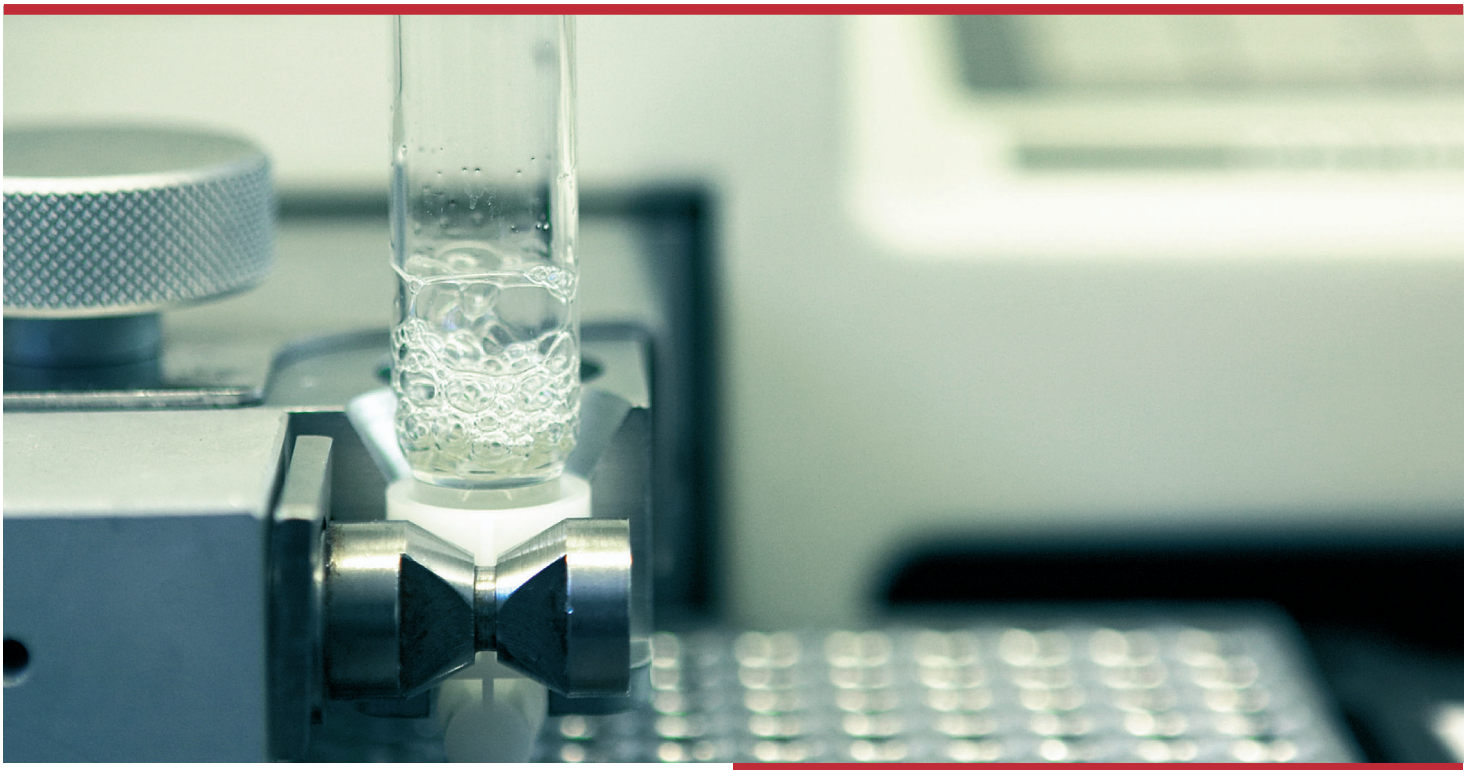
The 10th EURL-AR Proficiency Test enterococci, staphylococci and *E. coli* 2011



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THE 10th EURL-AR Proficiency Test
Enterococci, Staphylococci and *E. coli 2011*

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1. Introduction

This report describes the results of a proficiency test defined as External Quality Assurance System (EQAS) 2011 for antimicrobial susceptibility testing (AST) of enterococci, staphylococci and *Escherichia coli.* The results discussed in this report were obtained by National Reference Laboratories for Antimicrobial Resistance (NRL-AR) in Member States (MS) and in affiliated non-Member States of the European Union.

This is the 10th EQAS organized by the National Food Institute at the Technical University of Denmark (DTU Food) since its appointment as European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) by the European Commission (EC) in 2006. The EURL-AR is accredited by DANAK as provider of proficiency testing (accreditation no. 516); working with zoonotic pathogens and indicator organisms as bacterial isolates (identification, serotyping and antimicrobial susceptibility testing).

This EQAS aims to: i) monitor the quality of AST results produced by NRL-AR, ii) identify laboratories which may need assistance to improve their performance in AST, and iii) determine possible topics for further research or elaboration.
In reading this report, the following important considerations should be taken into account:

- Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determinations for all test strains in two different occasions at DTU-FOOD. These results were then verified by the United States Food and Drug Administration (FDA), Centre for Veterinary Medicine. Finally, a fourth MIC determination was performed at DTU-FOOD after preparation of the agar stab culture for shipment to participants to confirm that the vials contained the correct strains with the expected MIC values.

- Evaluation is based on interpretations of AST values determined by the participants. This is in agreement with the method used by MS to report AST data to EFSA, and complies with "the main objective of this EQAS to assess and improve the comparability of surveillance and antimicrobial susceptibility data reported to EFSA by the different NRLs", as stated in the protocol.

- Evaluation of a result as "deviating from the expected interpretation" should be carefully analyzed in a self-evaluation procedure performed by the participant. Since methods used for MIC determination have limitations, it is not considered a mistake to obtain a one-fold dilution difference in the MIC of a specific antimicrobial when testing the same strains. However, if the expected MIC is close to the breakpoint value for categorizing the strain as susceptible or resistant, a one-fold dilution difference, which is acceptable, may result in two different interpretations, i.e. the same strain will be categorized as susceptible and resistant, which will be evaluated as correct in one case and incorrect in the other if the evaluation is based on interpretation of MIC values. Since this report evaluates the interpretations of AST values, some

participants may find their results classified as wrong even though the actual MIC they reported is only one-fold dilution different from the expected MIC. In these cases, the participants should be confident about the good quality of their performance of AST. In the organization of the EQAS we try to avoid these situations by choosing test strains with MIC values distant from the breakpoints for resistance, which is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AR network unanimously established that if there are less than 75 % correct results for a specific strain/antimicrobial combination, the reasons for this situation must be further examined and, on selected occasions explained in details case by case, these results may subsequently be subtracted from the evaluation report.

- The EURL-AR network agreed on setting the accepted deviation level for laboratory performance to 5 %.

This report is approved in its final version by a technical advisory group composed by competent representatives from all NRLs who meet once a year at the EURL-AR workshop.
All conclusions presented in this report are publicly available. However, participating laboratories are identified by codes and each code is known only by the corresponding laboratory. The full list of laboratory codes is confidential information known only by relevant representatives of the EURL-AR and the EU Commission.

2. Materials and Methods

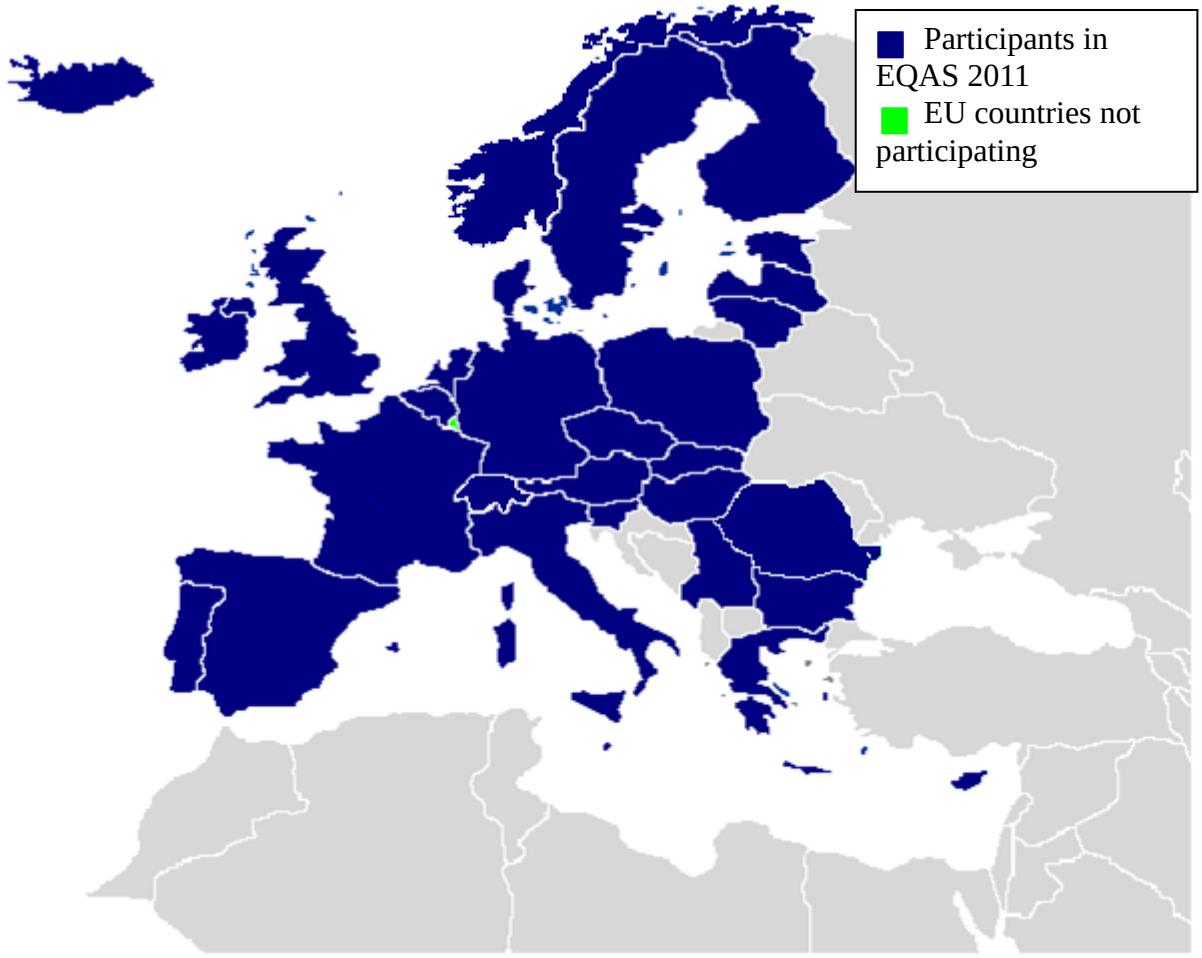
2.1 Participants in EQAS 2011

In May 2011, a pre-notification to announce the EQAS 2011 on antimicrobial susceptibility testing of enterococci, staphylococci and *E. coli* was sent by e-mail to the 32 European NRLs for antimicrobial resistance designated by the MS (App. 1). Eight additional laboratories (one from each of the following countries: Denmark, Iceland, Norway, Romania, Serbia, Spain, Switzerland and The Netherlands) were invited to take part in the EQAS 2011 on the basis of their participation in previous EQAS iterations and/or affiliation to the EU. Participants represented all EU countries except Luxembourg (App. 2). One of the three NRLs from Spain declined to participate, therefore only 31 out of 32 NRL-AR submitted results (Figure 1). Among the designated NRLs, 24 submitted results for the enterococci strains and 29 submitted results for the staphylococci and the *E. coli* strains. The level of participation was similar to EQAS 2010 in which 22, 28 and 29 laboratories submitted results for enterococci, staphylococci and *E. coli*, respectively. In addition, this report includes results from one laboratory for each of the following EU-affiliated country non-MS: Norway, Serbia and Switzerland (Figure 1).
To summarize, this report includes AST results of enterococci strains submitted by 27 laboratories, and AST results of staphylococci and *E. coli* strains submitted by 32 laboratories.

2.2 Strains

Bacterial strains included in this EQAS (eight enterococci, eight staphylococci and eight *E. coli*) were selected among the DTU Food strain collection on the basis of antimicrobial resistance profiles and MIC values. For quality assurance purposes, one strain per each bacterial species tested has been included in all EQAS iterations performed to date, which represents an internal control.

Figure 1. European map showing the countries participating in EQAS 2011



Antimicrobial susceptibility testing of the EQAS strains was performed at DTU Food by MIC determination using the Sensititre system from Trek Diagnostic Systems. The MIC values obtained (App. 3) were used as reference values for this EQAS trial after verification performed by the U.S. FDA. Upon agreement between DTU Food and FDA obtained MIC values, the strains were inoculated in agar as stab cultures and dispatched to the participating laboratories.

Reference strains *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were given to new participating laboratories with instructions to save and maintain them for quality assurance purposes and future EQAS trials.

2.3 Antimicrobials

The panels of antimicrobials recommended for AST are listed in Table 1.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2011 component

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

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

Guidelines for performing AST were set according to the Clinical and Laboratory Standards Institute (CLSI) document – M7-A8 (2009) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Eighth Edition".
MIC results were interpreted by using EUCAST epidemiological cut-off values (www.eucast.org), as recommended by EFSA and described in the protocol (App. 4). Results of ESBL detection tests were interpreted according to the recommendations reported in the EUCAST expert rules.
All the above-mentioned choices were made on the basis of agreements reached by NRL participants at EURL-AR workshops in previous years.

2.4 Distribution

Protocols and all relevant information were uploaded on the EURL-AR website (http://www.eurl-ar.eu), thereby EQAS participants could access necessary information at any time. In June 2011, bacterial strains in agar stab 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

Participants were instructed to keep the agar stab cultures refrigerated until performance of antimicrobial susceptibility tests (App. 4). In addition, instructions for interpretation of antimicrobial susceptibility test results were provided. For interpretation of MIC determination results, cut-off values were reported in the protocol (App. 4: Tables 1, 2 and 3). For interpretation of disk-diffusion (DD) method results, participants were advised to use interpretive breakpoints as in their routine methods (App. 5). In both cases, the EQAS test strains should have been categorized as resistant or susceptible, and the EURL-AR recommended interpreting intermediate results as susceptible.

Of note, the terms ‘susceptible’, ‘intermediate’ and ‘resistant’ should be reserved to categorize strains in relation to the therapeutic application of antimicrobial agents, while interpretation of AST results based on epidemiological cut-off values should result in categorization of bacterial strains in ‘wild-type’ or ‘non-wild-type’. However, due to different AST methods used by the participants and to simplify the interpretation of results, we will use the terms susceptible and resistant throughout this report even in the cases in which we refer to wild-type and non-wild-type strains.

All participating laboratories were invited to enter the obtained results into an electronic record sheet at the EURL-AR web-based database through a secured individual login and password. Alternatively, it was offered the possibility to fill-in a record sheet (provided with the protocol) and send it to the EURL-AR by fax, mail or email.
The record sheet contained also fields for reporting the results (zone diameters in millimeters or MIC values in µg/ml) obtained for the reference strains. These results were compared to the quality control ranges reported by CLSI in documents M31-A3 (2008) / M100-S21 (2011) (App. 6).
The website was accessible for data entry until 9th September 2011.

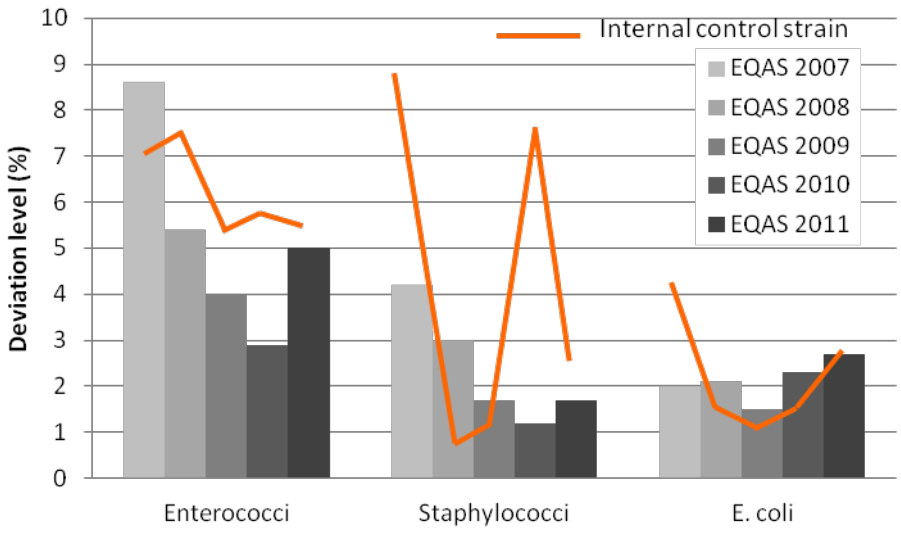
After the deadline for submission of results to the secured website, the participants were invited to login again to retrieve a database-generated individual report which contained an evaluation of the submitted results including possible deviations from the expected interpretations. Finally, participants were encouraged to complete an evaluation form available at the EURL-AR database with the aim to improve future EQAS trials

3. Results

3.1 EQAS 2011 compared to previous EQAS iterations

In EQAS 2011, the overall percentages of deviations from expected results were 5.04 %, 1.74 % and 2.65 % for enterococci, staphylococci and *E. coli*, respectively (Figure 2). These percentages are slightly higher compared to the ones observed in 2010. The internal control strains followed the same pattern and 5.04 %, 2.61 % and 2.73 % results deviating from the expected values were obtained for ENT-5.3, ST-5.1 and EC-5.5, respectively (Figure 2). Of note, these percentages do not include specific combinations strain/antimicrobial for which we observed less than 75 % reported results in agreement with the expected results (detailed explanation is provided in the paragraphs below).

Figure 2. Overview of the percentages of deviations from expected results obtained in different EQAS iterations for the three bacterial species tested. The internal control strain is represented by a line.



3.2 Deviations from expected results divided by species tested and AST method used

In the data analysis, results were grouped according to the methods used by the participants as follows. The agar diffusion method and MIC determination were evaluated together as they are both quantitative methods giving results corresponding to the minimum concentration of an antimicrobial which inhibits growth of the bacterial strain tested. The ROSCO and DD methods were evaluated together since they are based on the same principle of antimicrobial diffusion in the agar.

The highest percentage of results deviating from the expected values was obtained for the enterococci strains for which we observed 5 % of the overall results different from the expected

interpretations (Figure 3). Higher percentages of deviations from expected results were obtained by performing AST by disk diffusion methods than MIC determinations (Figure 3), which is similar to the pattern observed in previous EQAS iterations. Indeed, the percentage of deviations from expected results was 10 times higher for results obtained by DD compared to MIC in the enterococci and *E. coli* trial.

In EQAS 2011, 23, 25 and 27 participants performed AST by MIC determination for enterococci, staphylococci and *E. coli*, respectively, and four, seven and five participants performed AST by DD for enterococci, staphylococci and *E. coli*, respectively.

Overall, the percentage of results in agreement with the expected values ranged from a minimum of 92.1 % (strain ENT-5.7) to a maximum of 99.4 % (strains ST 5.5 and ST-5.7), as shown in Table 2. The staphylococci trial resulted in the highest percentages of results in agreement with the expected values.

Detailed analysis of the results obtained for each species and strain tested in EQAS 2011 are reported in the following paragraphs.

Figure 3. EQAS 2011: results deviating from the expected interpretation subdivided by tested species and antimicrobial susceptibility test method used

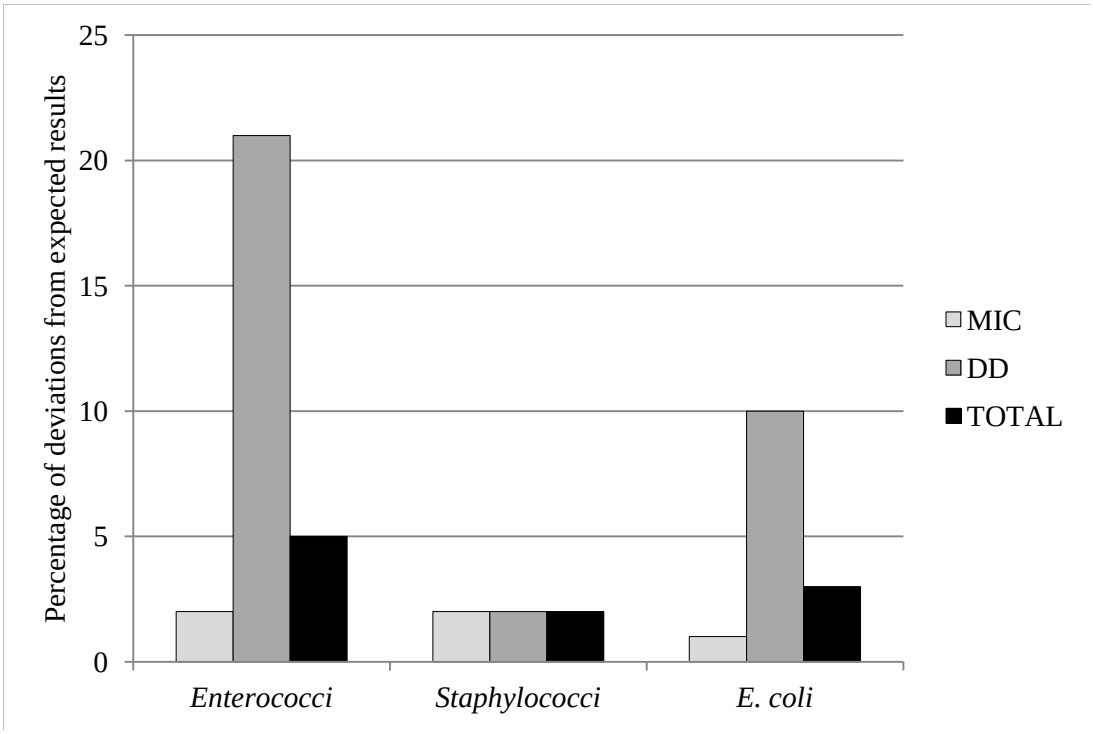


Table 2. Total number of antimicrobial susceptibility tests (AST) performed for each EQAS 2011 strain and percentage (%) of correct results

Strain*	No. AST	% correct	Strain*	No. AST	% correct	Strain*	No. AST	% correct
ENT-5.1	230	96.1	ST-5.1	269	97.7	EC-5.1	372	96.2
ENT-5.2	240	94.2	ST-5.2	327	96.0	EC-5.2	372	98.9
ENT-5.3	238	95.0	ST-5.3	328	97.9	EC-5.3	372	98.9
ENT-5.4	240	94.2	ST-5.4	327	99.1	EC-5.4	372	96.8
ENT-5.5	229	96.5	ST-5.5	328	99.4	EC-5.5	371	97.0
ENT-5.6	237	92.9	ST-5.6	326	98.8	EC-5.6	372	95.7
ENT-5.7	228	92.1	ST-5.7	327	99.4	EC-5.7	372	98.7
ENT-5.8	228	95.2	ST-5.8	329	98.8	EC-5.8	359	94.4

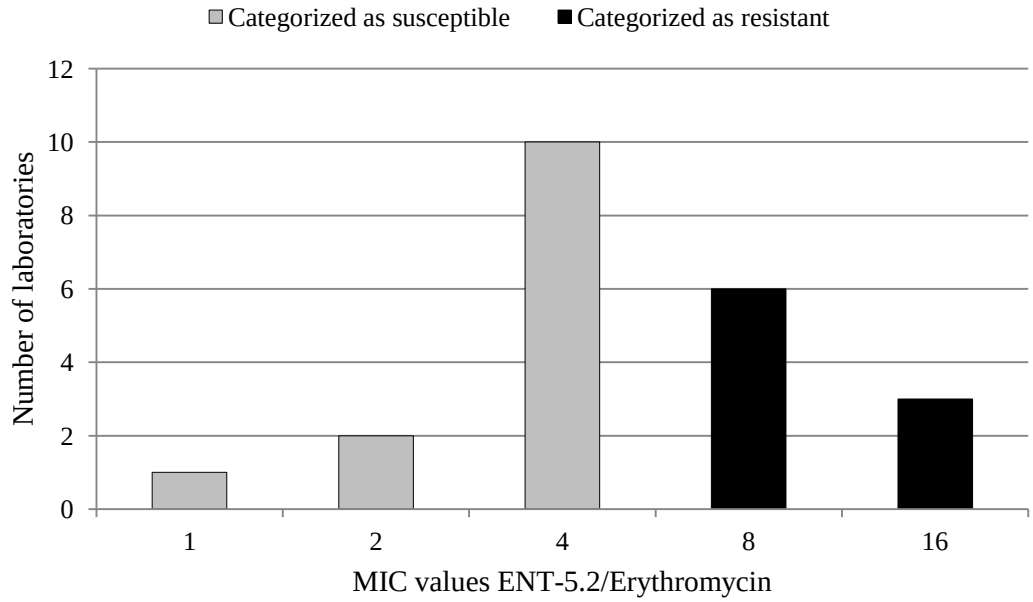
*ENT, enterococci; ST, staphylococci; EC, *Escherichia coli*.

3.2.1 Enterococci trial

As mentioned in the introduction, the EURL-AR network established that data should be individually examined and possibly subtracted from the general analysis if there are less than 75 % correct results for a strain/antimicrobial combination.

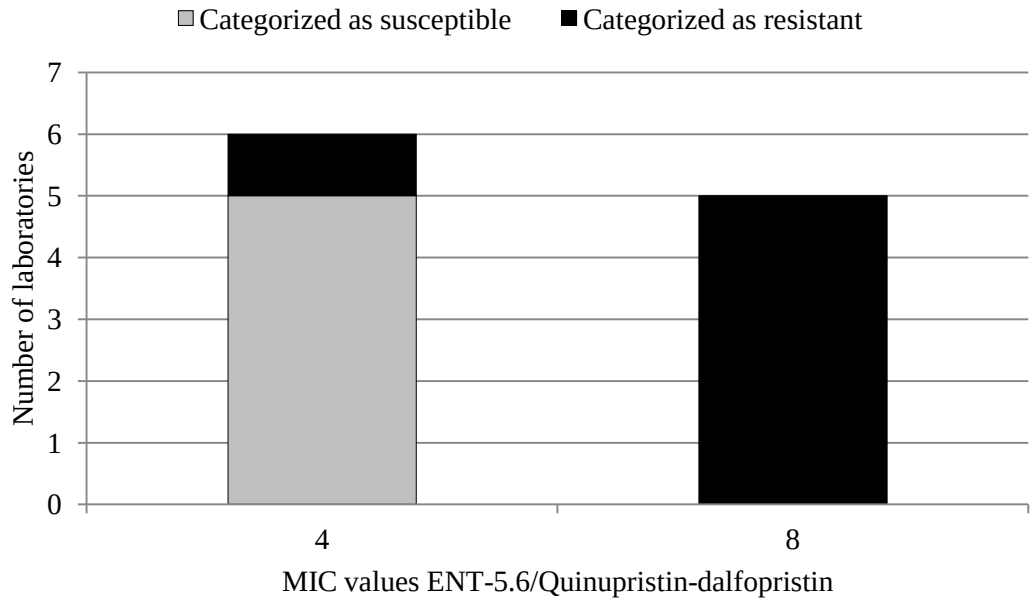
For ENT-5.2/Erythromycin, only 54 % of the results were in agreement with the expected interpretations and therefore the results were further examined. The distribution of MIC values obtained by participants performing MIC determination is reported in Figure 4. The expected MIC was 4 µg/ml, which results in categorization of the strain as susceptible. However, this value corresponds to the breakpoint for resistance (please refer to protocol, App. 4) and participants obtaining an MIC of 8 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as resistant and this was evaluated as an error. Ten participants obtained an MIC of 4 µg/ml and 6 participants obtained an MIC of 8 µg/ml (Figure 4). Among the four participants performing disk diffusion, three erroneously categorized the strain as resistant. All these results have been subtracted from the main analysis reported in this evaluation report since they cannot be representative of the quality of performance of the different participants in AST.

Figure 4. Distribution of MIC values obtained by participants performing MIC determination for the combination ENT-5.2/Erythromycin



Similarly, results for ENT-5,6/Quinupristin-dalfopristin were subtracted from the main analysis reported in this evaluation report. In this case, 62 % of the participants categorized the strain as resistant which is in agreement with the expected result. The distribution of MIC values obtained by participants performing MIC determination is reported in Figure 5. The expected MIC was 8 µg/ml which corresponds to a categorization of the strain as resistant. Participants obtaining an MIC of 4µg/ml reported the strain as susceptible according to the protocol, with one exception (Figure 5). However, performance of these participants is acceptable as the reported value is within one-fold dilution difference from the expected value. Two participants tested ENT-5.6/Quinupristin-dalfopristin by disk diffusion and correctly categorized the strain as resistant.

Figure 5. Distribution of MIC values obtained by participants performing MIC determination for the combination ENT-5.6/Quinupristin-dalfopristin



Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from ≤ 3 % to 7 % (Figure 6). The highest percentage (7 %) of disagreement with expected results was obtained for ENT-5.6 and ENT-5.7 (Figure 6). Percentage of disagreement with expected results was ≤ 3 % for ENT-5.1 and ENT-5.5 (Figure 6). Laboratories performing AST by disk diffusion reported results deviating from the expected categories in very high percentages ranging from 11 % (for ENT-5.1) to 29 % (for ENT-5.7), as shown in Figure 6. Out of 27 laboratories participating in the enterococci trial, four performed AST by disk diffusion.

Analysis of the results according to the tested antimicrobials showed that the highest percentages of deviation from expected interpretations were obtained in testing susceptibility to ampicillin (6 %), ciprofloxacin (8 %), gentamicin (8 %) and streptomycin (7 %) (Figure 7). Of note, ampicillin, gentamicin and streptomycin are among the EFSA-recommended antimicrobials (Table 1). Linezolid susceptibility tests resulted in 4 % of results in disagreement with expected values and tests of susceptibility to the remaining antimicrobials resulted in less than 3 % results deviating from the expected (Figure 7).
An overview of obtained and expected results is reported in Appendix 7a.

Figure 6. Enterococci trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used

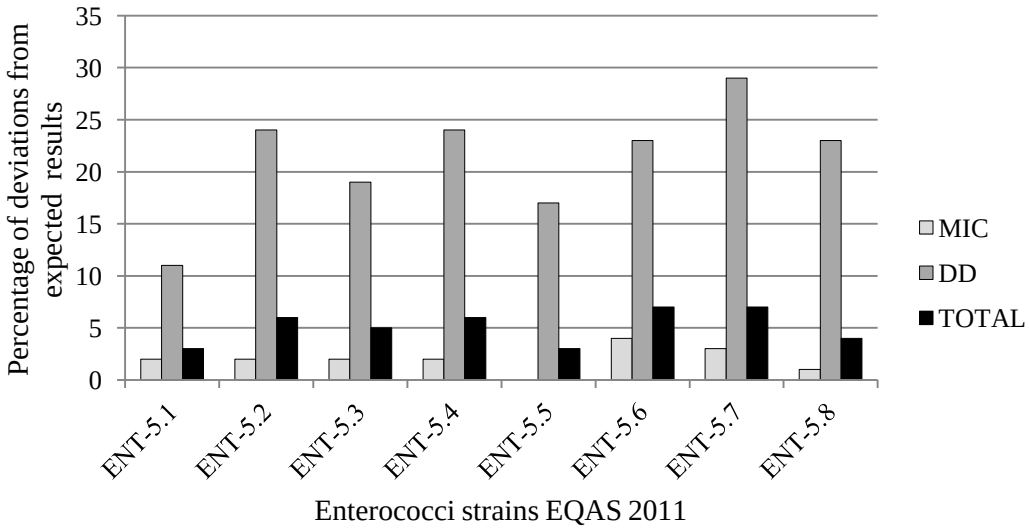
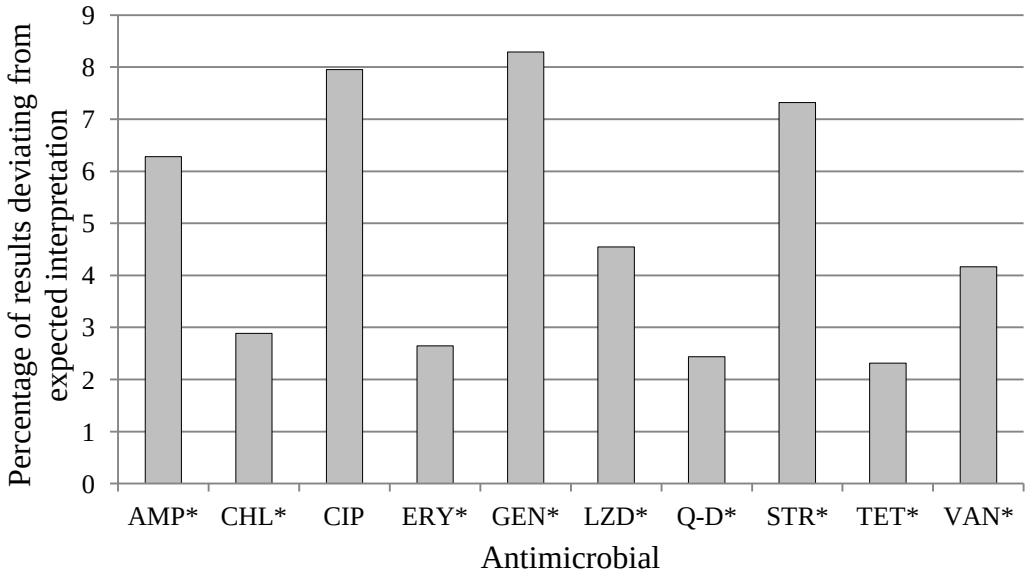


Figure 7. Enterococci trial: results deviating from expected interpretation according to the tested antimicrobial

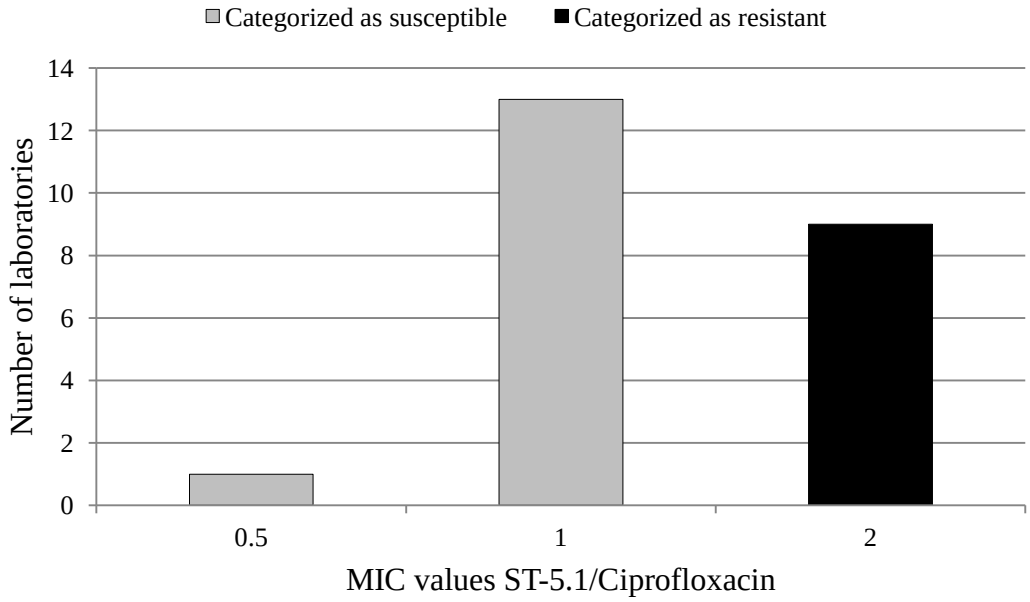


*Antimicrobials recommended by EFSA for antimicrobial resistance monitoring within EU

3.2.2 Staphylococci trial

Analysis of the different strain/antimicrobial combinations showed that ST-5.1/Ciprofloxacin was categorized in agreement with the expected category by only 34 % of the participants. According to the decision established by the EURL-AR network, further analysis was performed to examine the reason of this unsatisfactory result. The distribution of MIC values obtained by participants performing MIC determination is reported in Figure 8. The expected MIC was 2 µg/ml, which results in categorization of the strain as resistant. However, this value is one-step dilution above of the cut-off value (please refer to protocol, App. 4) and participants obtaining an MIC of 1 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as susceptible and this was evaluated as an error. Thirteen participants obtained an MIC of 1 µg/ml and 9 participants obtained an MIC of 2 µg/ml (Figure 8). Among the six participants performing disk diffusion, five erroneously categorized the strain as susceptible. All these results have been subtracted from the main analysis reported in this evaluation report since they cannot be representative of the quality of performance of the different participants in AST.

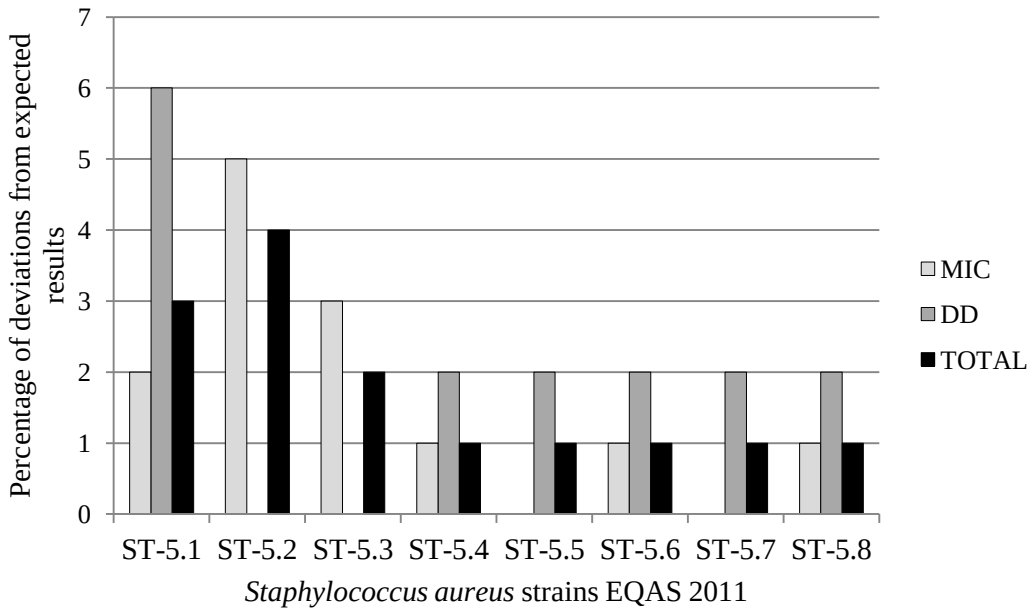
Figure 8. Distribution of MIC values obtained by participants performing MIC determination for the combination ST-5.1/Ciprofloxacin



Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from ≤ 1 % to 4 % (Figure 9). The highest percentage (4 %) of disagreement with expected results was obtained for ST-5.2 (Figure 9). Percentage of disagreement with expected results was ≤ 1 % for five out f the eight strains tested (Figure 9). Laboratories performing AST by disk diffusion obtained results deviating from the expected categories in percentages comparable to the ones obtained by MIC determination, as

shown in Figure 9. Out of 32 laboratories participating in the staphylococci trial, seven performed AST by disk diffusion.

Figure 9. Staphylococci trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used

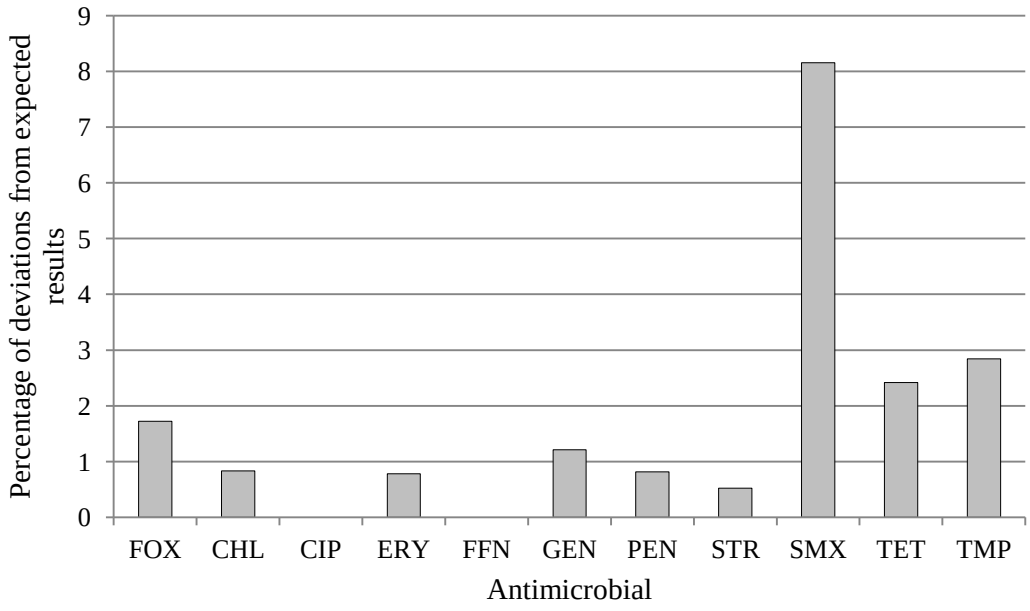


Analysis of the results according to the tested antimicrobials showed that the highest percentages of deviation from expected interpretations were obtained in testing susceptibility to sulphonamides (8 %) (Figure 10). Trimethoprim and tetracycline susceptibility testing resulted in deviations from expected results between 2 % and 3 %, while tests of susceptibility to the remaining antimicrobials resulted in less than 2 % results deviating from the expected (Figure 10).
An overview of obtained and expected results is reported in Appendix 7b.

Methicillin-resistant strain

Strains ST-5.1, ST-5.5 and ST-5.8 were methicillin-resistant. Among 32 participants testing staphylococci strains, one (# 41) did not report results concerning methicillin resistance.
Participant # 18 correctly reported ST-5.1 and ST-5.8 as methicillin-resistant but did not report any result for ST-5.5.
Participant # 4 reported strain ST-5.8 as methicillin-susceptible.
All remaining results were correct.

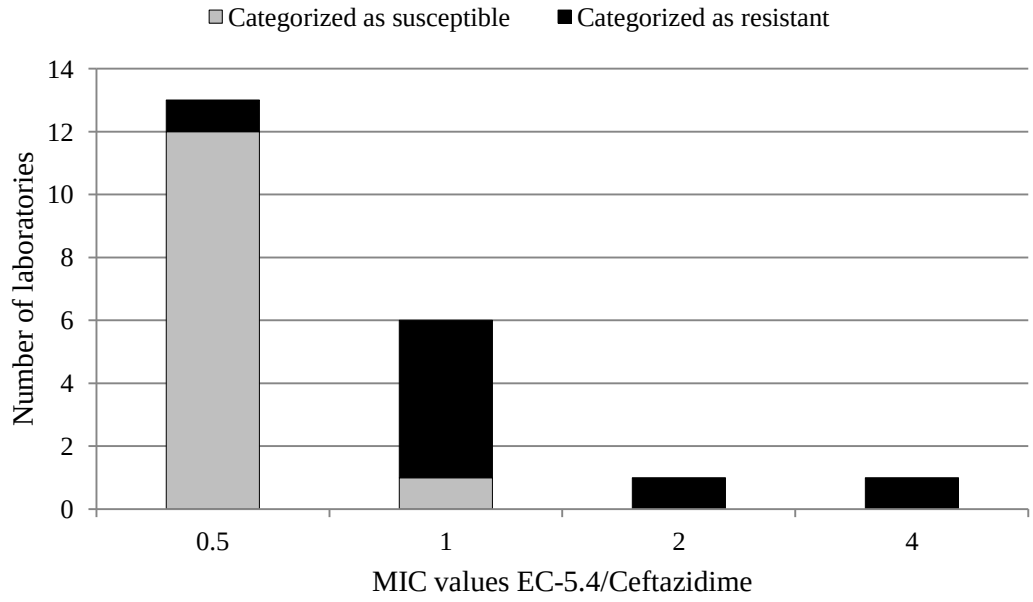
Figure 10. Staphylococci trial: results deviating from expected interpretation according to the tested antimicrobial



3.2.3 *Escherichia coli* trial

Analysis of the different strain/antimicrobial combinations showed that EC-5.4/Ceftazidime was categorized in agreement with the expected category by only 38 % of the participants. According to the decision established by the EURL-AR network, further analysis was performed to examine the reason of this unsatisfactory result. The distribution of MIC values obtained by participants performing MIC determination is reported in Figure 11. The expected MIC was 1 µg/ml, which results in categorization of the strain as resistant. However, this value is one-step dilution above of the cut-off value (please refer to protocol, App. 4) and participants obtaining an MIC of 0.5 µg/ml, which is acceptable as it is within one-fold dilution difference from the expected value, categorized the strain as susceptible and this was evaluated as an error. Thirteen participants obtained an MIC of 1 µg/ml and six participants obtained an MIC of 2 µg/ml (Figure 11). It should be stressed, however, that the phenotype of this isolate as regards ceftazidime is very specific due to the *bla*$_{\text{CTX-M-15}}$-gene. The isolate exhibits resistance (borderline) to this drug, but does not exhibit synergy when testing the combination of ceftazidime/clavulanic acid compared to ceftazidime. All the five participants performing disk diffusion erroneously categorized the strain as susceptible. All these results have been subtracted from the main analysis reported in this evaluation report since they cannot be representative of the quality of performance of the different participants in AST.

Figure 11. Distribution of MIC values obtained by participants performing MIC determination for the combination EC-5.4/Ceftazidime



As EC-5.8/Trimethoprim was categorized in agreement with the expected category by only 71 % of the participants, further analysis was performed to examine the reason of this unsatisfactory result. The distribution of MIC values obtained by participants performing MIC determination is reported in Figure 12. The expected MIC was ≤ 1 µg/ml, which results in categorization of the strain as susceptible. The cut-off value for categorizing *E. coli* as resistant to trimethoprim is > 2 µg/ml. Five and 12 participants determined an MIC ≤ 1 µg/ml and = 2 µg/ml, respectively, and correctly classified the strain as trimethoprim-susceptible (Figure 12). Six and two participants determined an MIC = 4 µg/ml and ≥ = 8 µg/ml, respectively, and categorized the strain as resistant (Figure 12). All the three participants performing disk diffusion correctly categorized the strain as susceptible. All these results have been included in this evaluation report since in this case evaluation based on MIC values and on interpretation of MIC values would lead to identical conclusions.

Analysis of results deviating from expected interpretation subdivided by strain showed that percentage of deviations from expected results ranged from ≤ 1 % to 6 % (Figure 13). The highest percentage (6 %) of disagreement with expected results was obtained for EC-5.8 (Figure 13). Percentage of disagreement with expected results was ≤ 1 % for EC-5.2, EC-5.3 and EC-5.7 (Figure 13). Laboratories performing AST by disk diffusion obtained results deviating from the expected categories in percentages higher than the ones obtained by MIC determination, as shown in Figure 13. These differences varied from two to seven times higher percentages of deviations in AST performed by DD compared to MIC in strains EC-5.3 and EC-5.8, respectively (Figure 13). Out of

32 laboratories participating in the *E. coli* trial, five performed AST by disk diffusion.
An overview of obtained and expected results is reported in Appendix 7c.

Figure 12. Distribution of MIC values obtained by participants performing MIC determination for the combination EC-5.8/Trimethoprim

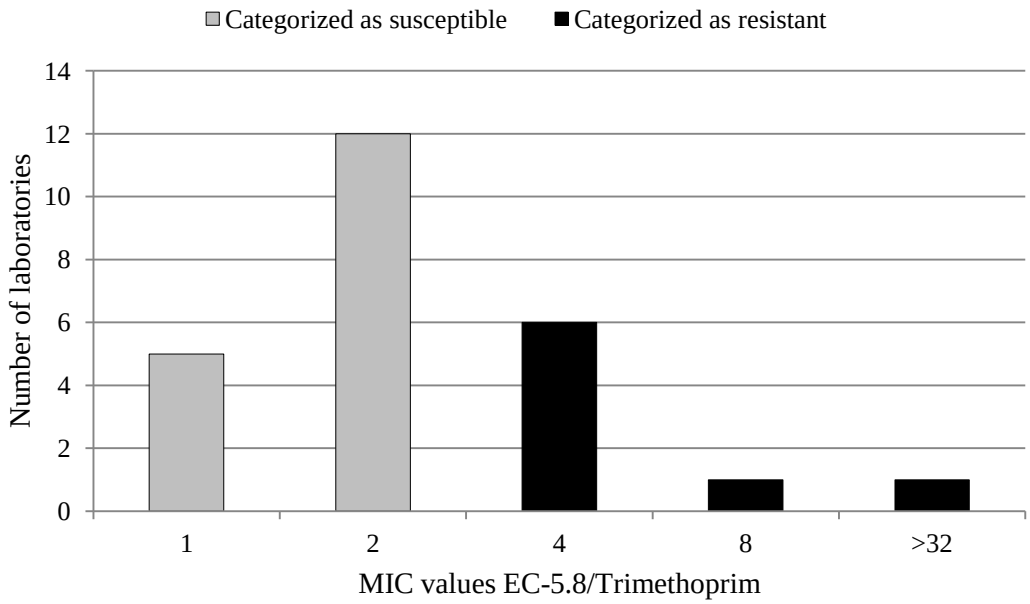
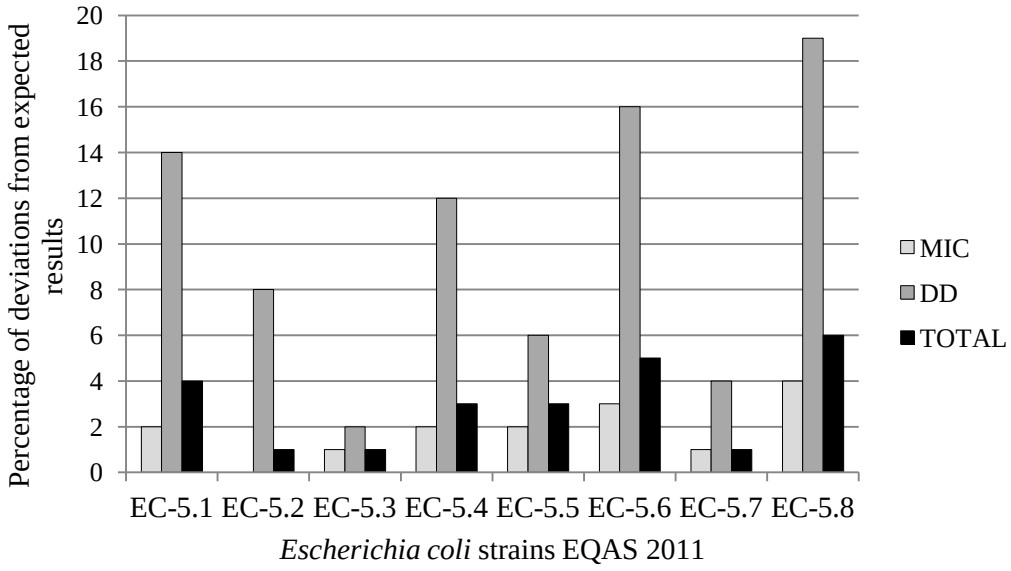


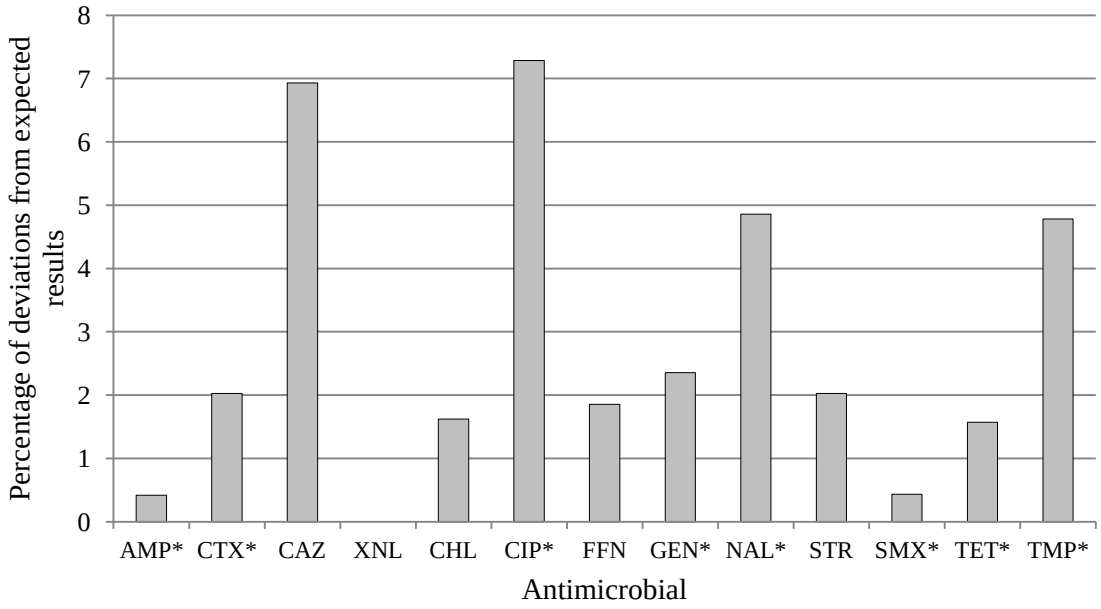
Figure 13. *Escherichia coli* trial: results deviating from the expected interpretation subdivided by tested strain and antimicrobial susceptibility test method used



Analysis of the results according to the tested antimicrobials showed that the highest percentages of deviation from expected interpretations were obtained in testing susceptibility to ceftazidime and

ciprofloxacin (ca. 7 %) (Figure 14). Nalidixic acid and trimethoprim susceptibility testing resulted in ca. 5 % and 3 % deviations from expected results, respectively, while tests of susceptibility to the remaining antimicrobials resulted in less than 2 % results deviating from the expected (Figure 14). No deviations were observed in ceftiofur susceptibility testing (Figure 14).
An overview of obtained and expected results is reported in Appendix 7c.

Figure 14. *Escherichia coli* trial: results deviating from expected interpretation according to the tested antimicrobial



*Antimicrobials recommended by EFSA for antimicrobial resistance monitoring within EU

Beta-lactamase-producing *E. coli*

As described in the protocol, MIC values and related interpretations for cefotaxime, ceftazidime and ceftiofur should be reported as found according to EUCAST expert rules. Ceftiofur susceptibility testing was performed 100 % correctly by the participants who tested this antimicrobial, while up to 7 % of results for ceftazidime susceptibility testing were in disagreement with the expected values.

Confirmation of beta-lactamase production is a mandatory component of this EQAS. All *E. coli* strains resistant to cefotaxime, ceftazidime and/or ceftiofur should undergo confirmatory tests for beta-lactamase production. Participant # 54 did not perform this component.
EC-5.4 and EC-5.5 were extended-spectrum beta-lactamase (ESBL) producers and EC-5.8 was an AmpC-producer.
Deviations from expected results were obtained as follows.
Participants # 22, # 46 and did not identify EC-5.4 as an ESBL producer as they did not obtained signs of synergy (please refer to protocol, App.4) by testing cefotaxime and cefotaxime+clavulanic acid.

Ten participants did not identify strain EC-5.8 as AmpC producer. Overall, these participants performed correct procedures except for the fact that they did not test for cefoxitin resistance despite having a strain resistant to cefotaxime, ceftazidime and/or ceftiofur and with no synergy by testing a cephalosporin in combination with clavulanic acid. One (# 41) of the participants not identifying EC-5.8 as AmpC producer classified instead the strain as ESBL producer since a phantom area was observed in the test with cefotaxime and cefotaxime+clavulanic acid. One participant (# 22) classified EC-5.8 as ESBL and AmpC-producer since an increase of the inhibition zone diameter ≥ 5 mm was observed by testing cefotaxime+clavulanic acid compared to cefotaxime alone.

Finally, two participants (# 21 and # 22) erroneously classified strain EC-5.6 as ESBL producer. In this case, participant # 21 categorized the strain as cefotaxime and ceftazidime resistant, performed confirmatory test and found synergy by testing cefotaxime and cefotaxime+clavulanic acid. Participant # 22 performed confirmatory test despite having correctly categorized EC-5.6 as cefotaxime and ceftazidime susceptible and classified the strain as ESBL producer because there was an enhancement of activity of cefotaxime+clavulanic acid compared to cefotaxime alone that was interpreted as synergy effect.

3.3 Deviations from expected results analyzed by participating laboratory

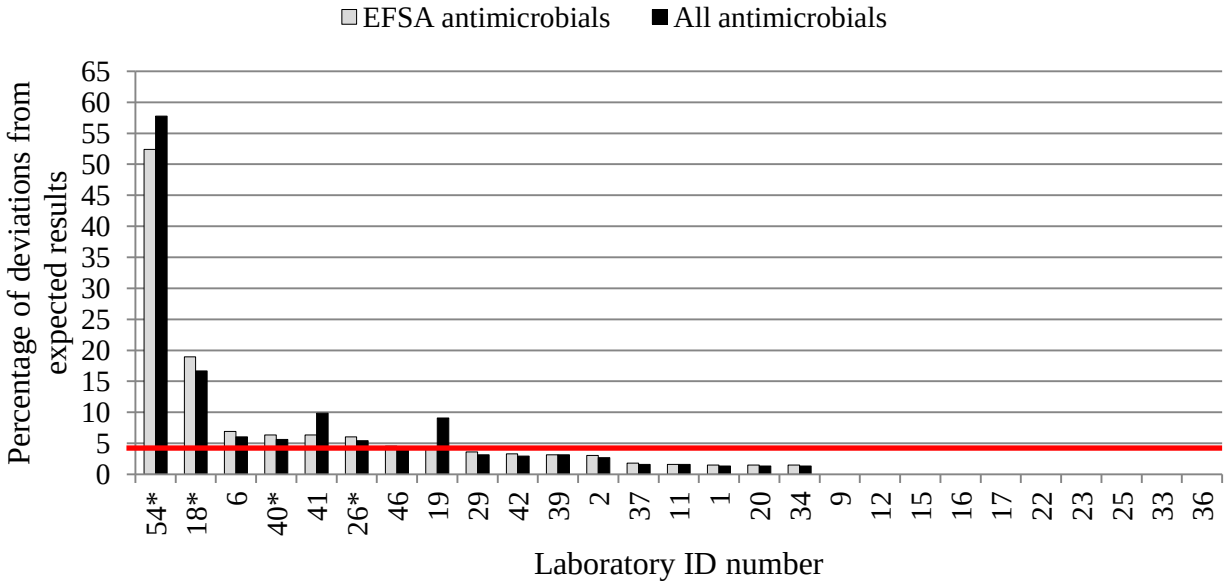
3.3.1 Enterococci trial

Analysis of laboratory performance of AST restricted to EFSA-recommended antimicrobials showed that six out of 27 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 15). Also by including all antimicrobials tested, six out of 27 participants obtained a percentage of deviations from expected results higher than 5 %, but one of these participants was actually below the 5 % threshold when considering EFSA-recommended antimicrobials only (Figure 15). All participants performing AST by disk diffusion obtained more than 5 % deviations from expected results (Figure 15).

Participant # 54 obtained deviations from expected results mainly in testing ampicillin (resistant strains classified as susceptible) and ciprofloxacin, gentamicin, linezolid and vancomycin (susceptible strains classified as resistant). Participant # 18 obtained deviations from expected results mainly in testing gentamicin and streptomycin (susceptible strains classified as resistant).

Participant # 41 obtained deviations from expected results mainly in testing ciprofloxacin (susceptible strains classified as resistant). In the remaining cases, deviations from expected results were observed for different antimicrobials and were represented both by classification of susceptible strains as resistant and vice versa (App. 8a).

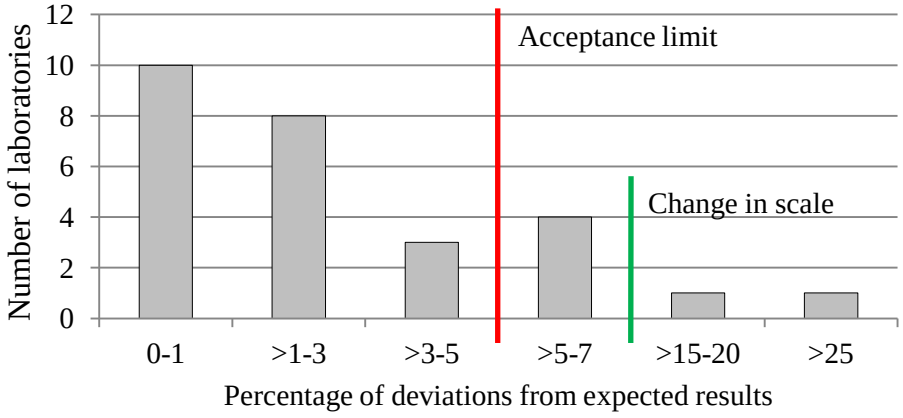
Figure 15. Percentage of deviations from expected results obtained by each laboratory in the enterococci trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing including EFSA-recommended antimicrobials only



*Laboratories performing AST by disc diffusion

In summary, 21 of 27 participants in the enterococci trial achieved the acceptance level by having less than 5 % of results deviating from the expected values (Figure 16). Among the six participants who did not meet the acceptance level, one was considered an outlier (#54) (Figure 16).
Deviations from expected results obtained by each participant in the enterococci trial are reported in Appendix 8a.

Figure 16. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing enterococci strains for susceptibility to EFSA-recommended antimicrobials

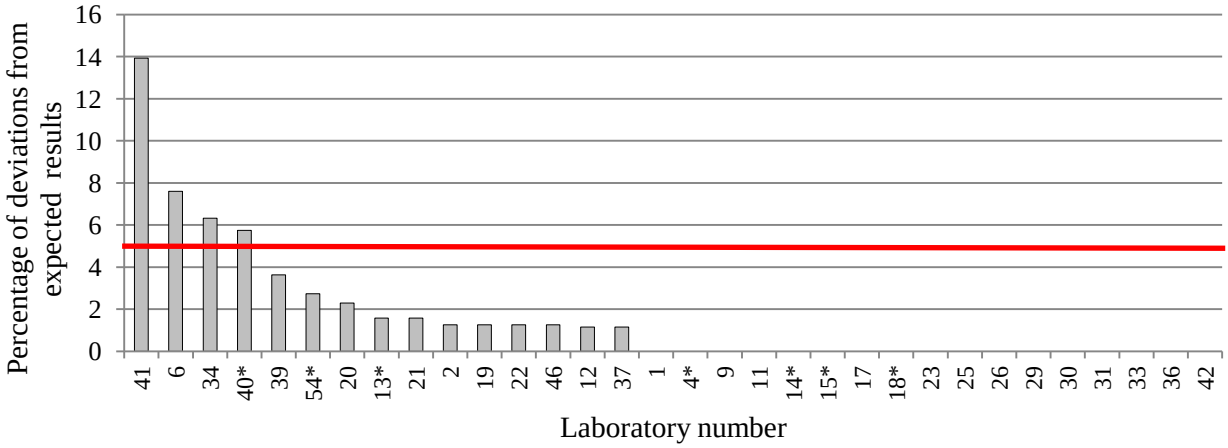


3.3.2 Staphylococci trial

Analysis of laboratory performance of AST showed that four out of 32 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 17). One out of seven participants performing AST by disk diffusion obtained more than 5 % deviations from expected results (Figure 17).

Participant # 6 obtained deviations from expected results mainly in testing sulphonamides (susceptible strains classified as resistant). Participant # 40 obtained deviations from expected results mainly in testing tetracycline (resistant strains classified as susceptible). Participant # 41 obtained deviations from expected results mainly in testing sulphonamides, tetracycline and trimethoprim (susceptible strains classified as resistant). Participant # 18 obtained deviations from expected results for different antimicrobials and both classification of susceptible strains as resistant and vice versa were observed (App. 8b).

Figure 17. Percentage of deviations from expected results obtained by each laboratory in the staphylococci trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing

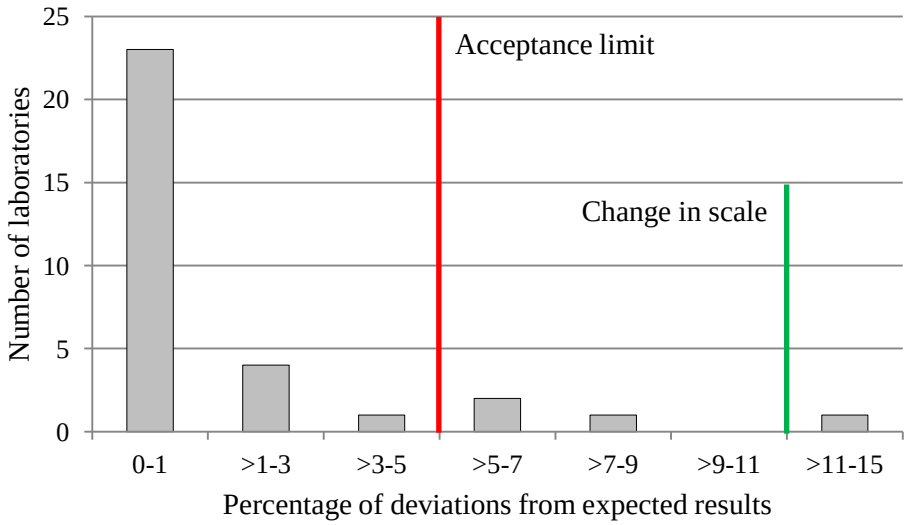


*Laboratories performing AST by disc diffusion

In summary, 28 of 32 participants in the staphylococci trial achieved the acceptance level by having less than 5 % of results deviating from the expected values (Figure 18). No outlier was identified among the four participants who did not meet the acceptance level (Figure 18). However, the participant (# 41) who did not report any information concerning methicillin resistance have been contacted by the EURL-AR to agree on possible supportive actions.

Deviations from expected results obtained by each participant in the staphylococci trial are reported in Appendix 8b.

Figure 18. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing staphylococci strains for antimicrobial susceptibility

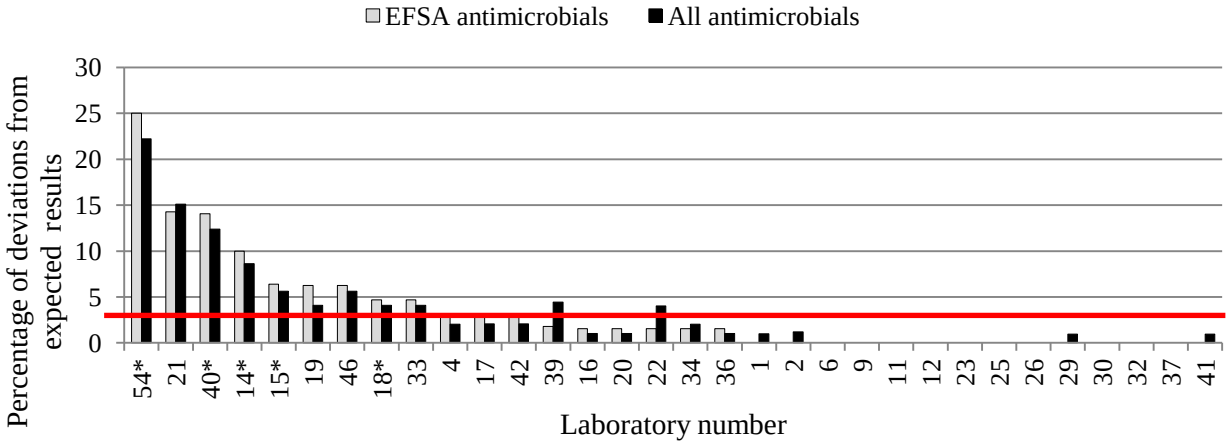


3.3.3 *Escherichia coli* trial

Analysis of laboratory performance of AST restricted to EFSA-recommended antimicrobials showed that seven out of 32 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 19). By including all antimicrobials tested, six out of 32 participants obtained a percentage of deviations from expected results higher than 5 % (Figure 19). Four out of five participants performing AST by disk diffusion obtained more than 5 % deviations from expected results (Figure 19).

Participant # 54 obtained deviations from expected results mainly in testing ceftazidime and ciprofloxacin (resistant strains classified as susceptible) and gentamicin (resistant strains classified as susceptible and vice versa). Participant # 21 obtained deviations from expected results for different antimicrobials including cefotaxime, ceftazidime, nalidixic acid and ciprofloxacin (susceptible strains classified as resistant). Participant # 40 obtained deviations from expected results for different antimicrobials including cefotaxime, ceftazidime, gentamicin and tetracycline (resistant strains classified as susceptible), and ciprofloxacin (resistant strains classified as susceptible and vice versa). Participants # 14 and 15 obtained deviations from expected results for different antimicrobials including cefotaxime and ceftazidime (resistant strains classified as susceptible), and nalidixic acid (resistant strains classified as susceptible). Participants # 19 obtained deviations from expected results mainly in testing ciprofloxacin (resistant strains classified as susceptible). In the remaining case, deviations from expected results were observed for different antimicrobials and were represented both by classification of susceptible strains as resistant and viceversa (App. 8c).

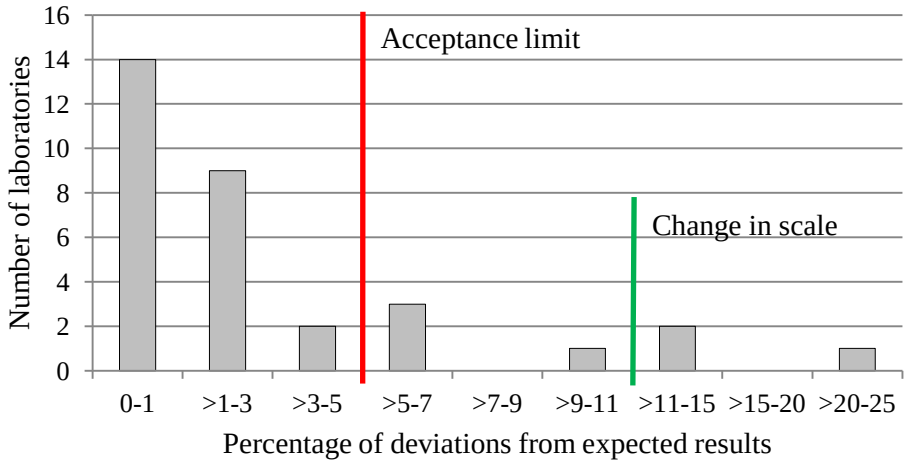
Figure 19. Percentage of deviations from expected results obtained by each laboratory in the *Escherichia coli* trial. The laboratories were ranked by decreasing percentage of deviations from expected results in antimicrobial susceptibility testing



*Laboratories performing AST by disc diffusion

In summary, 25 of 32 participants in the *E. coli* trial achieved the acceptance level by having less than 5 % of results deviating from the expected values (Figure 20). Among the seven participants who did not meet the acceptance level, one was considered an outlier (Figure 20).
Deviations from expected results obtained by each participant in the *E. coli* trial are reported in Appendix 8c.

Figure 20. Number of laboratories categorized according to the percentage of deviations from expected results obtained by testing *Escherichia coli* strains for susceptibility to EFSA-recommended antimicrobials



3.4 Deviations from expected results for the reference strains

The results for antimicrobial susceptibility testing of the reference strains have been evaluated according to the CLSI-established quality control (QC) ranges (App. 6).

3.4.1 *Enterococcus faecalis* ATCC 29212

Twenty-one participants performed AST of *E. faecalis* ATCC 29212 by MIC determination. One result out of the QC range was obtained for ampicillin susceptibility tests (Table 3). Two results out of the QC range were obtained for erythromycin and for tetracycline susceptibility tests (Table 3). In summary, out of 187 tests performed overall, 181 were correct.

Table 3. Antimicrobial susceptibility testing of *Enterococcus faecalis* ATCC 29212 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

***Enterococcus faecalis* ATCC 29212**				
Antimicrobial	**QC range (MIC)**	**Min. value**	**Max. value**	**No. of deviations from expected result/ Total no. of tests**
Ampicillin	0.5 - 2	1	4	1/19
Chloramphenicol	1 - 4	4	8	0/20
Ciprofloxacin	0.25 - 2	0.5	1	0/16
Erythromycin	1 - 4	0.25	4	2/21
Gentamicin	4 - 16	4	≤ 128	0/21
Linezolid	1 - 4	1	2	0/18
Quinu-dalfo-pristin	2 - 8	2	8	0/10
Streptomycin	n.a.*	32	512	0/20
Tetracycline	8 - 32	4	32	2/21
Vancomycin	1 - 4	1	4	0/21

*n.a., not applicable

3.4.2a *Staphylococcus aureus* ATCC 25923

Five participants performed AST of *S. aureus* ATCC 25923 by disk diffusion. One result out of the QC range was obtained for cefoxitin, chloramphenicol and gentamicin susceptibility tests (Table 4). Two results out of the QC range were obtained for penicillin susceptibility tests (Table 4). In summary, out of 40 tests performed overall, 35 were correct.

One participant performed AST of *S. aureus* ATCC 25923 by ROSCO method, and the results were not included in Table 4 because the quality control values were different from the ones used for disk diffusion. This participant obtained a result out of the QC range for erythromycin and gentamicin (obtained zone diameter smaller than the lowest acceptable value in the QC range).

Table 4. Antimicrobial susceptibility testing of *Staphylococcus aureus* ATCC 25923 by disk diffusion: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

***Staphylococcus aureus* ATCC 25923**				
Antimicrobial	**QC range (MIC)**	**Min. value**	**Max. value**	**No. of deviations from expected result/Total no. of tests**
Cefoxitin	23 - 29	26	33	1/6
Chloramphenicol	16 - 26	21	27	1/4
Ciprofloxacin	22 - 30	22	30	0/4
Erythromycin	22 - 30	23	30	0/5
Florfenicol	n. a.	22	27	n. a.
Gentamicin	19 - 27	19	28	1/5
Penicillin	26 - 37	32	40	2/5
Streptomycin	14 - 22	15	19	0/3
Sulfisoxazole	24 - 30	24	29	0/3
Tetracycline	24 - 34	28	32	0/4
Trimethoprim	19 - 26	20	24	0/2

3.4.2b *Staphylococcus aureus* ATCC 25913

Twenty-four participants performed AST of *S. aureus* ATCC 25913 by MIC determination (Table 5). Out of 204 tests performed overall, 203 were correct.

Table 5. Antimicrobial susceptibility testing of *Staphylococcus aureus* ATCC 25913 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

***Staphylococcus aureus* ATCC 25913**				
Antimicrobial	**QC range (MIC)**	**Min. value**	**Max. value**	**No. of deviations from expected result/Total no. of tests**
Cefoxitin	1 - 4	1	4	0/19
Chloramphenicol	2 - 8	2	8	0/23
Ciprofloxacin	0.12 - 0.5	0.25	0.5	0/23
Erythromycin	0.25 - 1	0.25	1	0/24
Florfenicol	2 - 8	4	8	0/9
Gentamicin	0.125 - 1	0.25	≤ 2	0/23
Penicillin	0.25 - 2	0.12	2	1/24
Sulfisoxazole	32 - 128	64	128	0/12
Tetracycline	0.125 - 1	0.25	1	0/24
Trimethoprim	1 - 4	1	4	0/23

3.4.3 *Escherichia coli* ATCC 25922

Four participants performed AST of *E. coli* ATCC 25922 by disk diffusion. One result out of the QC range was obtained for tetracycline susceptibility tests (Table 6). In summary, out of 42 tests performed overall, 41 were correct.

Table 6. Antimicrobial susceptibility testing of *Escherichia coli* ATCC 25922 by disk diffusion: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

***Escherichia coli* ATCC 25922**				
Antimicrobial	**QC range (MIC)**	**Min. value**	**Max. value**	**No. of deviations from expected result/Total no. of tests**
Ampicillin	16 - 22	19	20	0/2
Cefotaxime	29 - 35	30	35	0/4
Cefoxitin	23 - 29	26	28	0/4
Ceftazidime	25 - 32	26	32	0/3
Ceftiofur	26 - 31	26	29	0/3
Chloramphenicol	21 - 27	22	26	0/3
Ciprofloxacin	30 - 40	35	38	0/3
Gentamicin	19 - 26	20	26	0/4
Imipenem	26 - 32	30	30	0/1
Nalidixic acid	22 - 28	25	26	0/4
Streptomycin	12 - 20	16	18	0/2
Sulfisoxazole	15 - 23	21	23	0/2
Tetracycline	18 - 25	24	27	1/4
Trimethoprim	21 - 28	26	28	0/3

Twenty-six participants performed AST of *E. coli* ATCC 25922 by MIC determination. One result out of the QC range was obtained for ceftazidime susceptibility tests (Table 7). Two results out of the QC range were obtained for gentamicin and for sulfisoxazole susceptibility tests, and three results out of the QC range were obtained for ciprofloxacin susceptibility tests (Table 7). In summary, out of 287 tests performed overall, 279 were correct.

Table 7. Antimicrobial susceptibility testing of *Escherichia coli* ATCC 25922 by MIC determination: deviations from expected values and minimum and maximum values reported for each tested antimicrobial

***Escherichia coli* ATCC 25922**				
Antimicrobial	**QC range (MIC)**	**Min. value**	**Max. value**	**No. of deviations from expected result/Total no. of tests**
Ampicillin	2 - 8	2	8	0/26
Cefotaxime	0.03 - 0.125	0.06	0.125	0/26
Cefoxitin	2 - 8	2	8	0/8
Ceftazidime	0.06 - 0.5	0.25	1	1/23
Ceftiofur	0.25 - 1	0.25	0.5	0/4
Chloramphenicol	2 - 8	2	8	0/26
Ciprofloxacin	0.004 - 0.016	0.008	0.06	3/26
Gentamicin	0.25 - 1	0.25	2	2/26
Imipenem	0.06 - 0.25	0.12	0.5	0/4
Nalidixic acid	1 - 4	2	4	0/26
Streptomycin	4 - 16	4	8	0/25
Sulfisoxazole	8 - 32	8	128	2/17
Tetracycline	0.5 - 2	1	2	0/25
Trimethoprim	0.5 - 2	0.5	2	0/25

4. Discussion

4.1 General overview

In the overall analysis of results, it could be observed that the levels of deviations from the expected results were low and comparable to last year for AST of staphylococci and *E. coli*, while there was an increase in deviations from the expected results for AST of enterococci (Figure 2). The percentage of deviations from the expected results for AST of the internal control strains followed the general trend observed in the overall EQAS 2011 (Figure 2). Of note, results for the *S. aureus* internal control strain were considerably improved compared to last year (Figure 2).

It is important to consider that the number of EQAS participants changes from year to year, which implies that comparisons among different EQAS iterations are difficult to interpret. Results from three laboratories from EU–affiliated countries non-MS were included in this report, which is a novelty compared to reports issued in previous years. Among the NRLs designated by the MS, one declined to participate in all the three components of this EQAS 2011, and never participated in any of the enterococci, staphylococci and *E. coli* EQAS conducted to date.

The EURL-AR has emphasized the need for harmonization of AST methodology among NRLs, and has recommended MIC determination on several occasions. In this EQAS trial, the number of participants performing MIC determination is comparable to the high numbers observed last year. Of note, enterococci and *E. coli* AST performed by MIC determination resulted in significantly higher percentages of correct results compared to results obtained by DD over the different EQAS iterations. Therefore, the EURL-AR encourages participants using disk diffusion to test enterococci and *E. coli* to consider changing method and harmonizing with the majority of NRLs which perform MIC determination.

4.2 Enterococci trial

The percentages of results deviating from the expected interpretations varied from 3 % to 7 % among the different test strains (Figure 6). These relatively high percentages of deviations from expected results were mainly generated by participants performing AST by disk diffusion (Figure 6).

Similar problems were observed last year in EQAS 2010. Enterococci appear to be quite difficult to test correctly by disk diffusion, and several different reasons may be found. Unsatisfactory performance may be due to factors related to the strains as certain enterococci strains may require incubation times longer than overnight incubation. In addition, inoculum size and density may also represent a source of errors in AST performance.
Outcome of AST by disk diffusion is also influenced by factors related to the agar media like humidity, pH and volume. Finally, there may be factors related to the antimicrobial-containing disks like expiry date, humidity and concentration used.

Susceptibility tests to ampicillin, ciprofloxacin, gentamicin and streptomycin resulted in the highest percentages of results deviating from the expected interpretations (Figure 7). For ampicillin, ciprofloxacin and gentamicin, the incorrect classification was represented by susceptible strains reported as resistant, which was mainly obtained by participants # 54, # 41 and # 18 (App. 8a). For streptomycin, the incorrect classification was represented both by susceptible strains reported as resistant and vice versa, which was reported by various participants including # 54, # 46, # 18 and # 6 (App. 8a). Of note, ampicillin, gentamicin and streptomycin are among the EFSA-recommended antimicrobials, which implies that it is important that each participant who submitted incorrect results takes corrective actions.

The number of participants submitting more than 5 % results deviating from the expected interpretation was six, which is one more compared to last year. Of note, all the four participants testing enterococci by disk diffusion obtained percentages of results deviating from the expected interpretations above 5 %, which is considered the threshold for acceptable AST performance.
Among the six participants who did not meet the 5 % acceptance threshold, one was considered an outlier with deviations mainly due to susceptible strains classified as resistant to ampicillin, ciprofloxacin, gentamicin, linezolid and vancomycin. Another participant reported 17 % of results in disagreement with the expected values. Both participants have been contacted by the EURL-AR to identify possible causes of this unsatisfactory performance and to improve the quality of results.

The number of participants performing AST with 100 % agreement with the expected results was 10 (37 %), which is three laboratories less than last year.

AST of the quality control strain *E. faecalis* ATCC 29212 was quite satisfactory for the 21 participants that tested this strain by MIC determination (Table 3). In summary, out of 187 tests performed overall, 181 (97 %) were correct. Of note, the outlier identified in the enterococci trial did not report any value for the quality control strain.

4.3 Staphylococci trial

The percentages of results deviating from the expected interpretations ranged from 1 % to 4 % among the different test strains (Figure 9). As observed last year, the percentages of deviations from expected results generated by participants performing MIC determination and by participants performing DD were not considerably different (Figure 9). The number of participants performing MIC determination increased from 21 (EQAS 2010) to 25 participants.
The overall satisfactory results obtained in the staphylococci trial show a successful implementation of the new method for AST.

Identification of methicillin-resistant strains was generally satisfactory, which demonstrated that laboratories within the EURL-AR network correctly identify MRSA. However, few improvements

are necessary as participant # 41 did not report results concerning methicillin resistance, and participant # 18 did not report any result for ST-5.5 (but correctly reported ST-5.1 and ST-5.8 as methicillin-resistant). Participant # 4 reported erroneously strain ST-5.8 as methicillin-susceptible.
Of note, participant # 39 performed this test for the first time in this EQAS iteration and obtained 100 % correct results.

The number of participants submitting more than 5 % results deviating from the expected interpretation was four (Figure 10), which is two more compared to last year. No outliers were identified in the staphylococci trial. However, lab # 41 obtained 14 % results deviating from expected values, and the EURL-AR has contacted this participant to identify possible causes of this unsatisfactory performance and to improve the quality of results.

The number of participants performing AST with 100 % agreement with the expected results was 17 (53 %) and additional six (18 %) laboratories reported only 1 % of results deviating from expected values.

AST of the quality control strain *S. aureus* ATCC 25923 (for DD) resulted in 88 % correct tests (Table 4), and AST of the quality control strain *S. aureus* ATCC 29213 (for MIC determination) resulted in 99 % correct tests (Table 5). Overall, this performance was quite satisfactory.

4.4 *Escherichia coli* trial

The percentages of results deviating from the expected interpretations varied from 1 % to 6 % among the different test strains (Figure 13). These relatively high percentages of deviations from expected results were mainly generated by participants performing AST by disk diffusion (Figure 13).

Susceptibility tests to ceftazidime and ciprofloxacin resulted in the highest percentages (ca. 7 %) of results deviating from the expected interpretations (Figure 14). For ceftazidime, the incorrect classification was represented by susceptible strains reported as resistant, which was reported by various participants including # 21, # 29, # 34 and # 39 (App. 8c). For ciprofloxacin, the incorrect classification was represented both by susceptible strains reported as resistant and vice versa, which was obtained by various participants including # 4, # 19, # 21 and # 42 (App. 8c). These results indicate that increased attention should be paid to correctly categorize strains according to susceptibility to critically important antimicrobials like ciprofloxacin (fluoroquinolone) and ceftazidime (cephalosporin). In addition, susceptibility tests to nalidixic acid and trimethoprim resulted in higher percentages of results deviating from the expected interpretations compared to last year (≤ 3 % in EQAS 2010; ca. 5 % in EQAS 2011. Figure 14). Of note, nalidixic acid, ciprofloxacin and trimethoprim are among the EFSA-recommended antimicrobials, which implies that it is important that each participant who submitted incorrect results takes corrective actions.

The number of participants submitting more than 5 % results deviating from the expected interpretation was seven, which is much higher than last year when only two participants performed outside the acceptance level. Four out of five participants testing *E. coli* by disk diffusion obtained percentages of results deviating from the expected interpretations above the 5 % threshold for acceptable AST performance. Among the seven participants who did not meet the 5 % acceptance threshold, one was considered an outlier. This participant reported wrong results mainly for ciprofloxacin and gentamicin by categorizing resistant strains as susceptible, and will be soon contacted by the EURL-AR to identify possible causes of this unsatisfactory performance and to improve the quality of results.

The number of participants performing AST with 100 % agreement with the expected results was 14 (44 %).

Detection of beta-lactamases of the ESBL and AmpC-type should be further improved especially concerning identification of AmpC-type beta-lactamases. Participants did not show difficulties in correctly identifying cephalosporin resistance and a general improvement was observed compared to last year. However, there are limitations in the correct performance and interpretation of ESBL and AmpC confirmatory tests.

AST of the quality control strain *E. coli* ATCC 25922 resulted in 98 % and 97 % correct tests by DD and MIC determination, respectively (Tables 6 and 7). Overall, this performance was quite satisfactory. However, the majority of deviations was observed for tests of ciprofloxacin and this results must be improved in future trials since ciprofloxacin is among the critically important antimicrobials as defined by the WHO. Interestingly, the participants mentioned above reporting more than 5 % incorrect results for specific antimicrobials (ceftazidime and ciprofloxacin) had no values outside the QC range, which may indicate that the erroneous results were accidentally produced and there should not be any major issue in the methodology used. In any case, these participants are invited to review the causes of incorrect results for the *E. coli* test strains and possibly perform the test again. Of note, the outlier identified in the *E. coli* trial did not report any value for the quality control strain.

5. Conclusions

The number of laboratories not performing AST within the acceptable level (i.e. > 5 % results deviating from the expected values) was relatively low, but higher compared to last year especially in the enterococci trial. One participant was classified as an outlier both in the enterococci and in the *E. coli* trial. Since one of the tasks of the EURL-AR is to give specific recommendations targeting individual difficulties in performing acceptable AST, laboratories considered outliers have been contacted to assess the causes of inadequate AST performance and provide guideline to improve the methods used. These individual contacts should be taken as an opportunity to improve

knowledge on AST. Notably, participant # 39, who received a short visit by representatives of the EURL-AR last year, performed MRSA and ESBL confirmatory tests for the first time in this EQAS iteration and obtained excellent results.

Results obtained by MIC determination exhibited considerably higher level of agreement with the expected results compared to results obtained by disk diffusion both for the enterococci and the *E. coli* trials. As this situation was observed also in previous EQAS iterations, the EURL-AR strongly encourages participants to perform AST by MIC determination which seems to be more reliable and reproducible.

Additional improvements are needed to correctly identify *E. coli* producing beta-lactamases of the ESBL and AmpC-type as this is a priority area within the EURL-AR activities. We strongly encourage participants having problems in identifying these strains to perform a re-test as a training exercise and to contact us in case any discussion is needed.

Finally, the EURL-AR is open to suggestions to improve future EQAS trials and invites the entire network to contribute with ideas for training courses and specific focus areas to expand our knowledge in antimicrobial resistance.

National Food Institute

DFVF- M00-06-001/21.05.2010

Lyngby, 14 April 2011

EURL-AR EQAS 2011 FOR *E. COLI*, STAPHYLOCOCCI AND ENTEROCOCCI:
PRE-NOTIFICATION

The EURL-AR announces the launch of another EQAS, thus providing the opportunity for proficiency testing which is considered an essential tool for the generation of reliable laboratory results of consistently good quality.

This EQAS consists of antimicrobial susceptibility testing of eight *Escherichia coli* isolates, eight staphylococci and eight enterococci isolates. In addition, Quality Control (QC) strains *E. coli* ATCC 25922 (CCM 3954), *Enterococcus faecalis* ATCC 29212 (CCM 4224), *Staphylococcus aureus* ATCC 25923 (CCM 3953) (for disk diffusion) and *S. aureus* ATCC 29213 (CCM 4223) (for MIC) will be distributed to new participating laboratories.

This EQAS is specifically for NRLs on antimicrobial resistance. All laboratories receiving this pre-notification are automatically regarded as participants and do not need to sign up to participate. Participation is free of charge for all EU designated NRLs.

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
The content of the parcel is "Biological Substance Category B": eight *E. coli*, eight staphylococci, eight enterococci and, for new participants, the QC strains mentioned above. *Please inform me about documents or other information that can simplify custom procedures* (e.g., specific text that should be written on the accompanying letter). To avoid delays, I kindly ask you to send me this information already at this stage.

TIMELINE FOR EQAS 2011:

- Shipment of isolates and protocol. The isolates will be shipped in June 2011. The protocol for reviving the isolates and performing antimicrobial susceptibility tests will be available at www.eurl-ar.eu
- **Submission of results**. Results must be submitted to the National Food Institute **no later than 9 September 2011** via the password-protected website. Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.
- EQAS report. A report summarising and comparing results for all participants will be issued. A code which is known only to the National Food Institute and the EU Commission is assigned to participating laboratories, thus guaranteeing anonymity.
- Next EQAS. The next EURL-AR EQAS component will be the antimicrobial susceptibility testing of *Salmonella* and *Campylobacter,* and it will be carried out in October 2011.

Please do not hesitate to contact me for further information.

Yours sincerely,

Valeria Bortolaia

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EQAS 2011: list of participants including the strains analyzed by each laboratory

Institute	Country	*E. coli*	Staphylococci	Enterococci
Austrian Agency for Health and Food Safety	Austria	X	X	X
Institute of Public Health	Belgium	X	X	
NRL AR on food, Nayional Diagnostic and Research Veterinary Institute	Bulgaria	X	X	X
Veterinary Services	Cyprus	X	X	X
State Veterinary Institute Prague	Czech Republic	X	X	X
DTU National Food Institute	Denmark	X	X	X
Estonian Veterinary and Food Laboratory	Estonia	X	X	X
Finnish Food Safety Authority EVIRA	Finland	X	X	X
Agence nationale de sécurité sanitaire ANSES - Maisons Alfort - LERQAP	France		X	
Agence nationale de sécurité sanitaire ANSES - Ploufragan - LERAP	France	X	X	
Agence nationale de sécurité sanitaire ANSES - Lyon	France	X	X	X
Agence nationale de sécurité sanitaire ANSES - Fougères LERMVD	France	X		X
Federal Institute for Risk Assessment	Germany	X	X	X
Veterinary Laboratory of Chalkis	Greece	X	X	X
Central Agricultural Office Veterinary Diagnostic Directorate	Hungary	X	X	X
Central Veterinary Research Laboratory	Ireland	X	X	X
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy	X	X	
Institute of Food Safety, Animal Health and Enviroment "BIOR"	Latvia	X	X	X
National Veterinary Laboratory	Lithuania	X	X	X
Public Health Laboratory	Malta	X	X	X
Food and Consumer Product Safety Authority (VWA)	The Netherlands	X	X	X
Central Veterinary Institute of Wageningen UR	The Netherlands	X	X	X
Veterinærinstituttet	Norway	X	X	X
National Veterinary Research Institute	Poland	X	X	X
Laboratorio National de Investigacáo Veterinaria	Portugal	X	X	X
National Institute of R/D for Microbiology and Immunology “Cantacuzino”	Romania	X	X	X
Institute for Hygiene and Veterinary Public Health	Romania	X	X	X
Institute of Veterinary Medicine of Serbia	Serbia	X	X	X
State Veterinary and Food Institute (SVFI)	Slovakia	X	X	X
National Veterinary Institute	Slovenia	X	X	
Laboratorio Central de Sanidad, Animal de Santa Fe	Spain		X	
Laboratorio Central de Sanidad, Animal de Algete	Spain	X		
VISAVET Health Surveillance Center, Complutense University	Spain	X	X	X
*CN de Alimentacion. Agencia Espanola de Seguridad Alimentria y Nutricion	Spain			
National Veterinary Institute, SVA	Sweden	X	X	X
Vetsuisse Faculty Bern, Institute of Veterinary Bacteriology	Switzerland	X	X	X
Centre for Infections Health Protection Agency	United Kingdom	X	X	X
The Veterinary Laboratory Agency	United Kingdom	X	X	X

EQAS 2011 - *Enterococci* test strains: expected results (MIC values and related interpretations)

Isolate ID	Species	Antimicrobial									
		AMP	CHL	CIP	ERY	GEN	LZD	STR	Q-D	TET	VAN
EURL ENT 5.1	*E. faecalis*	≤ 2	= 64	= 1	> 32	> 1024	= 2	> 2048	-	> 32	≤ 1
EURL ENT 5.2	*E. faecium*	≤ 2	= 4	= 1	= 4	≤ 16	= 2	≤ 64	= 1	≤ 1	≤ 1
EURL ENT 5.3	*E. faecium*	= 4	= 8	≤ 0.5	= 2	≤ 16	= 2	≤ 64	= 2	> 32	> 32
EURL ENT 5.4	*E. faecium*	> 32	= 16	= 2	> 32	≤ 16	= 2	> 2048	= 0.5	> 32	= 16
EURL ENT 5.5	*E. faecalis*	≤ 2	= 8	≤ 0.5	> 32	≤ 16	= 1	> 2048	-	= 32	= 2
EURL ENT 5.6	*E. faecium*	≤ 2	= 8	= 1	> 32	≤ 16	= 1	= 1024	= 8	≤ 1	≤ 1
EURL ENT 5.7	*E. faecalis*	≤ 2	= 4	= 1	= 1	≤ 16	= 2	= 128	-	≤ 1	= 2
EURL ENT 5.8	*E. faecalis*	≤ 2	= 64	= 1	> 32	≤ 16	= 2	> 2048	-	> 32	≤ 1

Isolate ID	Species	Antimicrobial									
		AMP	CHL	CIP	ERY	GEN	LZD	STR	Q-D	TET	VAN
EURL ENT 5.1	*E. faecalis*	SUSC	RESIST	SUSC	RESIST	RESIST	SUSC	RESIST	not applicable	RESIST	SUSC
EURL ENT 5.2	*E. faecium*	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC
EURL ENT 5.3	*E. faecium*	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST
EURL ENT 5.4	*E. faecium*	RESIST	SUSC	SUSC	RESIST	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST
EURL ENT 5.5	*E. faecalis*	SUSC	SUSC	SUSC	RESIST	SUSC	SUSC	RESIST	not applicable	RESIST	SUSC
EURL ENT 5.6	*E. faecium*	SUSC	SUSC	SUSC	RESIST	SUSC	SUSC	RESIST	RESIST	SUSC	SUSC
EURL ENT 5.7	*E. faecalis*	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	not applicable	SUSC	SUSC
EURL ENT 5.8	*E. faecalis*	SUSC	RESIST	SUSC	RESIST	SUSC	SUSC	RESIST	not applicable	RESIST	SUSC

RESIST

AMP, ampicillin
CHL, chloramphenicol
CIP, ciprofloxacin
ERY, erythromycin
GEN, gentamicin
LZD, linezolid
STR, streptomycin
Q-D, quinupristin-dalfopristin (synercid)
TET, tetracyclin
VAN, vancomycin

EQAS 2011 - *Staphylococcus aureus* test strains: expected results (MIC values and related interpretations)

Isolate ID	Antimicrobial											*mecA*
	CHL	CIP	ERY	FFN	FOX	GEN	PEN	STR	SMX	TET	TMP	
EURL ST 5.1	= 4	= 2	≤ 0.25	= 4	= 8	> 16	> 16	> 64	= 512	= 32	≤ 0.5	pos.
EURL ST 5.2	> 64	= 0.5	> 16	> 64	= 4	≤ 0.25	> 16	> 64	≤ 32	≤ 0.5	= 1	neg.
EURL ST 5.3	= 8	= 0.5	= 0.5	= 4	= 2	≤ 0.25	≤ 0.06	≤ 4	≤ 32	≤ 0.5	= 1	neg.
EURL ST 5.4	= 8	= 0.5	> 16	= 4	= 2	≤ 0.25	= 16	> 64	≤ 32	= 32	= 1	neg.
EURL ST 5.5	= 8	> 8	= 0.5	= 4	= 16	≤ 0.25	= 8	> 64	≤ 32	> 32	> 32	pos.
EURL ST 5.6	= 4	= 0.5	≤ 0.25	= 2	= 2	≤ 0.25	= 0.5	= 8	≤ 32	= 8	≤ 0.5	neg.
EURL ST 5.7	= 8	= 0.5	= 0.5	= 4	= 4	≤ 0.25	= 4	> 64	≤ 32	> 32	> 32	neg.
EURL ST 5.8	= 8	= 0.5	= 0.5	= 4	= 16	= 0.5	= 16	> 64	≤ 32	> 32	> 32	pos.

Isolate ID	Antimicrobial											*mecA*
	CHL	CIP	ERY	FFN	FOX	GEN	PEN	STR	SMX	TET	TMP	
EURL ST 5.1	SUSC	RESIST	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	SUSC	pos.
EURL ST 5.2	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	RESIST	RESIST	SUSC	SUSC	SUSC	neg.
EURL ST 5.3	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	neg.
EURL ST 5.4	SUSC	SUSC	RESIST	SUSC	SUSC	SUSC	RESIST	RESIST	SUSC	RESIST	SUSC	neg.
EURL ST 5.5	SUSC	RESIST	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST	SUSC	RESIST	RESIST	pos.
EURL ST 5.6	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	SUSC	SUSC	RESIST	SUSC	neg.
EURL ST 5.7	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	SUSC	RESIST	RESIST	neg.
EURL ST 5.8	SUSC	SUSC	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST	SUSC	RESIST	RESIST	pos.

CHL, chloramphenicol
CIP, ciprofloxacin
ERY, erythromycin
FFN, florfenicol
FOX, cefoxitin
GEN, gentamicin
PEN, penicillin
STR, streptomycin
STX, sulphamethoxazole
TET, tetracycline
TMP, trimethoprim

RESIST

EQAS 2011 - *Escherichia coli* test strains: expected results (MIC values and related interpretations)

Isolate ID	MIC (µg/mL)																	NOTE
	AMP	CAZ	CHL	CIP	CTX	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CAZ+CLA	CTX/CTX+CLA	IP/IPI	
EURL EC 5.1	= 4	= 0.25	= 8	= 0.5	≤ 0.12	= 8	= 4	= 1	= 16	≤ 64	> 128	> 32	≤ 1	≤ 0.5				
EURL EC 5.2	> 32	= 0.125	= 4	≤ 0.015	≤ 0.12	= 4	= 4	= 16	≤ 4	≤ 64	> 128	> 32	≤ 1	≤ 0.5				
EURL EC 5.3	> 32	= 0.25	> 64	= 0.25	≤ 0.12	= 8	= 4	= 1	> 64	> 1024	> 128	> 32	> 32	≤ 0.5				
EURL EC 5.4	> 32	= 1	= 4	= 0.5	> 16	= 8	= 4	≥ 16	= 16	> 1024	> 128	> 32	> 32	> 8	MIC ratio < 8	MIC ratio ≥ 8	MIC ratio < 8	**ESBL**
EURL EC 5.5	> 32	= 4	= 4	≤ 0.015	= 32	= 4	= 4	= 1	≤ 4	≤ 64	≤ 8	≤ 2	≤ 1	> 8	MIC ratio ≥ 8	MIC ratio ≥ 8	MIC ratio < 8	**ESBL**
EURL EC 5.6	> 32	= 0.064	= 32	= 0.06	≤ 0.12	= 8	= 4	= 16	= 32	> 1024	= 128	> 32	> 32	≤ 0.5				
EURL EC 5.7	= 4	= 0.125	= 8	< 0.015	≤ 0.12	= 8	= 4	= 1	≤ 4	≤ 64	≤ 8	≤ 2	≤ 1	≤ 0.5				
EURL EC 5.8	> 32	= 2	= 8	= 2	= 1	= 8	> 8	≤ 0.5	> 64	≤ 64	≤ 8	≤ 2	≤ 1	= 1	MIC ratio < 8		MIC ratio < 8	**AmpC**

Isolate ID	MIC (µg/mL)																	NOTE
	AMP	CAZ	CHL	CIP	CTX	FFN	FOX	GEN	NAL	SMX	STR	TET	TMP	XNL	CAZ/CAZ+CLA	CTX/CTX+CLA	IP/IPI	
EURL EC 5.1	SUSC	SUSC	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	SUSC	SUSC				
EURL EC 5.2	RESIST	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	SUSC	SUSC	RESIST	RESIST	SUSC	SUSC				
EURL EC 5.3	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST	SUSC				
EURL EC 5.4	RESIST	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	RESIST	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST	no synergy	phantom	no synergy	**ESBL**
EURL EC 5.5	RESIST	RESIST	SUSC	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	RESIST	phantom	synergy	no synergy	**ESBL**
EURL EC 5.6	RESIST	SUSC	RESIST	RESIST	SUSC	SUSC	SUSC	RESIST	RESIST	RESIST	RESIST	RESIST	RESIST	SUSC				
EURL EC 5.7	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC	SUSC				
EURL EC 5.8	RESIST	RESIST	SUSC	RESIST	RESIST	SUSC	RESIST	SUSC	RESIST	SUSC	SUSC	SUSC	SUSC	SUSC	no synergy		no synergy	**AmpC**

AMP, ampicillin
CAZ, ceftazidime
CHL, chloramphenicol
CIP, ciprofloxacin
CTX, cefotaxime
FFN, florfenicol
FOX, cefoxitin
GEN, gentamicin
NAL, nalidixic acis
SMX, sulphamethoxazole
STR, streptomycin
TET, tetracycline
TMP, trimethoprim
XNL, ceftiofur
CAZ+CLA, ceftazidime + clavulanic acid
CTX+CLA, cefotaxime + clavulanic acid
IP, imipenem
IPI, imipenem + EDTA

RESIST

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PROTOCOL

For antimicrobial susceptibility testing of *Escherichia coli*, enterococci and staphylococci

1. Introduction

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *E. coli*, enterococci and staphylococci is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The EC/Ent/Staph EQAS 2011 will include AST of eight *E. coli*, eight enterococci and eight staphylococci strains and AST of 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).

The above-mentioned reference strains are included in the parcel only for new participants in the EQAS who did not receive them previously. The reference strains are original certified cultures and are free of charge. Please take proper care of these strains, and handle and maintain them according to the instructions reported in the manual 'Subculture and Maintenance of QC Strains'. Please use the reference strains for future internal quality control when performing AST in your laboratory.

2. Objectives

This EQAS aims to support laboratories to assess and, if necessary, to improve the quality of results obtained by AST of pathogens of food- and animal-origin, with special regard to *E. coli*, enterococci and staphylococci. Further objectives are to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *E. coli*, enterococci and staphylococci reported

to EFSA by different laboratories, and to harmonise the breakpoints for antimicrobial susceptibility used within the EU.

3. Outline of the EC/Ent/Staph EQAS 2011

3.1 Shipping, receipt and storage of strains

In June 2011, the EU-appointed National Reference Laboratories for Antimicrobial Resistance will receive a parcel containing eight *E. coli*, eight enterococci and eight staphylococci strains from the National Food Institute, Denmark. This parcel will also contain reference strains, but only for participants who did not receive them previously. All strains are non-toxin-producing human pathogens Class II, and extended-spectrum beta-lactamase (ESBL)-producing strains and methicillin-resistant *Staphylococcus aureus* (MRSA) strains could be included.
The reference strains are shipped lyophilised, while the test strains are stab cultures. On arrival, the stab cultures must be subcultured and all cultures should be kept refrigerated until testing. Lyophilised reference strains should be revived by following the procedure reported in the link below.

3.2 Suggested procedure for reconstitution of the lyophilised reference strains

Please refer to the document 'Instructions for opening and reviving lyophilised cultures' reported on the EURL-AR website (www.eurl-ar.eu).

3.3 Antimicrobial susceptibility testing

The strains should be tested for susceptibility to the antimicrobials listed in tables 1, 2 and 3 by using the method implemented in your laboratory for performing monitoring for EFSA.

Participants performing minimum inhibitory concentration (MIC) determination should use the values listed in tables 1, 2 and 3 for interpretation of results. These values represent the epidemiological cut-off values developed by EUCAST (www.eucast.org), and allow categorisation of bacterial strains into two categories: resistant and susceptible. A categorization as intermediate is not accepted, and "**intermediate strains" should be interpreted as susceptible**.

Participants using disk diffusion are recommended to interpret the results according to the breakpoints used routinely. Strains must be categorised into resistant and susceptible. Also in this case, a categorization as intermediate is not accepted, and "**intermediate strains" should be interpreted as susceptible**.

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TABLE 1.
Antimicrobials recommended for AST of *Escherichia coli* and interpretative breakpoints

Antimicrobials for *E. coli* AST	MIC (µg/mL) **R is >**
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Cefoxitin, FOX	8
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	8*
Sulfonamides, SMX	256**
Tetracycline, TET	8
Trimethoprim, TMP	2

*Based on studies performed by the EURL-AR network (manuscript accepted for publication in Microbial Drug Resistance)

**CLSI M100 Table 2A

Important notes*: beta-lactam resistance*

Confirmatory tests for ESBL production is mandatory on all strains resistant to cefotaxime (CTX), ceftazidime (CAZ) and/or ceftiofur (XNL).

Confirmatory test for ESBL production requires use of both cefotaxime (CTX) and ceftazidime (CAZ) alone and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC CTX : CTX/CL or CAZ : CAZ/CL ratio ≥ 8) or ii) a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Confirmatory test for Metallo-beta-lactamase (MBL) production requires use of imipenem (IMI) and IMI/EDTA. Synergy is defined as a ≥ 3 twofold concentration decrease in the MIC for the combination IMI/EDTA vs. MIC for IMI alone (E-test 3 dilution steps difference, MIC IMI : IMI/EDTA ratio ≥ 8; CLSI M100, Table 2A; Enterobacteriaceae). The presence of synergy indicates MBL production.

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Detection of AmpC-type beta-lactamases can be performed by testing the bacterium for susceptibility to cefoxitin (FOX). Resistance to FOX could indicate the presence of an AmpC-type beta-lactamase, that should be verified by PCR and sequencing.

The EURL-AR aims to harmonise with EUCAST expert rules. Accordingly, MIC values and relative interpretation of cefotaxime, ceftazidime and/or ceftiofur used for detection of beta-lactamase-producing strains in this EQAS should be reported as found.

TABLE 2.
Antimicrobials recommended for AST of *Enterococcus* spp. and interpretative breakpoints

Antimicrobials for enterococci AST	MIC (µg/mL) **R is >**	MIC (µg/mL) **R is >**
	E. faecium	*E. faecalis*
Ampicillin, AMP	4	4
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Erythromycin, ERY	4	4
Gentamicin, GEN	32	32
Linezolid, LZD	4	4
Streptomycin, STR	128	512
Quinupristin-dalfopristin (Synercid), SYN	4*	Not applicable
Tetracycline, TET	4	4
Vancomycin, VAN	4	4

*DANMAP 2009 (www.danmap.org)

Important notes: *identity of the test strains*
Please refer to the test forms for the species (*E. faecalis* or *E. faecium*) of the test strains.

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TABLE 3.
Antimicrobials recommended for AST of *Staphylococcus aureus* and interpretative breakpoints

Antimicrobials for *S. aureus* AST	MIC (µg/mL) **R is >**
Cefoxitin, FOX	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

*CLSI M100 Table 2C

Important notes: *MRSA*
Some test strains may be methicillin-resistant. **Confirmation of *mec*A presence is mandatory** in this EQAS. For this purpose, you are welcome to use the method you prefer, and upload the result as 'positive' or 'negative'. According to CLSI recommendations (M100, Table 2C), all MRSA should be regarded as resistant to all β-lactam antibiotics.

4. Reporting of results and evaluation

Please write your results in the test forms, and enter your results into the interactive web database. In addition, we kindly ask you to report in the database the tested MIC range and/or antimicrobial disk content. Finally, if **you did not use the cut-off values recommended in the protocol for interpretation of AST results, please report the breakpoints used in the database.**

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. **Results must be submitted no later than 9 September 2011.** After the deadline, the database will be closed and you will be able to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

If you do not have access to the Internet, or if you experience difficulties in entering your results, please return the completed test forms by e-mail, fax or mail to the National Food Institute, Denmark.

All results will be summarised in a report available to all participants. The data in the report will be presented with laboratory codes. A laboratory code is only known to the individual laboratory, while the complete list of laboratories and their respective codes is confidential and only known to the EURL-AR and the EU Commission. All conclusions will be public.

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

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5. How to enter results in the interactive database

Please read carefully this paragraph before entering the web page.
Remember that you need by your side the completed test forms and the breakpoint values you used.

Enter the EURL-AR EQAS 2011 start web page (http://thor.dfvf.dk/crl), write your username and password in lower-cases and press enter. Your username and password are the same used in the previous EQAS's arranged by The National Food Institute, Denmark. Do not hesitate to contact us if you experience problems with the login.

You can browse back and forth by using the back and forward keys and by clicking on the EURL logo.

Click on either "*E. coli* test results", "enterococci test results" or "staphylococci test results" based on the results you are going to upload. The description reported below is based on *Salmonella* test result entry, but it is the exact same procedure for entering *E. coli*, enterococci and staphylococci test results.

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

In the next page, you can navigate among fields with the Tab-key and the mouse.

Complete the fields related to the method used for antimicrobial susceptibility testing of *Salmonella* and the brand of discs, tablets, MIC trays, etc.

Fill in the fields related to either antimicrobial disk content or tested MIC range. If you used disk diffusion, please upload the breakpoints used for interpretation of results.

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Click on "save and go to next page"

In the data entry pages, enter the obtained values and the interpretation (R, resistant or S, susceptible) for each *E. coli*, enterococcus and staphylococcus strain.

For *E. coli* strains, remember to report also the results for the ESBL detection tests.
For *S. aureus* strains, remember to report also the results for presence/absence of *mecA*.

If you did not test for susceptibility to a given antimicrobial, please leave the field empty.

Click on "save and go to next page"

When uploading data on the reference strains, please enter the zone diameters in mm and MIC values in µg/ml. Remember to use the operator keys to show symbols like "equal to", etc... If you do not use CLSI guidelines for AST of the reference strains, please add a comment on the method used.

Click on "save and go to next page"

This page is a menu that allows you to review the input pages and approve your input.

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

Please complete the evaluation form.

Before approving your input, please be sure that you have filled in all the relevant fields because YOU CAN ONLY APPROVE ONCE! The approval blocks your data entry in the interactive database.

Appendix 5a. Breakpoints used routinely in disk diffusion - Enterococci

Antimicrobial	Lab. no.	R <= (mm)	S >= (mm)
Ampicillin, AMP	15	16	19
	18	8	10
	26	16	17
	40	16	17
Ciprofloxacin, CIP	18	18	19
	26	15	21
	40	15	21
Erythromycin, ERY	15	17	22
	18	17	18
	26	13	23
	40	13	23
Gentamicin, GEN	15	17	17
	18	13	14
	26	12	15
	40	12	15
Linezolid, LZD	15	24	24
	18	18	19
	26	20	23
	40	20	23
Streptomycin, STR	15	12	14
	18	11	12
	26	6	10
	40	11	15
Quinupristin-dalfopristin, Q-D	18	21	22
	26	15	19
Tetracycline, TET	15	17	19
	18	16	17
	26	14	19
	40	14	16
Vancomycin, VAN	15	17	17
	18	11	12
	26	14	17
	40	14	17

Appendix 5b. Breakpoints used routinely in disk diffusion - Staphylococci

Antimicrobial	Lab. no.	R <= (mm)	S >= (mm)
Chloramphenicol, CHL	4	18	
	14	22	23
	15	19	22
	18	17	18
	40	12	18
Ciprofloxacin, CIP	4	19	
	13	15	21
	14	18	22
	15	22	22
	18	18	19
	40	15	21
Erythromycin, ERY	4	18	21
	13	16	22
	14	18	22
	15	17	22
	18	18	21
	40	13	23
Florfenicol, FFN	15	15	19
	18	17	18
Gentamicin, GEN	4	18	
	13	12	15
	14		20
	15	20	20
	18	17	18
	40	12	15
Penicillin, PEN	13	28	29
	14		29
	15	29	29
	18	28	29
	40	28	29
Streptomycin, STR	13	12	15
	15	13	15
	18	11	12
	40	11	15
Sulfamethoxazole, SMX	13	12	17
	14	11	17
	18	15	16
	40	12	17
Tetracycline, TET	4	19	22
	13	14	19
	14	20	23
	15	17	19
	18	19	22
	40	14	19
Trimethoprim, TMP	4	14	17
	14	15	20
	18	14	17
	40	10	16

Appendix 5c. Breakpoints used routinely in disk diffusion - *Escherichia coli*

Antimicrobial	Lab. no.	R <= (mm)	S >= (mm)
Ampicillin, AMP	14		19
	15	14	21
	18	13	14
	40	13	17
Cefotaxime, CTX	14		26
	15	22	26
	18	17	21
	40	14	23
Ceftazidime, CAZ	14		26
	15	18	26
	18	17	21
	40	14	18
Ceftiofur, XNL	14		21
	15	17	21
	18	19	20
Chloramphenicol, CHL	14		23
	15	18	22
	18	16	17
	40	12	18
Ciprofloxacin, CIP	14		25
	15	21	25
	18	18	22
	40	15	21
Florfenicol, FFN	15	14	19
	18	16	17
Gentamicin, GEN	14		18
	15	15	18
	18	13	17
	40	12	18
Nalidixic acid, NAL	14		20
	15	14	20
	18	15	16
	40	13	19
Streptomycin, STR	15	12	15
	18	10	11
	40	11	15
Sulfamethoxazole, SMX	14		17
	15	9	16
	18	13	14
	40	12	17
Tetracycline,TET	14		19
	15	16	19
	18	14	15
	40	11	15
Trimethoprim, TMP	14		20
	15	11	16
	18	14	18
	40	10	16

Appendix 6. Acceptable ranges for the quality control strains

Enterococcus 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
Quinupristin-dalfopristin, Q-D	2 - 8
Tetracycline, TET	8 - 32
Tigecycline, TGC	0.03 - 0.12
Vancomycin, VAN	1 - 4

	Staphylococcus aureus		
	ATCC 25923		**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
Sulphonamides, SMX	24 - 30	26 - 34	32 - 128
Tetracycline, TET	24 - 34	23 - 33	0.12 - 1
Trimethoprim, TMP	19 - 26	19 - 25	1-4

Escherichia coli ATCC 25922		
Antimicrobial	**Disk diffusion***	**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
Florfenicol, 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 (in µg/ml) and disk diffusion ranges (in mm) according to CLSI M100-S21 with the exception of the MIC range for streptomycin which is according to Sensititre. In addition, the range for ciprofloxacin is extended to include 0.016 µg/ml

Appendix 7a. Summary of obtained and expected results in the enterococci trial

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL ENT 5.1	Ampicillin , AMP	S	4	96	23	1
	Chloramphenicol, CHL	R	88	12	23	3
	Ciprofloxacin , CIP	S	5	95	21	1
	Erythromycin, ERY	R	100	0	27	0
	Gentamicin, GEN	R	100	0	27	0
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	-	50	50	-	2
	Streptomycin, STR	R	100	0	26	0
	Tetracycline, TET	R	100	0	27	0
	Vancomycin, VAN	S	4	96	26	1
	TOTAL				221	9
EURL ENT 5.2	Ampicillin , AMP	S	8	92	22	2
	Chloramphenicol, CHL	S	0	100	26	0
	Ciprofloxacin , CIP	S	9	91	20	2
	Erythromycin, ERY	S	46	54	14	12
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	S	0	100	14	0
	Streptomycin, STR	S	15	85	22	4
	Tetracycline, TET	S	4	96	26	1
	Vancomycin, VAN	S	0	100	27	0
	TOTAL				221	9
EURL ENT 5.3	Ampicillin , AMP	S	8	92	22	2
	Chloramphenicol, CHL	S	0	100	26	0
	Ciprofloxacin , CIP	S	5	95	21	1
	Erythromycin, ERY	S	7	93	25	2
	Gentamicin, GEN	S	4	96	24	1
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	S	8	92	12	1
	Streptomycin, STR	S	8	92	23	2
	Tetracycline, TET	R	96	4	26	1
	Vancomycin, VAN	R	96	4	26	1
	TOTAL				221	9
EURL ENT 5.4	Ampicillin , AMP	R	96	4	23	1
	Chloramphenicol, CHL	S	4	96	25	1
	Ciprofloxacin , CIP	S	14	86	19	3
	Erythromycin, ERY	R	96	4	26	1
	Gentamicin, GEN	S	15	85	22	4
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	S	0	100	14	0
	Streptomycin, STR	R	100	0	25	0
	Tetracycline, TET	R	100	0	27	0
	Vancomycin, VAN	R	89	11	24	3
	TOTAL				221	9

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL ENT 5.5	Ampicillin , AMP	S	4	96	23	1
	Chloramphenicol, CHL	S	0	100	26	0
	Ciprofloxacin , CIP	S	5	95	21	1
	Erythromycin, ERY	R	100	0	27	0
	Gentamicin, GEN	S	8	92	24	2
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	-	50	50	-	2
	Streptomycin, STR	R	100	0	26	0
	Tetracycline, TET	R	100	0	27	0
	Vancomycin, VAN	S	4	96	26	1
	TOTAL				221	9
EURL ENT 5.6	Ampicillin , AMP	S	13	87	20	3
	Chloramphenicol, CHL	S	0	100	26	0
	Ciprofloxacin , CIP	S	14	86	19	3
	Erythromycin, ERY	R	100	0	27	0
	Gentamicin, GEN	S	8	92	23	2
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	R	62	38	8	5
	Streptomycin, STR	R	80	20	20	5
	Tetracycline, TET	S	4	96	26	1
	Vancomycin, VAN	S	4	96	26	1
	TOTAL				221	9
EURL ENT 5.7	Ampicillin , AMP	S	4	96	23	1
	Chloramphenicol, CHL	S	0	100	26	0
	Ciprofloxacin , CIP	S	9	91	20	2
	Erythromycin, ERY	S	7	93	25	2
	Gentamicin, GEN	S	12	88	22	3
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	-	50	50	-	2
	Streptomycin, STR	S	15	85	22	4
	Tetracycline, TET	S	7	93	25	2
	Vancomycin, VAN	S	4	96	26	1
	TOTAL				221	9
EURL ENT 5.8	Ampicillin , AMP	S	4	96	23	1
	Chloramphenicol, CHL	R	92	8	24	2
	Ciprofloxacin , CIP	S	5	95	21	1
	Erythromycin, ERY	R	100	0	27	0
	Gentamicin, GEN	S	12	88	22	3
	Linezolid, LZD	S	5	95	21	1
	Quinu-dalfo-pristin, Q-D	-	50	50	-	2
	Streptomycin, STR	R	100	0	26	0
	Tetracycline, TET	R	100	0	27	0
	Vancomycin, VAN	S	4	96	26	1
	TOTAL				221	9

Suceptibility tests resulted in deviations from expected results

Appendix 7b. Summary of obtained and expected results in the staphylococci trial

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL ST 5.1	Cefoxitin, FOX	R	97	3	28	1
	Chloramphenicol, CHL	S	3	97	29	1
	Ciprofloxacin, CIP	R	34	66	10	19
	Erythromycin, ERY	S	0	100	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	R	97	3	30	1
	Penicillin, PEN	R	100	0	31	0
	Streptomycin, STR	R	100	0	24	0
	Sulfamethoxazole, SMX	R	87	13	20	3
	Tetracycline, TET	R	97	3	30	1
	Trimethoprim, TMP	S	0	100	27	0
	TOTAL				272	26
EURL ST 5.2	Cefoxitin, FOX	S	3	97	28	1
	Chloramphenicol, CHL	R	97	3	29	1
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	R	97	3	31	1
	Florfenicol, FFN	R	100	0	11	0
	Gentamicin, GEN	S	3	97	30	1
	Penicillin, PEN	R	97	3	30	1
	Streptomycin, STR	R	100	0	24	0
	Sulfamethoxazole, SMX	S	23	77	17	5
	Tetracycline, TET	S	3	97	30	1
	Trimethoprim, TMP	S	7	93	25	2
	TOTAL				285	13
EURL ST 5.3	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	3	97	30	1
	Penicillin, PEN	S	0	100	30	0
	Streptomycin, STR	S	0	100	24	0
	Sulfamethoxazole, SMX	S	13	88	21	3
	Tetracycline, TET	S	3	97	30	1
	Trimethoprim, TMP	S	7	93	25	2
	TOTAL				292	7
EURL ST 5.4	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	R	100	0	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	0	100	31	0
	Penicillin, PEN	R	100	0	31	0
	Streptomycin, STR	R	100	0	24	0
	Sulfamethoxazole, SMX	S	4	96	22	1
	Tetracycline, TET	R	97	3	30	1
	Trimethoprim, TMP	S	4	96	25	1
	TOTAL				295	3

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL ST 5.5	Cefoxitin, FOX	R	97	3	28	1
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	R	100	0	30	0
	Erythromycin, ERY	S	3	97	31	1
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	0	100	31	0
	Penicillin, PEN	R	100	0	31	0
	Streptomycin, STR	R	100	0	24	0
	Sulfamethoxazole, SMX	S	0	100	23	0
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	R	100	0	26	0
	TOTAL				296	2
EURL ST 5.6	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	0	100	31	0
	Penicillin, PEN	R	97	3	29	1
	Streptomycin, STR	S	0	100	24	0
	Sulfamethoxazole, SMX	S	4	96	22	1
	Tetracycline, TET	R	97	3	30	1
	Trimethoprim, TMP	S	4	96	25	1
	TOTAL				293	4
EURL ST 5.7	Cefoxitin, FOX	S	0	100	29	0
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	0	100	31	0
	Penicillin, PEN	R	100	0	31	0
	Streptomycin, STR	R	100	0	24	0
	Sulfamethoxazole, SMX	S	4	96	22	1
	Tetracycline, TET	R	97	3	30	1
	Trimethoprim, TMP	R	100	0	26	0
	TOTAL				296	2
EURL ST 5.8	Cefoxitin, FOX	R	97	3	28	1
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	S	0	100	30	0
	Erythromycin, ERY	S	0	100	32	0
	Florfenicol, FFN	S	0	100	11	0
	Gentamicin, GEN	S	0	100	31	0
	Penicillin, PEN	R	100	0	31	0
	Streptomycin, STR	R	96	4	23	1
	Sulfamethoxazole, SMX	S	4	96	22	1
	Tetracycline, TET	R	100	0	31	0
	Trimethoprim, TMP	R	100	0	26	0
	TOTAL				295	3

Suceptibility tests resulted in deviations from expected results

Appendix 7c. Summary of obtained and expected results in the *Escherichia coli* trial

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL EC 5.1	Ampicillin, AMP	S	0	100	30	0
	Cefotaxime, CTX	S	0	100	31	0
	Ceftazidime, CAZ	S	7	93	27	2
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	S	0	100	31	0
	Ciprofloxacin, CIP	R	90	10	28	3
	Florfenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	32	0
	Nalidixic acid, NAL	S	16	84	26	5
	Streptomycin, STR	R	94	6	29	2
	Sulfamethoxazole, SMX	S	0	100	29	0
	Tetracycline, TET	R	97	3	31	1
	Trimethoprim, TMP	S	3	97	28	1
	TOTAL				358	14
EURL EC 5.2	Ampicillin, AMP	R	100	0	30	0
	Cefotaxime, CTX	S	0	100	31	0
	Ceftazidime, CAZ	S	0	100	29	0
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	S	0	100	31	0
	Ciprofloxacin, CIP	S	0	100	31	0
	Florfenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	R	94	6	30	2
	Nalidixic acid, NAL	S	0	100	31	0
	Streptomycin, STR	R	100	0	31	0
	Sulfamethoxazole, SMX	S	0	100	29	0
	Tetracycline, TET	R	97	3	31	1
	Trimethoprim, TMP	S	0	100	29	0
	TOTAL				368	4
EURL EC 5.3	Ampicillin, AMP	R	100	0	30	0
	Cefotaxime, CTX	S	0	100	31	0
	Ceftazidime, CAZ	S	3	97	28	1
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	R	100	0	31	0
	Ciprofloxacin, CIP	R	94	6	29	2
	Florfenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	32	0
	Nalidixic acid, NAL	R	100	0	31	0
	Streptomycin, STR	R	100	0	31	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	Tetracycline, TET	R	100	0	32	0
	Trimethoprim, TMP	R	97	3	28	1
	TOTAL				368	4
EURL EC 5.4	Ampicillin, AMP	R	100	0	30	0
	Cefotaxime, CTX	R	100	0	31	0
	Ceftazidime, CAZ	R	38	62	11	18
	Ceftiofur, XNL	R	100	0	9	0
	Chloramphenicol, CHL	S	3	97	30	1
	Ciprofloxacin, CIP	R	90	10	28	3
	Florfenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	R	97	3	31	1
	Nalidixic acid, NAL	S	16	84	26	5
	Streptomycin, STR	R	100	0	31	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	Tetracycline, TET	R	100	0	32	0
	Trimethoprim, TMP	R	100	0	29	0
	TOTAL				343	29

Strain	Antimicrobial	Expected results	% R	% S	Number of expected results	Number of results deviating from expected
EURL EC 5.5	Ampicillin, AMP	R	100	0	30	0
	Cefotaxime, CTX	R	100	0	31	0
	Ceftazidime, CAZ	R	90	10	26	3
	Ceftiofur, XNL	R	100	0	9	0
	Chloramphenicol, CHL	S	3	97	30	1
	Ciprofloxacin, CIP	S	3	97	30	1
	Florfenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	3	97	31	1
	Nalidixic acid, NAL	S	3	97	30	1
	Streptomycin, STR	S	3	97	30	1
	Sulfamethoxazole, SMX	S	3	97	28	1
	Tetracycline, TET	S	3	97	31	1
	Trimethoprim, TMP	S	4	96	27	1
	TOTAL				360	11
EURL EC 5.6	Ampicillin, AMP	R	100	0	30	0
	Cefotaxime, CTX	S	3	97	30	1
	Ceftazidime, CAZ	S	7	93	27	2
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	R	94	6	29	2
	Ciprofloxacin, CIP	R	77	23	24	7
	Florfenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	R	94	6	30	2
	Nalidixic acid, NAL	R	97	3	30	1
	Streptomycin, STR	R	100	0	31	0
	Sulfamethoxazole, SMX	R	100	0	29	0
	Tetracycline, TET	R	100	0	32	0
	Trimethoprim, TMP	R	100	0	29	0
	TOTAL				356	16
EURL EC 5.7	Ampicillin, AMP	S	3	97	29	1
	Cefotaxime, CTX	S	0	100	31	0
	Ceftazidime, CAZ	S	3	97	28	1
	Ceftiofur, XNL	S	0	100	9	0
	Chloramphenicol, CHL	S	0	100	31	0
	Ciprofloxacin, CIP	S	3	97	30	1
	Florfenicol, FFN	S	4	96	26	1
	Gentamicin, GEN	S	0	100	32	0
	Nalidixic acid, NAL	S	0	100	31	0
	Streptomycin, STR	S	3	97	30	1
	Sulfamethoxazole, SMX	S	0	100	29	0
	Tetracycline, TET	S	0	100	32	0
	Trimethoprim, TMP	S	0	100	29	0
	TOTAL				367	5
EURL EC 5.8	Ampicillin, AMP	R	100	0	29	0
	Cefotaxime, CTX	R	87	13	26	4
	Ceftazidime, CAZ	R	82	18	23	5
	Ceftiofur, XNL	S	0	100	7	0
	Chloramphenicol, CHL	S	0	100	30	0
	Ciprofloxacin, CIP	R	97	3	29	1
	Florfenicol, FFN	S	0	100	27	0
	Gentamicin, GEN	S	0	100	31	0
	Nalidixic acid, NAL	R	100	0	30	0
	Streptomycin, STR	S	3	97	29	1
	Sulfamethoxazole, SMX	S	0	100	28	0
	Tetracycline, TET	S	3	97	30	1
	Trimethoprim, TMP	S	29	71	20	8
	TOTAL				339	20

Suceptibility tests resulted in deviations from expected results

Appendix 8a. Deviations from expected results in the enterococci trial

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[4]	Method used for AST
1	EURL ENT 5.2	Erythromycin, ERY[1]	R	8.0	S	= 4	MIC
	EURL ENT 5.4	Gentamicin, GEN	R	64	S	<=16	
	EURL ENT 5.6	Quinu-dalfo-pristin, Q-D	S	4.00	R	= 8	
2	EURL ENT 5.1	Chloramphenicol, CHL	S	32	R	= 64	MIC
	EURL ENT 5.2	Erythromycin, ERY[1]	R	8	S	= 4	
	EURL ENT 5.3	Vancomycin, VAN	S	<=1	R	> 32	
	EURL ENT 5.6	Quinu-dalfo-pristin, Q-D	S	4	R	= 8	
6	EURL ENT 5.1	Quinu-dalfo-pristin, Q-D[2]		<=16	-	-	MIC
	EURL ENT 5.2	Erythromycin, ERY[1]	R	>8	S	= 4	
	EURL ENT 5.2	Streptomycin, STR	R	<=512	S	<=64	
	EURL ENT 5.5	Quinu-dalfo-pristin, Q-D[2]		<=8	-	-	
	EURL ENT 5.6	Quinu-dalfo-pristin, Q-D	S	<=4	R	= 8	
	EURL ENT 5.7	Erythromycin, ERY	R	>8	S	= 1	
	EURL ENT 5.7	Quinu-dalfo-pristin, Q-D[2]		<=8	-	-	
	EURL ENT 5.7	Streptomycin, STR	R	>2048	S	= 128	
	EURL ENT 5.7	Tetracycline, TET	R	>32	S	<=1	
	EURL ENT 5.8	Quinu-dalfo-pristin, Q-D[2]		<=8	-	-	
9	EURL ENT 5.2	Erythromycin, ERY[1]		4	S	= 4	MIC
	EURL ENT 5.6	Ampicillin , AMP[3]		8	S	<=2	
11	EURL ENT 5.2	Erythromycin, ERY[1]	R	8	S	= 4	MIC
	EURL ENT 5.6	Streptomycin, STR	S	128	R	= 1024	
12	EURL ENT 5.2	Erythromycin, ERY[1]	R	16	S	= 4	MIC
16	EURL ENT 5.1	Quinu-dalfo-pristin, Q-D[2]		8	-	-	MIC
	EURL ENT 5.5	Quinu-dalfo-pristin, Q-D[2]		8	-	-	
	EURL ENT 5.6	Quinu-dalfo-pristin, Q-D	S	4	R	= 8	
	EURL ENT 5.7	Quinu-dalfo-pristin, Q-D[2]		8	-	-	
	EURL ENT 5.8	Quinu-dalfo-pristin, Q-D[2]		4	-	-	
17	EURL ENT 5.2	Erythromycin, ERY[1]	R	>8	S	= 4	MIC
18	EURL ENT 5.2	Erythromycin, ERY[1]	R	13	S	= 4	DD
	EURL ENT 5.2	Gentamicin, GEN	R	9	S	<=16	
	EURL ENT 5.2	Streptomycin, STR	R	6	S	<=64	
	EURL ENT 5.3	Streptomycin, STR	R	6	S	<=64	
	EURL ENT 5.4	Gentamicin, GEN	R	11	S	<=16	
	EURL ENT 5.4	Vancomycin, VAN	S	16	R	= 16	
	EURL ENT 5.5	Gentamicin, GEN	R	10	S	<=16	
	EURL ENT 5.6	Gentamicin, GEN	R	9	S	<=16	
	EURL ENT 5.6	Tetracycline, TET	R	11	S	<=1	
	EURL ENT 5.7	Gentamicin, GEN	R	9	S	<=16	
	EURL ENT 5.7	Streptomycin, STR	R	6	S	= 128	
	EURL ENT 5.8	Gentamicin, GEN	R	9	S	<=16	
19	EURL ENT 5.2	Erythromycin, ERY[1]	R	8	S	= 4	MIC
	EURL ENT 5.3	Erythromycin, ERY	R	4	S	= 2	
	EURL ENT 5.4	Ciprofloxacin , CIP	R	8	S	= 2	
	EURL ENT 5.6	Ciprofloxacin , CIP	R	8	S	= 1	
	EURL ENT 5.6	Streptomycin, STR	S	32	R	= 1024	
	EURL ENT 5.7	Ciprofloxacin , CIP	R	8	S	= 1	
20	EURL ENT 5.1	Chloramphenicol, CHL	S	32	R	= 64	MIC
23	EURL ENT 5.6	Quinu-dalfo-pristin, Q-D	S	4	R	= 8	MIC
26	EURL ENT 5.1	Quinu-dalfo-pristin, Q-D[2]	R	14	-	-	DD
	EURL ENT 5.4	Vancomycin, VAN	S	17	R	= 16	
	EURL ENT 5.5	Quinu-dalfo-pristin, Q-D[2]	R	13	-	-	
	EURL ENT 5.6	Streptomycin, STR	S	16	R	= 1024	
	EURL ENT 5.7	Gentamicin, GEN	R	12	S	<=16	
	EURL ENT 5.7	Quinu-dalfo-pristin, Q-D[2]	R	14	-	-	
	EURL ENT 5.8	Chloramphenicol, CHL	S	12	R	= 64	
	EURL ENT 5.8	Quinu-dalfo-pristin, Q-D[2]	R	16	-	-	

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[4]	Method used for AST
29	EURL ENT 5.3	Ampicillin , AMP	R	8	S	= 4	MIC
	EURL ENT 5.6	Streptomycin, STR	S	16	R	= 1024	
33	EURL ENT 5.2	Erythromycin, ERY[1]	R	8	S	= 4	MIC
34	EURL ENT 5.1	Quinu-dalfo-pristin, Q-D[2]	S	16	-	-	MIC
	EURL ENT 5.3	Quinu-dalfo-pristin, Q-D	R	2	S	= 2	
	EURL ENT 5.5	Quinu-dalfo-pristin, Q-D[2]	S	8	-	-	
	EURL ENT 5.7	Quinu-dalfo-pristin, Q-D[2]	S	8	-	-	
	EURL ENT 5.8	Quinu-dalfo-pristin, Q-D[2]	S	8	-	-	
36	EURL ENT 5.2	Erythromycin, ERY[1]	R	8	S	= 4	MIC
37	EURL ENT 5.1	Chloramphenicol, CHL	S	32	R	= 64	MIC
39	EURL ENT 5.3	Tetracycline, TET	S	64	R	> 32	MIC
	EURL ENT 5.8	Gentamicin, GEN	R	128	S	<=16	
40	EURL ENT 5.2	Erythromycin, ERY[1]	R	12	S	= 4	DD
	EURL ENT 5.2	Streptomycin, STR	R	11	S	<=64	
	EURL ENT 5.4	Erythromycin, ERY	S	17	R	> 32	
	EURL ENT 5.4	Vancomycin, VAN	S	23	R	= 16	
	EURL ENT 5.8	Chloramphenicol, CHL	S	18	R	= 64	
41	EURL ENT 5.2	Ampicillin , AMP	R	8	S	<=2	MIC
	EURL ENT 5.2	Ciprofloxacin , CIP	R	8	S	= 1	
	EURL ENT 5.2	Tetracycline, TET	R	>16	S	<=1	
	EURL ENT 5.4	Ciprofloxacin , CIP	R	>8	S	= 2	
	EURL ENT 5.6	Ampicillin , AMP	R	8	S	<=2	
	EURL ENT 5.6	Ciprofloxacin , CIP	R	>8	S	= 1	
	EURL ENT 5.7	Tetracycline, TET	R	8	S	<=1	
42	EURL ENT 5.1	Quinu-dalfo-pristin, Q-D[2]		16	-	-	MIC
	EURL ENT 5.2	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.3	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.4	Gentamicin, GEN	R	256	S	<=16	
	EURL ENT 5.5	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.5	Quinu-dalfo-pristin, Q-D[2]		16	-	-	
	EURL ENT 5.6	Ampicillin , AMP	R	16	S	<=2	
	EURL ENT 5.6	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.7	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.7	Quinu-dalfo-pristin, Q-D[2]		32	-	-	
	EURL ENT 5.8	Gentamicin, GEN[3]		<=128	S	<=16	
	EURL ENT 5.8	Quinu-dalfo-pristin, Q-D[2]		8	-	-	
46	EURL ENT 5.4	Ampicillin , AMP	S	0.5	R	> 32	MIC
	EURL ENT 5.6	Streptomycin, STR	S	128	R	= 1024	
	EURL ENT 5.7	Streptomycin, STR	R	1024	S	= 128	

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[4]	Method used for AST
54	EURL ENT 5.1	Ampicillin , AMP	R	14	S	<=2	DD
	EURL ENT 5.1	Ciprofloxacin , CIP	R	0	S	= 1	
	EURL ENT 5.1	Linezolid, LZD	R	15	S	= 2	
	EURL ENT 5.1	Vancomycin, VAN	R	11	S	<=1	
	EURL ENT 5.2	Ampicillin , AMP	R	11	S	<=2	
	EURL ENT 5.2	Ciprofloxacin , CIP	R	11	S	= 1	
	EURL ENT 5.2	Erythromycin, ERY[1]	R	11	S	= 4	
	EURL ENT 5.2	Gentamicin, GEN	R	10	S	<=16	
	EURL ENT 5.2	Linezolid, LZD	R	19	S	= 2	
	EURL ENT 5.2	Streptomycin, STR	R	0	S	<=64	
	EURL ENT 5.3	Ampicillin , AMP	R	10	S	= 4	
	EURL ENT 5.3	Ciprofloxacin , CIP	R	14	S	<=0.5	
	EURL ENT 5.3	Erythromycin, ERY	R	12	S	= 2	
	EURL ENT 5.3	Gentamicin, GEN	R	10	S	<=16	
	EURL ENT 5.3	Linezolid, LZD	R	19	S	= 2	
	EURL ENT 5.3	Streptomycin, STR	R	0	S	<=64	
	EURL ENT 5.4	Chloramphenicol, CHL	R	12	S	= 16	
	EURL ENT 5.4	Ciprofloxacin , CIP	R	11	S	= 2	
	EURL ENT 5.4	Gentamicin, GEN	R	10	S	<=16	
	EURL ENT 5.4	Linezolid, LZD	R	19	S	= 2	
	EURL ENT 5.5	Ampicillin , AMP	R	14	S	<=2	
	EURL ENT 5.5	Ciprofloxacin , CIP	R	15	S	<=0.5	
	EURL ENT 5.5	Gentamicin, GEN	R	11	S	<=16	
	EURL ENT 5.5	Linezolid, LZD	R	18	S	= 1	
	EURL ENT 5.5	Vancomycin, VAN	R	13	S	= 2	
	EURL ENT 5.6	Ampicillin , AMP	R	0	S	<=2	
	EURL ENT 5.6	Ciprofloxacin , CIP	R	0	S	= 1	
	EURL ENT 5.6	Gentamicin, GEN	R	11	S	<=16	
	EURL ENT 5.6	Linezolid, LZD	R	17	S	= 1	
	EURL ENT 5.6	Vancomycin, VAN	R	14	S	<=1	
	EURL ENT 5.7	Ampicillin , AMP	R	15	S	<=2	
	EURL ENT 5.7	Ciprofloxacin , CIP	R	11	S	= 1	
	EURL ENT 5.7	Erythromycin, ERY	R	12	S	= 1	
	EURL ENT 5.7	Gentamicin, GEN	R	11	S	<=16	
	EURL ENT 5.7	Linezolid, LZD	R	17	S	= 2	
	EURL ENT 5.7	Streptomycin, STR	R	0	S	= 128	
	EURL ENT 5.7	Vancomycin, VAN	R	14	S	= 2	
	EURL ENT 5.8	Ampicillin , AMP	R	15	S	<=2	
	EURL ENT 5.8	Ciprofloxacin , CIP	R	12	S	= 1	
	EURL ENT 5.8	Gentamicin, GEN	R	12	S	<=16	
	EURL ENT 5.8	Linezolid, LZD	R	17	S	= 2	
	EURL ENT 5.8	Vancomycin, VAN	R	13	S	<=1	

[1], not included in the evaluation report (please refer to paragraph 3.2.1)

[2], not included in the evaluation report because there are no recommendations for quinupristin-dalfopristin susceptibility testing in *E. faecalis* (please refer to protocol, Appendix 4)

[3], not included in the evaluation report because no interpretation was reported by the participant

[4], expected values were not calculated for disk diffusion method and participants performing disk diffusion were invited to apply the interpretive breakpoins routinely used in their laboratories

Appendix 8b. Deviations from expected results in the staphylococci trial

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[2]	Method used for AST
2	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.2	Sulfamethoxazole, SMX	R	512	S	<=32	
4	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	22.43	R	= 2	DD
	EURL ST 5.8	Methicillin resistant	Neg		Pos		
6	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	<=1	R	= 2	MIC
	EURL ST 5.2	Sulfamethoxazole, SMX	R	>512	S	<=32	
	EURL ST 5.3	Gentamicin, GEN	R	>16	S	<=0.25	
	EURL ST 5.3	Sulfamethoxazole, SMX	R	>512	S	<=32	
	EURL ST 5.4	Sulfamethoxazole, SMX	R	>512	S	<=32	
	EURL ST 5.7	Sulfamethoxazole, SMX	R	<=256	S	<=32	
	EURL ST 5.8	Sulfamethoxazole, SMX	R	>512	S	<=32	
11	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	0.5	R	= 2	MIC
12	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.2	Cefoxitin, FOX	R	8	S	= 4	
13	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	23	R	= 2	DD
	EURL ST 5.1	Sulfamethoxazole, SMX	S	15	R	= 512	
18	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	22	R	= 2	DD
19	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.2	Sulfamethoxazole, SMX	R	512	S	<=32	
20	EURL ST 5.2	Sulfamethoxazole, SMX	R	>512	S	<=32	MIC
	EURL ST 5.3	Sulfamethoxazole, SMX	R	>512	S	<=32	
21	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.1	Sulfamethoxazole, SMX	S	128	R	= 512	
22	EURL ST 5.1	Cefoxitin, FOX	S	4	R	= 8	MIC
	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	
23	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
26	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
29	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
34	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.2	Chloramphenicol, CHL	S	8	R	> 64	
	EURL ST 5.2	Erythromycin, ERY	S	<=0.25	R	> 16	
	EURL ST 5.2	Penicillin, PEN	S	<=0.12	R	> 16	
	EURL ST 5.3	Trimethoprim, TMP	R	4	S	= 1	
	EURL ST 5.8	Streptomycin, STR	S	>32	R	> 64	
36	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
37	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.1	Sulfamethoxazole, SMX	S	128	R	= 512	
39	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	1	R	= 2	MIC
	EURL ST 5.2	Trimethoprim, TMP	R	4	S	= 1	
	EURL ST 5.6	Penicillin, PEN	S	0.5	R	= 0.5	
40	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	28	R	= 2	DD
	EURL ST 5.1	Gentamicin, GEN	S	15	R	> 16	
	EURL ST 5.1	Tetracycline, TET	S	16	R	= 32	
	EURL ST 5.4	Tetracycline, TET	S	16	R	= 32	
	EURL ST 5.6	Tetracycline, TET	S	21	R	= 8	
	EURL ST 5.7	Tetracycline, TET	S	16	R	> 32	

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[2]	Method used for AST
41	EURL ST 5.2	Gentamicin, GEN	R	4	S	<=0.25	MIC
	EURL ST 5.2	Sulfamethoxazole, SMX	R	>512	S	<=32	
	EURL ST 5.2	Tetracycline, TET	R	2	S	<=0.5	
	EURL ST 5.2	Trimethoprim, TMP	R	8	S	= 1	
	EURL ST 5.3	Sulfamethoxazole, SMX	R	>512	S	<=32	
	EURL ST 5.3	Tetracycline, TET	R	2	S	<=0.5	
	EURL ST 5.3	Trimethoprim, TMP	R	16	S	= 1	
	EURL ST 5.4	Trimethoprim, TMP	R	16	S	= 1	
	EURL ST 5.5	Erythromycin, ERY	R	4	S	= 0.5	
	EURL ST 5.6	Sulfamethoxazole, SMX	R	256	S	<=32	
	EURL ST 5.6	Trimethoprim, TMP	R	16	S	<=0.5	
46	EURL ST 5.1	Chloramphenicol, CHL	R	8	S	= 4	MIC
54	EURL ST 5.1	Ciprofloxacin, CIP[1]	S	24	R	= 2	DD
	EURL ST 5.5	Cefoxitin, FOX	S	17	R	= 16	
	EURL ST 5.8	Cefoxitin, FOX	S	17	R	= 16	

[1], not included in the evaluation report (please refer to paragraph 3.2.2)

[2], expected values were not calculated for disk diffusion method and participants performing disk diffusion were invited to apply the interpretive breakpoins routinely used in their laboratories

Appendix 8c. Deviations from expected results in the *Escherichia coli* trial

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[2]	Method used for AST
1	EURL EC 5.7	Streptomycin, STR	R	16	S	<=8	MIC
4	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
	EURL EC 5.6	Ciprofloxacin, CIP	S	0.06	R	= 0.06	
	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	
6	EURL EC 5.4	Ceftazidime, CAZ[1]	S	<=0.5	R	= 1	MIC
9	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
14	EURL EC 5.1	Nalidixic acid, NAL	R	13	S	= 16	DD
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	28	R	= 1	
	EURL EC 5.4	Nalidixic acid, NAL	R	14	S	= 16	
	EURL EC 5.6	Ciprofloxacin, CIP	S	30	R	= 0.06	
	EURL EC 5.8	Cefotaxime, CTX	S	29	R	= 1	
	EURL EC 5.8	Ceftazidime, CAZ	S	26	R	= 2	
15	EURL EC 5.1	Nalidixic acid, NAL	R	11	S	= 16	DD
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	27	R	= 1	
	EURL EC 5.4	Nalidixic acid, NAL	R	14	S	= 16	
	EURL EC 5.5	Ceftazidime, CAZ	S	26	R	= 4	
	EURL EC 5.8	Cefotaxime, CTX	S	30	R	= 1	
	EURL EC 5.8	Ceftazidime, CAZ	S	28	R	= 2	
16	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	MIC
17	EURL EC 5.1	Nalidixic acid, NAL	R	32	S	= 16	MIC
	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	
18	EURL EC 5.1	Ciprofloxacin, CIP	S	28	R	= 0.5	DD
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	27	R	= 1	
	EURL EC 5.4	Ciprofloxacin, CIP	S	22	R	= 0.5	
	EURL EC 5.8	Cefotaxime, CTX	S	28	R	= 1	
	EURL EC 5.8	Ceftazidime, CAZ	S	26	R	= 2	
19	EURL EC 5.1	Nalidixic acid, NAL	R	16	S	= 16	MIC
	EURL EC 5.3	Ciprofloxacin, CIP	S	0.032	R	= 0.25	
	EURL EC 5.4	Ciprofloxacin, CIP	S	0.002	R	= 0.5	
	EURL EC 5.6	Ciprofloxacin, CIP	S	0.032	R	= 0.06	
20	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	
21	EURL EC 5.4	Chloramphenicol, CHL	R	>64	S	= 4	MIC
	EURL EC 5.4	Nalidixic acid, NAL	R	64	S	= 16	
	EURL EC 5.5	Chloramphenicol, CHL	R	64	S	= 4	
	EURL EC 5.5	Ciprofloxacin, CIP	R	0.25	S	<=0.015	
	EURL EC 5.5	Gentamicin, GEN	R	16	S	= 1	
	EURL EC 5.5	Nalidixic acid, NAL	R	64	S	<=4	
	EURL EC 5.5	Streptomycin, STR	R	128	S	<=8	
	EURL EC 5.5	Sulfamethoxazole, SMX	R	1024	S	<=64	
	EURL EC 5.5	Tetracycline, TET	R	32	S	<=2	
	EURL EC 5.5	Trimethoprim, TMP	R	32	S	<=1	
	EURL EC 5.6	Cefotaxime, CTX	R	4	S	<=0.12	
	EURL EC 5.6	Ceftazidime, CAZ	R	1	S	= 0.064	
22	EURL EC 5.1	Streptomycin, STR	S	128	R	> 128	MIC
	EURL EC 5.3	Trimethoprim, TMP	S	<0.5	R	> 32	
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	
23	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
25	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
26	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
29	EURL EC 5.1	Ceftazidime, CAZ	R	8	S	= 0.25	MIC
30	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
32	EURL EC 5.4	Ceftazidime, CAZ[1]	S	<=0.5	R	= 1	MIC
33	EURL EC 5.1	Nalidixic acid, NAL	R	32	S	= 16	MIC
	EURL EC 5.4	Nalidixic acid, NAL	R	>16	S	= 16	
	EURL EC 5.6	Chloramphenicol, CHL	S	32	R	= 32	
	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	

Laboratory ID	Strain	Antimicrobial	Obtained interpretation	Obtained value (µg/ml if MIC; mm if DD)	Expected interpretation	Expected MIC (µg/ml)[2]	Method used for AST
34	EURL EC 5.4	Nalidixic acid, NAL	R	32	S	= 16	MIC
	EURL EC 5.6	Ceftazidime, CAZ	R	1	S	= 0.064	
36	EURL EC 5.8	Trimethoprim, TMP	R	4	S	<=1	MIC
39	EURL EC 5.1	Ceftazidime, CAZ	R	2	S	= 0.25	MIC
	EURL EC 5.1	Trimethoprim, TMP	R	1	S	<=1	
	EURL EC 5.3	Ceftazidime, CAZ	R	1	S	= 0.25	
	EURL EC 5.7	Ceftazidime, CAZ	R	1	S	= 0.125	
40	EURL EC 5.1	Ciprofloxacin, CIP	S	32	R	= 0.5	DD
	EURL EC 5.1	Streptomycin, STR	S	12	R	> 128	
	EURL EC 5.1	Tetracycline, TET	S	12	R	> 32	
	EURL EC 5.2	Gentamicin, GEN	S	18	R	= 16	
	EURL EC 5.2	Tetracycline, TET	S	12	R	> 32	
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	24	R	= 1	
	EURL EC 5.5	Ceftazidime, CAZ	S	20	R	= 4	
	EURL EC 5.6	Chloramphenicol, CHL	S	19	R	= 32	
	EURL EC 5.6	Ciprofloxacin, CIP	S	32	R	= 0.06	
	EURL EC 5.6	Gentamicin, GEN	S	15	R	= 16	
	EURL EC 5.6	Nalidixic acid, NAL	S	16	R	= 32	
	EURL EC 5.7	Ciprofloxacin, CIP	R	28	S	< 0.015	
	EURL EC 5.8	Cefotaxime, CTX	S	30	R	= 1	
	EURL EC 5.8	Ceftazidime, CAZ	S	26	R	= 2	
41	EURL EC 5.4	Ceftazidime, CAZ[1]	S	1	R	= 1	MIC
42	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
	EURL EC 5.6	Ciprofloxacin, CIP	S	0.06	R	= 0.06	
	EURL EC 5.8	Trimethoprim, TMP	R	8	S	<=1	
46	EURL EC 5.4	Ceftazidime, CAZ[1]	S	0.5	R	= 1	MIC
	EURL EC 5.6	Ciprofloxacin, CIP	S	<=0.125	R	= 0.06	
	EURL EC 5.7	Ampicillin,Â AMP	R	64	S	= 4	
	EURL EC 5.8	Streptomycin, STR	R	128	S	<=8	
	EURL EC 5.8	Tetracycline, TET	R	64	S	<=2	
	EURL EC 5.8	Trimethoprim, TMP	R	>32	S	<=1	
54	EURL EC 5.1	Ciprofloxacin, CIP	S	23	R	= 0.5	DD
	EURL EC 5.2	Florfenicol, FFN	R	11	S	= 4	
	EURL EC 5.2	Gentamicin, GEN	S	24	R	= 16	
	EURL EC 5.3	Ciprofloxacin, CIP	S	23	R	= 0.25	
	EURL EC 5.4	Ceftazidime, CAZ[1]	S	23	R	= 1	
	EURL EC 5.4	Ciprofloxacin, CIP	S	23	R	= 0.5	
	EURL EC 5.4	Florfenicol, FFN	R	10	S	= 8	
	EURL EC 5.4	Gentamicin, GEN	S	26	R	>= 16	
	EURL EC 5.5	Ceftazidime, CAZ	S	22	R	= 4	
	EURL EC 5.6	Ciprofloxacin, CIP	S	26	R	= 0.06	
	EURL EC 5.6	Florfenicol, FFN	R	11	S	= 8	
	EURL EC 5.6	Gentamicin, GEN	S	25	R	= 16	
	EURL EC 5.7	Florfenicol, FFN	R	15	S	= 8	
	EURL EC 5.8	Ceftazidime, CAZ	S	23	R	= 2	
	EURL EC 5.8	Ciprofloxacin, CIP	S	17	R	= 2	

[1], not included in the evaluation report (please refer to paragraph 3.2.3)

[2], expected values were not calculated for disk diffusion method and participants performing disk diffusion were invited to apply the interpretive breakpoins routinely used in their laboratories

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