRL EC.2,8	Ciprofloxacin	S	23,5	R	0.12	DD
29	CRL EC.2,8	Florphenicol	R	0	S	4	DD
30	CRL EC.2,2	Streptomycin	R	6	S	16	DD
30	CRL EC.2,1	Amoxicillin cl	R	14	S	8	DD
30	CRL EC.2,5	Nalidixic acid	R	14	S	8	DD
33	CRL EC.2,2	Streptomycin	R	64	S	16	MIC
33	ATCC 25922	Ciprofloxacin	0.03		0.004-0.016		MIC
37	CRL EC.2,1	Amoxicillin cl	R	16/2	S	8	AGA
37	CRL EC.2,2	Amoxicillin cl	R	16/2	S	8	AGA
37	CRL EC.2,2	Streptomycin	R	32	S	16	AGA
37	CRL EC.2,3	Amoxicillin cl	R	32/2	S	4	AGA
37	CRL EC.2,3	Ciprofloxacin	S	≤ 0.015	R	0.06	AGA
37	CRL EC.2,5	AmoxicillinÂ cl	R	64/2	S	8	AGA
37	CRL EC.2,7	Amoxicillin cl	R	64/2	S	4	AGA
37	CRL EC.2,8	Amoxicillin cl	R	16/2	S	4	AGA
37	ATCC 25922	Sulfisoxazole	128		8-32		AGA

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
40	CRL EC.2,1	Ceftazidime	S	18	R	8	DD
40	CRL EC.2,3	Chloramphenicol	S	13	R	32	DD
40	CRL EC.2,3	Ciprofloxacin	S	28	R	0.06	DD
40	CRL EC.2,3	Gentamicin	S	13	R	16	DD
40	CRL EC.2,4	Cefotaxime	S	15	R	>4.0	DD
40	CRL EC.2,4	Ceftazidime	S	15	R	16	DD
40	CRL EC.2,5	Cefotaxime	S	16	R	>4.0	DD
40	CRL EC.2,5	Ceftazidime	S	21	R	1.0	DD
40	CRL EC.2,5	Ciprofloxacin	S	22	R	0.5	DD
40	CRL EC.2,5	Gentamicin	S	14	R	>16.0	DD
40	CRL EC.2,6	Ampicillin	R	14	S	4	DD
40	CRL EC.2,8	Cefotaxime	S	18	R	0.5	DD
40	CRL EC.2,8	Ceftazidime	S	15	R	8	DD
40	CRL EC.2,8	Ciprofloxacin	S	24	R	0.12	DD
40	ATCC 25922	Ceftiofur	22		26-31		DD
40	ATCC 25922	Ceftazidime	24		25-32		DD
40	ATCC 25922	Cefotaxime	27		29-35		DD
40	ATCC 25922	Nalidixic acid	21		22-28		DD
40	ATCC 25922	Sulfisoxazole	6		15-23		DD
40	ATCC 25922	Trimethoprim	20		21-28		DD
40	ATCC 25922	TMP+SMX	22		23-29		DD

Percentage of resistant and sensitive enterococci

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.2,1	Ampicillin, AMP	S	12	88	22	3
	Avilamycin, AVI	S	0	100	5	0
	Chloramphenicol, CHL	S	4	96	24	1
	Ciprofloxacin, CIP	S	6	94	16	1
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	S	22	78	18	5
	Florfenicol, FFN	S	0	100	13	0
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	6	94	16	1
	Streptomycin, STR	S	13	87	20	3
	Synacid, SYN	R	82	18	9	2
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	R	100	0	24	0
	TOTAL				**222**	**17**
CRL ENT.2,2	Ampicillin, AMP	S	0	100	25	0
	Avilamycin, AVI	S	0	100	5	0
	Chloramphenicol, CHL	S	8	92	22	2
	Ciprofloxacin, CIP	S	11	89	16	2
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	25	0
	Florfenicol, FFN	S	8	92	12	1
	Gentamicin, GEN	R	96	4	22	1
	Linezolid, LZD	S	6	94	16	1
	Streptomycin, STR	R	100	0	22	0
	Synacid, SYN	S	40	60	6	4
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	20	80	4	1
	Vancomycin, VAN	S	13	88	21	3
	TOTAL				**225**	**15**
CRL ENT.2,3	Ampicillin, AMP	S	0	100	25	0
	Avilamycin, AVI	R	100	0	5	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	6	94	17	1
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	S	0	100	25	0
	Florfenicol, FFN	S	0	100	13	0
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	S	23	77	17	5
	Synacid, SYN	S	0	100	11	0
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	R	100	0	24	0
	TOTAL				**234**	**7**

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.2,4	Ampicillin, AMP	S	60	40	10	15
	Avilamycin, AVI	R	100	0	5	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	35	65	11	6
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	25	0
	Florfenicol, FFN	S	0	100	13	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	6	94	16	1
	Streptomycin, STR	R	32	68	7	15
	Synacid, SYN	R	100	0	11	0
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	0	100	24	0
	TOTAL				**201**	**39**
CRL ENT.2,5	Ampicillin, AMP	S	0	100	25	0
	Avilamycin, AVI	R	100	0	5	0
	Chloramphenicol, CHL	S	20	80	20	5
	Ciprofloxacin, CIP	S	18	82	14	3
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	25	0
	Florfenicol, FFN	S	8	92	12	1
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	R	100	0	24	0
	Synacid, SYN	S	22	78	9	2
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	4	96	23	1
	TOTAL				**229**	**13**
CRL ENT.2,6	Ampicillin, AMP	S	8	92	23	2
	Avilamycin, AVI	R	100	0	5	0
	Chloramphenicol, CHL	S	4	96	24	1
	Ciprofloxacin, CIP	S	22	78	14	4
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	24	0
	Florfenicol, FFN	S	0	100	13	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	R	100	0	24	0
	Synacid, SYN	R	100	0	10	0
	Tetracycline, TET	R	100	0	26	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	0	100	24	0
	TOTAL				**232**	**9**

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.2,7	Ampicillin, AMP	S	0	100	25	0
	Avilamycin, AVI	S	0	100	5	0
	Chloramphenicol, CHL	S	4	96	24	1
	Ciprofloxacin, CIP	S	11	89	16	2
	Daptomycin, DAP	S	33	67	2	1
	Erythromycin, ERY	S	4	96	24	1
	Florfenicol, FFN	S	0	100	13	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	6	94	16	1
	Streptomycin, STR	S	17	83	19	4
	Synacid, SYN	S	64	36	4	7
	Tetracycline, TET	S	12	88	23	3
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	0	100	24	0
	TOTAL				**220**	**22**
CRL ENT.2,8	Ampicillin, AMP	S	0	100	25	0
	Avilamycin, AVI	S	0	100	5	0
	Chloramphenicol, CHL	S	16	84	21	4
	Ciprofloxacin, CIP	S	11	89	16	2
	Daptomycin, DAP	S	67	33	1	2
	Erythromycin, ERY	R	100	0	25	0
	Florfenicol, FFN	S	8	92	12	1
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	6	94	16	1
	Streptomycin, STR	S	17	83	19	4
	Synacid, SYN	R	91	9	10	1
	Tetracycline, TET	R	96	4	25	1
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	0	100	24	0
	TOTAL				**224**	**18**

Antimicrobials producing deviations

Percentage of resistant and sensitive staphylococci

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.2,1	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	R	38	62	11	18
	Erythromycin, ERY	S	0	100	28	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	R	96	4	25	1
	Penicillin, PEN	R	100	0	26	0
	Streptomycin, STR	R	100	0	21	0
	Sulfamethoxazole, SMX	R	95	5	18	1
	Tetracycline, TET	R	100	0	30	0
	Trimethoprim, TMP	S	4	96	24	1
	TOTAL				**226**	**21**
CRL ST.2,2	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	0	100	27	0
	Penicillin, PEN	S	0	100	27	0
	Streptomycin, STR	S	5	95	19	1
	Sulfamethoxazole, SMX	S	0	100	19	0
	Tetracycline, TET	S	3	97	30	1
	Trimethoprim, TMP	S	0	100	24	0
	TOTAL				**248**	**2**
CRL ST.2,3	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	R	100	0	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	4	96	26	1
	Penicillin, PEN	R	77	23	20	6
	Streptomycin, STR	R	95	5	20	1
	Sulfamethoxazole, SMX	S	16	84	16	3
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	R	100	0	25	0
	TOTAL				**240**	**11**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.2,4	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	R	100	0	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	4	96	26	1
	Penicillin, PEN	R	100	0	26	0
	Streptomycin, STR	S	10	90	18	2
	Sulfamethoxazole, SMX	S	16	84	16	3
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	R	96	4	24	1
	TOTAL				**243**	**7**
CRL ST.2,5	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	4	96	25	1
	Penicillin, PEN	S	4	96	25	1
	Streptomycin, STR	R	90	10	19	2
	Sulfamethoxazole, SMX	S	21	79	15	4
	Tetracycline, TET	S	3	97	30	1
	Trimethoprim, TMP	S	4	96	24	1
	TOTAL				**240**	**10**
CRL ST.2,6	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	R	100	0	27	0
	Penicillin, PEN	R	88	12	22	3
	Streptomycin, STR	S	10	90	18	2
	Sulfamethoxazole, SMX	R	100	0	19	0
	Tetracycline, TET	R	52	48	16	15
	Trimethoprim, TMP	R	100	0	25	0
	TOTAL				**229**	**20**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.2,7	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	R	100	0	28	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	4	96	26	1
	Penicillin, PEN	R	96	4	24	1
	Streptomycin, STR	S	5	95	19	1
	Sulfamethoxazole, SMX	S	16	84	16	3
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	S	12	88	22	3
	TOTAL				**239**	**9**
CRL ST.2,8	Chloramphenicol, CHL	S	0	100	27	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	29	0
	Florfenicol, FFN	S	0	100	16	0
	Gentamicin, GEN	S	4	96	26	1
	Penicillin, PEN	R	96	4	25	1
	Streptomycin, STR	S	60	40	8	12
	Sulfamethoxazole, SMX	S	0	100	19	0
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	R	100	0	25	0
	TOTAL				**236**	**14**

Antimicrobials producing deviations

Percentage of resistant and sensitive *E. coli*

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.2,1	Amoxicillin cl., AUG	S	23	77	10	3
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	100	0	29	0
	Ceftazidime, CAZ	R	91	9	20	2
	Ceftiofur, XNL	R	100	0	14	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	30	0
	Nalidixic acid, NAL	S	0	100	30	0
	Streptomycin, STR	S	0	100	29	0
	Sulfamethoxazole, SMX	S	0	100	29	0
	TMP+SMX, SXT	S	0	100	14	0
	Tetracycline, TET	S	0	100	30	0
	Trimethoprim, TMP	S	4	96	27	1
	TOTAL				**376**	**6**
CRL EC.2,2	Amoxicillin cl., AUG	S	8	92	11	1
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	22	0
	Ceftiofur, XNL	S	0	100	12	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	R	100	0	29	0
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	30	0
	Nalidixic acid, NAL	R	100	0	30	0
	Streptomycin, STR	S	86	14	4	24
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	S	0	100	13	0
	Tetracycline, TET	R	100	0	30	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**352**	**25**
CRL EC.2,3	Amoxicillin cl., AUG	S	8	92	12	1
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	22	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	R	97	3	28	1
	Ciprofloxacin, CIP	R	71	29	20	8
	Florphenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	R	97	3	28	1
	Nalidixic acid, NAL	R	100	0	30	0
	Streptomycin, STR	R	100	0	29	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	R	100	0	14	0
	Tetracycline, TET	R	100	0	30	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**367**	**12**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.2,4	Amoxicillin cl., AUG	S	0	100	13	0
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	97	3	28	1
	Ceftazidime, CAZ	R	91	9	20	2
	Ceftiofur, XNL	R	100	0	14	0
	Chloramphenicol, CHL	S	3	97	28	1
	Ciprofloxacin, CIP	R	100	0	29	0
	Florphenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	S	0	100	30	0
	Nalidixic acid, NAL	R	100	0	30	0
	Streptomycin, STR	S	3	97	28	1
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	R	100	0	14	0
	Tetracycline, TET	S	0	100	30	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**376**	**6**
CRL EC.2,5	Amoxicillin cl., AUG	S	46	54	7	6
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	97	3	28	1
	Ceftazidime, CAZ	R	82	18	18	4
	Ceftiofur, XNL	R	100	0	14	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	R	83	17	24	5
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	R	97	3	29	1
	Nalidixic acid, NAL	S	21	79	23	6
	Streptomycin, STR	R	100	0	29	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	R	100	0	14	0
	Tetracycline, TET	R	100	0	30	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**358**	**23**
CRL EC.2,6	Amoxicillin cl., AUG	S	0	100	13	0
	Ampicillin, AMP	S	3	97	28	1
	Cefotaxime, CTX	S	0	100	29	0
	Ceftazidime, CAZ	S	0	100	22	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	30	0
	Nalidixic acid, NAL	S	0	100	30	0
	Streptomycin, STR	S	0	100	29	0
	Sulfamethoxazole, SMX	S	0	100	29	0
	TMP+SMX, SXT	S	0	100	14	0
	Tetracycline, TET	S	0	100	30	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**380**	**1**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.2,7	Amoxicillin cl., AUG	S	8	92	12	1
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	S	3	97	28	1
	Ceftazidime, CAZ	S	0	100	22	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	S	0	100	29	0
	Ciprofloxacin, CIP	S	0	100	29	0
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	R	100	0	30	0
	Nalidixic acid, NAL	S	0	100	30	0
	Streptomycin, STR	R	100	0	29	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	S	0	100	14	0
	Tetracycline, TET	R	100	0	30	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**379**	**2**
CRL EC.2,8	Amoxicillin cl., AUG	S	8	92	12	1
	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	93	7	27	2
	Ceftazidime, CAZ	R	91	9	20	2
	Ceftiofur, XNL	R	69	31	9	4
	Chloramphenicol, CHL	R	100	0	29	0
	Ciprofloxacin, CIP	R	79	21	22	6
	Florphenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	R	100	0	30	0
	Nalidixic acid, NAL	S	3	97	28	1
	Streptomycin, STR	R	100	0	29	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	TMP+SMX, SXT	R	100	0	14	0
	Tetracycline, TET	S	0	100	30	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**362**	**17**

Antimicrobials producing deviations

Antimicrobial test range for MIC (µg/mL) - Enterococci

	Laboratory number											
Antimicrobial	**2**	**9**	**11**	**12**	**17**	**20**	**21**	**22**	**24**	**26**	**33**	**35**
Ampicillin	0.25-32	0.5-32	0.25-32	0.25-32	0.5-32	0.5-32	0.12-8	4	0.25-32	1-128	0.25-32	2-32
Avilamycin	1-128	-	-	-	-	-	-	-	0.25-32	-	-	0.5-32
Chloramphenicol	4-256	2-64	0.5-64	0.5-64	2-256	2-64	4-16	32	1-128	2-64	0.5-64	2-32
Ciprofloxacin	0.25-32	0.008-8	-	0.06-4	0.008-64	0.008-8	-	4	0.5-64	0.5-64	-	0.25-8
Daptomycin	-	-		-	-	0.5-16	-	-	-	-	-	-
Erythromycin	0.5-64	0.03-4	0.5-64	0.25-64	0.12-16	0.5-8	0.25-4	4	1-128	1-128	0.5-64	0.12-64
Florfenicol	-	2-64	-	4-32	2-64	2-64	-	-	-	-	-	-
Gentamicin	4-48	0.25-32	2-256	2-256	0.25-64	0.25-32 &128-1024	-	32	128-1024	128-1024	2-256	500
Linezolid	-	-	0.5-16	0.5-16	1-16	0.5-8	0.1-8	4	0.25-32	0.25-32	0.5-16	-
Streptomycin	16-2048	2-128	8-1024	8-1024	2-128	2-128 &512-2048	-	128/512	512-2038	512-2048	8-1024	2-64;2000
Synacid	0.5-128	-	-	-	0.5-8	1-32	0.25-2	-	0.5-64	0.5-32	-	0.5-32
Tetracycline	0.5-64	1-64	0.5-64	0.5-64	1-64	1-64	0.2-8	2	0.5-64	0.5-64	0.5-64	1-64
Tigecycline	-	-	-	-	-	0.015-0.5	-	-	-	-	-	-
Vancomycin	1-64	0.25-32	1-128	1-128	2-32	0.5-32	0.5-16	4	0.5-64	0.5-64	1-128	0.5-64

Antimicrobial test range for MIC (µg/mL) - Staphylococci

	Lab no.											
Antimicrobial	**1**	**11**	**12**	**17**	**20**	**21**	**22**	**24**	**25**	**26**	**33**	**35**
Chloramphenicol	2-64	0.5-64	0.5-64	2-256	2-32	0.25-2	16	1-128	-	2-64	0.5- 64	2-32
Ciprofloxacin	0.12-8	0.06-4	0.06-4	0.008-64	0.008-8	01-4	1	0.5-64	-	0.008-8	0.06- 4	0.25-8
Erythromycin	0.12-16	0.25-32	0.25-32	0.12-16	0.5-8	0.25-4	1	1-128	0.125-16	0.25-4	0.25-32	0.12-64
Florfenicol	1-64	-	4-32	2-64	2-64	-	-	-	-	2-64	-	-
Gentamicin	-	0.5-64	0.5-64	0.25-64	0.25-32	02-8	2	128-1024	-	0.25-32	0.5- 64	-
Penicillin	0.06-16	0.03-4	0.03-4	0.5-8	0.5-16	0.06-8	0.125	-	0.06-8	0.06-8	0.03- 4	0.03-16
Streptomycin	2-128	-	4-32	2-128	2-128	-	-	512-2048	0.5-64	2-128	-	2-64
Sulfamethoxazole	8-512	-	16-2048	8-1024	8-1024	-	-	-	-	-	-	-
Tetracycline	0.5-32	0.5-64	0.5-64	1.64	1-64	02-8	1	0.5-64	0.125-16	1-64	0.5- 64	1-64
Trimethoprim	1-32	0.5-32	0.5-32	0.5-32	0.5-32	-	4	-	-	0.5-32	0.5- 32	4-32

Antimicrobial test range for MIC (µg/mL) - *E. coli*

Antimicrobial	Laboratory number												
	1	**2**	**9**	**11**	**12**	**17**	**20**	**21**	**24**	**26**	**32**	**33**	**35**
Amoxicillin+clavulanic	2-32	-	-	-	-	-	1/0.5-32/16	-	-	-	-	-	-
Ampicillin	1-32	0.5-64	0.5-32	0.25-32	0.25-32	0.5-32	0.5-32	0.5-32	0.5-32	0.5-32	0.5 - 32	0.5- 64	0.05-32
Cefotaxime	0.125-4	0.06-128	0.06-4	1-128	0.06-2	0.06-4	0.06-4	0.06-4	0.06-4	0.06-4	0.06 - 4	0.06-8	0.06-4
Ceftazidime	-	-	0.25-16	-	-	0.25-16	0.25-16	0.25-16	0.25-16	0.25-16	0.25 - 16	-	0.25-16
Ceftiofur	0.5-8	-	-	0.12-16	0.12-16	-	0.25-8	-	-	-	-	-	-
Chloramphenicol	2-64	2-256	2-64	1-128	1-128	2-64	2-64	2-64	2-64	2-64	2-64	2- 256	2-64
Ciprofloxacin	0.015-4	0.008-8	0.008-8	0.08-1	0.008-1	0.008-8	0.015-8	0.008-8	0.008-8	0.008-8	0.008 - 8	0.008- 8	0.008-8
Florphenicol	2-64	-	2-64	4-32	4-32	2-64	2-64	2-64	2-64	2-64	2-64	2-32	2-64
Gentamicin	0.5-16	0.25-32	0.25-32	0.5-64	0.5-64	0.25-32	0.25-32	0.25-32	0.25-32	0.25-32	0.25 - 32	0.25- 32	0.25-32
Nalidixic acid	4-64	2-256	4-64	1-128	1-128	4-64	0.5-64	4-64	4-64	4-64	4-64	2- 256	4-64
Streptomycin	8-128	2-256	2-128	2-256	2-256	2-128	2-128	2-128	2-128	2-128	2 - 128	2- 256	2-128
Sulfamethoxazole (SMX)	64-1024	8-1024	8-1024	16-2048	16-2048	8-1024	8-1024	8-1024	8-1024	8-1024	8 - 1024	8-1024	8-1024
Tetracycline	2-32	0.5-64	1-64	0.5-64	0.5-64	1-64	1-64	1-64	1-64	1-64	1-64	0.5- 64	1-64
TMP+SMX	-	-	-	-	0.5/9.5-4/76	-	0.012/2.38-4/76	-	-	-	-	-	-
Trimethoprim (TMP)	1-32	0.25-16	0.5-32	0.25-32	0.25-32	0.5-32	0.5-32	0.5-32	0.5-32	0.5-32	0.5 - 32	0.25- 32	0.5-32

Appendix 10

Comments received:

- To add one step to the validation were you can check easily before validation
- In the test report, add a column "obtained value" in the final page of "test deviation"
- Some names of the agents could be improved. (Florphenicol, Quinpristin-dalfopristin (in the letter)
- We found very important to be able to produce ourselves a file with the results of the EQAS that would be stored at our computer. Actually, only a paper copy is possible
- Would like to know what type of *Staphylococcus* you send, to help making the right evaluations of methicillin resistance (Oxa MIC) and further actions (PCR for MRS).

Database related issues will be pasted on to the system developer for improvement and we would aim at producing a pdf file for saving the results.

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