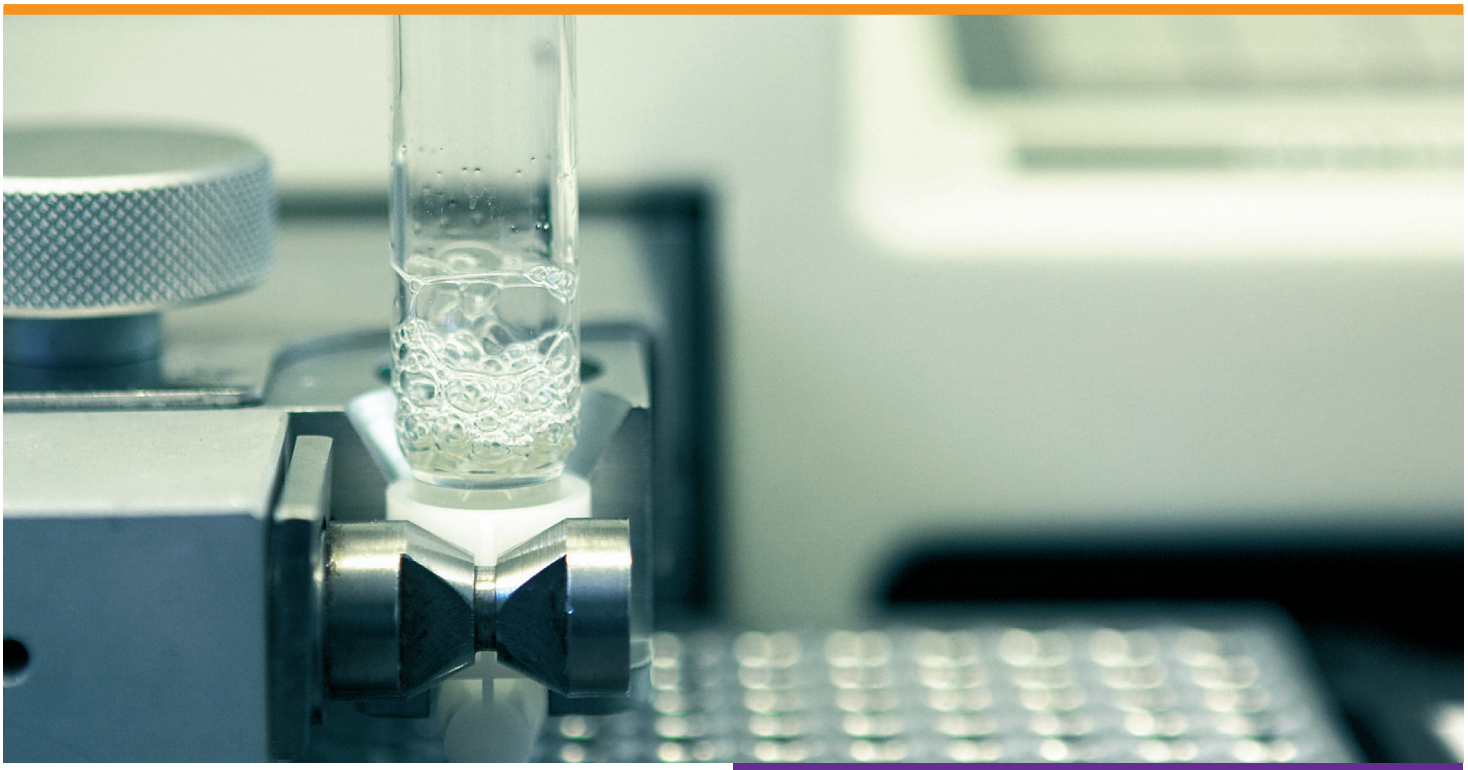
The 6th EURL-AR Proficiency Testing enterococci, staphylococci and *E. coli* 2009



Lourdes Garcia-Migura
Rene Hendriksen
Susanne Karlsmose
Frank Aarestrup

DTU Food
National Food Institute

EU Reference Laboratory – Antimicrobial Resistance

THE 6th EURL-AR Profiency Testing enterococci, staphylococci and *E. coli* 2009

1. edition, May 2010

Photo: Mikkel Adsbøl
ISBN: 978-87-92158-73-4

The report is available at
www.food.dtu.dk

National Food Institute
Technical University of Denmark
Kemitorvet, Building 204
DK-2800 Lyngby

1. INTRODUCTION

This report summarises the results of the proficiency trial in antimicrobial susceptibility testing (AST) also known as External Quality Assurance System (EQAS 2009) concerning *Escherichia coli*, enterococci and staphylococci. The National Food Institute (DTU Food) was appointed as the European Union Reference Laboratory on Antimicrobial Resistance (EURL-AR) by the European Commission (EC) in 2006. Since then, this has been the 6th EQAS trial carried out within the EURL-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 EURL and the EU Commission. All conclusions are public.

The technical advisory group for the EURL EQAS scheme consists of competent representatives from all NRL's, who meet once a year at the EURL-workshop. During the last EURL-AR Workshop (2009), the network agreed upon lowering the accepted deviation level for laboratory performance from 7% to 5% for the EQAS 2009. As in previous EQAS, incorrect results under a 75% threshold for a test strain/antimicrobial combination have been further analysed in this report, and if no reason was observed that could explain these deviations, the results were subtracted from the evaluation report.

The EURL-AR is accredited by DANAK as provider of proficiency testing; zoonotic pathogens and indicator organisms in bacterial isolates (serotyping, identification and antimicrobial susceptibility testing).

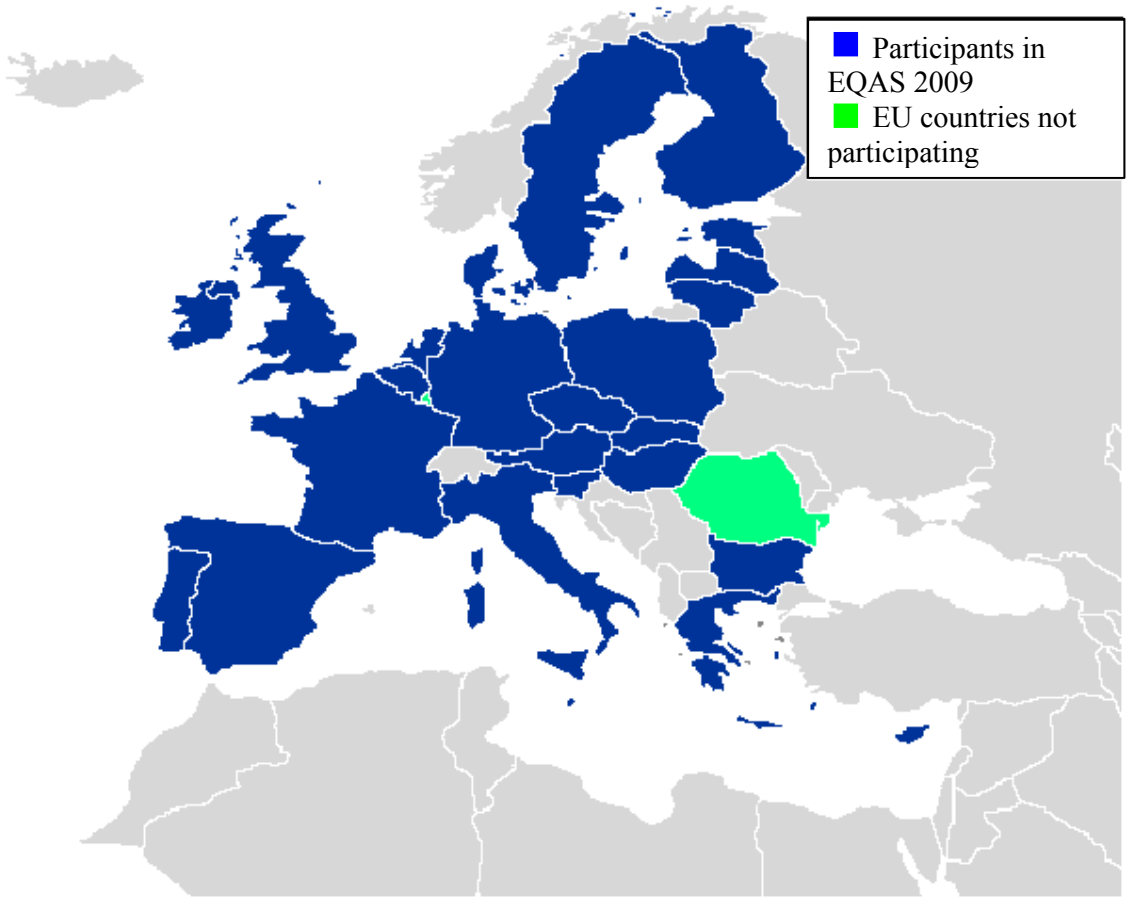
2. MATERIALS AND METHODS

2.1 Participants in EQAS 2009

In May 2009, a pre-notification to announce the EQAS 2009 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). Five additional laboratories from Spain, Romania, Denmark, Switzerland and Norway were enrolled by the EURL-AR to make up a total of 37 participating laboratories, although results from these laboratories were not included in the evaluation. Participants represented all EU countries except for Luxembourg (App. 2). One of the three NRLs from Spain and the NRL from Romania declined to participate, therefore out of 32

participating laboratories, a total of 30 submitted results (Figure 1). Of those, 23, 27 and 28 laboratories analysed the enterococci, staphylococci and the *E. coli* strains, respectively. Similar number of participation compared to EQAS 2008 when 23, 28 and 27 laboratories submitted results for enterococci, staphylococci and *E. coli*, respectively.

Figure 1. European map illustrating the participating countries in the EQAS trial 2009.



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 profiles. For quality assurance purposes, three internal control strains have been repeatedly included in every EQAS performed to date, one for each of the bacterial species tested. Antimicrobial susceptibility testing of the strains was performed at DTU Food and the MIC values obtained were used as reference for the EQAS trial (App. 3). 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.

Table 1. Panel of antimicrobials used for susceptibility testing in each of the organisms examined in the EQAS 2009.

Enterococci trial	**Staphylococci trial***	***E. coli* trial**
Ampicillin†	Cefoxitin	Ampicillin†
Avilamycin	Chloramphenicol	Cefotaxime†
Chloramphenicol†	Ciprofloxacin	Ceftazidime
Ciprofloxacin	Erythromycin	Ceftiofur
Daptomycin	Florfenicol	Chloramphenicol†
Erythromycin†	Gentamicin	Ciprofloxacin†
Gentamicin†	Penicillin	Florfenicol
Linezolid†	Streptomycin	Gentamicin†
Streptomycin†	Sulfonamides	Nalidixic acid†
Quinupristin-dalfopristin†	Tetracycline	Streptomycin†
Tetracycline†	Trimethoprim	Sulphonamides†
Tigecycline		Tetracycline†
Vancomycin†		Trimethoprim†

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

AST guidelines were set according to the Clinical and Laboratory Standards Institute (CLSI) document M7-A7 (2006) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Seventh Edition". MIC determination including extended spectrum β-lactamase (ESBL) was performed at the EURL-AR using the Sensititre system from Trek diagnostics Ltd. In addition, for ESBL confirmation, cefotaxime + clavulanic acid, ceftazidime + clavulanic acid, imipenem and imipenem + EDTA were tested by E-test (AB-Biodisk). The MIC results were interpreted using the epidemiological cut off values set by EUCAST (www.eucast.org), recommended by EFSA and described in the protocol (App. 4).

However, results of the ESBL detection were interpreted according to the recommendations from CLSI.

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

2.4 Distribution

The protocols and other relevant material were made available to all participants from the EURL-AR website (http://crl-ar.eu). In June, 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 UN3373, biological substances category B.

2.5 Procedure

Upon arrival and prior to performing the antimicrobial susceptibility test, the laboratories were instructed to store 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 susceptible. The EURL-AR recommended interpreting intermediate results as susceptible.

The EURL-AR is aware that there are two different types of interpretative criteria of results, clinical breakpoints and epidemiological cut-off values. The terms 'susceptible', 'intermediate' and 'resistant' should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. When reporting data using epidemiological cut-off values, bacteria should be reported as 'wild-type' or 'non-wild-type' (Schwarz *et al.*, 2010). Due to the different methods of AST used by the participants and also to simplify the interpretation of results, throughout this report, we will still maintain the terms susceptible and resistance, even in the cases where we are referring to wild-type and non-wild-type strains.

The 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-S19 (2009), Trek Diagnostic Sensititre System (App. 6).

All participating laboratories were advised to enter the results into an electronic record sheet at the EURL-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, mail or email to EURL-AR. The website was open for data entry until the 30th of September 2009.

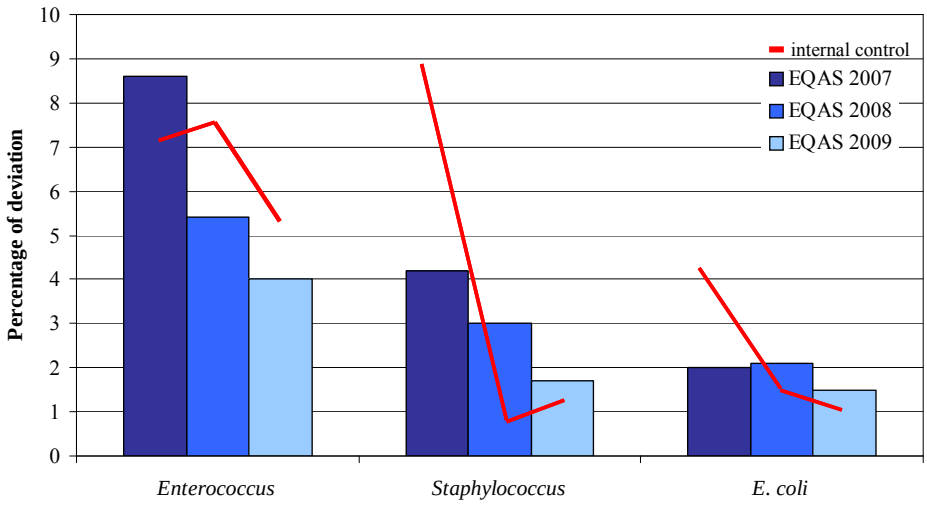
After submitting the data to the secured web site, the laboratories were instructed to retrieve an instantly generated individual report evaluating the submitted results where all deviations from the expected interpretations were reported. In addition and with the aim to improve future EQAS trials, participants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

3. RESULTS

3.1 EQAS 2009 versus previous EQAS

The percentages of deviation obtained for the enterococci, staphylococci and *E. coli* trials have decreased over the three years period from 8.6% to 4%, 4.2% to 1.7% and 2% to 1.5%, respectively (Figure 2). This decrease has also been followed by results obtained in the three internal control strains. These internal control strains have been repeatedly included in every EQAS to date. As illustrated in Figure 2, it has been a major improvement in the quality of the results between 2007, when the first trial took part and 2009, especially for the enterococci and staphylococci trials.

Figure 2. Comparison of results between EQAS 2007, EQAS 2008 and EQAS 2009 illustrating the deviation levels for the different species tested.



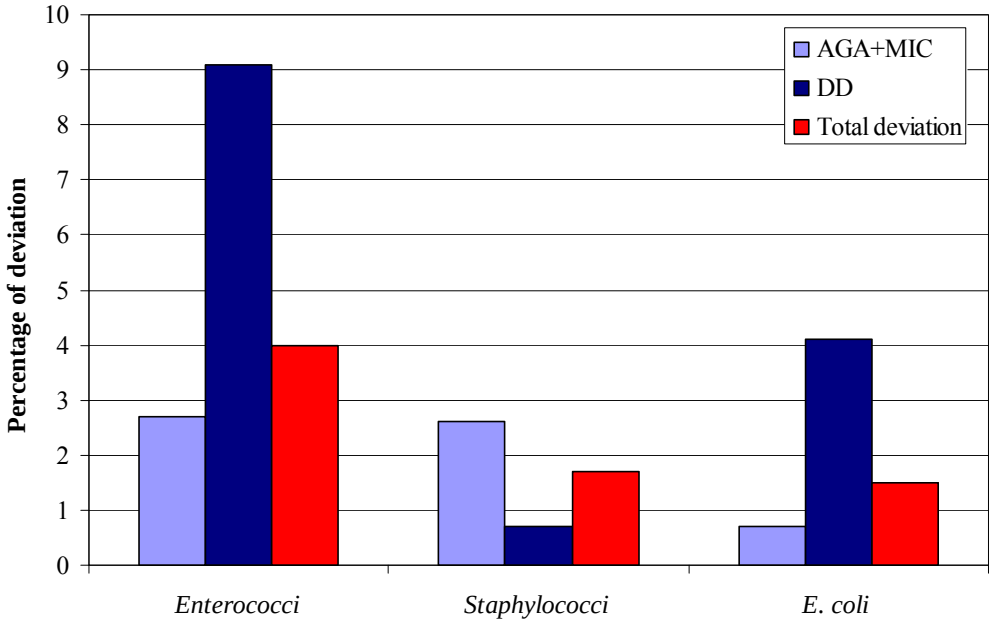
3.2 Deviations by species and method

When analysing the data, agar dilution (AGA) methods and MIC determination have been evaluated together. They are both quantitative methods and the obtained values are the concentrations at which the antimicrobials inhibit the growth of the microorganisms. On the other hand, the ROSCO method used for AST of staphylococci has been considered a disk diffusion (DD) method, since the antimicrobial would diffuse in the agar in the same way as from a disk. Contrarily to other years, none of the participants performed e-test as the routine method for AST of *E. coli* strains.

As observed in previous years, analysing the deviating results for the individual species (Figure 3), enterococci produced the highest deviation by comparison to the other two species, mainly caused by the laboratories performing disk diffusion for AST. This method produced 9.1% of the deviations when compared to MIC methods which deviated only 2.7%. Similar results were observed for *E. coli* trial, with a deviation of 4.1% caused by participants using disk diffusion by comparison to 0.7% caused by participants performing MIC. On the other hand, for the staphylococci trial, it appeared that laboratories performing disk diffusion methods generated lower number of errors (0.7%) than those using MIC (2.6%)

Thus, looking retrospectively from 2007 to 2009, the number of participants performing MIC has increased from 15 to 18, 14 to 16 and 15 to 22 for the enterococci, staphylococci and *E. coli* trials, respectively. In the same order, the number of laboratories performing disk diffusion has declined from 11 to 5, 17 to 15 and 15 to 6.

Figure 3. Percentage of deviations for the different strains in comparison with the different methods used for AST.



As shown in Table 2, the percentage of correct results per strain ranged from 94.1% to 99.2% depending of strain. The best results were obtained for the staphylococci and *E. coli* trials, in which none of the strains showed values below 96%. The enterococci trial was slightly less successful with three strains out of eight exhibiting deviations between 5.3% and 5.9%.

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.3,1	210	96.7%	ST.3,1	225	98.7%	EC.3,1	336	99.1%
ENT.3,2	209	97.6%	ST.3,2	250	98.8%	EC.3,2	335	97.9%
ENT.3,3	211	97.6%	ST.3,3	250	99.2%	EC.3,3	335	99.4%
ENT.3,4	209	96.7%	ST.3,4	250	98%	EC.3,4	336	98.8%
ENT.3,5	190	94.7%	ST.3,5	250	99.2%	EC.3,5	336	99.1%
ENT.3,6	205	94.1%	ST.3,6	250	96.8%	EC.3,6	336	98.5%
ENT.3,7	210	95.7%	ST.3,7	250	99.2%	EC.3,7	226	96.9%
ENT.3,8	207	94.7%	ST.3,8	249	96%	EC.3,8	335	97.9%

The following sections of this report describe in detail the deviations obtained for each one of the three species in this EQAS carried out in 2009 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 EURL meetings, when the percentage of correct results was lower than 75% the data should be further analysed and possible subtracted from the analysis. As the percentage of correct results for the combination of strains ENT.3,5 ciprofloxacin and daptomycin, and ENT.3,6 with daptomycin were below the 75%, results were not included in the evaluation (Table 3). In all cases, the expected MIC and the cut off value to determine if the strain was resistant were within one fold dilution difference. In addition for 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. However, to see the total percentage of positive results for each strain and antimicrobial tested refer to Appendix 7a.

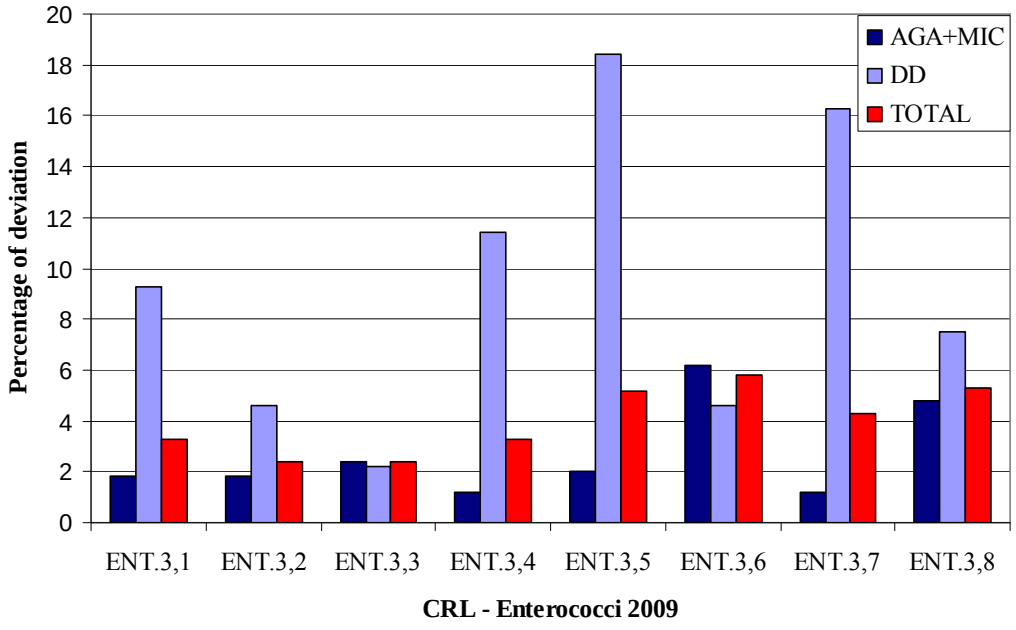
Table 3. Enterococci strain and antimicrobial combination omitted from the EQAS evaluation.

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off Value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
ENT.3,5	Ciprofloxacin	R	**41%**	>4	4	**10/12**	0/5
ENT.3,5	Daptomycin	R	**33%**	8	4	**2/3**	0/0
ENT.3,6	Daptomycin	S	**33%**	4	4	**2/3**	0/0

[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

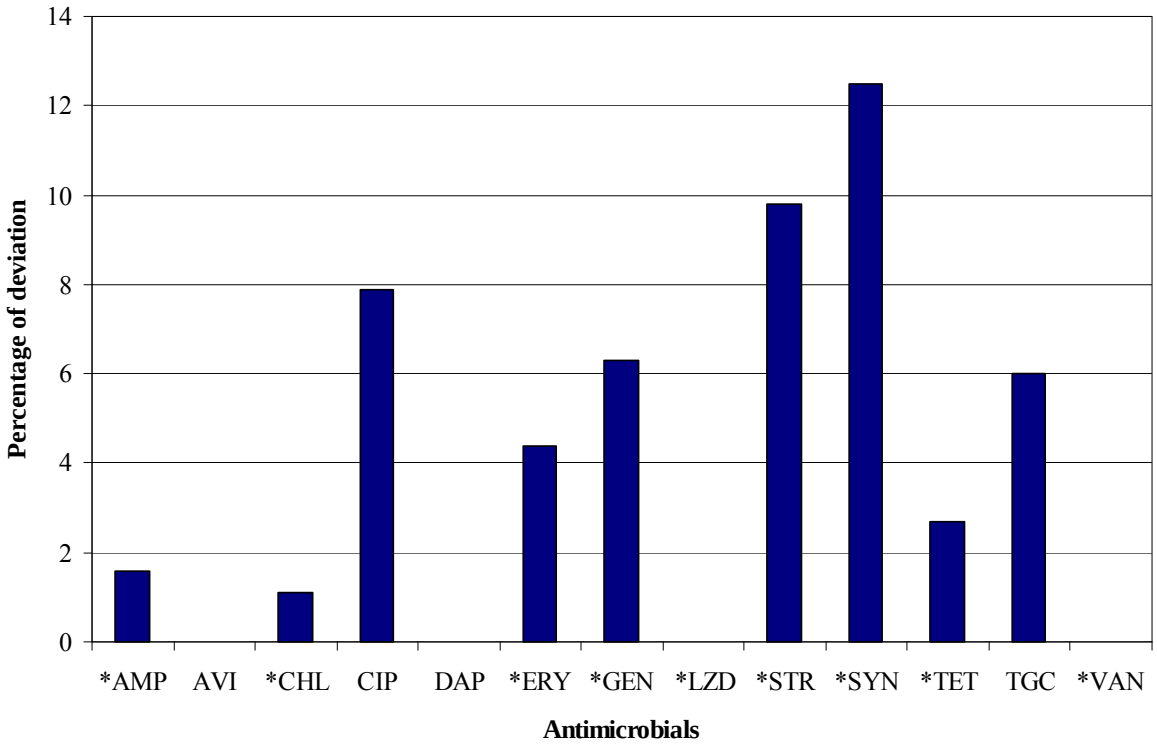
As illustrated in Figure 4, strains ENT.3,5, ENT.3,6 and ENT.3,8 exhibited the highest deviation values of 5.2%, 5.8% and 5.3% respectively. Out of the 23 laboratories taking part in the enterococci trial, 18 performed MIC methods whereas five conducted disk diffusion. In general, when comparing the different methods for AST used in each of the strains, the highest number of deviations were observed in participating laboratories performing disk diffusion. Furthermore, significant difference was 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 represented in Figure 5, showed deviations with values of 12.5% for synacid, 9.8% for streptomycin, 7.9% for ciprofloxacin, 6.3% for gentamicin and 6.0% for tigecycline. Synacid, streptomycin and gentamicin 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

Regarding the staphylococci strains, only one combination of strain/antimicrobial produced more than 25% incorrect results, and subsequently it was extracted from the evaluation. This combination was ST.3,1 and ciprofloxacin (Table 4). The expected result for this antimicrobial (MIC = 2 mg/L) and the cut off value (1 mg/L) to categorise the strain as resistant were within one fold dilution. Thus, producing results within the correct range (± one fold dilution) could conclude in the wrong outcome. These differences in the obtained results appeared to be caused by participants using MIC as well as disk diffusion.

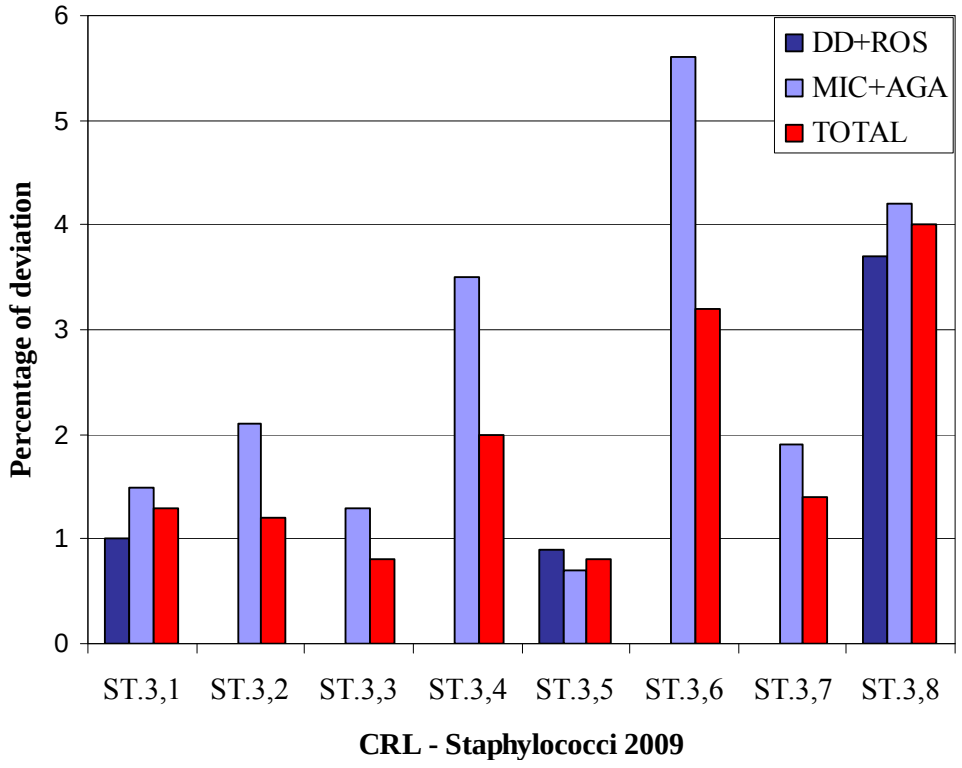
Table 4. Staphylococci strain and antimicrobial combination omitted from the EQAS evaluation.

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
ST.3,1	Ciprofloxacin	R	**33%**	2	1	**8/13**	**8/11**

[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

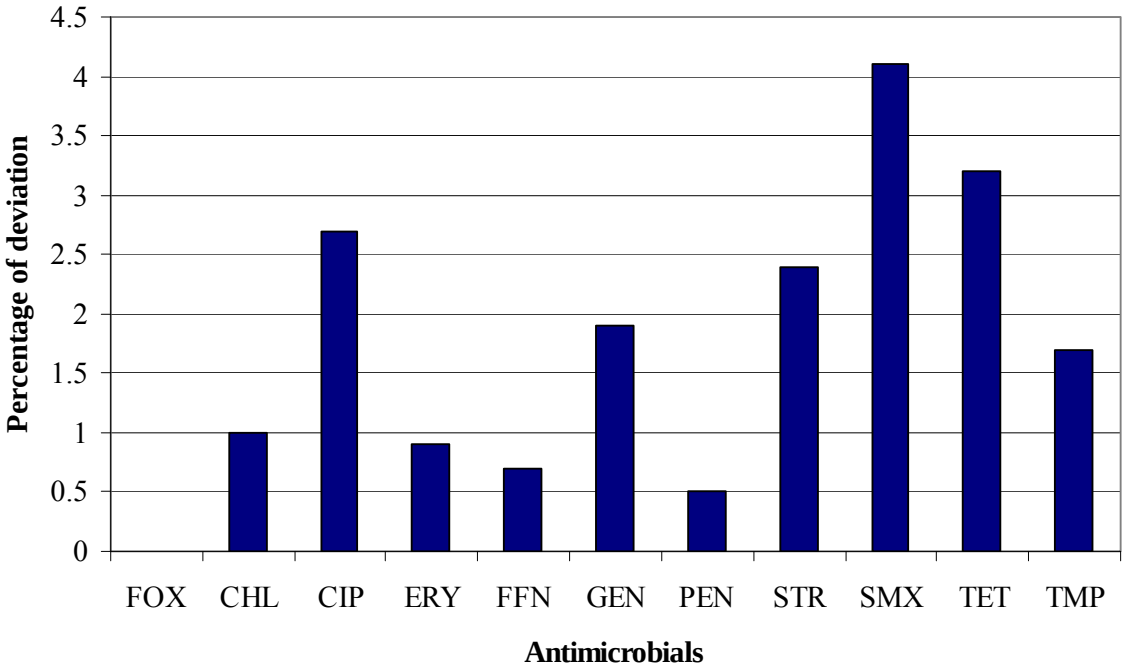
The results of the staphylococci trial were very satisfactory, with strain ST.3,8 presenting the highest deviation percentage of 4% followed by ST.3,6 with 3.2% (Figure 6). For the 27 laboratories involved in the staphylococci trial, 16 used MIC determination, 10 disk diffusion and one the ROSCO method. On the contrary to what it has been observed in the enterococci trial, significantly higher deviations were observed by participants performing MIC compared to disk diffusion methods ($p = 0.003$).

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 rosco method.



When analyzing the deviations in the staphylococci proficiency trial with respect to the antimicrobial tested (Figure 7), all deviations recorded were below 5%, and sulfamethoxazole was the antimicrobial with the higher percentage of deviation (4.1%). To see 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.3,1, ST.3,3 and ST.3,5 were confirmed to be methicillin resistant. As it was agreed in the EURL-AR workshop held in Copenhagen 2009, in the present EQAS, confirmation of *mecA* presence was mandatory for all participants. Therefore, a misidentification of methicillin resistant staphylococci would count as a deviation. Out of 208 tests performed for confirmation of *mecA* in the eight strains, nine were incorrect, resulting in a deviation of 4.3%.

3.2.3 *E. coli* trial

Regarding the analysis of the *E. coli* data, four combinations of strain/antimicrobial were subtracted from the evaluation for producing a low percentage of positive results. These combinations were the result of testing strain EC.3,7 against the following antimicrobials, chloramphenicol, florfenicol, gentamicin and streptomycin. In all cases, the expected MIC was one fold dilution below the cut off value for the antimicrobial.

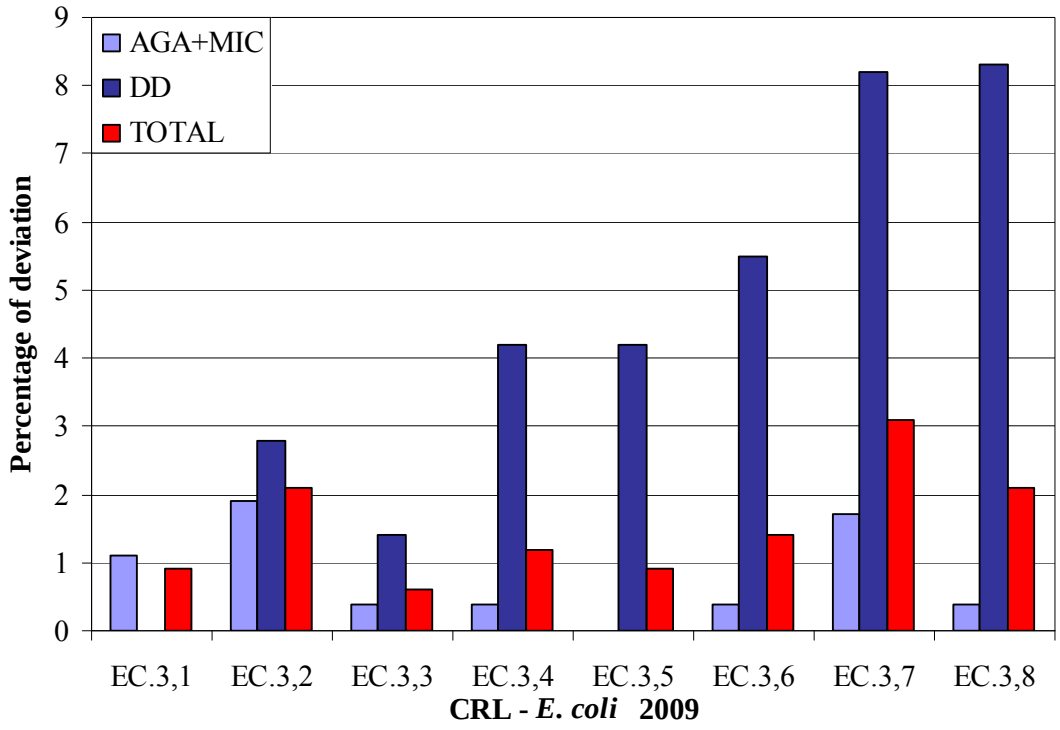
Table 5. *E. coli* strain and antimicrobial combination omitted from the EQAS evaluation.

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
EC.3,7	Chloramphenicol	S	**71%**	16	16	**6/22**	**2/6**
EC.3,7	Florfenicol	S	**64%**	16	16	**7/21**	**2/4**
EC.3,7	Gentamicin	S	**54%**	2	2	**13/22**	0/6
EC.3,7	Streptomycin	S	**58%**	8	16	**9/22**	**2/4**

[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

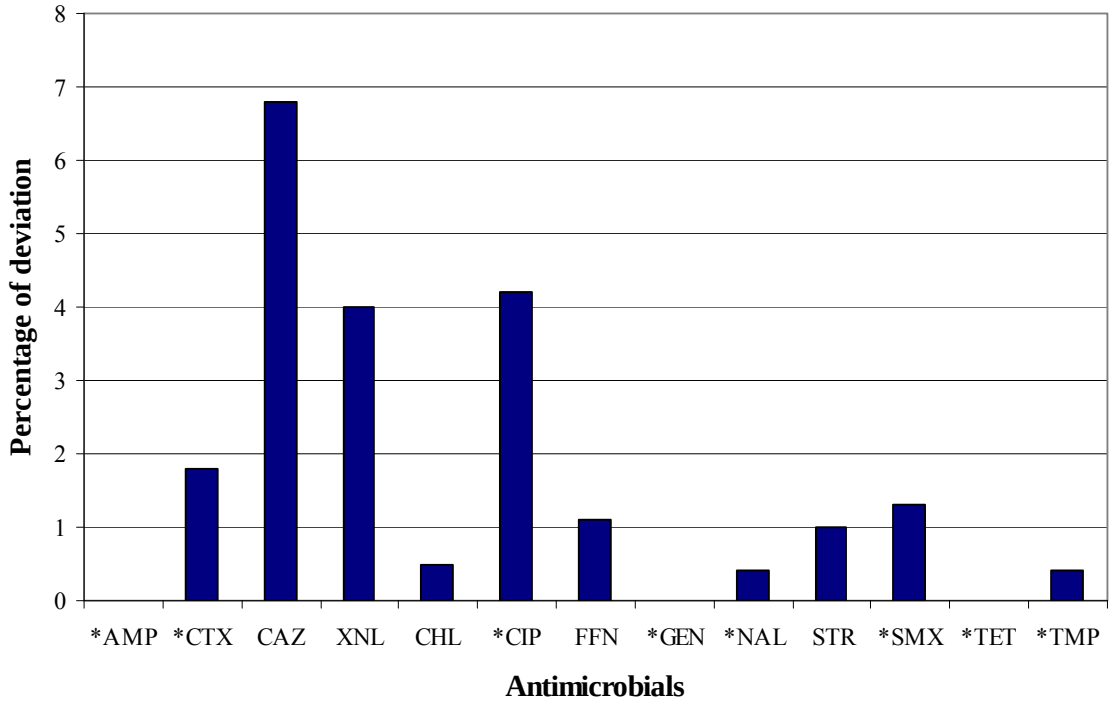
All of the deviation values obtained in terms of total deviation range are between 3.1% and 0.6% (Figure 8), resulting in the most successful trial out of the three species tested in this EQAS. The *E. coli* trial was performed by 28 laboratories of which 22 conducted MIC determination and six disk diffusion. Thus, when analyzing the results based on the different methods used for AST, the deviation values achieved performing disk diffusion were significantly higher than when using MIC ($p < 0.01$). For instance, participants using disk diffusion obtained deviations of 5.5%, 8.2% and 8.3% for the strains EC.3,6, EC.3,7 and EC.3,8 whereas participants performing MIC obtained deviations of 0.4%, 1.7% and 0.4%, respectively. For more details in all deviations per antimicrobial refer to Appendix 7c.

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



As illustrated in Figure 9, the highest deviation per antimicrobial in the *E. coli* trial was obtained for ceftazidime (CAZ). On the other hand, the deviation level for the antimicrobials recommended by EFSA for monitoring antimicrobial resistance, remained low. Thus, ciprofloxacin is one of those antimicrobials, and the total percentage of deviation resulted in 4.2%. This deviation was generated by laboratories performing disk diffusion in strains EC.3,4, EC.3,6 and EC.3,8. The three strains exhibited low levels of resistance to ciprofloxacin with values of 0.250 mg/L, 0.120 mg/L and 0.250 mg/L, respectively. Laboratories performing disk diffusion categorised those strains as susceptible instead of resistant (cut off value for ciprofloxacin is 0.032 mg/L)

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



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

Extended spectrum betalactamase (ESBL) producing strains

With regards to the panel of cephalosporins selected to identify possible ESBL producing strains, cefotaxime (CTX), ceftazidime (CAZ) and ceftiofur (XNL), the highest deviation value was obtained for ceftazidime (6.7%). This deviation was mainly caused by participants #40, #23 and #18 performing disk diffusion. Furthermore, five out of 20 laboratories performing AST against ceftazidime for strain EC.3,7, reported this *E. coli* strain as susceptible. Since this strain exhibited resistance to cefotaxime and ceftiofur, it should have been reported as resistant for ceftazidime.

Regarding ceftiofur, the deviations were mainly caused by laboratory #29. This participant, despite reporting MIC as AST method, has introduced mm values as obtained results. In addition, the three resistant strains for this antimicrobial were reported as susceptible.

Out of the eight *E. coli* strains selected for the EQAS 2009, EC.3,2, EC.3,5 and EC.3,7 were "true ESBL's" and harboured bla_{TEM52}, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$ genes, respectively. Of the 28 laboratories that took part in the *E. coli* trial this year, 23 identified the ESBL producing strains correctly. Participants #23 and #39 did not perform any of the confirmatory tests in any of the three ESBL strains.

Laboratories #20, #29, and #40 failed to identify strain EC3,7 as an ESBL producer. In particular, laboratory #40 obtained susceptible results for this strain against all cephalosporins tested; therefore they did not perform any ESBL confirmatory tests. For participant #29, the problem was the interpretation of the confirmatory test (CAZ:CAZ/CL), where they obtained synergy, but still reported the strain as a none ESBL producer. Finally, laboratory #20 did not obtain synergy in any of the two confirmatory tests (CAZ:CAZ/CL and CTX:CTX/CL) but results for ceftiofur, cefotaxime and cefoxitin were correct.

In addition, E.C,3.8 yielded the *bla*$_{CMY-2}$ gene and therefore was an ampC strain. This strain was resistant to cefotaxime (MIC > 4 mg/L), ceftazidime (MIC = 8 mg/L), ceftiofur (MIC = 8 mg/L) and cefoxitin (MIC ≥ 16 mg/L). Seven participants exhibited deviations for this particular strain. Laboratory #4 confirmed E.C,3.8 as ESBL producer instead of ampC. The results from the two MIC ratio confirmatory tests (CAZ:CAZ/CL and CTX:CTX/CL) were ≥ 8. This laboratory did not perform MIC for cefoxitin. Participants #22 and #37 confirmed the strain to be ESBL together with ampC. Both participants obtained synergy for the CTX:CTX/CL confirmatory test. On the other hand, four participants (#23, #24, #30 and #39) failed to identify E:C,3.8 as ampC positive. Participant #23 obtained susceptible values for the cephalosporins tested in their panel (ceftazidime and ceftiofur). Laboratories #24 and #39 despite finding E.C,3.8 resistant to two cephalosporin compounds, they did not perform confirmatory test nor cefoxitin susceptibility testing. Finally, laboratory #30 obtained correct results for all tests, including cefoxitin resistance, and confirmatory tests (CAZ:CAZ/CL and CTX:CTX/CL), but failed to interpret the results correctly.

3.3 Deviations by laboratory

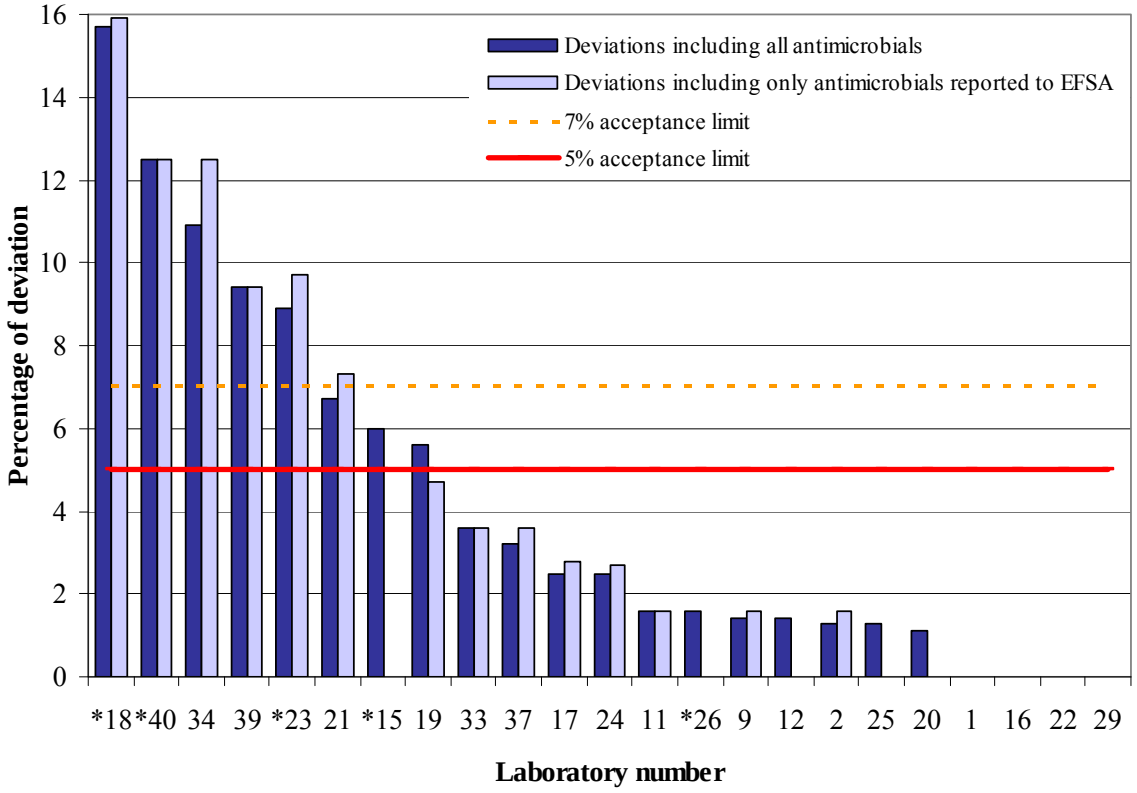
3.3.1 Enterococci trial

When analysing laboratory performance to only those antimicrobials reported to EFSA, out of the 23 participating laboratories, six obtained deviations greater than the recently agreed 5% acceptance limit (Figure 10). On the other hand, when evaluating all antimicrobials, the number of participants deviating above the 5% increased from six to eight. In addition, the number of laboratories performing 100% correctly also decreased from nine to four.

The percentage of deviations differed widely between the laboratories with a maximum of 15.7% and a minimum of 0%. Furthermore, laboratories #15 and #19 increased their deviation percentage from 0% to 6% and from 4.7%to5.6%, when evaluating all antimicrobials tested instead of those recommended by EFSA. In both cases, the deviations were caused by ciprofloxacin. On the other hand, laboratories #18, #34, #23 and #21 obtained slightly higher deviation percentages when

analysing only the antimicrobials recommended by EFSA. For laboratories #18 and #23, the deviations appeared to be caused mainly by two antimicrobials, gentamicin and streptomycin. Laboratories #40 and #34 seemed to have problems with streptomycin, whereas laboratory #21 failed five out of the eight tests performed in synacid. Laboratory #39 has deviated in five different antimicrobials without any particular pattern.

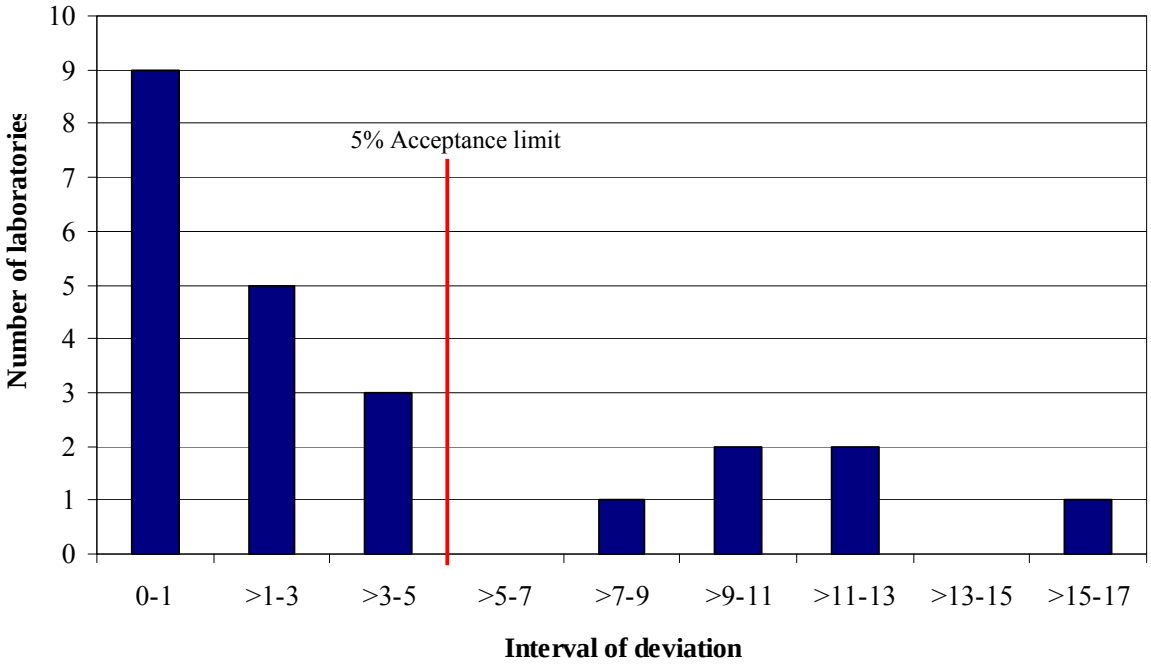
Figure 10. Individual deviations per laboratory in percentage of their total number of enterococci tests. The laboratories were ranked by decreasing percentage of deviations.



*Laboratories performing DD for AST

As shown in Figure 11, a total of 17 laboratories out of the 23 taking part, achieved the acceptance level of performance lower than 5%. In addition, of the nine participants that generated 100% correct results, seven performed MIC determination and the remaining two performed disk diffusion. Based on these results, in this enterococci EQAS trial, none of the laboratories were identified as outliers. However, the participant that obtained 15.9% deviation will be contacted by the EURL with the aim to identify possible causes of deviations and improve the quality of their results. Appendix 8a summarises all deviations by laboratory.

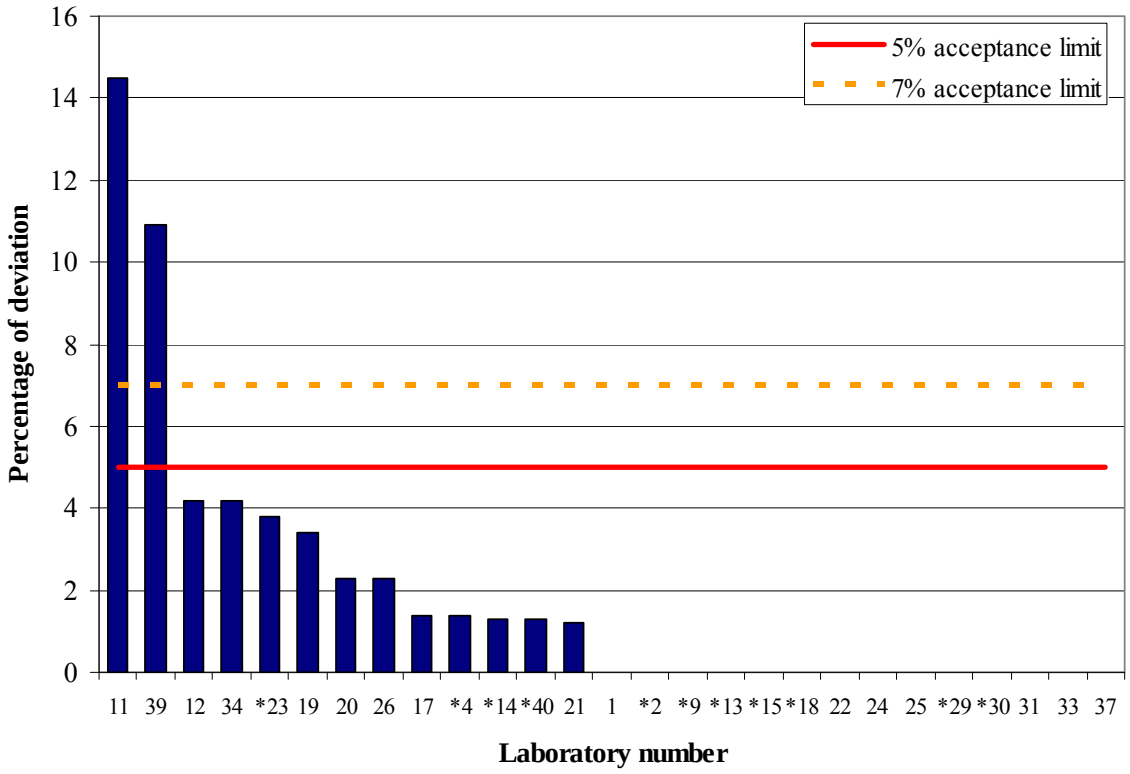
Figure 11. The number of laboratories listed in intervals of percent of total deviations in antimicrobials recommended by EFSA. The vertical line marks the acceptance limit set by the EURL at 5%.



3.3.2 Staphylococci trial

In this EQAS staphylococci trial, two laboratories exceeded the 5% acceptance limit of deviation. However, the percentage of deviation per individual laboratory was lower than in previous trials, with values equivalent to 14.5% and 10.9% for laboratories #11 and #39, respectively (Figure 12). For laboratory #11, deviations were caused mainly by the antimicrobials tetracycline and gentamicin. In both cases, susceptible strains were categorised as resistant. Participant #39 had problems with ciprofloxacin, and five susceptible strains were interpreted as resistant.

Figure 12. Individual deviations per laboratory in percentage of their total number of staphylococci tests. The laboratories were ranked by decreasing percentage of deviations.

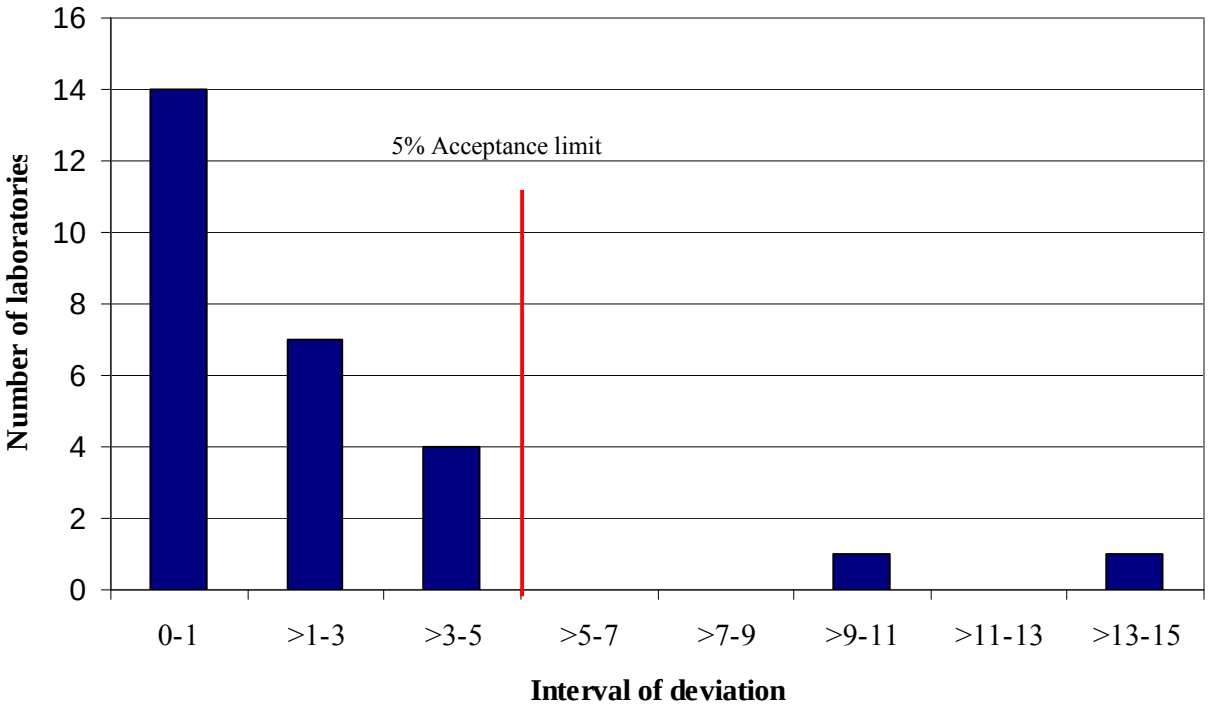


*Laboratories performing DD for AST

In total, 14 laboratories out of the 27 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).

When clustering the laboratories in intervals of deviation as illustrated in Figure 13, only two participants obtained deviations higher than 5%. On the contrary, 14 out of 27 laboratories obtained deviations in the lowest interval between 0% and 1%. No outliers were identified in this staphylococci trial, but the one participant that obtained a high percentage of deviation in this trial will be contacted in the near future.

Figure 13. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the 5% acceptance limit set by the EURL.

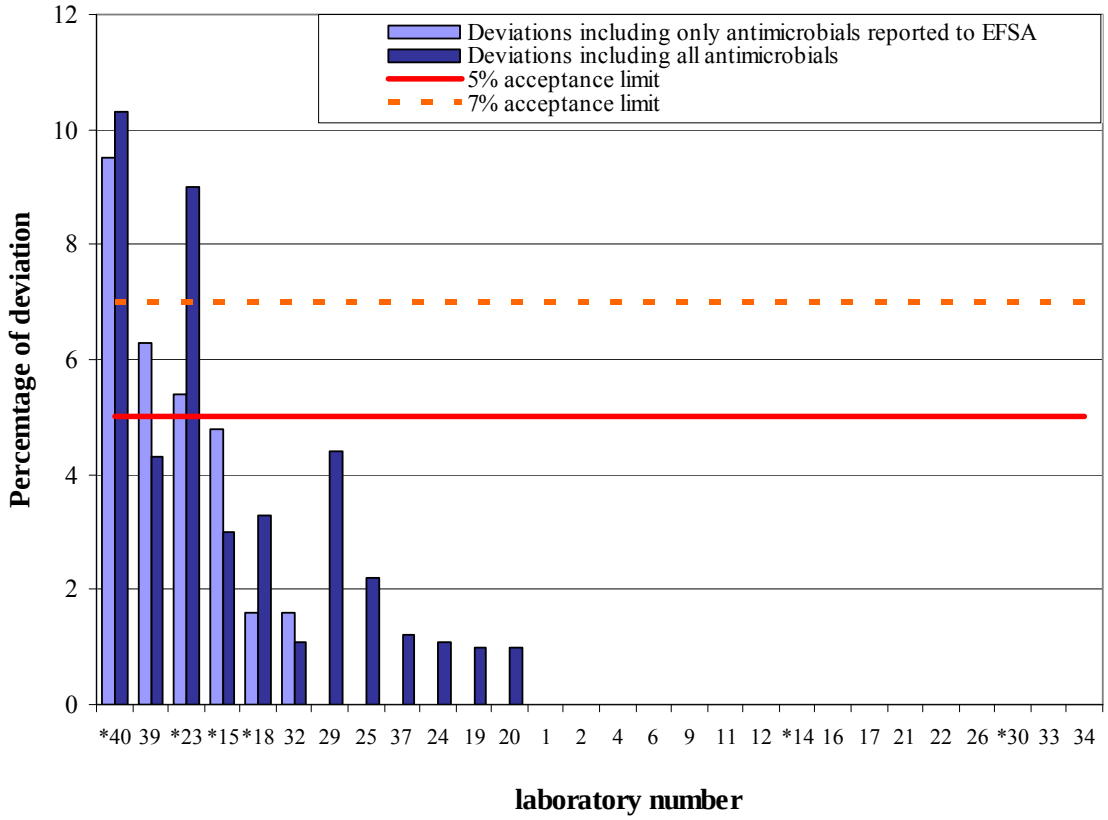


3.3.3 *E. coli* trial

As illustrated in Figure 14, analysing results based only on the antimicrobials recommended by EFSA, out of the 28 participating laboratories, three obtained deviations above the stipulated 5%. For laboratory #40, with the highest deviation in this trial, the 9.5 % deviation was caused by the cephalosporin cefotaxime together with sulfamethoxazole. For participant #39, the 6.3% deviation does not appear to be caused by any particular antimicrobial. On the other hand, for participant #23, the 5.4% deviation was caused by ciprofloxacin.

When analysing results of all the antimicrobials tested instead of just those recommended by EFSA, laboratory #23 increased the deviation percentage considerably from 5.4% to 9%. This increase was the result of including ceftazidime in the evaluation. Similar results were observed for participant #29, that increased the deviation percentage from 0% when evaluating only antimicrobials recommended by EFSA to 4.4%. Most of their incorrect results were obtained for ceftiofur, antimicrobial not listed in the EFSA panel.

Figure 14. Individual deviations per laboratory in percentage of their total number of *E. coli* tests. The laboratories were ranked by decreasing percentage of deviations.

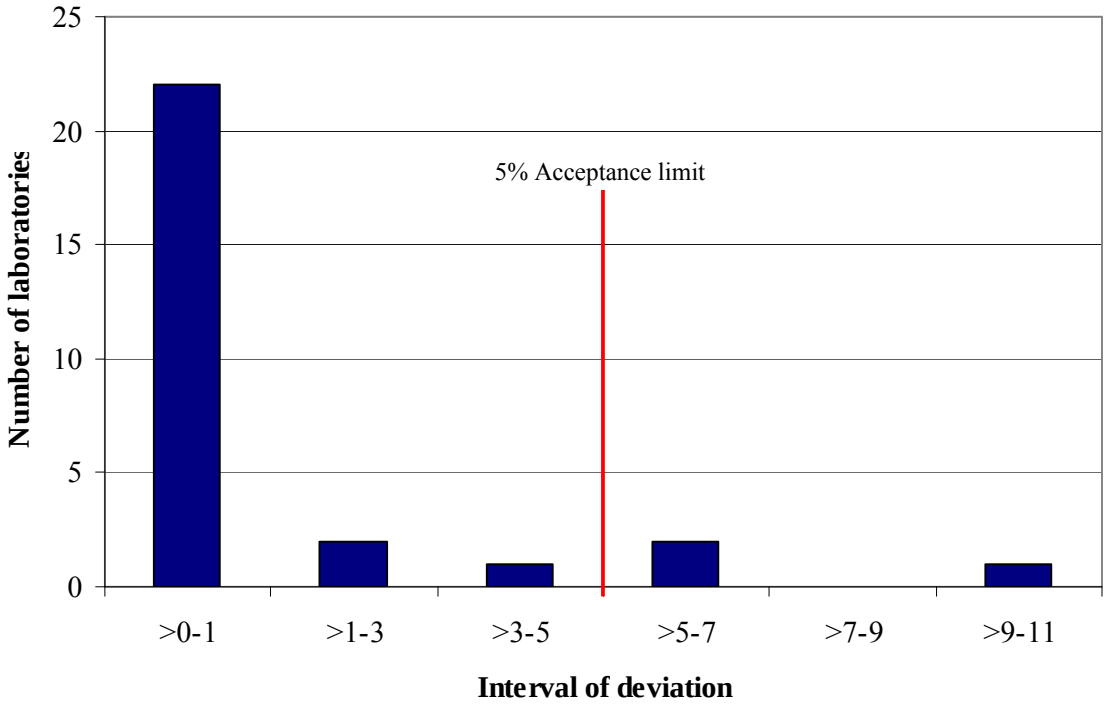


*Laboratories performing DD for AST

Out of 22 laboratories performing 100% of correct results in the antimicrobials reported to EFSA, 20 conducted MIC determination for AST instead of disk diffusion. To see the deviations for each individual laboratory refer to Appendix 8c.

As illustrated in Figure 15, the majority of the laboratories obtained deviations in the interval between 0% and 1%. Only two laboratories clustered outside the 5% threshold. For this *E. coli* trial, none of the participants were identified as outliers. However, once again, the one participant obtaining the highest deviation percentage will be contacted by the EURL with the aim of improving the quality of their performance. Appendix 8 is a summary of all the deviations obtained per participating laboratory.

Figure 15. The number of laboratories listed in intervals of percent of total deviations. The vertical line marks the 5% acceptance limit set by the EURL.



3.4 Deviations by reference strains

As the majority of the participants performing AST by disk diffusion methods have followed CLSI guidelines, the results for the reference strains have been evaluated according to them (the quality control ranges can be found in Appendix 6).

3.4.1 Enterococci

The 17 participating laboratories that carried out MIC determination in the reference strain *E. faecalis* ATCC 29212 obtained 99.3% of results within range (Table 7). This is 154 correct tests out of a total of 155 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	0/17	0.5 - 2	0.5	2
Avilamycin,	0/3	0.5 - 4	1	4
Chloramphenicol	0/17	4 - 16	4	8
Ciprofloxacin	0/10	0.25 - 2	0.5	2
Daptomycin	0/3	1 - 8	1	2
Erythromycin	0/17	1 - 4	1	4
Gentamicin	0/17	4 - 16	4	≤128
Linezolid	**1/11**	1 - 4	**0.5**	2
Streptomycin	0/16	0-256	32	128
Synacid	0/8	2 - 8	4	8
Tetracycline	0/17	8 - 32	8	32
Tigecycline	0/3	0.03 - 0.12	0.12	0.1
Vancomycin	0/16	1 - 4	2	4

As CLSI has not published a QC range for *E. faecalis* ATCC 29212 using disk diffusion, the three laboratories that have entered data for the reference strain performing this method for AST could not be evaluated.

3.4.2 Staphylococci

A total of 10 laboratories performed disk diffusion in the reference strain *S. aureus* ATCC 25923. Table 8 shows the results and the deviations obtained by antimicrobial. Only two of the values for cefoxitin and one value for gentamicin were out of range when compared to the expected results. The total number of tests performed with this reference strain was 76, of which 73 were within range.

In addition, one participant performed ROSCO method in this reference strain and the results have not been included in Table 8, since the quality control values were different to those used for disk diffusion. This participant exhibited deviations in four antimicrobials out of the eight antimicrobials

tested against *S. aureus* ATCC 25923; these were chloramphenicol, erythromycin, gentamicin, and trimethoprim.

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

Antimicrobial	Deviation/Total no. of test	QC range	Min value	Max value
Cefoxitin	**2/10**	23-29	26	**31**
Chloramphenicol	0/9	16-26	18	26
Ciprofloxacin	0/10	22-30	23	29
Erythromycin	0/10	22-30	22	28.5
Florfenicol	0/7	None	20	29
Gentamicin	**1/10**	19-27	19	**34**
Penicillin	0/10	26-37	30	37
Sulfisoxazole	0/10	24-30	24.5	26

The 14 laboratories that tested the reference strain *S. aureus* ATCC 25913 conducting MIC and agar dilution methods produced three deviations for penicillin, tetracycline and trimethoprim. This means a total of 104 correct results out of 108 tests performed in this strain. (Table 9).

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

Antimicrobial	Deviation/Total no. of test	QC range	Min value	Max value
Cefoxitin	0/7	1-4	2	4
Chloramphenicol	0/13	2-8	4	8
Ciprofloxacin	**1/13**	0.12-0.5	0.12	**1**
Erythromycin	0/14	0.25-1	0.25	1
Florfenicol	0/6	2-8	4	8
Gentamicin	0/13	0.12-1	0.25	≤2
Penicillin	**1/12**	0.25-2	**0.125**	2
Sulfisoxazole	0/4	32-128	32	128
Tetracycline	**1/14**	0.12-1	0.5	**8**
Trimethoprim	**1/12**	1-4	**0.5**	4

3.4.3 *E. coli*

Five laboratories carried out disk diffusion on the reference strain *E. coli* ATCC 25922. The total number of test performed in this strain was 48 and only one was out of range. (Table 10).

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

Antimicrobial	Deviation/Total no of test	QC range	Min value	Max value
Cefotaxime, CTX	0/3	16-22	32	32
Ceftazidime, CAZ	0/4	29-35	27	29
Ceftiofur, XNL	**1/5**	26-31	**24**	28
Chloramphenicol, CHL	0/5	21-27	22	26.3
Ciprofloxacin, CIP	0/4	30-40	34	40
Florfenicol, FFN	0/2	22-28	23	27
Gentamicin, GEN	0/5	19-26	20	24.4
Nalidixic acid, NAL	0/5	22-28	25	27
Sulfisoxazole, FIS	0/5	15-23	18	23
Tetracycline, TET	0/5	18-25	20	25
Trimethoprim, TMP	0/5	21-28	21	26

Finally, 21 laboratories tested the reference strain using MIC determination. They performed a total of 237 tests of which four were incorrect causing an average deviation of 1.7%. The deviations in this strain were produced by three antimicrobials, ampicillin, ciprofloxacin and sulfisoxazole (Table 11).

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

Antimicrobial	Deviation/Total no of test	QC range	Min value	Max value
Ampicillin, AMP	**1/21**	2-8	**1**	8
Cefotaxime, CTX	0/21	0.03-0.12	0.06	0.12
Ceftazidime, CAZ	0/13	0.06-0.5	0.12	0.25
Ceftiofur, XNL	0/6	0.25-1	0.25	0.5
Chloramphenicol, CHL	0/21	2-8	4	8
Ciprofloxacin, CIP	**2/21**	0.004-0.016	0.008	**0.03**
Florfenicol, FFN	0/19	2-8	4	8
Gentamicin, GEN	0/21	0.25-1	0.25	1
Nalidixic acid, NAL	0/21	1-4	1	4
Streptomycin, STR	0/19	4-16	4	8
Sulfisoxazole, FIS	**1/14**	8-32	16	**64**
Tetracycline, TET	0/21	0.5-2	1	2
Trimethoprim, TMP	0/19	0.5-2	0.5	1

4. DISCUSSION

4.1 General overview

Analysing the deviating results for each one of the species tested in the enterococci, staphylococci and *E. coli* EQAS retrospectively; the decrease in percentage of deviations suggests that the quality of the results has improved over the three years' period. As illustrated in Figure 2, for enterococci and staphylococci, the deviations have decreased considerably between 2007 and 2009. However, these results should be interpreted with care, since the number of participants and the designated NRL's have changed over these years, and also the tested strains have been different. On the other hand, the degree of difficulty of the EQAS has also increased over the years, introducing more challenging resistance patterns as well as confirmatory tests for MRSA strains and ESBL identification; tests that nowadays are mandatory, albeit not evaluated as part of the overall deviation level. In addition, in the last year, the number of NRL's performing antimicrobial susceptibility testing in each one of the three trials appeared to have stabilised. There are still two NRL's that have declined to participate in all enterococci, staphylococci and *E. coli* EQAS conducted to date. However, recent correspondence suggests their participation in the forthcoming EQAS 2010.

During these years, the EURL-AR has worked towards harmonization of methodology between NRL's and towards agreement in the use of the same defined breakpoints. Since 2007 when the first enterococci, staphylococci and *E. coli* EQAS were conducted, the number of laboratories performing MIC methods for antimicrobial susceptibility testing has increased, especially for the enterococci and *E. coli* trials (App. 9 shows the different MIC ranges used by laboratory). For these two micro-organisms, the participants performing disk diffusion obtained significantly higher deviations than those performing MIC, therefore, the EURL-AR still encourage participants using disk diffusion to harmonise methods towards those that deliver a better quality of results, in the case of these two species, MIC.

For the first time in the three years that this ring trial has been conducted, none of the participants have been identified as outliers. The EURL-AR has followed up participants that in previous EQAS did not perform according to the agreed standards. For instance, from the EQAS 2008 to the EQAS 2009 laboratories #40, #29 and #23 have decreased the deviation percentage on the enterococci trial from 39% to 12.5%, 34% to 0% and 18% to 9%, respectively. Same results for the staphylococci trial, with a reduction of the deviations from 14% to 1% and 9.9% to 3.9% for laboratories #40 and #23, respectively. There is a visible improvement in the results achieved by these participants. Whereas some of them have attended training courses provided by the EURL-AR, others have been given specific recommendations to target their individual difficulties.

4.2 Enterococci trial

Deviations in the eight enterococci strains fall bellow 6% and were mainly generated by participants performing disk diffusion for AST rather than MIC. Furthermore, all laboratories with 100% correct results performed MIC determination. Still, this year when compared to 2008, a decreased in the deviation caused by laboratories performing disk diffusion from 16% to 9.1% was observed.

Synacid was the antimicrobial exhibiting the highest deviation percentage. These deviations were mainly generated by laboratory #21 that reported five of the strains incorrectly. As this participant has not entered obtained values in the database, it is not possible to establish if the problem was interpretation of the obtained values or the methodology used.

For streptomycin, the main source of the deviation was strain *E. faecium* ENT.3.7. This strain exhibited an MIC equal to 128 mg/L. As the breakpoint value for this compound is 128 mg/L, results within one fold dilution over the MIC, resulted in the incorrect outcome. In addition, enterococci are intrinsically resistant to aminoglycosides, therefore, laboratories performing disk

diffusion and are not using streptomycin disks at a concentration of 300 µg would not be able to detect high level resistance to aminoglycosides.

For gentamicin, the main reason for the deviating results was laboratory #18 performing disk diffusion. This participant obtained resistance values in the seven strains that were susceptible, whereas the reference strain tested against gentamicin produced the expected results. As these three antimicrobials, synacid, streptomycin and gentamicin are recommended by EFSA, the importance of these deviations is slightly higher than those that are not used for EFSA's report. However, it appears that the problems are in individual NRL's conducting disk diffusion. When performing this method for AST, it is important to consider the different factors that may influence 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.

When analyzing results for all antimicrobials tested, the number of laboratories deviating more than the 5% acceptance limit was eight. On the other hand, when the analysis only included those antimicrobials reported to EFSA, the deviating laboratories decreased to six. Overall, the percentage of correct results for the enterococci trial was 96%, results that demonstrate a great improvement when compared to 91.4% from 2007. Also MIC determination of the quality control strain *E. faecalis* ATCC 29212 showed only one deviation (against linezolid). This is a total of 154 correct results out of a total of 155 tests performed in this strain. The analysis of the reference strains was used as a quality assessment to monitor the excellence of the laboratories procedures.

4.2 Staphylococci trial

Contrarily to what it has been observed for the enterococci and *E. coli* trials, participants performing disk diffusion reported significantly better results than those performing MIC. This is mainly caused by laboratories #11 and #39 performing MIC and producing a high number of deviations, the first one testing against gentamicin and tetracycline and the second testing against ciprofloxacin.

All of the strains and antimicrobials tested presented deviations below 5%. The antimicrobial with the higher deviation percentage was sulfamethoxazole (4.1%). This compound has a bacteriostatic effect and sometimes the reading of results can be difficult. When conducting disk diffusion, the bacteriostatic effect of the antimicrobial may produce a double halo on the plate whereas performing MIC it is normal to observe growth in all wells due to survival of the micro-organisms instead of active division of the bacterium. Still, only two laboratories clustered outside the 5%

limit and most of the participants grouped in the deviation interval between 0 and 1, which is a very positive result.

Out of the eight staphylococci strains selected for this EQAS, ST.3,1, ST.3,3 and ST.3,5 harboured the *mecA* gene and therefore were methicillin resistant *S. aureus*. Deviations in identification of the *mecA* gene were low (4.3%), and caused by two laboratories. It appeared that one of the participants have not performed the PCR test in any of the eight strains. In addition, one NRL seemed to forget to enter the *mecA* entry for strain ST.3.1, since the results for the remaining seven strains were in accordance with the expected results. By comparison to the last year's EQAS, where 30% of the laboratories failed to identify methicillin resistance, this year trial has been a great success.

For the reference strains, only one laboratory performing the ROSCO method appeared to have deviated in four out of eight antimicrobials. The obtained values were in all cases higher than expected. Both, laboratories performing disk diffusion on strain *S. aureus* ATCC 25923 and laboratories using MIC methods on strain *S. aureus* ATCC 25913 produced the same percentage of deviation (4%). Those laboratories obtaining deviations in the reference strains are recommended to take action and assess possible factors that may have a negative influence on the quality of the results.

4.3 *E. coli* trial

In this EQAS, participants have obtained the best results for *E. coli* when compared to previous EQAS. It has been a decrease in the average deviation for the *E. coli* trial, from 2.1% from EQAS 2008 to 1.5% this year. All of the deviations for the strains fell below 3.5%. Of those antimicrobials recommended by EFSA for monitoring antimicrobial resistance, the highest deviation values were registered for ciprofloxacin as in previous years. Strains EC.3,4, EC.3,6 and EC.3,8 that exhibited low-level resistance to ciprofloxacin were reported as susceptible by some of the participants performing disk diffusion for AST. These laboratories followed CLSI guidelines for interpretation of zone diameters. As there is a clear discrepancy on the cut off values recommended by EUCAST ($R > 0.032$ mg/L) and those recommended by CLSI ($R \geq 4$ mg/L), participants following CLSI procedures reported the wrong results. Identification of low level ciprofloxacin resistance is an important issue, since the use of ciprofloxacin in strains with low susceptibility may induce the emergence of more resistant strains by additional mutations. For those participants performing disk diffusion, the EURL-AR recommends the use of a low concentration of ciprofloxacin (1 µg) in the disks as it appeared to increase the sensitivity of the assay (Cavaco & Aarestrup, 2009).

The majority of the laboratories clustered in the interval of deviation between 0 and 1%, and only three exhibited deviations higher than 5% when evaluating only antimicrobials reported to EFSA. Two of these laboratories performed disk diffusion and encountered the same type of problems; identification of low level ciprofloxacin resistant strains as well as cephalosporin resistance.

Regarding the reference strain *E. coli* ATCC 25922, the percentage of results within range for all tests performed by disk diffusion was 98%. Similar percentage of correct results was obtained by participants performing MIC determination (98.3%).

Extended spectrum betalactamase (ESBL)

With regards to cephalosporin antimicrobials, the highest deviation value was reported for ceftazidime. This was mainly caused by participants misinterpreting the results obtained for these compounds. As stated in the protocol, "concerning **cefotaxime, ceftazidime and/or ceftiofur** used when detecting ESBL-producing strains in the EQAS: **If** a microorganism is **resistant to one or two of these drugs**, **it should be regarded resistant to all three (this does not include cefoxitin, as ampC's are resistant to cefoxitin and 'true ESBLs' are not).**"

Three of the EQAS strains exhibited resistance to cephalosporins and were confirmed to be "true ESBL strains". Five laboratories failed to identify some of the strains or misread the results obtained in the confirmatory tests. Similar results were obtained for the ampC strain E.C.3,8. Seven participants produced deviations on this strain. As stated in the protocol, "AmpC detection can be performed by testing the microorganism to cefoxitin, resistance to cefoxitin could indicate ampC."

In general, participants obtaining deviations in this part of the test should pay attention to this issue, as ESBL producing strains are a major concern, and failing to report the correct outcome may bring major consequences in terms of human health and therapeutic treatment. These laboratories are encouraged to revise their procedures for ESBL identification and confirmation, and ensure the implementation of a better detection system.

5. CONCLUSIONS

Since the EURL-AR was appointed, one of the main aims has been to increase the quality of the results obtained by all NRL's reporting to EFSA and carrying out AST at the National level for the monitoring programmes implemented by the European Commission. As agreed in the EURL-AR workshop, the deviation margin per laboratory was decreased from 7% to 5%. Under this new threshold, results obtained in this year EQAS showed that the number of laboratories performing

below 5% deviation was higher than in previous years. In addition, for enterococci, staphylococci and *E. coli*, this is the first EQAS that does not identify any participants as outliers. From these results we can conclude that there has been a great improvement in the quality and reliability of the data when compared to results from previous EQAS. However, those participants that have obtained a high percentage of deviation for any of the three trials will be contacted by the EURL to try to assess the causes of the deviations and provide guidelines to improve the results.

However, there is still a significant difference in the results obtained by participants performing disk diffusion when compared to those performing MIC determination, especially for the enterococci and the *E. coli* trials. The EURL-AR encourages participants conducting disk diffusion in enterococci and *E. coli* to harmonise towards MIC methods that produce more reliable and reproducible data.

Out of the three species tested in this EQAS, enterococci produced the highest number of deviations. Furthermore, there are still antimicrobials recommended by EFSA, such as synacid, streptomycin and gentamicin that need special attention. As advice, it is important to be aware of the antimicrobials with a bacteriostatic effect, and for those participants performing disk diffusion it is necessary to use the correct antimicrobial concentration in the disk not to miss strains exhibiting high level of resistance to aminoglycosides.

Regarding ESBL producing *E. coli*, they are still considered a priority area for the EURL-AR. This year, the number of laboratories failing to identify the strains resistant to cephalosporins has been remarkably high, especially for the ampC strain. We encourage NRL's obtaining deviating results in these strains to perform a re-test as a training exercise and contact us in case of any doubts in the interpretation of results.

Finally, the EURL-AR is always willing to improve the quality of work and we encourage all participants to bring forward any ideas to make a better forthcoming EQAS 2010.

6. REFERENCE LIST

[1] Cavaco LM & Aarestrup FM. (2009) Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac(6')Ib-cr*, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. *J Clin Microbiol* 47: 2751-2758.

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

DTU Food
National Food Institute

Community Reference Laboratory Antimicrobial Resistance

CRL-AR EQAS pre-notification

DFVF- M00-06-001/31.10.2008

EQAS 2009 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 2009.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

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

Returning of results: Results must be returned to the National Food Institute, by September 1st, 2009. When you enter your results via a password-protected website, an evaluation report of your results will be generated immediately.

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

Next EQAS: The next CRL EQAS that we will have is on antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* which will be carried out in October 2009.

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

Sincerely,

Susanne Karlsmose
EQAS-Coordinator

Participant List

Ent.	Staph.	*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	Veterinary Services	Cyprus
X	X	X	State Veterinary Institute Prague	Czech Republic
X	X	X	The National Food Institute	Denmark
X	X	X	DTU Veterinærinstituttet	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
X	X	X	Public Health Laboratory	Malta/UK
X	X	X	Food and Consumer Product Safety Authority (VWA)	Netherlands
X	X	X	Central Veterinary Institute of Wageningen UR	Netherlands
X	X	X	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
X	X	X	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	Vetsuisse faculty Bern, Institute of veterinary bacteriology	Switzerland
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 declined to participate

Enterococci test strains and reference values (MIC)

Strain	Species	AMP	AVI	CHL	CIP	DAP	ERY	GEN	KAN	LZD	STR	SYN	TET	TGC	VAN
ENT.3,1	*E. faecium*	≤2	≤4	4	1	4	1	≤16	256	2	≤64	<0.3	≤1	0.06	≤1
ENT.3,2	*E. faecalis*	4	≤4	8	1	2	>32	32	>2048	1	>2048	8	>32	0.120	2
ENT.3,3	*E. faecalis*	≤2	≤4	64	1	2	>32	1024	>2048	2	>2048	16	>32	0.120	≤1
ENT.3,4	*E. faecalis*	≤2	≤4	64	2	1	>32	≤16	>2048	1	>2048	8	>32	0.120	>32
ENT.3,5	*E. faecium*	≤2	≤4	4	>4	8	1	≤16	256	2	≤64	2	≤1	0.06	≤1
ENT.3,6	*E. faecium*	>32	≤4	4	>4	4	>32	≤16	>2048	1	>2048	0.5	>32	0.030	≤1
ENT.3,7	*E. faecium*	>32	>32	8	4	4	>32	≤16	512	2	128	16	≤1	0.06	2
ENT.3,8	*E. faecium*	4	≤4	8	1	1	2	≤16	≤128	2	≤16	2	>32	0.06	>32

Strain	Species	AMP	AVI	CHL	CIP	DAP	ERY	GEN	KAN	LZD	STR	SYN	TET	TGC	VAN
ENT.3,1	*E. faecium*	S	S	S	S	S	S	S	S	S	S	S	S	S	S
ENT.3,2	*E. faecalis*	S	S	S	S	S	R	S	R	S	R	S	R	S	S
ENT.3,3	*E. faecalis*	S	S	R	S	S	R	R	R	S	R	S	R	S	S
ENT.3,4	*E. faecalis*	S	S	R	S	S	R	S	R	S	R	S	R	S	R
ENT.3,5	*E. faecium*	S	S	S	R	R	S	S	S	S	S	R	S	S	S
ENT.3,6	*E. faecium*	R	S	S	R	S	R	S	R	S	R	S	R	S	S
ENT.3,7	*E. faecium*	R	R	S	S	S	R	S	S	S	S	R	S	S	S
ENT.3,8	*E. faecium*	S	S	S	S	S	S	S	S	S	S	R	R	S	R

AMP:ampicillin
AVI: avilamycin
CHL: chloramphenicol
CIP: ciprofloxacin
DAP: daptomycin
ERY: erythromycin
GEN: gentamicin
KAN: kanamycin
LZD: linezolid
STR: streptomycin
SYN:synacid
TET: tetracycline
TGC: tigecycline
VAN: vancomycin

Resistant

Staphylococci test strains and reference values (MIC)

Strain	*mecA*	CHL	CIP	ERY	FFN	FOX	GEN	MRS	PEN	STR	SMX	TET	TMP	SXT
ST.3,1	Yes	4	2	0.25	4	8	>16	+	>16	>64	256	32	0.5	0.25
ST.3,2		4	0.25	0.25	2	2	0.25	-	0.06	4	32	0.5	1	0.25
ST.3,3	Yes	16	0.5	>16	8	16	0.25	+	8	>64	32	>32	>32	0.5
ST.3,4		8	0.5	0.25	4	4	0.5	-	>16	>64	32	0.5	>32	0.25
ST.3,5	Yes	8	0.5	0.5	4	16	0.25	+	8	>64	32	>32	>32	0.5
ST.3,6		8	0.5	>16	4	4	0.25	-	>16	>64	32	0.5	>32	0.5
ST.3,7		8	0.5	>16	4	4	0.25	-	2	4	32	>32	>32	0.25
ST.3,8		>64	0.25	>16	>64	4	0.5	-	>16	>64	32	0.5	1	0.25

Strain		CHL	CIP	ERY	FFN	FOX	GEN	MRS	PEN	STR	SMX	TET	TMP	SXT
ST.3,1	*S. aureus*	S	R	S	S	R	R	+	R	R	R	R	S	S
ST.3,2	*S. aureus*	S	S	S	S	S	S	-	S	S	S	S	S	S
ST.3,3	*S. aureus*	S	S	R	S	R	S	+	R	R	S	R	R	S
ST.3,4	*S. aureus*	S	S	S	S	S	S	-	R	R	S	S	R	S
ST.3,5	*S. aureus*	S	S	S	S	R	S	+	R	R	S	R	R	S
ST.3,6	*S. aureus*	S	S	R	S	S	S	-	R	R	S	S	R	S
ST.3,7	*S. aureus*	S	S	R	S	S	S	-	R	S	S	S	R	S
ST.3,8	*S. aureus*	R	S	R	R	S	S	-	R	R	S	S	S	S

Resistant

CHL: chloramphenicol
CIP: ciprofloxacin
ERY: erythromycin
FFN: florfenicol
GEN: gentamicin
MRS: methicillin resistant
PEN: penicillin
STR: streptomycin
SMX: Sulphamethoxazole
TET: tetracyclin
TMP: trimethoprim
SXT: Sulphamethoxazole/trimethoprim

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

Strain	AMP	CAZ	CHL	CIP	CTX	ESBL gene	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL
EC.3,1	4	0.125	8	0.030	≤0.120		8	2	≤0.5	≤4	≤64	≤8	≤2	≤1	≤0.5
EC.3,2	>32	4	16	>4	>4	ESBL	16	16	≤0.5	>64	>1024	≤8	>32	>32	>8
EC.3,3	2	0.125	8	0.030	≤0.120		8	4	≤0.5	≤4	>1024	32	>32	≤1	≤0.5
EC.3,4	4	0.125	4	0.250	≤0.120		4	4	≤0.5	>64	≤64	≤8	>32	≤1	≤0.5
EC.3,5	>32	2	4	≤0.015	>4	ESBL	4	4	1	≤4	≤64	≤8	≤2	≤1	>8
EC.3,6	>32	0.125	8	0.120	≤0.120		8	4	>16	>64	>1024	128	>32	≤1	≤0.5
EC.3,7	>32	0.5	16	>4	4	ESBL	16	8	2	>64	>1024	≤8	>32	>32	>8
EC.3,8	>32	8	4	0.250	>4	AmpC	4	>16	≤0.5	>64	>1024	>128	>32	>32	8

Strain	AMP	CAZ	CHL	CIP	CTX	ESBL gene	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CLV	CTX/CLV
EC.3,1	S	S	S	S	S		S	S	S	S	S	S	S	S	S		
EC.3,2	R	R	S	R	R	*bla* $_{\text{TEM52}}$	S	S	S	R	R	S	R	R	R	Synergy	Synergy
EC.3,3	S	S	S	S	S		S	S	S	S	R	R	R	S	S		
EC.3,4	S	S	S	R	S		S	S	S	R	S	S	R	S	S		
EC.3,5	R	R	S	S	R	*bla* $_{\text{CTX-M-1}}$	S	S	S	S	S	S	S	S	R	Synergy	Synergy
EC.3,6	R	S	S	R	S		S	S	R	R	R	R	R	S	S		
EC.3,7	R	R	S	R	R	*bla* $_{\text{CTX-M-9}}$	S	S	S	R	R	S	R	R	R	Synergy	Synergy
EC.3,8	R	R	S	R	R	*bla* $_{\text{CMY-2}}$	S	R	S	R	R	R	R	R	R		

Ampicillin, AMP
CAZ: ceftazidime
CHL: chloramphenicol
Ceftiofur, XNL
CIP: ciprofloxacin,
CTX: cefotaxime
FFN: florphenicol
FOX: cefoxitin
GEN: gentamicin
NAL: nalidixic acid
STR: streptomycin
FIS: sulfisoxazole
TET: tetracycline
TMP: trimethoprim
CLV: clavulanic acid

*Synergy when CAZ/CLV and CTX/CLV ≥ 8

Resistant

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

PROTOCOL

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

1 INTRODUCTION

One of the tasks as the EU Community Reference Laboratory for Antimicrobial Resistance is to organise and conduct an External Quality Assurance System (EQAS) on susceptibility testing of *E. coli*, enterococci and staphylococci. The EC/Ent/Staph EQAS 2009 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.

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

2 OBJECTIVES

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

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 2009

3.1 Shipping, receipt and storage of strains

In June 2009 the 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 and MRSA 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' on the CRL-website (see www.crl-ar.eu).

3.3 Susceptibility testing

The strains should be susceptibility tested towards as many as possible of the following antimicrobials by the method used in the laboratory when performing monitoring for EFSA. For MIC, the cut off values listed in tables 3.3.1; 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; therefore **intermediary results should be interpreted as susceptible**. Interpretations in concordance with the expected value will be categorised as 'correct', whereas interpretations that deviate from the expected interpretation will be categorised as 'incorrect'.

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

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

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

3.3.1 *E. coli*

Antimicrobials for *E. coli*	MIC (µg/mL) **R is >**
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

ESBL production

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

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

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

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

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

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

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
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 forms showing which test strains are *E. faecium* and *E. faecalis* respectively.

3.3.3 Staphylococci

Antimicrobials for *S. aureus*	MIC (µg/mL) **R is >**
Cefoxitin	4
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. **This year confirmation of *mec*A presence is mandatory**. The strains may be tested by any method that you prefer. The result must be uploaded as 'positive' or

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

'negative'. According to the CLSI recommendations (M100-S19, table 2C), all MRSA should be regarded resistant for all β-lactam antibiotics.

4 REPORTING OF RESULTS AND EVALUATION

Fill in your results in the test forms, and enter your results into the interactive web database. Please read the detailed description below before entering your results. When you enter the results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print an evaluation report of your results. Please submit results by latest **September 1st, 2009**.

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

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

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

Susanne Karlsmose
The National Food Institute
Technical University of Denmark
27 Bülowsvej, DK-1790 Copenhagen V
Denmark
Tel: +45 3588 6601
Fax: +45 3588 6001
E-mail: suska@food.dtu.dk

5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

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

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

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

Click on either "*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.

EU Community Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2009

DTU Food
National Food Institute

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.

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

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

Click on "save and go to next page"

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

Click on "save and go to next page"

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

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.

Breakpoints used in daily routine (disk diffusion) - Enterococci

Antimicrobial	Lab. no.	R≤ mm	S≥ mm
Ampicillin, AMP	15	16	19
	18	16	17
	23	16	17
	26	16	17
	40	16	17
Chloramphenicol, CHL	15	19	23
	18	12	18
	23	12	18
	26	12	18
	40	12	18
Ciprofloxacin, CIP	18	15	21
	23	15	21
	26	15	21
	40	15	21
Erythromycin, ERY	15	17	22
	18	13	23
	23	13	23
	26	13	23
	40	13	23
Gentamicin, GEN	15	11	17
	18	12	15
	23	12	15
	40	12	15
Linezolid, LZD	15	24	24
	18	20	23
	23	20	23
	26	20	23
	40	20	23
Streptomycin, STR	15	12	14
	18	11	12
	23	11	15
	40	11	15
Tetracycline, TET	15	17	19
	18	14	19
	23	14	19
	26	14	19
	40	14	16
Tigecycline, TGC	26	20	21
Vancomycin, VAN	15	17	17
	18	14	17
	23	14	17
	26	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	22	23
	15	19	22
	18	12	18
	23	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
	23	15	21
	29	15	21
	30	15	21
	40	15	21
Erythromycin, ERY	2	13	23
	9	13	23
	13	16	22
	14	18	22
	15	17	22
	18	13	23
	23	13	23
	29	13	23
	30	13	23
	40	13	23
Florfenicol, FFN	9	12	18
	14	18	22
	15	14	19
	18	12	18
	23	12	18
	29	14	19
	30	14	19
Gentamicin, GEN	2	12	15
	9	12	15
	13	12	15
	14	19	
	15	20	20
	18	12	15
	23	12	15
	29	12	16
	30	12	15
	40	12	15

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Penicillin, PEN	2	28	29
	9	28	29
	13	28	29
	14		29
	15	29	29
	18	28	29
	23	28	29
	29	28	29
	30	28	29
	40	28	29
Streptomycin, STR	9	11	15
	13	12	15
	15	13	15
	18	11	12
	23	11	15
	29	11	15
	40	11	15
Sulfamethoxazole, SMX	2	12	17
	9	12	17
	13	12	17
	14	11	17
	18	12	17
	23	12	17
	29	12	17
	30	12	17
	40	12	17
Tetracycline, TET	2	14	19
	9	14	19
	13	14	19
	14	20	23
	15	17	19
	18	14	19
	23	14	19
	29	14	19
	30	14	19
	40	14	19
Trimethoprim, TMP	2	10	16
	9	10	16
	14	15	20
	18	10	16
	23	10	16
	30	10	16
	40	10	16

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

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Ampicillin, AMP	14		19
	15	15	21
	18	13	17
	23	13	17
	30	13	17
	40	16	17
Cefotaxime, CTX	14		26
	15	22	26
	18	27	
	30	14	23
	40	14	23
Ceftazidime, CAZ	14	20	
	15	18	26
	18	22	
	23	14	18
	30	14	18
	40	14	18
Ceftiofur, XNL	14		21
	15	17	21
	23	17	18
Chloramphenicol, CHL	14		23
	15	18	22
	18	12	18
	23	12	18
	30	12	18
	40	12	18
Ciprofloxacin, CIP	15	21	25
	18	15	21
	23	15	21
	30	15	21
	40	15	21
Florphenicol, FFN	14		19
	15	14	19
	18	12	18
	23	12	18
	30	14	19

Antimicrobial	Lab. no.	R ≤ mm	S ≥ mm
Gentamicin, GEN	14		18
	15	15	18
	18	12	15
	23	12	15
	30	12	15
	40	12	18
Nalidixic acid, NAL	14		20
	15	14	20
	18	13	19
	23	13	19
	30	13	19
	40	13	19
Streptomycin, STR	15	12	15
	18	11	15
	23	11	15
	30	11	15
	40	11	15
Sulfamethoxazole, SMX	14		17
	15	11	17
	18	12	17
	23	12	17
	30	12	17
	40	12	17
Tetracycline,TET	14		19
	15	16	19
	18	11	15
	23	11	15
	30	11	15
	40	11	15
Trimethoprim, TMP	14		20
	15	11	16
	18	10	16
	23	10	16
	30	10	16
	40	10	16

Quality control ranges for the control strains

E. faecalis ATCC 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**	**ROSCO**	**MIC**
Chloramphenicol, CHL	16 - 26	None	2 - 8
Ciprofloxacin, CIP	22 - 30	21 - 29	0.12 - 0.5
Erythromycin, ERY	22 - 30	26 - 33	0.25 - 1
Florfenicol, FFN	None	None	2 - 8
Gentamicin, GEN	19 - 27	25 - 32	0.12 - 1
Penicillin, PEN	26 - 37	None	0.25 - 2
Streptomycin, STR	14 - 22	None	None
Suphonamides, SMX	24 - 30	26 - 34	32 - 128
Tetracycline, TET	24 - 34	23 - 33	0.12 - 1
Trimethoprim, TMP	19 - 26	19 - 25	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
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	None	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-S19 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.

Percentage of resistant and sensitive enterococci

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.3,1	Ampicillin , AMP	S	0	100	23	0
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	S	0	100	23	0
	Ciprofloxacin , CIP	S	0	100	16	0
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	S	4	96	22	1
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	S	18	82	18	4
	Synacid, SYN	S	0	100	9	0
	Tetracycline, TET	S	4	96	22	1
	Tigecycline, TGC	S	0	100	4	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**203**	**7**
CRL ENT.3,2	Ampicillin , AMP	S	0	100	23	0
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	S	0	100	22	0
	Ciprofloxacin , CIP	S	6	94	16	1
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	23	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	R	100	0	21	0
	Synacid, SYN	S	11	89	8	1
	Tetracycline, TET	R	100	0	23	0
	Tigecycline, TGC	S	25	75	3	1
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**204**	**5**
CRL ENT.3,3	Ampicillin , AMP	S	0	100	23	0
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	R	91	9	21	2
	Ciprofloxacin , CIP	S	6	94	16	1
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	23	0
	Gentamicin, GEN	R	100	0	22	0
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	R	100	0	21	0
	Synacid, SYN	S	22	78	7	2
	Tetracycline, TET	R	100	0	23	0
	Tigecycline, TGC	S	0	100	5	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**206**	**5**

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.3,4	Ampicillin , AMP	S	0	100	23	0
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	R	100	0	23	0
	Ciprofloxacin , CIP	S	12	88	15	2
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	23	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	0	100	16	0
	Streptomycin, STR	R	100	0	21	0
	Synacid, SYN	S	22	78	7	2
	Tetracycline, TET	R	100	0	23	0
	Tigecycline, TGC	S	25	75	3	1
	Vancomycin, VAN	R	100	0	22	0
	TOTAL				**202**	**7**
CRL ENT.3,5	Ampicillin , AMP	S	4	96	22	1
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	S	0	100	23	0
	Erythromycin, ERY	S	5	95	21	1
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	S	18	82	18	4
	Synacid, SYN	R	78	22	7	2
	Tetracycline, TET	S	4	96	22	1
	Tigecycline, TGC	S	0	100	4	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**180**	**10**
CRL ENT.3,6	Ampicillin , AMP	R	100	0	23	0
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	S	0	100	23	0
	Ciprofloxacin , CIP	R	80	20	12	3
	Erythromycin, ERY	R	87	13	20	3
	Gentamicin, GEN	S	10	90	19	2
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	R	100	0	22	0
	Synacid, SYN	S	11	89	8	1
	Tetracycline, TET	R	87	13	20	3
	Tigecycline, TGC	S	0	100	4	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**193**	**12**

Strain	Antimicrobial	Expected result	%R	%S	Number expected results	Number deviating results
CRL ENT.3,7	Ampicillin , AMP	R	100	0	23	0
	Avilamycin, AVI	R	100	0	3	0
	Chloramphenicol, CHL	S	0	100	23	0
	Ciprofloxacin , CIP	S	13	88	14	2
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	R	100	0	23	0
	Gentamicin, GEN	S	9	91	20	2
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	S	23	77	17	5
	Synacid, SYN	R	100	0	9	0
	Tetracycline, TET	S	0	100	23	0
	Tigecycline, TGC	S	0	100	4	0
	Vancomycin, VAN	S	0	100	22	0
	TOTAL				**201**	**9**
CRL ENT.3,8	Ampicillin , AMP	S	9	91	21	2
	Avilamycin, AVI	S	0	100	3	0
	Chloramphenicol, CHL	S	0	100	22	0
	Ciprofloxacin , CIP	S	0	100	16	0
	Daptomycin, DAP	S	0	100	3	0
	Erythromycin, ERY	S	14	86	18	3
	Gentamicin, GEN	S	5	95	21	1
	Linezolid, LZD	S	0	100	17	0
	Streptomycin, STR	S	18	82	18	4
	Synacid, SYN	R	89	11	8	1
	Tetracycline, TET	R	100	0	23	0
	Tigecycline, TGC	S	0	100	4	0
	Vancomycin, VAN	R	100	0	22	0
	TOTAL				**196**	**11**

Antimicrobials producing deviations

Percentage of resistant and sensitive staphylococci

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.3,1	Cefoxitin, FOX	R	100	0	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Erythromycin, ERY	S	0	100	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	R	100	0	26	0
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	100	0	21	0
	Sulfamethoxazole, SMX	R	89	11	17	2
	Tetracycline, TET	R	100	0	27	0
	Trimethoprim, TMP	S	5	95	21	1
	TOTAL				**222**	**3**
CRL ST.3,2	Cefoxitin, FOX	S	0	100	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	0	100	26	0
	Erythromycin, ERY	S	0	100	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	4	96	25	1
	Penicillin, PEN	S	4	96	22	1
	Streptomycin, STR	S	0	100	21	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	S	4	96	26	1
	Trimethoprim, TMP	S	0	100	22	0
	TOTAL				**247**	**3**
CRL ST.3,3	Cefoxitin, FOX	R	100	0	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	25	1
	Erythromycin, ERY	R	100	0	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	4	96	25	1
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	100	0	21	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	100	0	27	0
	Trimethoprim, TMP	R	100	0	22	0
	TOTAL				**248**	**2**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.3,4	Cefoxitin, FOX	S	0	100	18	0
	Chloramphenicol, CHL	S	4	96	24	1
	Ciprofloxacin, CIP	S	0	100	26	0
	Erythromycin, ERY	S	0	100	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	4	96	25	1
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	100	0	21	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	S	7	93	25	2
	Trimethoprim, TMP	R	95	5	21	1
	TOTAL				**245**	**5**
CRL ST.3,5	Cefoxitin, FOX	R	100	0	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	25	1
	Erythromycin, ERY	S	0	100	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	100	0	21	0
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	96	4	26	1
	Trimethoprim, TMP	R	100	0	22	0
	TOTAL				**248**	**2**
CRL ST.3,6	Cefoxitin, FOX	S	0	100	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	25	1
	Erythromycin, ERY	R	93	7	25	2
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	90	10	19	2
	Sulfamethoxazole, SMX	S	6	94	17	1
	Tetracycline, TET	S	4	96	26	1
	Trimethoprim, TMP	R	95	5	21	1
	TOTAL				**242**	**8**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL ST.3,7	Cefoxitin, FOX	S	0	100	18	0
	Chloramphenicol, CHL	S	0	100	25	0
	Ciprofloxacin, CIP	S	4	96	25	1
	Erythromycin, ERY	R	100	0	27	0
	Florfenicol, FFN	S	0	100	17	0
	Gentamicin, GEN	S	0	100	26	0
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	S	5	95	20	1
	Sulfamethoxazole, SMX	S	0	100	18	0
	Tetracycline, TET	R	100	0	27	0
	Trimethoprim, TMP	R	100	0	22	0
	TOTAL				**248**	**2**
CRL ST.3,8	Cefoxitin, FOX	S	0	100	18	0
	Chloramphenicol, CHL	R	96	4	24	1
	Ciprofloxacin, CIP	S	4	96	25	1
	Erythromycin, ERY	R	100	0	27	0
	Florfenicol, FFN	R	94	6	16	1
	Gentamicin, GEN	S	4	96	24	1
	Penicillin, PEN	R	100	0	23	0
	Streptomycin, STR	R	95	5	20	1
	Sulfamethoxazole, SMX	S	17	83	15	3
	Tetracycline, TET	S	7	93	25	2
	Trimethoprim, TMP	S	0	100	22	0
	TOTAL				**239**	**10**

Antimicrobials producing deviations

Percentage of resistant and sensitive *E. coli*

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.3,1	Ampicillin, AMP	S	0	100	28	0
	Cefotaxime, CTX	S	4	96	26	1
	Ceftazidime, CAZ	S	0	100	21	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	S	4	96	26	1
	Florphenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	S	0	100	28	0
	Streptomycin, STR	S	0	100	27	0
	Sulfamethoxazole, SMX	S	0	100	28	0
	Tetracycline, TET	S	0	100	28	0
	Trimethoprim, TMP	S	4	96	26	1
	TOTAL				**333**	**3**
CRL EC.3,2	Ampicillin, AMP	R	100	0	28	0
	Cefotaxime, CTX	R	100	0	27	0
	Ceftazidime, CAZ	R	90	10	18	2
	Ceftiofur, XNL	R	92	8	11	1
	Chloramphenicol, CHL	S	4	96	27	1
	Ciprofloxacin, CIP	R	100	0	27	0
	Florphenicol, FFN	S	8	92	24	2
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	R	100	0	28	0
	Streptomycin, STR	S	4	96	26	1
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**328**	**7**
CRL EC.3,3	Ampicillin, AMP	S	0	100	28	0
	Cefotaxime, CTX	S	0	100	27	0
	Ceftazidime, CAZ	S	0	100	20	0
	Ceftiofur, XNL	S	0	100	12	0
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Florphenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	S	0	100	28	0
	Streptomycin, STR	R	96	4	26	1
	Sulfamethoxazole, SMX	R	96	4	27	1
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**333**	**2**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.3,4	Ampicillin, AMP	S	0	100	28	0
	Cefotaxime, CTX	S	0	100	27	0
	Ceftazidime, CAZ	S	0	100	20	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	R	89	11	24	3
	Florphenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	R	100	0	28	0
	Streptomycin, STR	S	0	100	27	0
	Sulfamethoxazole, SMX	S	4	96	27	1
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**332**	**4**
CRL EC.3,5	Ampicillin, AMP	R	100	0	28	0
	Cefotaxime, CTX	R	100	0	27	0
	Ceftazidime, CAZ	R	85	15	17	3
	Ceftiofur, XNL	R	100	0	12	0
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	S	0	100	27	0
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	S	0	100	28	0
	Streptomycin, STR	S	0	100	27	0
	Sulfamethoxazole, SMX	S	0	100	28	0
	Tetracycline, TET	S	0	100	28	0
	Trimethoprim, TMP	S	0	100	28	0
	TOTAL				**333**	**3**
CRL EC.3,6	Ampicillin, AMP	R	100	0	28	0
	Cefotaxime, CTX	S	0	100	27	0
	Ceftazidime, CAZ	S	0	100	20	0
	Ceftiofur, XNL	S	0	100	13	0
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	R	89	11	24	3
	Florphenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	R	100	0	28	0
	Nalidixic acid, NAL	R	96	4	27	1
	Streptomycin, STR	R	100	0	27	0
	Sulfamethoxazole, SMX	R	96	4	27	1
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	S	0	100	27	0
	TOTAL				**331**	**5**

Strain	Antimicrobial	Expected results	%R	%S	Number expected results	Number deviating results
CRL EC.3,7	Ampicillin, AMP	R	100	0	28	0
	Cefotaxime, CTX	R	96	4	26	1
	Ceftazidime, CAZ	R	75	25	15	5
	Ceftiofur, XNL	R	92	8	11	1
	Ciprofloxacin, CIP	R	100	0	27	0
	Nalidixic acid, NAL	R	100	0	28	0
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**219**	**7**
CRL EC.3,8	Ampicillin, AMP	R	100	0	28	0
	Cefotaxime, CTX	R	93	7	25	2
	Ceftazidime, CAZ	R	95	5	19	1
	Ceftiofur, XNL	R	83	17	10	2
	Chloramphenicol, CHL	S	0	100	28	0
	Ciprofloxacin, CIP	R	93	7	25	2
	Florphenicol, FFN	S	0	100	26	0
	Gentamicin, GEN	S	0	100	28	0
	Nalidixic acid, NAL	R	100	0	28	0
	Streptomycin, STR	R	100	0	27	0
	Sulfamethoxazole, SMX	R	100	0	28	0
	Tetracycline, TET	R	100	0	28	0
	Trimethoprim, TMP	R	100	0	28	0
	TOTAL				**328**	**7**

Antimicrobials producing deviations

Deviations per laboratory for the enterococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
2	CRL ENT.3,6	Synacid	R	2	S	0.5	MIC
9	CRL ENT.3,7	Streptomycin	R	256	S	128	MIC
11	CRL ENT.3,8	Erythromycin	R	8	S	2	MIC
12	CRL ENT.3,6	Ciprofloxacin	S	4	R	>4	MIC
15	CRL ENT.3,2	Ciprofloxacin	R	18	S	1	DD
15	CRL ENT.3,3	Ciprofloxacin	R	17	S	1	DD
15	CRL ENT.3,4	Ciprofloxacin	R	17	S	2	DD
15	CRL ENT.3,7	Ciprofloxacin	R	13	S	4	DD
17	CRL ENT.3,3	Synacid	R	>8	S	16	MIC
17	CRL ENT.3,4	Synacid	R	>8	S	8	MIC
18	CRL ENT.3,1	Gentamicin	R	6	S	16	DD
18	CRL ENT.3,1	Streptomycin	R	6	S	64	DD
18	CRL ENT.3,2	Gentamicin	R	6	S	32	DD
18	CRL ENT.3,4	Gentamicin	R	11	S	16	DD
18	CRL ENT.3,5	Gentamicin	R	12	S	16	DD
18	CRL ENT.3,5	Streptomycin	R		S	64	DD
18	CRL ENT.3,6	Gentamicin	R	6	S	16	DD
18	CRL ENT.3,7	Ciprofloxacin	R	15	S	4	DD
18	CRL ENT.3,7	Gentamicin	R	6	S	16	DD
18	CRL ENT.3,7	Streptomycin	R	6	S	128	DD
18	CRL ENT.3,8	Streptomycin	R	6	S	16	DD
19	CRL ENT.3,5	Synacid	S	1	R	2	MIC
19	CRL ENT.3,6	Ciprofloxacin	S	4	R	>4	MIC
19	CRL ENT.3,6	Erythromycin	S	0.25	R	>32	MIC
19	CRL ENT.3,6	Tetracycline	S	1	R	>32	MIC
20	CRL ENT.3,2	Tigecycline	R	0.5	S	0.12	MIC
21	CRL ENT.3,2	Synacid	R		S	8	MIC
21	CRL ENT.3,3	Synacid	R		S	16	MIC
21	CRL ENT.3,4	Synacid	R		S	8	MIC
21	CRL ENT.3,5	Synacid	S		R	2	MIC
21	CRL ENT.3,8	Synacid	S		R	2	MIC
23	CRL ENT.3,1	Streptomycin	R	6	S	64	DD
23	CRL ENT.3,5	Streptomycin	R	6	S	64	DD
23	CRL ENT.3,6	Gentamicin	R	12	S	16	DD
23	CRL ENT.3,7	Gentamicin	R	12	S	16	DD
23	CRL ENT.3,7	Streptomycin	R	6	S	128	DD
23	CRL ENT.3,8	Streptomycin	R	6	S	16	DD
24	CRL ENT.3,6	Erythromycin	S	≤1	R	>32	MIC
24	CRL ENT.3,6	Tetracycline	S	≤0.5	R	>32	MIC
25	CRL ENT.3,6	Ciprofloxacin	S	4	R	>4	MIC
26	CRL ENT.3,4	Tigecycline	R	19	S	0.12	DD
33	CRL ENT.3,6	Erythromycin	S	≤0.5	R	>32	MIC
33	CRL ENT.3,6	Tetracycline	S	≤0.5	R	>32	MIC
34	CRL ENT.3,5	Streptomycin	R	>128	S	64	MIC
34	CRL ENT.3,7	Streptomycin	R	>128	S	128	MIC
34	CRL ENT.3,8	Ampicillin	R	128	S	4	MIC
34	CRL ENT.3,8	Erythromycin	R	>128	S	2	MIC
34	CRL ENT.3,8	Gentamicin	R	>32	S	16	MIC
34	CRL ENT.3,8	Streptomycin	R	>128	S	16	MIC
37	CRL ENT.3,3	Chloramphenicol	S	32	R	64	AGA
37	CRL ENT.3,8	Ampicillin	R	8	S	4	AGA

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
39	CRL ENT.3,1	Erythromycin	R	32	S	1	MIC
39	CRL ENT.3,1	Streptomycin	R	256	S	64	MIC
39	CRL ENT.3,1	Tetracycline	R	8	S	1	MIC
39	CRL ENT.3,2	Gentamicin	R	>256	S	32	MIC
39	CRL ENT.3,3	Chloramphenicol	S	32	R	64	MIC
39	CRL ENT.3,8	Erythromycin	R	>64	S	2	MIC
40	CRL ENT.3,1	Streptomycin	R	10	S	64	DD
40	CRL ENT.3,4	Ciprofloxacin	R	10	S	2	DD
40	CRL ENT.3,4	Gentamicin	R	11	S	16	DD
40	CRL ENT.3,5	Ampicillin	R	6	S	2	DD
40	CRL ENT.3,5	Erythromycin	R	6	S	1	DD
40	CRL ENT.3,5	Streptomycin	R	6	S	64	DD
40	CRL ENT.3,5	Tetracycline	R	6	S	1	DD
40	CRL ENT.3,7	Streptomycin	R	8	S	128	DD
40	CRL ENT.3,8	Streptomycin	R	8	S	16	DD

Deviations per laboratory for the staphylococci strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
4	CRL ST.3,8	Streptomycin	S	14	R	>64	ROS
11	CRL ST.3,2	Gentamicin	R	4	S	0.25	MIC
11	CRL ST.3,2	Penicillin	R	2	S	0.06	MIC
11	CRL ST.3,2	Tetracycline	R	4	S	0.5	MIC
11	CRL ST.3,3	Gentamicin	R	4	S	0.25	MIC
11	CRL ST.3,4	Gentamicin	R	8	S	0.5	MIC
11	CRL ST.3,6	Tetracycline	R	2	S	0.5	MIC
11	CRL ST.3,8	Gentamicin	R	4	S	0.5	MIC
11	CRL ST.3,8	Tetracycline	R	2	S	0.5	MIC
12	CRL ST.3,6	Erythromycin	S	0.5	R	>16	MIC
12	CRL ST.3,6	Streptomycin	S	≤4	R	>64	MIC
12	CRL ST.3,6	Trimethoprim	S	4	R	>32	MIC
14	CRL ST.3,8	Sulfamethoxazole	R	16	S	32	DD
17	CRL ST.3,7	Streptomycin	R	32	S	4	MIC
19	CRL ST.3,5	Ciprofloxacin	R	2	S	0.5	MIC
19	CRL ST.3,6	Erythromycin	S	0.25	R	>16	MIC
19	CRL ST.3,6	Streptomycin	S	2	R	>64	MIC
20	CRL ST.3,4	Tetracycline	R	>64	S	0.5	MIC
20	CRL ST.3,4	Trimethoprim	S	2	R	>32	MIC
21	CRL ST.3,8	Sulfamethoxazole	R	512	S	32	MIC
23	CRL ST.3,5	Tetracycline	S	6	R	>32	DD
23	CRL ST.3,8	Chloramphenicol	S	20	R	>64	DD
23	CRL ST.3,8	Florfenicol	S	20	R	>64	DD
26	CRL ST.3,6	Sulfamethoxazole	R	>512	S	32	MIC
26	CRL ST.3,8	Sulfamethoxazole	R	>512	S	32	MIC
34	CRL ST.3,1	Sulfamethoxazole	S	128	R	256	MIC
34	CRL ST.3,4	Chloramphenicol	R	16	S	8	MIC
34	CRL ST.3,8	Tetracycline	R	64	S	0.5	MIC
39	CRL ST.3,1	Trimethoprim	R	1	S	0.5	MIC
39	CRL ST.3,3	Ciprofloxacin	R	>32	S	0.5	MIC
39	CRL ST.3,4	Tetracycline	R	2	S	0.5	MIC
39	CRL ST.3,6	Ciprofloxacin	R	>32	S	0.5	MIC
39	CRL ST.3,7	Ciprofloxacin	R	>32	S	0.5	MIC
39	CRL ST.3,8	Ciprofloxacin	R	>32	S	0.25	MIC
40	CRL ST.3,1	Sulfamethoxazole	S	19	R	256	DD

Deviations per laboratory for the *E. coli* strains

Lab no.	Strain	Antimicrobial	Obtained interpretation	Obtained value	Expected interpretation	Expected MIC	Method used
15	CRL EC.3,4	Ciprofloxacin	S	26	R	0.25	DD
15	CRL EC.3,6	Ciprofloxacin	S	27	R	0.12	DD
15	CRL EC.3,8	Ciprofloxacin	S	25	R	0.25	DD
18	CRL EC.3,5	Ceftazidime	S	27	R	2	DD
18	CRL EC.3,7	Ceftazidime	S	30	R	0.5	DD
18	CRL EC.3,8	Cefotaxime	S	24	R	>4	DD
19	CRL EC.3,2	Streptomycin	R	32	S	8	MIC
20	CRL EC.3,7	Ceftazidime	S	≤0.25	R	0.5	MIC
23	CRL EC.3,2	Ceftazidime	S	18	R	4	DD
23	CRL EC.3,4	Ciprofloxacin	S	30	R	0.25	DD
23	CRL EC.3,5	Ceftazidime	S	21	R	2	DD
23	CRL EC.3,6	Ciprofloxacin	S	26	R	0.12	DD
23	CRL EC.3,7	Ceftazidime	S	25	R	0.5	DD
23	CRL EC.3,8	Ceftazidime	S	18	R	8	DD
23	CRL EC.3,8	Ceftiofur	S	18	R	8	DD
23	CRL EC.3,8	Ciprofloxacin	S	22	R	0.25	DD
24	CRL EC.3,7	Ceftazidime	S	0.5	R	0.5	MIC
25	CRL EC.3,2	Chloramphenicol	R	32	S	16	MIC
25	CRL EC.3,2	Florphenicol	R	32	S	16	MIC
29	CRL EC.3,2	Ceftiofur	S	20	R	>8	MIC
29	CRL EC.3,3	Streptomycin	S	32	R	32	MIC
29	CRL EC.3,7	Ceftiofur	S	23	R	>8	MIC
29	CRL EC.3,8	Ceftiofur	S	21	R	8	MIC
32	CRL EC.3,4	Sulfamethoxazole	R	≤1024	S	64	MIC
37	CRL EC.3,2	Florphenicol	R	32	S	16	AGA
39	CRL EC.3,1	Cefotaxime	R	0.5	S	0.12	MIC
39	CRL EC.3,1	Ciprofloxacin	R	1	S	0.03	MIC
39	CRL EC.3,1	Trimethoprim	R	16	S	1	MIC
39	CRL EC.3,6	Nalidixic acid	S	8	R	>64	MIC
40	CRL EC.3,2	Ceftazidime	S	17	R	4	DD
40	CRL EC.3,3	Sulfamethoxazole	S	15	R	>1024	DD
40	CRL EC.3,4	Ciprofloxacin	S	30	R	0.25	DD
40	CRL EC.3,5	Ceftazidime	S	21	R	2	DD
40	CRL EC.3,6	Ciprofloxacin	S	31	R	0.12	DD
40	CRL EC.3,6	Sulfamethoxazole	S	19	R	>1024	DD
40	CRL EC.3,7	Cefotaxime	S	16	R	4	DD
40	CRL EC.3,7	Ceftazidime	S	24	R	0.5	DD
40	CRL EC.3,8	Cefotaxime	S	16	R	>4	DD

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

Antimicrobial	Laboratory number													
	1	**2**	**9**	**11**	**12**	**17**	**19**	**20**	**21**	**22**	**24**	**25**	**33**	**35**
Ampicillin	2-32	0.25-32	0.5-32	0.25-32	0.25-32	0.5-32	0.5-32	0.5-32	0.12-8	4	1-128	1-128	0.25-32	2-32
Avilamycin	4-32	1-128	-	-	-	-	-	-	-	-	-	-	-	0.5-32
Chloramphenicol	2-64	4-256	2-64	0.5-64	0.5-64	2-256	2-32	2-64	2-64	0.5-64	1-128	1-128	0.5-64	2-32
Daptomycin	0.25-16	-	-		-	-	-	0.5-16	-	-	-	-	-	-
Erythromycin	0.5-32	0.5-64	0.5-64	0.5-64	0.5-64	0.12-16	0.25-8	0.5-8	0.25-4	0.5-64	1-128	1-128	0.5-64	0.12-64
Gentamicin	16-1024	4-2048	2-256	2-256	2-256	0.25-64	16-1024	0.25-32 &128-1024	0.25-32	2-256	4-512	4-512	2-256	500
Linezolid	0.5-8	-	-	0.5-16	0.5-16	1-16	-	0.5-8	-	0.5-16	0.25-32	0.25-32	-	-
Streptomycin	64-2048	16-2048	8-1024	8-1024	8-1024	2-128	32-2048	2-128 &512-2048	2-128	8-1024	8-1024	8-1024	8-1024	2-64;2000
Synacid	0.25-16	0.5-128	-	-	-	0.5-8	-	1-32	0.25-2	-	0.25-32	0.5-32	-	0.5-32
Tetracycline	1-32	0.5-64	1-64	0.5-64	0.5-64	1-64	1-32	1-64	1-64	0.5-64	0.5-64	0.5-64	0.5-64	1-64
Tigecycline	0.015-2	-	-	-	-	-	-	0.015-0.5	-	-	-	-	-	-
Vancomycin	1-32	1-64	1-128	1-128	1-128	2-32	0.25-32	0.5-32	-	1-128	0.5-64	0.5-64	1-128	0.5-64

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

Antimicrobial	Lab no.													
	1	**11**	**12**	**17**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**31**	**33**	**39**
Chloramphenicol	2-64	0.5-64	0.5-64	2-256	2-16	2-64	2-64	0.5-64	1-128	-	2-64	8	0.5- 64	-
Ciprofloxacin	0.12-8	0.06-4	0.06-4	0.008-64	1-2	0.008-8	0.008-8	0.06-4	0.5-64	-	0.12-8	0.12-2	0.06- 4	0.5-64
Erythromycin	0.12-16	0.25-32	0.25-32	0.12-16	0.25-4	0.5-8	0.12-128	0.25-32	1-128	0.125-16	0.25-16	0.5-4	0.25-32	0.25-32
Florfenicol	1-64	-	4-32	2-64		2-64	-	-	1-64	-	1-64	-	-	0.25-32
Gentamicin	-	0.5-64	0.5-64	0.25-64	0.25-16	0.25-32	0.12-128	0.5-64	4-512	-	0.25-16	2-8	0.5- 64	-
Penicillin	0.06-16	0.03-4	0.03-4	-	0.06-8	0.5-16	-	0.03-4	-	0.06-8	0.06-16	0.12-8	0.03- 4	0.5-64
Streptomycin	2-128	-	4-32	2-128	4-1024	2-128	0.12-128	-	8-1024	0.5-64	4-64	1000	-	0.03-4
Sulfamethoxazole	8-512	-	16-2048	8-1024		8-1024	8-1024	-	-	-	32-512	-	-	-
Tetracycline	0.5-32	0.5-64	0.5-64	1-64	1-16	1-64	1-64	0.5-64	0.5-64	0.125-16	0.5-32	4-16	0.5- 64	0.5-64
Trimethoprim	1-32	0.5-32	0.5-32	0.5-32	-	0.5-32	0.5-32	0.5-32	-	-	0.5-32	8	0.5- 32	0.5-32

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

Antimicrobial	Laboratory number																	
	1	**2**	**4**	**6**	**9**	**11**	**12**	**17**	**19**	**20**	**21**	**22**	**24**	**25**	**26**	**32**	**33**	**39**
Ampicillin	1-32	0.5-64	0.5-32	0.5-32	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	0.5-32	0.5-32	0.5 - 32	0.5- 64	0.25-32
Cefotaxime	0.125-4	0.06-128	0.05-4	0.06-4	0.06-4	0.06-2	0.06-2	0.06-4	0.06-4	0.06-4	0.06-4	0.06-4	0.06-4	0.06-4	0.06-4	0.06 - 4	0.06-8	0.06-2
Ceftazidime	-	-	0.25-16	0.25-16	0.25-16	-	-	0.25-16	0.2-16	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	-	-	0.12-8	-	-	-	-	-	-	0.12-16	0.12-16
Chloramphenicol	2-64	2-256	2-64	2-64	2-64	1-128	1-128	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2- 256	1-128
Ciprofloxacin	0.015-4	0.008-8	0.005-8	0.008-8	0.008-8	0.08-1	0.008-1	0.008-8	0.008-8	0.008-8	0.008-8	0.008-8	0.008-8	0.008-8	0.008-8	0.008 - 8	0.008- 8	0.008-1
Florfenicol	2-64	-	2-64	2-64	2-64	4-32	4-32	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2-64	2-32	2-32
Gentamicin	0.5-16	0.25-32	0.5-32	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	0.25 - 32	0.25- 32	0.5-64
Nalidixic acid	4-64	2-256	4-64	4-64	4-64	1-128	1-128	4-64	4-64	4-64	4-64	4-64	4-64	4-64	4-64	4-64	2- 256	1-128
Streptomycin	8-128	2-256	2-128	2-128	2-128	2-256	2-256	2-128	2-128	2-128	2-128	0.25-32	2-128	2-128	2-128	2 - 128	2- 256	2-256
Sulfamethoxazole	64-1024	8-1024	8-1024	8-1024	8-1024	16-2048	16-2048	8-1024	8-1024	8-1024	8-1024	8-1024	8-1024	8-1024	8-1024	8 - 1024	8-1024	16-2018
Tetracycline	2-32	0.5-64	1-64	1-64	1-64	0.5-64	0.5-64	1-64	1-64	1-64	1-64	1-64	1-64	1-64	1-64	1-64	0.5- 64	0.5-64
Trimethoprim	1-32	0.25-16	0.5-32	0.5-32	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	0.5-32	0.5-32	0.5 - 32	0.25- 32	0.25-32

National Food Institute
Technical University of Denmark
Mørkhøj Bygade 19
DK - 2860 Søborg

Tel. 35 88 70 00
Fax 35 88 70 01

www.food.dtu.dk

ISBN: 978-87-92158-73-4