DTU

The 30th EURL-AR Proficiency Test
Escherichia coli, Salmonella, Campylobacter and *Staphylococcus aureus* (2022)

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DANAK
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Community Reference Laboratory Antimicrobial Resistance

The 30th EURL-AR Proficiency Test *Escherichia coli, Salmonella, Campylobacter* and *Staphylococcus aureus* (2022)

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli, Salmonella, Campylobacter* and *S. aureus*
2022, final version, 1. ed

Table of Contents

List of Appendices

- Appendix 1: Pre-notification
- Appendix 2a: Protocol
- Appendix 2b: Test Forms
- Appendix 3a: Cover Letter
- Appendix 3b: Instructions for opening and reviving lyophilised cultures
- Appendix 3c: Subculture and maintenance of Quality Control strains
- Appendix 4: Expected MIC values and phenotype interpretation
- Appendix 5: List of deviations

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus* 2022, final version, 1. ed

1. Introduction

This report describes and summarizes the results of the 30th proficiency test (PT) conducted by the National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). This PT focuses on antimicrobial susceptibility testing (AST) of four bacterial species or genera: *Escherichia coli*, *Salmonella*, *Campylobacter and Staphylococcus aureus.* For *E. coli* this is the 15th PT, for *Salmonella* and *Campylobacter* it is the 17th, whereas for *S. aureus*, it is the 13th. The PT includes:

 a) The categorisation of the relevant *E. coli* and *Salmonella* strains as ESBL-, AmpC- and carbapenemase-phenotypes.

 b) The identification of the species of the *Campylobacter* strains as *C. jejuni* or *C. coli*.

 c) The identification of the *S. aureus* strains as *mecA/mecC* positive or negative.

The current PT aims to: i) monitor the quality of AST results produced by National Reference Laboratories (NRL-AR), ii) identify laboratories which may need assistance to improve their performance in AST, and iii) determine possible topics for further research or collaboration. The expected results were generated by performing Minimum Inhibitory Concentration (MIC) determinations for all test strains in two different occasions at the Technical University of Denmark, National Food Institute (DTU Food). The expected results were verified by an internationally recognized reference laboratory (Statens Serum Institut, SSI, Denmark). Finally, MIC determination was performed at DTU Food after preparation of the agar stab culture/charcoal swab for shipment to participants, to confirm that the vials contained the correct strains corresponding to the expected MIC values.

The evaluation of the submitted results is based on interpretations of MIC values determined by the participants. This agrees with the method used by Member States (MS) to report AST data to the European Food Safety Authority (EFSA) and complies with the main objective of this PT, i.e., to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *E. coli*, *Salmonella, Campylobacter* and *S. aureus* reported to EFSA by different laboratories, as stated in the protocol. The criteria used for the evaluation of the submitted data are:

 1) The EURL-AR network agreed on setting the acceptable deviation level for each laboratory performance on AST to 5% for each microorganism tested (*E. coli*, *Salmonella, Campylobacter* and *S. aureus*).

 2) In 2008, 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.

Evaluation of a result as "deviating from the expected interpretation" should be carefully analysed in a self-evaluation procedure, performed by the participants, including also considerations related to any corrective actions introduced in the laboratory. Note that it is not considered a mistake to obtain a one two-fold dilution difference in the MIC value of a specific antimicrobial, when testing the same strains, since methods used for MIC determination have limitations. In case the expected MIC is close to the breakpoint value for categorising the strain as susceptible or resistant, one two-fold dilution difference - which is acceptable - may result in two different interpretations, *i.e.*, the same strain can be categorised as susceptible or resistant. The present report is based on evaluation of AST interpretations; therefore, some participants may find their results classified as incorrect even though the actual MIC value they reported is only one two-fold dilution away from the expected MIC value. These cases are referred to as "breakpoint issues". For each strain-antimicrobial combination with less than 75% correct results, the deviations were further examined and for cases where most of the deviations were due to "breakpoint issues", all scores for the particular strain-antimicrobial combination were blanked. In the organisation of the PT, the aim is to avoid these situations by selecting test strains with MIC values distant from the epidemiological cut offs (ECOFF) values.

This report is approved in its final version by a technical advisory group composed by competent representatives from all NRL-ARs. This group meets annually at the EURL-AR workshop. All

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

conclusions presented in this report are publicly available. Participating laboratories are identified by codes and each code is known only by the corresponding laboratory. The full list of laboratory codes is confidential and known only by relevant representatives of the EURL-AR and the EU Commission. The EURL-AR is accredited by DANAK as provider of PT (accreditation no. 516); working with zoonotic pathogens and indicator organisms as bacterial isolates (identification, serotyping and antimicrobial susceptibility testing).

1.1. Participants in PT 2022

A pre-notification (Appendix 1) to announce the EURL-AR PT on AST of *E. coli*, *Salmonella*, *Campylobacter* and *S. aureus* was distributed on 30 June 2022 by e-mail to the 45 laboratories in the EURL-AR-network contact list including all EU countries and, in addition, Iceland, North Macedonia, Norway, Serbia, Switzerland, Turkey and the United Kingdom. Participating laboratories from non-EU countries or laboratories not designated as NRL-AR of their country were charged a fee for their participation, whereas the NRLs from EU Member States (one per MS) participated free of charge.

Figure 1. Participating countries in the 30th EURL-AR PT (n=33) that performed antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and Staphylococcus (S. aureus). The map was created on MapChart.

Data from 33 countries are presented in this report, each represented by one laboratory, except for France, for which the microorganisms were analysed by two laboratories (one analysed *E. coli*, *Salmonella* and *S. aureus* and the other analysed *Campylobacter*). The results evaluated and presented in this report are from the NRLs designated by the MS (n=27) and NRLs in affiliated non-MS (n=6) (Iceland, North Macedonia, Norway, Serbia, Switzerland and the United Kingdom). All the countries

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

signed up for all organisms included in the present PT, except for Serbia, which signed up for *E. coli* and *Salmonella* and North Macedonia which signed up for *E. coli*. The level of participation per country is presented in Figure 1. In total, this report evaluates 33, 32, 31 and 32 sets of results from the *E. coli*, *Salmonella, Campylobacter* and *S. aureus* trials respectively.

1.2. Strains

Eight strains for each organism (*E. coli*, *Salmonella*, *Campylobacter* and *S. aureus*) were selected among isolates from the strain collection at DTU Food, based on their antimicrobial resistance (AMR) profiles and their MIC values to the tested antimicrobials. The methicillin resistant *S. aureus* strain ST-17.7 (*mecC* positive) was kindly provided by the National Veterinary Institute, Uppsala, Sweden. For quality assurance purposes, one strain per bacterial species has been included in all PT iterations performed to date, representing an internal control.

Prior to distribution of the test strains, DTU Food performed AST on the test strains and the AST profiles of all test strains were verified by the Statens Serum Institut, SSI, Denmark. When MIC values from the different tests were not in agreement but varied +/- one two-fold dilution step, the latest value obtained by DTU Food was selected as the reference value. Quality assurance reference strains *E. coli* CCM 3954 (ATCC 25922), *C. jejuni* CCM 6214 (ATCC 33560) and *S. aureus* ATCC 29213 had been forwarded to all participating laboratories when they were new participants with instructions to store and maintain them for quality assurance purposes and future PT trials. Moreover, the EURL-AR has distributed *Acinetobacter baumannii* (2012-70-100-69) as well as *C. coli* (2012-70-443-2) for the purpose of performing internal method QC when performing ASTs for *E. coli, Salmonella,* or *Campylobacter* respectively. The obtained results from the EURL-AR internal method QC strains were captured in the webtool and are presented in the laboratories' individual evaluation report. However, no further overall analysis of the EURL-AR internal method QC strain results is performed for the purpose of this PT report.

1.3. Antimicrobials

The antimicrobials tested in this PT are listed in the protocol (Appendix 2). The antimicrobials tested correspond to the panel of antimicrobials listed in the Commission Implementing Decision 2020/1729/EU (international reference method ISO standard 20776-1:2019). The method applied for the AST was the ISO standard, ISO 20776-1 "Clinical laboratory testing and *in vitro* diagnostic test system – Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices", and, in addition, the following guidelines/standards from the Clinical and Laboratory Standards Institute (CLSI) were applied: Document M7-A11 (2019) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Eleventh Edition"; document M100, 32nd ed. (2022) "Performance Standards for Antimicrobial Susceptibility Testing", document VET01 (2018) "Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals" – Fifth Edition; and document VET06 (2017) "Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated from Animals" – First Edition.

MIC results were interpreted by using the interpretative criteria listed the PT protocol (Appendix 2) which represent epidemiological cut-off values developed by EUCAST (www.eucast.org), where these were not available, tentative values were applied (Appendix 2). Results for beta-lactam resistance mechanisms were interpreted according to the most recent EFSA recommendations also included as an appendix in the PT protocol (Appendix 2). MIC determination of the *E. coli* and *Salmonella* test strains was performed using the Sensititre system EUVSEC3 and EUVSEC2, while for *Campylobacter* and *S. aureus* MIC determination was performed using Sensititre EUCAMP3 and EUST2, respectively (Trek Diagnostic Systems Ltd, UK). The panels of antimicrobials included in EURL-AR PT 2022 are

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

presented in Table 1.

1.4. Distribution

In September 2022, bacterial strains in agar stab cultures (*E. coli*, *Salmonella* and *S. aureus*) or charcoal swabs in transport media (Stuarts) (*Campylobacter* spp.), together with a cover letter (Appendix 3) were dispatched in double pack containers (class UN 6.2) to the participating laboratories. The shipment (UN3373, biological substances category B) was sent according to International Air Transport Association (IATA) regulations.

Table 1. Panels of antimicrobials used in this EURL-AR proficiency test 2022.

E. coli & Salmonella		Campylobacter	S. aureus
Panel 1 (EUVSEC3)	**Panel 2 (EUVSEC2)**	**Panel 1 (EUCAMP3)**	**Panel 1 (EUST2)**
Amikacin (AMI) Ampicillin (AMP) Azithromycin (AZI) Cefotaxime (FOT or Ceftazidime (TAZ or Chloramphenicol (CHL) Ciprofloxacin (CIP) Colistin (COL) Gentamicin (GEN) Meropenem (MERO) Nalidixic acid (NAL) Sulfonamides (SMX) Tetracycline (TET) Tigecycline (TGC) Trimethoprim (TMP)	Cefepime (FEP) Cefotaxime (FOT or CTX) Cefotaxime+clavulanic acid (F/C) Cefoxitin (FOX) Ceftazidime (TAZ or CAZ) Ceftazidime+clavulanic acid Ertapenem (ETP) Imipenem (IMI) Meropenem (MERO) Temocillin (TRM)	Chloramphenicol Ciprofloxacin (CIP) Ertapenem (ETP) Erythromycin (ERY) Gentamicin (GEN) Tetracycline (TET)	Cefoxitin (FOX) Chloramphenicol (CHL) Ciprofloxacin (CIP) Clindamycin (CLI) Erythromycin (ERY) Fusidic acid (FUS) Gentamicin (GEN) Kanamycin (KAN) Linezolid (LZD) Mupirocin (MUP) Penicillin (PEN) Quinupristin-dalfopristin

1.5. Procedure

Protocols and all relevant information were uploaded on the EURL-AR website (http://www.eurl-ar.eu), thereby PT participants could access necessary information at any time. Participants were instructed to subculture charcoal swabs immediately and store the agar stabs at 4ºC (dark) until performance of AST. Information related to the handling of the test strains and reference strains was made available (Appendix 2 and 3). The participants were instructed to apply the interpretative criteria listed in the protocol (Appendix 2). Instructions for interpretation of AST results allowed for categorisation of strains as resistant or susceptible. Categorisation as 'intermediate' was not accepted.

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

As regards the method for performing the antimicrobial susceptibility testing, the protocol referred to the Commission Implementing Decision 2020/1729/EU and instructed participants to perform the international reference method for antimicrobial susceptibility testing, *i.e.*, dilution methods performed according to the methods described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), accepted as the international reference method ISO standard 20776-1:2019.

A mandatory part of the PT was to detect ESBL-, AmpC- and carbapenemase-producing strains and interpret results according to the most recent EFSA recommendations as described in the protocol.

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

Results for QC reference strains were MIC values for the reference strains *E. coli* (ATCC 25922) (for both the *E. coli* and the *Salmonella* trial), *C. jejuni* (ATCC 33560) and *S. aureus* (ATCC 29213). The results were evaluated towards the quality control ranges according to the relevant guidelines, *i.e.*, the CLSI documents VET06 (2017) or M100, 32nd ed. (2022).

All participating laboratories were invited to submit the obtained results into an electronic record sheet at the EURL-AR webtool through a secured individual login and password. In addition, participants were encouraged to complete an evaluation form with the aim to improve future PT trials. The database was finally closed, and evaluations were made available to participants on 15 March 2023. After this date, the participants were invited to login to retrieve an individual, database-generated report which contained an evaluation of the submitted results including possible deviations from the expected interpretations. Deviations in interpretation (resistant or susceptible) were categorised as 'incorrect' (score=0), as were also deviations concerning confirmation of an isolate as extended spectrum beta-lactamase- (ESBL-), AmpC- or carbapenemase-producer, deviations in relation to the species detection of *Campylobacter* and deviations of the identification of *mecA* or *mecC* genes in the *S. aureus* test strains.

2. Results and Discussion

The participants were requested to report AST results, i.e., MIC values and the phenotype interpretation as resistant or susceptible, based on the ECOFF values provided in the protocol. Only the phenotype interpretation was evaluated, whereas the MIC values were used as supplementary information. The percent deviation level was calculated for each strain-antimicrobial combination, per trial. For strain-antimicrobial combinations with more than or equal to 25% deviation level, the discrepancies were further analysed to evaluate if they were caused by a break point issue (see Introduction). In cases for which the high deviation level was mainly due to a breakpoint issue, the results were omitted from the evaluation, i.e. all scores (both 1 and 0) for the particular strain-antimicrobial combination were blanked. The overview of all the strain-antimicrobial combinations that were omitted from the evaluation are presented in Table 2. The percent deviation level was calculated for each participating laboratory per trial, after blanking the scores for the specific strain-antimicrobial combinations, as described above.

The overall performance of the participants for each trial, as well as the performance for the internal control strains were expressed as percent deviation level and are presented in Figure 2, along with historical data from previous EQAS iterations. The AST data from 2022 reflect a high level of performance, with the overall deviation level for each trial ranging from 0.7% (*S. aureus* trial) to 1.2% (*Salmonella* trial), and for the internal control strains ranging from 0.0% (*Campylobacter* trial) to 1.6% (*E. coli* trial).

The data from ESBL-phenotype identification were evaluated based on the submitted MIC values for relevant antimicrobials. With the aim of concluding on the strains' ESBL-, AmpC- and carbapenemase phenotype, two antimicrobial panels were included in the testing of the *E. coli* and *Salmonella* strains as also specified in the EU regulation 2020/1729/EU. Test strains found resistant to FOT, TAZ or MERO on the first panel (see 2020/1729/EU, Table 1) were additionally tested on the second panel (see 2020/1729/EU, Table 4), according to the protocol indications. In the following sections, the performance of the participants is presented and discussed in detail, individually for each trial (*E. coli*, *Salmonella*, *Campylobacter* and *S. aureus*).

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

Table 2. Strain-antimicrobial combinations that were omitted from the evaluation.

Trial	Strain/antimicrobial combination omitted from the evaluation (Scores blanked)	%Deviation level
E. coli	EC-17.1/Cefoxitin	30.3
E. coli	EC-17.7/Chloramphenicol	53.1
E. coli	EC-17.7/Imipenem	48.5
Salmonella	S-17.1/Amikacin	31.3
Salmonella	S-17.2/Tigecycline	31.3
Salmonella	S-17.3/Imipenem	67.7
Campylobacter	C-17.8/Chloramphenicol	86.7
S. aureus	ST-17.1/Ciprofloxacin	34.6
S. aureus	ST-17.6/Quinopristin-dalfopristin	68.0
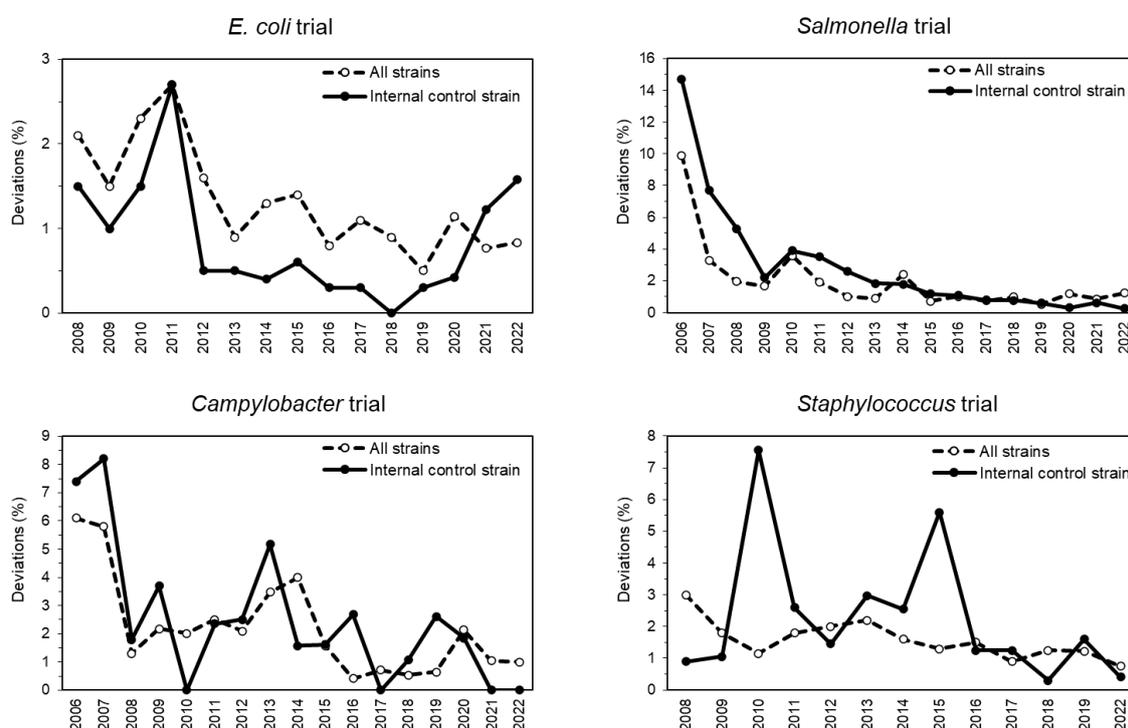
Figure 2. A comparison between the EURL-AR proficiency tests since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing for each trial (Top Left: E. coli, Top Right: Salmonella, Bottom Left: Campylobacter, Bottom Right: S. aureus).

2.1. *E. coli* trial

2.1.1. AST data

Expected MIC values and phenotype interpretation (R/S) for the strains included in the *E. coli* trial are presented in Appendix 4. The percent deviation level for each laboratory in the *E. coli* trial is presented in Figure 3 and was generally low. One third of the participants (n=11) reported all result correctly, and approximately 36% (n=12) reported only one incorrect result (deviation level 0.5%). Laboratories #26 and #64 obtained the highest deviation level slightly below the 5% limit (4.8 and 4.3% respectively). Approximately one forth of the participants (n=8) reported two or three incorrect results (deviation level from 1.0 to 2.7 percent. Similarly, the deviation level for each strain-antimicrobial combination was

generally low (maximum 12%) with the exception of EC-17.3/SMX, which obtained a deviation level of 24% (Figure 4).. From the nine laboratories that reported an incorrect phenotype interpretation for EC-17.3/SMX (R instead of S), eight reported MIC values which were 16 to 64 times higher than the expected value (8 mg/L). One laboratory reported an MIC value of 16 mg/L, which is one two-fold dilution higher than the expected value, and therefore within the method limitations. According to the international guidelines for reading the MIC values for broth microdilution, the MIC for SMX should be read at the lowest concentration that inhibits ≥80 % of growth as compared to the growth control. Not following the above guideline could explain the higher MIC values reported for EC-17.3/SMX by eight laboratories. Lastly, the following strain antimicrobial combinations were omitted from the analysis (scores blanked) as the percent deviation level for these combinations was greater than 25% and was mainly due to breakpoint issues: EC-17.1/FOX (30.3%), EC-17.7/CHL (53.1%) and EC-17.7/IMI (48.5%) – see also Table 2 and text above.
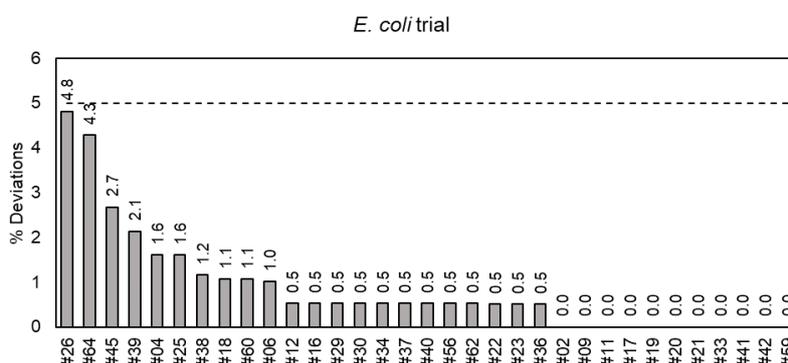

Figure 3. Percent deviation level for each laboratory, AST results, E. coli trial.

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed
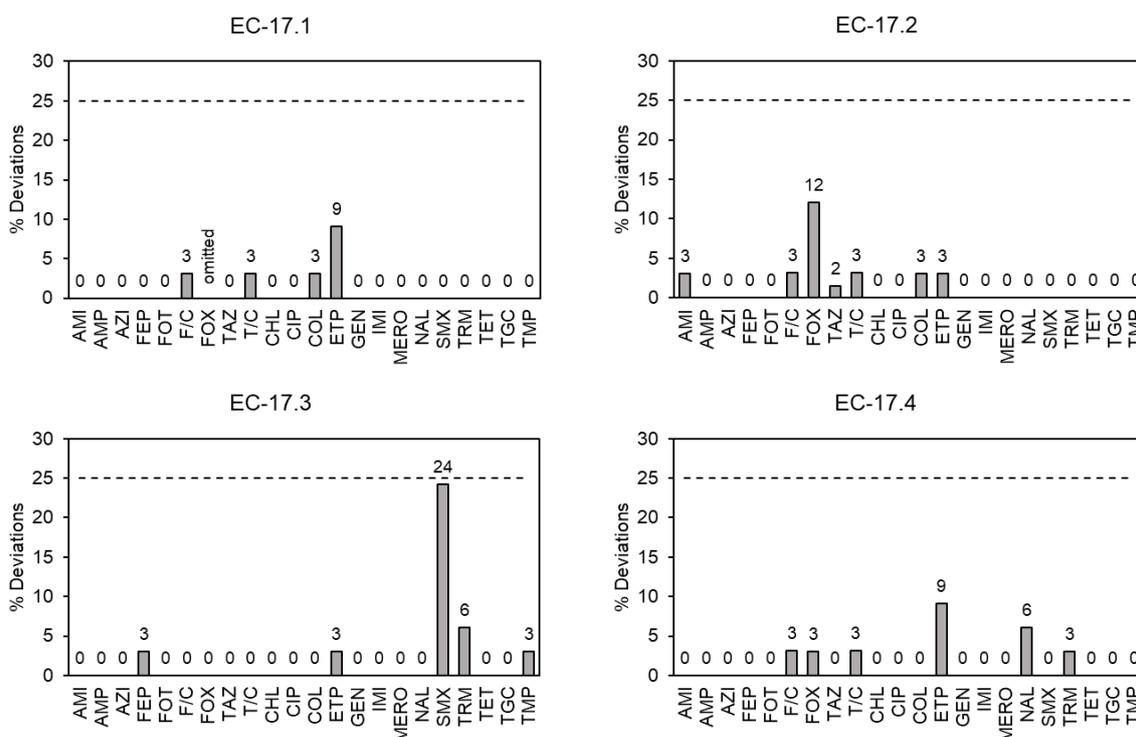
Figure continues from previous page.
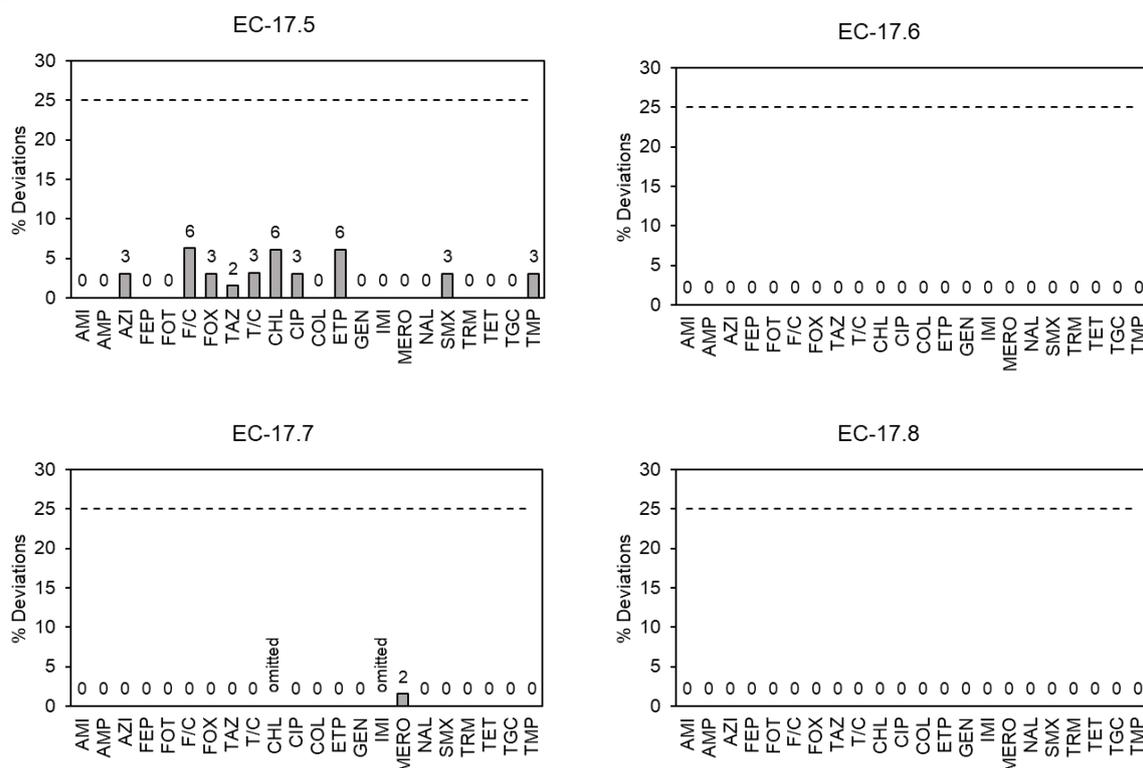
Figure 4. Percent deviation level for each strain-antimicrobial combination, E. coli trial.

2.1.2. ESBL phenotype identification

The expected ESBL phenotypes for each strain of the *E. coli* trial are presented in *Table 3*. All laboratories tested the ESBL-, carbapenemase- and AmpC-phenotype strains to panel 2. All participants identified the expected ESBL phenotype for EC-17.5 (ESBL), EC-17.6 (susceptible) and EC-17.7 (carbapenemase).

For strain EC-17.1, aproximately half of the participants categorised it as ESBL+AmpC, which was the expected phenotype. Around 27% of the participants (n=9) reported an ESBL phenotype instead for this strain. From these nine laboratories, the majority (n=8) reported an MIC value for FOX of 8 mg/L, while an MIC value of 16 mg/L was expected. The reported value is within the method limitations (one two-fold dilution lower), and this strain/antimicrobial combination was omitted from the evaluation (Table 2); however, when $MIC_{FOX} \leq 8$, the strain is categorized as ESBL. Therefore, both ESBL+AmpC as well as ESBL phenotypes were accepted for EC-17.1. Around one fifth of the participants (n=7) reported a phenotype "Other" for this strain, which is also accepted, as MERO$\leq$0.12 and ETP>ECOFF (see criteria for interpretation of *E. coli* and *Salmonella* panel 2 results from EFSA).

For strain EC-17.2, four laboratories reported MIC_{FOX} value of 16mg/L, which is one two-fold dilution higher than the expected value (8 mg/L), however this lead to an unexpected phenotype interpretation for this strain by these laboratories (ESBL+AmpC instead of ESBL). This appears as a mistake at the individual evaluation report of these laboratories, however it should be handled internally as a discrepancy due to method limitations. All but one laboratories reported the expected phenotype for EC-17.3 (AmpC); however, one categorized the strain as "Other" even though the submitted MIC results from this laboratory fully support an AmpC phenotype, and at the same time do not support any of the "Other" phenotypes in the EFSA criteria for phenotype interpretation.

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

Table 3. Expected ESBL phenotype for E. coli trial.

Strain	Criteria	Expected phenotype	Genotype
EC-17.1	FOT>1, TAZ>1, MEM≤0.12, FOX>8, SYN F/C, SYN T/C, ETP>ECOFF	ESBL+AmpC-phenotype **or** ESBL or Other	$bla_{CTX-M-55}$
EC-17.2	FOT>1, TAZ>1, MERO≤0.12, FOX<=8, SYN F/C, SYN T/C	ESBL-phenotype	$bla_{CTX-M-14}$
EC-17.3	FOT>1, TAZ>1, MEM≤0.12, FOX>8, No SYN F/C, No SYN T/C	AmpC-phenotype	ampC promoter (g.-42C>T)
EC-17.4	FOT>1, MERO≤0.12, FOX<=8, SYN F/C, ETP>ECOFF	ESBL-phenotype **or** Other	$bla_{CTX-M-65}$, $bla_{TEM-207}$
EC-17.5	FOT>1, TAZ>1, MEM≤0.12, FOX>8, SYN F/C, SYN T/C	ESBL-phenotype	bla_{CTX-M1}
EC-17.6	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
EC-17.7	MERO>0.12	Carbapenemase-phenotype	bla_{OXA-48}, $bla_{CTX-M-27}$, bla_{DHA-1}
EC-17.8	MERO>0.12	Carbapenemase-phenotype	$bla_{OXA-181}$, bla_{NDM-1}, bla_{CMY-6}
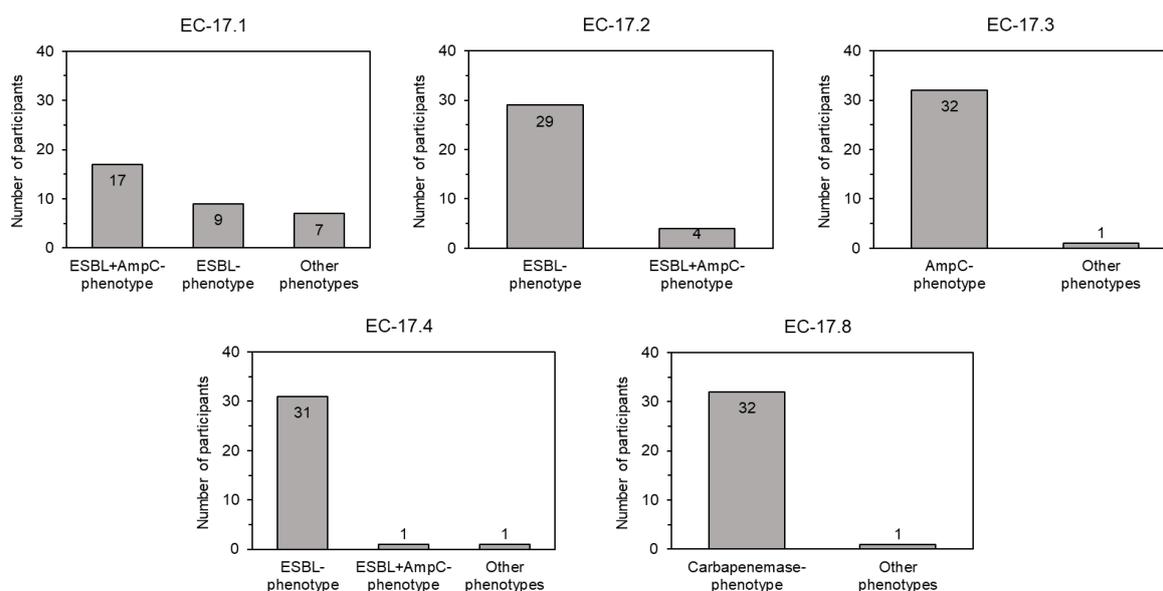
Figure 5. Obtained ESBL phenotypes for strains EC-17.1, EC-17.2, EC-17.3 EC-17.4 and EC-17.8.

Almost all the participants (31/33) reported the expected phenotype (ESBL) for strain EC-17.4. One participant reported an MIC value for FOX of 16, while a value of 8 mg/L was expected, and that lead to reporting an ESBL+AmpC phenotype for this strain. Even though this was treated as a mistake in the individual evaluation report, it should be handled as a discrepancy due to method limitations. One laboratory categorized the strain as "Other" phenotypes, because MERO≤0.12 and ETP>ECOFF, which is also accepted. Lastly, one participant categorized strain EC-17.8 as "Other" even though the reported MIC values do not support this phenotype, and was therefore handled as a mistake. The rest of the participants (32/33) reported the expected phenotype for strain EC-17.8 (carbapenemase producer).

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

2.1.3. Reference strain results

For the reference strain *E. coli* ATCC25922 results, deviations were defined as submitted MIC values that were outside the quality control (QC) acceptance intervals (Table 4), as defined in the CLSI M100 32nd edition. No QC range was available for AZI, F/C, T/C and TRM. The deviations for the reference strain results are presented in Figure 6. Almost all participants reported MIC_{FOT}<=0.25, which is one two-fold dilution higher than than the high limit of the QC range (0.03-0.125) but that was the lowest concentration in the EUVSEC panels, so it was accepted.

Table 4. Quality control (QC) acceptance intervals for the reference strain E. coli ATCC25922.

Antimicrobial	MIC range
AMI	0.5-4
AMP	2-8
AZI	none
FEP	0.016-0.125
FOT	0.03-0.125
F/C	none
FOX	2-8
TAZ	0.06-0.5
T/C	none
CHL	2-8
CIP	0.004-0.016
COL	0.25-2
ETP	0.004-0.016
GEN	0.25-1
IMI	0.06-0.25
MERO	0.008-0.06
NAL	1-4
SMX	8-32
TRM	none
TET	0.5-2
TGC	0.03-0.25
TMP	0.5-2

Figure 6. Percent deviation level for each antimicrobial for the E. coli ATCC25922 reference strain, E. coli trial.
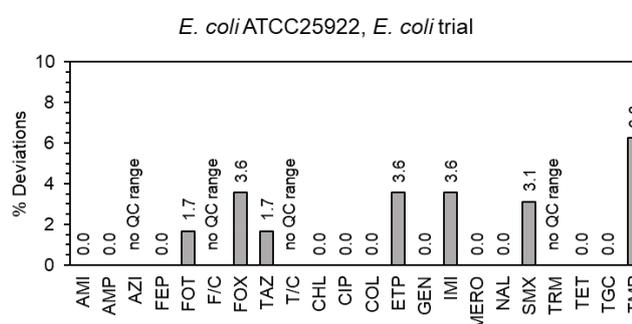
2.2. *Salmonella* trial

2.2.1. AST data

Expected MIC values and phenotype interpretation (R/S) for the strains included in the *Salmonella* trial are presented in Appendix 4. The percent deviation level for each laboratory in the *Salmonella* trial is presented in Figure 7 and was generally low. Approximately one fourth of the participants (n=8) reported all result correctly. Approximately 70% of the participants (n=23) reported one to seven incorrect result (deviation level 0.5 to 4.1%). One participant (#45) obtained a deviation level of 6.4% (13 incorrect results) which is above the 5% limit. The incorrect results were mainly reported for strain S-17.8 (10/13) so maybe this was due to incorrect reporting.

The deviation level for each strain-antimicrobial combination was less than 13% (Figure 8), with three exceptions: S-17.1/FOX (22%), S-17.1/TRM (16%) and S-17.2/AMI (19%). For S-17.1/FOX the high deviation level was due to a breakpoint issue, i.e. seven laboratories reported an MIC value for FOX of 8 mg/L, which is one two-fold dilution lower than the expected value (16mg/L). That lead to an unexpected phenotype interpretation for these labs (S instead of R) as the ECOFF for FOX is 8 mg/L. For S-17.1/TRM the obtained deviations were also mainly caused by a breakpoint issue. Four participants reported an MIC value for TRM of 16 mg/L, which is one two-fold dilution lower from the expected value (32 mg/L), thus leading to an unexpected phenotype interpretation (S instead of R) as the ECOFF for TRM is 16 mg/L. One laboratory reported an MIC of 32 mg/L but still reported a

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

susceptible phenotype, which is a mistake, based on the set ECOFF value. Lastly, six participants reported a MIC value for S-17.2/AMI of 8 mg/L, which is one two-fold dilution higher than the expected value (4 mg/L) and led to an unexpected phenotype interpretation (R instead of S) based on the ECOFF value (4 mg/L). The fact that the above mentioned deviations were due to breakpoint issues should be taken into account when performing self evaluation. Finally, the following strain antimicrobial combinations were omitted from the analysis (scores blanked) as the percent deviation level for these combinations was greater than 25% and was mainly due to breakpoint issues: S-17.1/AMI (31.3%), S-17.2/TGC (31.3%) and S-17.3/IMI (67.7%) – see also Table 2 and text above.
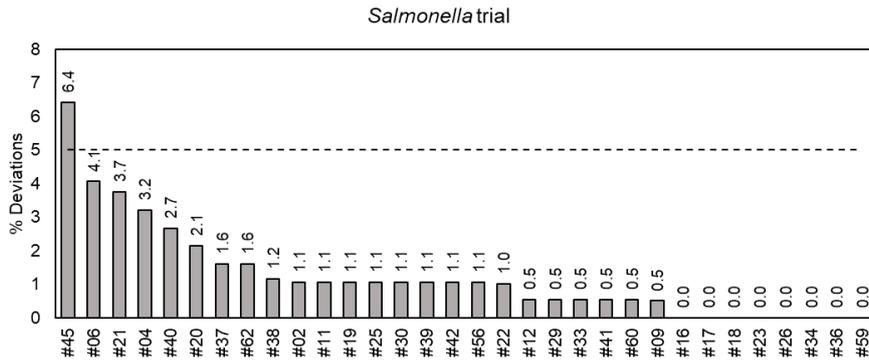
Figure 7. Percent deviation level for each laboratory, AST data, Salmonella trial.
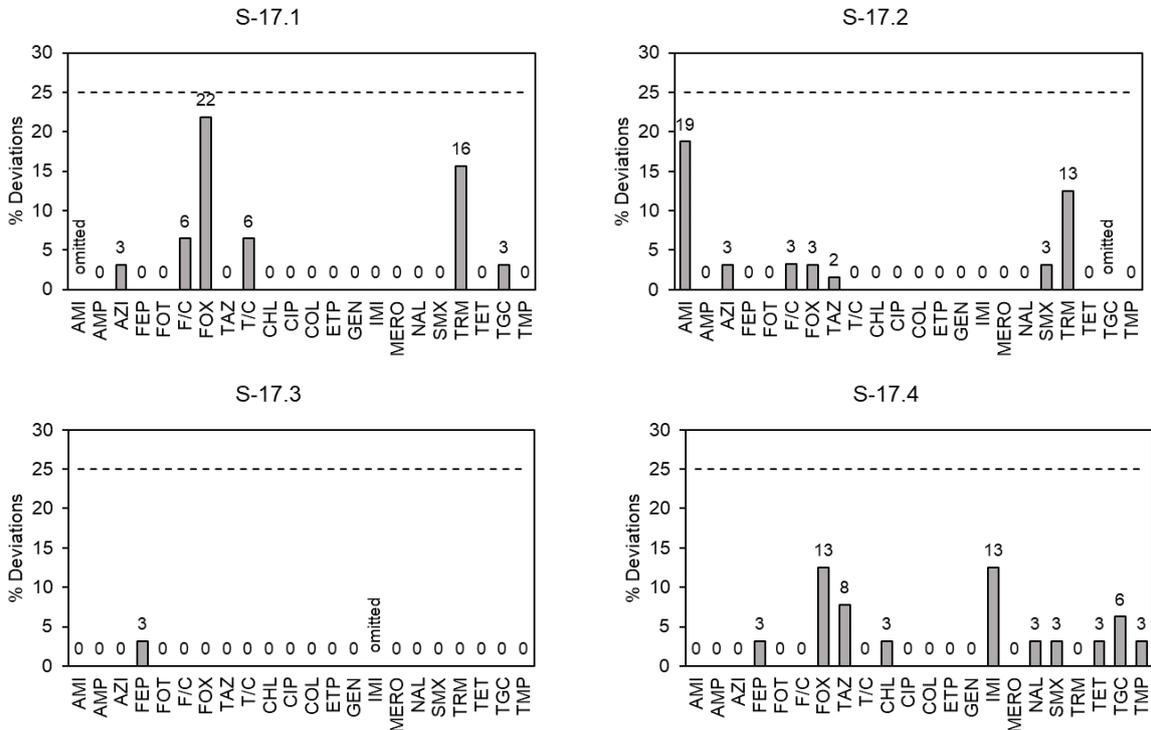
Figure continues on next page

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testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

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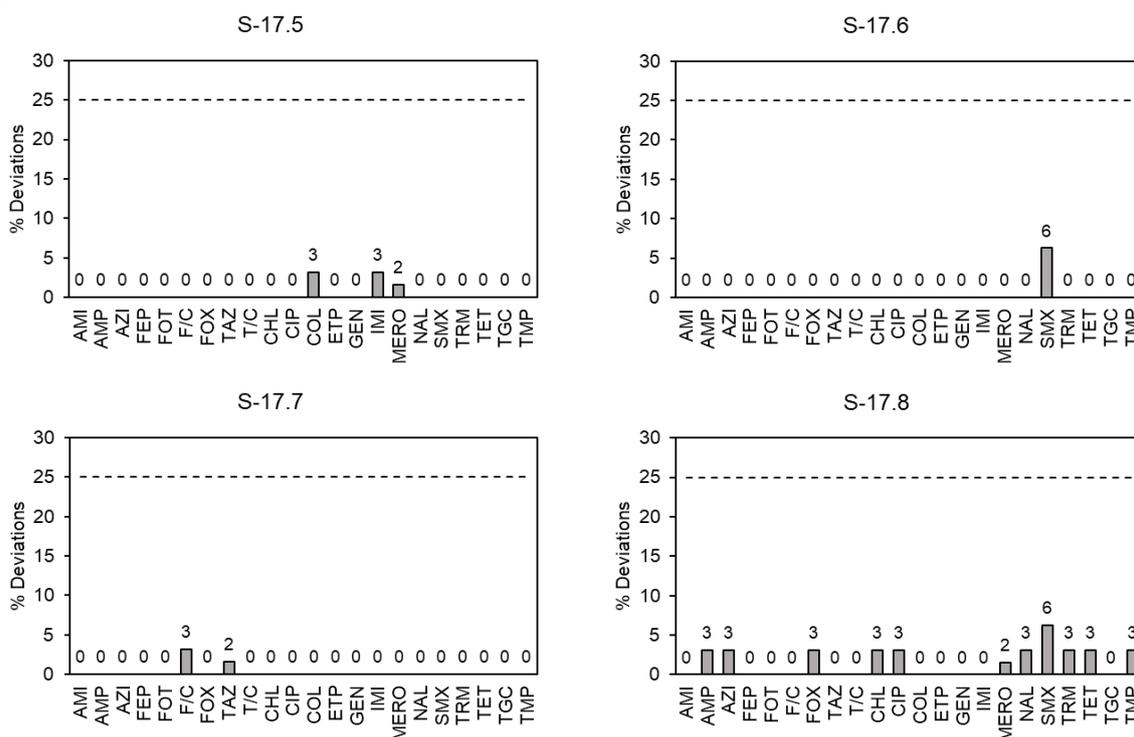
Figure 8. Percent deviation level for each strain-antimicrobial combination, Salmonella trial.

2.2.2. ESBL phenotype identification

The expected ESBL phenotypes for each strain of the *Salmonella* trial are presented in *Table 5*. All laboratories tested the ESBL-phenotype strains to panel 2. All participants reported susceptible phenotypes to FOT, TAZ, FOX and MERO and the expected phenotype "Susceptible" for strain S-17.6. Moreover all participants submitted the expected phenotypes for strains S-17.3 and S-17.4 (both carbapenemase phenotype) as well as S-17.7 (ESBL phenotype). For strain S-17.1, approximately one fourth of the participants (n=8) reported an unexpected phenotype (ESBL instead of ESBL+AmpC). For the majority of these participants (7/8) this was due to a breakpoint issue for FOX, where a MIC value of 8 mg/L was reported, while a value of 16 mg/L was expected. Therefore, the strain qualified to be categorised as ESBL for these participants. One participant (#36) reported the expected MIC for FOX, so the submited phenotype as "ESBL" is incorrect according to the EFSA guidelines.

Table 5. Performance of participants in the identification of ESBL phenotype for Salmonella trial.

Strain	Criteria	Expected ESBL phenotype	Genotype
S-17.1	FOT>1, TAZ>1, MEM≤0.12, FOX>8 SYN F/C, SYN T/C	ESBL+AmpC-phenotype	Unknown
S-17.2	FOT>1, TAZ>1, MERO≤0.12, FOX<=8 SYN F/C, SYN T/C	ESBL-phenotype	Unknown
S-17.3	MEM>0.12	Carbapenemase-phenotype	bla_{OXA-48}
S-17.4	MEM>0.12	Carbapenemase-phenotype	bla_{OXA-48}
S-17.5	MEM>0.12	Carbapenemase-phenotype	bla_{NDM-1}, bla_{CMY-16}
S-17.6	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible (to panel 2 antimicrobials)	No ESBL genes
S-17.7	FOT>1, MEM≤0.12, FOX<=8, SYN F/C	ESBL-phenotype	$bla_{CTX-M-9}$
S-17.8	FOT>1, TAZ>1, MEM≤0.12, FOX=2, SYN F/C, SYN T/C	ESBL-phenotype	Unknown

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed
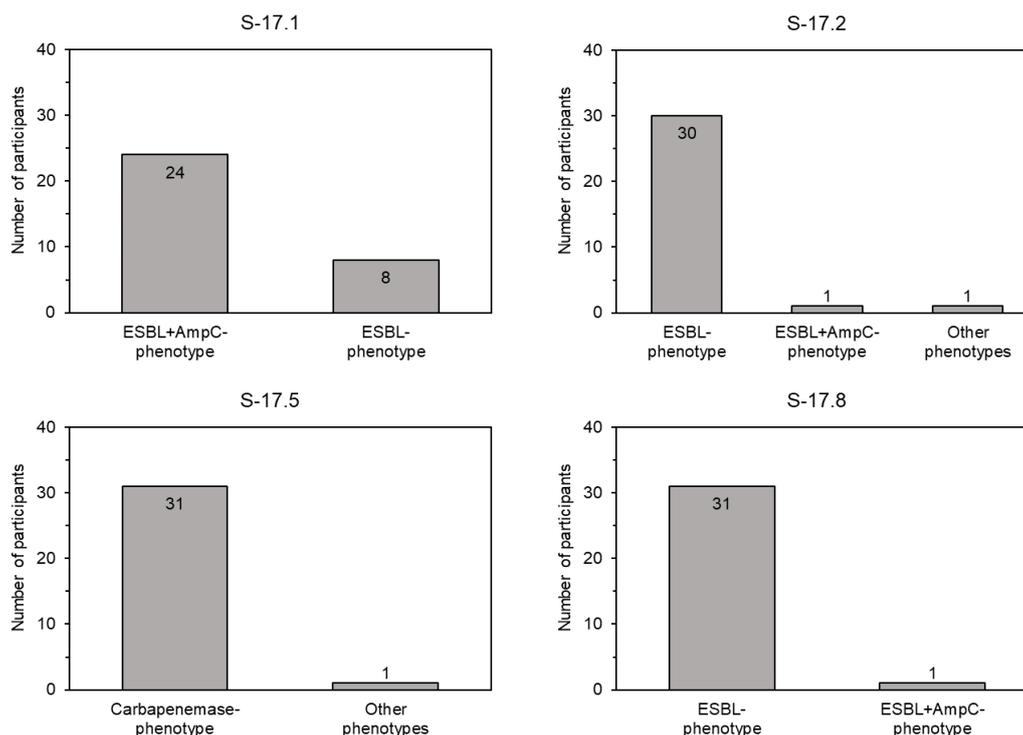
Figure 9. Obtained ESBL phenotypes for strains S-17.1, S-17.2, S-17.5 and S-17.8.

For strain S-17.2, all participants except for two, reported the expected phenotype (ESBL). One participant (#22) submitted an "ESBL+AmpC" phenotype for this strain, as they reported a MIC value for FOX of 64 mg/L instead of the expected value of 8 mg/L. Another participant (#38) reported an "Other" phenotype for strain S-17.2, which is not in agreement with this laboratory's submitted MIC data for this strain. Participant #40 was the only to report an unexpected phenotype ("Other") for strain S-17.5, and that was also not in agreement with the submitted MIC data for this strain. Lastly, for strain S-17.8, there was only one deviation from laboratory #45 which reported an ESBL+AmpC phenotype for this strain, and not the expected phenotype (ESBL). This is because this laboratory reported an MIC value for FOX of 16 mg/L, which is 8 times higher than the expected value (2 mg/L), and this was handled as a mistake.

2.2.3. Reference strain results

For the reference strain *E. coli* ATCC25922 results, deviations were defined as submitted MIC values that were outside the quality control (QC) acceptance intervals (*Table 6*) as defined in the CLSI M100 32nd edition. No QC range is available for AZI, F/C, T/C and TRM. Deviations were defined as submitted MIC values that are outside the QC acceptance intervals. The deviations for the reference strain are presented in Figure 10. Almost all participants reported $MIC_{FOT}\leq0.25$ mg/L, which is one two-fold dilution higher than the upper QC limit (0.125 mg/L), but is the lowest concentration in the panel, so it is accepted.

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

*Table 6. Quality control (QC) acceptance
intervals for the reference strain E. coli ATCC25922.*

Antimicrobial	MIC range
AMI	0.5-4
AMP	2-8
AZI	none
FEP	0.016-0.125
FOT	0.03-0.125
F/C	none
FOX	2-8
TAZ	0.06-0.5
T/C	none
CHL	2-8
CIP	0.004-0.016
COL	0.25-2
ETP	0.004-0.016
GEN	0.25-1
IMI	0.06-0.25
MERO	0.008-0.06
NAL	1-4
SMX	8-32
TRM	none
TET	0.5-2
TGC	0.03-0.25
TMP	0.5-2

*Figure 10. Percent deviation level for each antimicrobial for the
E. coli ATCC25922 reference strain, Salmonella trial.*
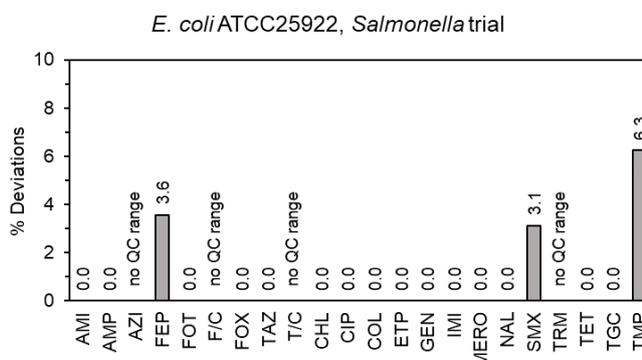
2.3. *Campylobacter* trial

2.3.1. AST data

Expected MIC values and phenotype interpretation (R/S) for the *Campylobacter* trial are presented in
Appendix 4. The percent deviation level for each laboratory in the *Campylobacter* trial is presented in
Figure 11 and was below 5%, with the majority of participant (n=18) reporting 100% correct results. The
overall performance for each strain/antimicrobial combination included in the *Campylobacter* trial is
presented in Figure 12. The percent deviation level for each strain/antimicrobial combination ranged
from zero to seven percent, apart from C-17.2/ETP which obtained a deviation level of 20%. Seven
participants reported MIC value for C-17.2/ETP of 0.5 mg/L, while a value of 1 mg/L was expected. The
ECOFF for ETP for *C. coli* is 0.5 mg/L, therefore the above-mentioned laboratories reported an
unexpected phenotype (S instead of R). This is handled as a mistake in the individual evaluation report,
but at the laboratory's internal evaluation it should be attributed to method limitations. The strain-
antimicrobial combination C-17.8/CHL was omitted from the analysis as it acquired a deviation level of
86.7%, which was mainly due to breakpoint issue – see Table 2 and text above.
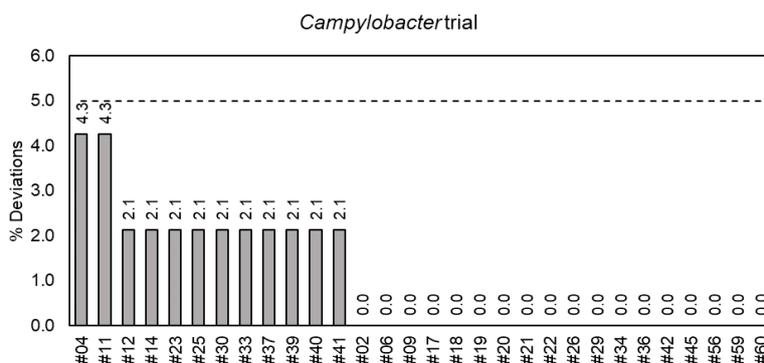
*Figure 11. Percent deviation level for each laboratory, AST data, Campylobacter trial.*
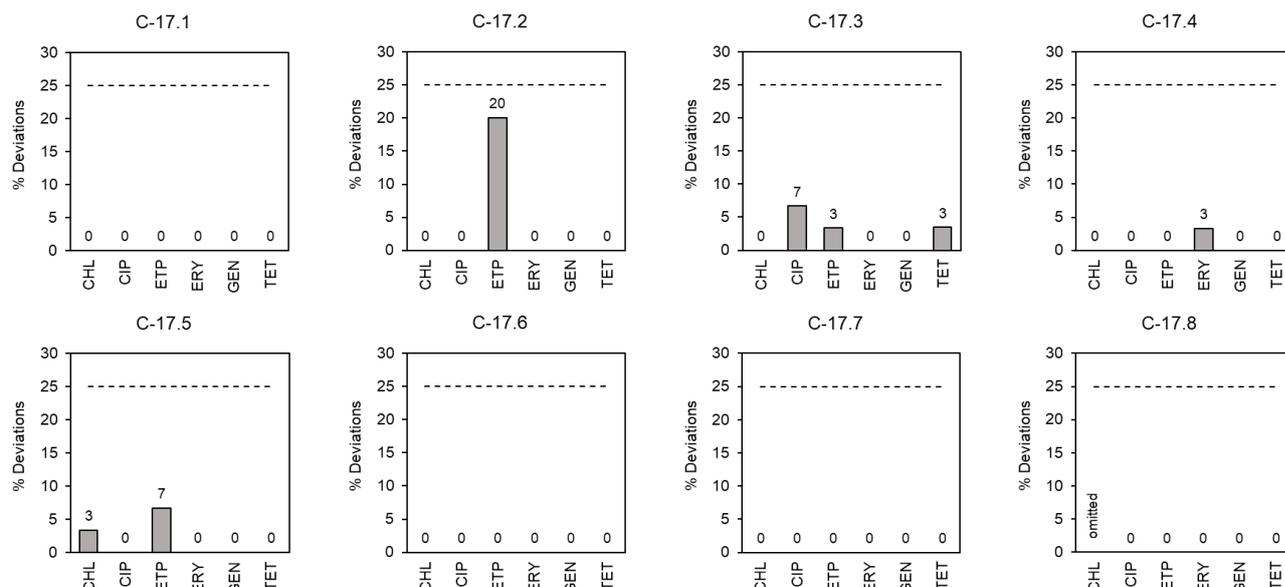
Figure 12. Percent deviation level for each strain-antimicrobial combination, Campylobacter trial.

2.3.2. Species identification results

The participants were requested to perform species identification of the *Campylobacter* test strains, using in-house methods or adopting the protocol available on the EURL-AR website. The expected species for each strain is presented in Table 7. All participants, except for one, achieved 100% correct results for species identification (Figure 13). Laboratory #18 reported a deviating result for strain C-17.2 (*C. jejuni* instead of *C. coli*).

Table 7. Expected species identification for each strain of the Campylobacter trial.

Campylobacter	Species
C-17.1	*C. jejuni*
C-17.2	*C. coli*
C-17.3	*C. coli*
C-17.4	*C. coli*
C-17.5	*C. jejuni*
C-17.6	*C. jejuni*
C-17.7	*C. coli*
C-17.8	*C. coli*
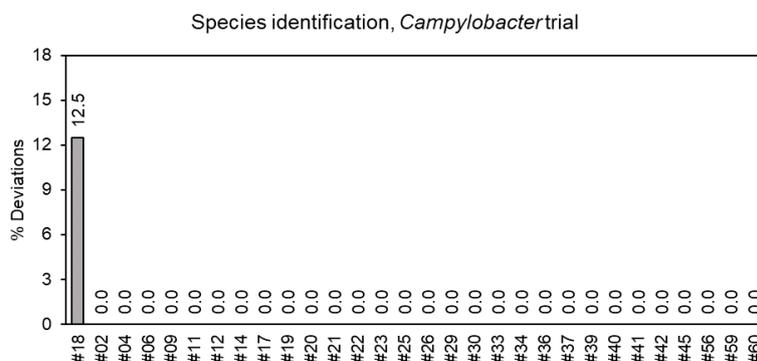
Figure 13. Percent deviation per laboratory, species identification, Campylobacter trial.

2.3.3. Reference strain results

For the reference strain *C. jejuni* ATCC 33560 results deviations were defined as submitted MIC values that are outside the quality control (QC) acceptance intervals (Table 8). Two deviations were reported: one for GEN where a MIC value of 0.25 was reported (lab #02, 36-37ºC/48h) and one for TET where a MIC of 2 was reported (#18, 42ºC/24h).

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

Table 8.QC ranges for reference strain C. jejuni ATCC 33560. Ranges are according to CLSI (VET06, 1st ed.)

Antimicrobial	Microbroth (36-37°C/48h)	Microbroth (42°C/24h)
CHL	1-8	1-4
CIP	0.06-0.25	0.03-0.125
ERY	0.5-2	0.25-2
ETP	No data	No data
GEN	0.5-2	0.25-2
TET	0.25-2	0.25-1

Figure 14. Percent deviation level for each antimicrobial for the C. jejuni (ATCC 33560) reference strain, Campylobacter trial.
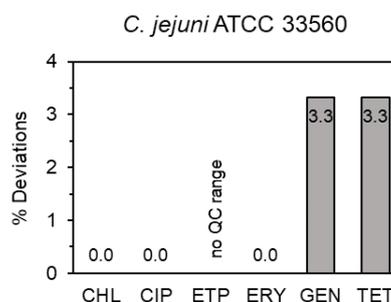
2.4. *S. aureus* trial

2.4.1. AST data

Expected MIC values and phenotype interpretation (R/S) for the *S. aureus* trial are presented in Appendix 4. The performance of the individual laboratories is presented in Figure 15. The deviation level among participants ranged from 0.0% to 4.7%, thus all laboratories complied to the 5% maximum deviation limit. The overall performance for each strain/antimicrobial included in the *S. aureus* trial is presented in Figure 16. The deviation level generally ranged from 0 to 7%, except for ST-17.3/TMP, ST-17.3/SMX and ST-17.8/LZD, which obtained deviation levels of 19, 15 and 12 percent respectively. Most of the deviations were due to breakpoint issues, however for ST-17.3/SMX the deviations were obtained by four participants who reported a MIC value of 512 mg/L, while a value of 64 mg/L was expected. That led to an unexpected phenotype interpretation (R instead of S) as the ECOFF is 128 mg/L. For the *S. aureus* trial, two strain antimicrobial combinations were omitted from the evaluation (scores blanked) as they acquired a deviation level greater than 25% (ST-17.1/CIP 34.6% and ST-17.6/SYN 68.0%) – see also Table 2 and text above.
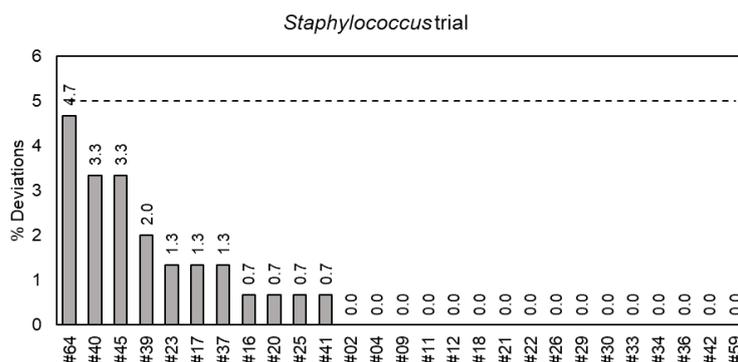
Figure 15. Percent deviation level for each laboratory, AST data, S. aureus trial.

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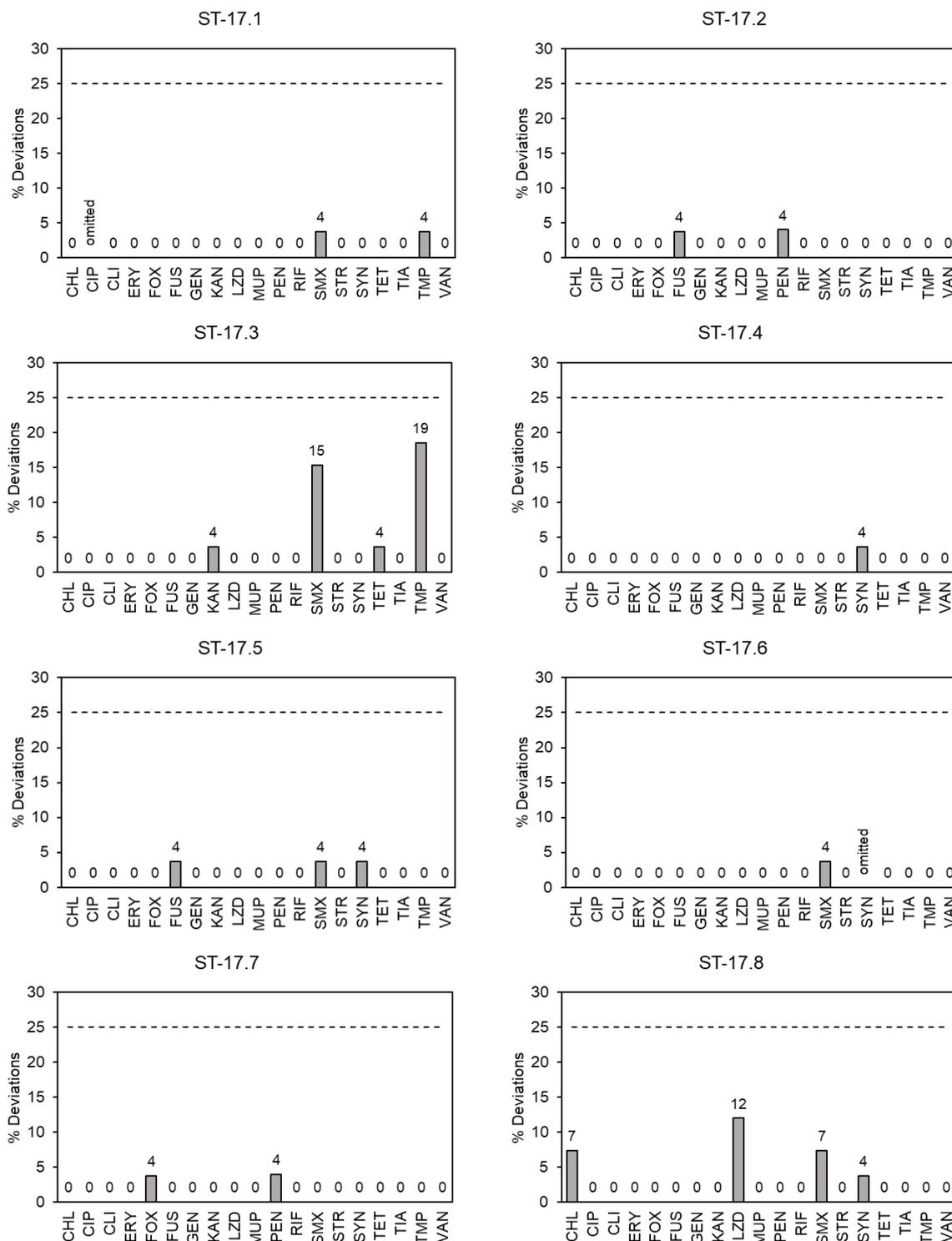
Figure 16. Percent deviation level for each strain-antimicrobial combination, S. aureus trial.

2.4.2. MRSA identification

The participants were requested to identfy MRSA (*mecA* or *mecC*) among the *S. aureus* test strains. From the 27 participants of the *S. aureus* trial, all identified the correct species for all strains, except for ST-17.2 and ST-17.7 for which one and two laboratories respectively reported unexpected phenotypes

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed

(*mecA*/*mecC* negative instead of positive, for both strains).

MRSA identification for each strain.

Strain	*mecA/mecC*
ST-17.1	*mecA*
ST-17.2	*mecA*
ST-17.3	*Negative*
ST-17.4	*mecA*
ST-17.5	*mecA*
ST-17.6	*Negative*
ST-17.7	*mecC*
ST-17.8	*mecA*
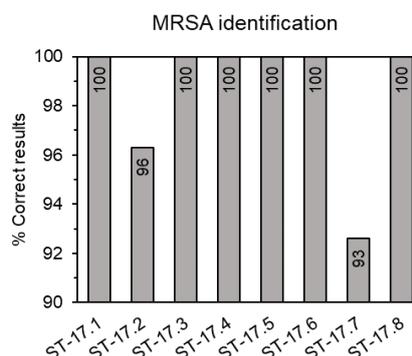
. Percent correct results per laboratory, MRSA identification.

2.4.3. Reference strain results

For the reference strain *S. aureus* ATCC 29213 results, deviations were defined as submitted MIC values that are outside the quality control (QC) acceptance intervals (Table 9). The percent deviation level for each antimicrobial is presented in Figure 17. All submitted results were correct for the majority of antimicrobials, apart from FUS and PEN for which a deviation level of 11.5 and 7.7 percent were obtained respectively. For FUS, three participants submitted an MIC value of 0.5 mg/L and for PEN two participants submitted an MIC value of 0.06 mg/L.

Table 9. Quality control (QC) acceptance intervals for the reference strain S. aureus ATCC 29213.

Figure 17. Percent deviation level for each antimicrobial for the S. aureus ATCC 29213 reference strain.

Antimicrobial	MIC
FOX	1-4
CHL	2-16
CIP	0.12-0.5
CLI	0.06-0.25
ERY	0.25-1
FUS	0.06-0.25
GEN	0.12-1
KAN	1-4
LZD	1-4
MUP	None
PEN	0.25-2
SYN	0.25-1
RIF	0.004-0.016
STR	None
SMX	None
TET	0.12-1
TIA	None
TRM	1-4
VAN	0.5-2

EURL-AR

The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility
testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *S. aureus*
2022, final version, 1. ed
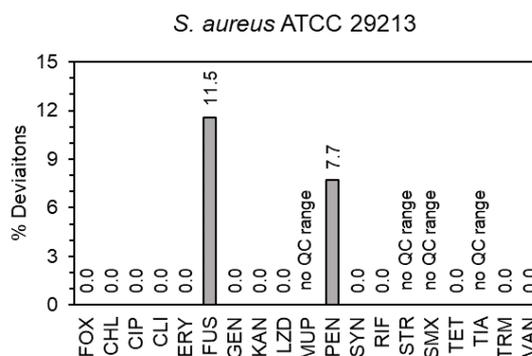
3. Concluding remarks

The goal of the EURL-AR EQAs is to have each participating NRL perform antimicrobial susceptibility testing of the test strains with a deviation level below 5% per organism. This year, this goal was reached for all participants, apart from one case: laboratory #45 obtained a deviation level of 6.4% in the *Salmonella* trial. The test covering the identification of the phenotype of *E. coli* and *Salmonella* strains producing beta-lactamases of the ESBL-, AmpC, and carbapenemase type obtained more that 90 percent correct categorisations for each trial. This is a priority area within the EURL-AR activities, and the focus on identifying ESBL-, AmpC-, and carbapenemase-producing organisms is encouraged. The deviations were mainly due to the fact that the strains had MIC values for the relevant antimicrobials that determine the ESBL-categorisation close to the cutoff values for the classification. Finally, the EURL-AR is open to suggestions to improve future PT trials and invites the entire network to contribute with ideas for training courses and specific focus areas to expand the network's knowledge in antimicrobial resistance.

4. References

European Commission Implementing Decision 2020/1729/EU (international reference method ISO standard 20776-1:2019) on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

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

G00-06-001/26.10.2020

EQAS 2022 for *E. coli*, *Salmonella*, *Campylobacter* and *Staphylococcus aureus*

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 *Salmonella* isolates, eight *Campylobacter* isolates and eight *Staphylococcus aureus* isolates. Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954), *C. jejuni* ATCC 33560 (CCM 6214), and *S. aureus* ATCC 29213 (CCM 4223) will be distributed to new participants.

It is the recipients' responsibility to comply with national legislation, rules and regulation regarding the correct use and handling of the provided strains and to possess the proper equipment and protocols to handle these strains.

This EQAS is specifically for NRL's on antimicrobial resistance (NRL-AR). Laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. You may contact the EQAS-coordinator if you wish to inform of changes in relation to your level of participation in comparison to previous years. The EURL-AR will be able to cover the expenses for one parcel, only, per EU Member State. Therefore, countries with more than one laboratory registered on the EURL-AR contact-list will be contacted directly to confirm which laboratory will be included for participation free of charge.

The invitation to participate in the proficiency test is extended to additional participants besides official NRLs and to participants from laboratories which are involved in the network but are not designated NRLs (cost for participation will be 100 EUR).

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY
The content of the parcel is "UN3373, Biological Substance Category B": Eight *E. coli* strains, eight *Salmonella* strains, eight *Campylobacter,* eight *Staphylococcus aureus* strains, and for new participants the QC strains mentioned above. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g. specific text that should be written on the proforma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped late September 2022. The protocol for this proficiency test will be available for download from the website (https://www.eurl-ar.eu/eqas.aspx).

Submission of results: Results must be submitted to the National Food Institute **no later than 9 December 2022** via the password-protected webtool.
Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected webtool once again to download an automatically generated evaluation report.

EQAS report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity as to the participants'

obtained results. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

<u>Next EQAS</u>: The next EQAS provided by the EURL-AR is planned to be carried out in October 2022 and will be the DTU Genomic PT 2022 aiming to facilitate harmonization and standardization in whole genome sequencing and data analysis of *Escherichia coli*, *Enterococcus faecium/faecalis* and *Staphylococcus aureus* isolates, with the aim to produce comparable data for monitoring and research purposes.

After this, in November 2022, the EQAS on selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from meat and caecal samples (Matrix EQAS) is planned to be carried out.

Please contact me if you have comments or questions regarding the EQAS.

Sincerely,

Susanne Karlsmose Pedersen (suska@food.dtu.dk)
EURL-AR EQAS-coordinator

PROTOCOL

For antimicrobial susceptibility testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *Staphylococcus*

1   INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *Escherichia coli, Salmonella*, *Campylobacter* and *Staphylococcus* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The current EQAS 2022 will include AST of eight *E. coli, Salmonella*, *Campylobacter and Staphylococcus* strains and AST of reference strains *E. coli* ATCC 25922 (CCM 3954), *Campylobacter jejuni* ATCC 33560 (CCM 6214) and *Staphylococcus. aureus* ATCC 29213 (CCM 4223) together with AST of the internal EURL reference strain, *Acinetobacter baumannii* (2012-70-100-69).

The reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The ATCC reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The EURL-AR QC-strains are provided for the purpose of additional QC

of the broth microdilution plates. The reference strains will not be included in the years to come and we therefore encourage you to take proper care of these strains for example by handling and maintaining them as suggested in the manual 'Subculture and Maintenance of Quality Control Strains' available on the EURL-AR website (see https://www.eurl-ar.eu/eqas.aspx).

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

2   OBJECTIVES

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, Salmonella, Campylobacter* and *Staphylococcus*. Further objectives are to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *E. coli*, *Salmonella*, *Campylobacter* and *Staphylococcus* reported to EFSA by different laboratories.

3   OUTLINE OF THE EC/SALM/CAMP/ENT EQAS 2022

3.1   Shipping, receipt and storage of strains

In September 2022, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, *Salmonella, Campylobacter* and staphylococci strains, respectively from the National Food Institute (see Table 1). For participants who did not receive them previously, this parcel will also contain reference strains.

Table 1: Codes for the test strains included in the current EQAS

E. coli	*Salmonella*	*Campylobacter*	*Staphylococcus*
2022 EC-17.1	2022 S-17.1	2022 C-17.1	2022 ST-17.1
2022 EC-17.2	2022 S-17.2	2022 C-17.2	2022 ST-17.2
2022 EC-17.3	2022 S-17.3	2022 C-17.3	2022 ST-17.3
2022 EC-17.4	2022 S-17.4	2022 C-17.4	2022 ST-17.4
2022 EC-17.5	2022 S-17.5	2022 C-17.5	2022 ST-17.5
2022 EC-17.6	2022 S-17.6	2022 C-17.6	2022 ST-17.6
2022 EC-17.7	2022 S-17.7	2022 C-17.7	2022 ST-17.7
2022 EC-17.8	2022 S-17.8	2022 C-17.8	2022 ST-17.8

All strains belong to UN3373, Biological substance, category B. Extended spectrum beta-lactamase (ESBL)-producing strains as well as carbapenemase producing strains are included in the selected material. It is the recipients' responsibility to comply with national legislation, rules and regulation regarding the correct use and handling of the provided strains and to possess the proper equipment and protocols to handle these strains.

All test strains will be shipped as swabs of pure cultures in transport media and new laboratories to the network will receive lyophilised ATCC reference strains. Upon arrival to your laboratory, store the strains in a dark place at 5°C to 25°C until microbiological analysis. The *Campylobacter* test strains must be subcultured immediately upon arrival. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

3.2 QC reference strains

Include the ATCC reference strains as well as the internal EURL reference strain for the MIC testing and report results of these together with the isolates obtained from the EQAS samples. I.e. for the *E. coli* and *Salmonella* testing, include *E. coli* ATCC 25922 (CCM 3954) together with *Acinetobacter baumannii* (2012-70-100-69).

Note that, for the testing of the *E. coli* ATCC25922 reference strain, the two compounds, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole.

For a suggested procedure for reconstitution of the lyophilised cultures, please refer to the document 'Instructions for opening and reviving lyophilised cultures' on the EURL-AR-website (see https://www.eurl-ar.eu/eqas.aspx).

3.3 Antimicrobial susceptibility testing

Participants should perform minimum inhibitory concentration (MIC) determination using the methods stated in the Commission Implementing Decision 2020/1729/EU (international reference method ISO standard 20776-1:2019). **Results should be produced according to the laboratory's routine procedures for antimicrobial susceptibility testing by MIC determination.** For interpretation of the results, please use the cut-off values listed in Tables 2, 3, 4, 5, 6 and 7 in this document. Except where specifically indicated, these values represent the current epidemiological cut-off values developed by EUCAST (www.eucast.org), and allow categorisation of bacterial

isolates into two categories: resistant and susceptible. A categorisation as intermediate is not accepted.

As the current regulation and recommendations focus on broth microdilution testing only, results obtained by other methods cannot be submitted for evaluation.

<u>Beta-lactam and carbapenem resistance</u>
Confirmatory tests for ESBL/AmpC/carbapenemase production are mandatory on all *E.coli* and *Salmonella* test strains resistant to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) and should be performed by testing the second panel of antimicrobials (Table 3 and Table 5 of this document corresponding to Table 5 in Commission Implementing Decision 2020/1729/EU).

Confirmatory test for AmpC-, ESBL- and carbapenemase production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. the MIC of the agent when tested alone (MIC FOT:FOT/Cl or TAZ:TAZ/Cl ratio ≥ 8) (CLSI M100 Table 3A, Tests for ESBLs). The presence of synergy indicates ESBL production.

Confirmatory test for carbapenemase production requires the testing of meropenem (MERO).

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.

The classification of the phenotypic beta-lactam resistance results should be based on the most recent EFSA recommendations (see appendix to this protocol). Importantly: Note that for *both E. coli* and *Salmonella*, two cut-off values apply for cefotaxime and ceftazidime: the EUCAST cut-off values, those that define R/S (see Tables 2, 3, 4 and 5), and the screening cut-off values (cefotaxime >1 and ceftazidime >1) which are those applied to categorise bacterial phenotypes as ESBL, AmpC, carbapenemase, etc., based on panel 2 results (see Appendix). Likewise this is the situation for the *E.coli* meropenem cut-off values/screening cut-off value.

3.3.2 *E. coli*

The interpretative criteria that should be applied for categorizing the *E. coli* test strain as resistant or susceptible are those listed in Tables 2 and 3.

Table 2: Panel 1 antimicrobials recommended for AST of *E. coli* spp. and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (17.08.2022) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	8
Ampicillin (AMP)	8
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.25
Ceftazidime (TAZ or CAZ)	0.5
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.064
Colistin (COL)	2
Gentamicin (GEN)	2
Meropenem (MERO or MEM)	0.06
Nalidixic acid (NAL)	8
Sulfonamides (SMX)	64*
Tetracycline (TET)	8
Tigecycline (TGC)	0.5
Trimethoprim (TMP)	2

* EFSA Technical Report (doi: 10.2903/sp.efsa.2021.EN-6652)

Table 3: Panel 2 antimicrobials recommended for AST of *E. coli* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (17.08.2022)

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.25
Cefotaxime (FOT or CTX)	0.25
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.25
Cefoxitin (FOX)	8
Ceftazidime (TAZ or CAZ)	0.5
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	0.5
Ertapenem (ETP)	0.03
Imipenem (IMI)	0.5
Meropenem (MERO or MEM)	0.06
Temocillin (TRM)	16

3.3.3 *Salmonella*

The interpretative criteria that should be applied for categorizing the *Salmonella* test strain as resistant or susceptible are those listed in Tables 4 and 5.

Table 4: Panel 1 antimicrobials recommended for AST of *Salmonella* spp. and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (17.08.2022) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	4
Ampicillin (AMP)	4
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.5
Ceftazidime (TAZ or CAZ)	2
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.064
Colistin (COL)	2*
Gentamicin (GEN)	2
Meropenem (MERO or MEM)	0.125*
Nalidixic acid (NAL)	8
Sulfonamides (SMX)	256*
Tetracycline (TET)	8
Tigecycline (TGC)	0.5*
Trimethoprim (TMP)	2

* EFSA Technical Report (doi: 10.2903/sp.efsa.2021.EN-6652)

Table 5: Panel 2 antimicrobials recommended for AST of *Salmonella* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (17.08.2022) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.25
Cefotaxime (FOT or CTX)	0.5
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.5*
Cefoxitin (FOX)	8
Ceftazidime (TAZ or CAZ)	2
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	2*
Ertapenem (ETP)	0.06*
Imipenem (IMI)	1
Meropenem (MERO or MEM)	0.125*
Temocillin (TRM)	16*

* EFSA Technical Report (doi: 10.2903/sp.efsa.2021.EN-6652)

3.3.4 *Campylobacter*

The interpretative criteria to be applied for categorizing the *Campylobacter* test strain as resistant or susceptible are those listed in Table 6.

The obtained values of the *C. jejuni* QC reference strain will be evaluated according to the values listed in the CLSI document VET06, 1st ed., i.e. based on incubation at 36-37ºC for 48 hours or 42ºC for 24 hours.

Table 6: Antimicrobials recommended for AST of *Campylobacter jejuni* and *C. coli* and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (17.08.2022) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.2

Antimicrobial	*C. jejuni*	*C. coli*
	MIC (µg/mL) (R>)	MIC (µg/mL) (R>)
Chloramphenicol (CHL)	16	16
Ciprofloxacin (CIP)	0.5	0.5
Ertapenem (ETP)	0.5*	0.5*
Erythromycin (ERY)	4	8
Gentamicin (GEN)	2	2
Tetracycline (TET)	1	2

* EFSA Technical Report (doi: 10.2903/sp.efsa.2021.EN-6652)

Identification of *Campylobacter* species

Species identification of the *Campylobacter* test strains must be performed by the NRLs using in-house methods or adopting the protocol available on the EURL-AR website under: http://eurl-ar.eu/233-protocols.htm.

3.3.6 *Staphylococci*

The interpretative criteria that should be applied for categorizing the Staphylococci test strain as resistant or susceptible are those listed in Table 7.

Table 7. Antimicrobials recommended for AST of *Staphylococcus aureus* and interpretive criteria ((T)ECOFFs) according to latest updates from EUCAST

Antimicrobials for *S. aureus*	MIC (µg/mL) R is >
Cefoxitin, FOX	4
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Clindamycin, CLN	0.25
Erythromycin, ERY	1
Fusidic acid, FUS	0.5
Gentamicin, GEN	2
Kanamycin, KAN	8
Linezolid, LZD	4
Mupirocin, MUP	1
Penicillin, PEN (benzylpenicillin)	0.125
Quin.-Dalf. (Synercid), SYN	1
Rifampicin, RIF	0.032
Streptomycin, STR	16
Sulfamethoxazole, SMX	128
Tetracycline, TET	1
Tiamulin (TIA)	2
Trimethoprim, TMP	2
Vancomycin, VAN	2

Identification of MRSA

Confirmation of *mec*A and/or *mecC* presence is mandatory in this EQAS and should be performed by the NRLs using in-house methods or adopting the protocol available on the EURL-AR website at www.eurl-ar.eu/233-protocols.htm. Test strains for which mecA and/or mecC have been confirmed should be reported as 'mecA/mecC positive' whereas test strains for which neither mecA nor mecC have been confirmed should be reported as 'mecA/mecC negative'.

5   REPORTING OF RESULTS AND EVALUATION

Test forms are available for recording your results before you enter them into the web tool.

We recommend reading carefully the web tool manual before submitting your results.

Results must be submitted no later than December 9th 2022.

After the deadline, when all participants have uploaded results, you will be able to login to the webtool once again 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'.

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

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

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National Food Institute,
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby
Denmark
Tel: +45 3588 6601
E-mail: suska@food.dtu.dk

6   HOW TO SUBMIT RESULTS VIA THE WEBTOOL

The 'guideline for submission of results via webtool' is available for download directly from the EURL-AR website (https://www.eurl-ar.eu/eqas.aspx).

Access the webtool using this address: https://amr-eqas.dtu.dk. Please follow the guideline carefully and **remember to access the webtool via an 'incognito' website**.

When you submit your results, remember to have by your side the completed test forms.

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting your input for *E. coli*, *Salmonella*, *Campylobacter* and staphylococci, respectively, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once for each organism!** 'Final submit' blocks data entry.

⇨ <u>About login to the webtool</u>:

When first given access to login to the webtool, your **personal** loginID and password were sent to you by email. This is relevant for two email addresses connected to each NRL-AR (the EURL-AR defined a primary and a secondary contact).
Note that:
a) If the EURL-AR has only one contact person for an NRL, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact suska@food.dtu.dk
b) If your laboratory has two or more contact points on the EURL-AR contact list, two have been defined as the primary and secondary contact. Should you like to make changes to the primary and secondary contact or should you like more than the two persons to be able to access the webtool, please contact suska@food.dtu.dk.

All participants registered with an account in the submission webtool will receive a separate email presenting further information related to the personal username and password. The email will be sent by the time when the webtool has gone through internal quality control and has been approved for user access. The EQAS Coordinator will let all participants know when to look out for it.

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APPENDIX

Criteria for interpretation of *E. coli* and *Salmonella*, panel 2 results

SUSCEPTIBLE
CTX - CAZ – FOX- MEM ≤ ECOFF

1. ESBL-Phenotype
- CTX or CAZ > 1 mg/L   AND
- MEM ≤ 0.12 mg/L   AND
- FOX ≤ 8 mg/L   AND
- SYN CTX/CLA and/or CAZ/CLA

2. AmpC-Phenotype
- CTX or CAZ > 1 mg/L   AND
- MEM ≤ 0.12 mg/L   AND
- FOX > 8 mg/L   AND
- NO SYN CTX/CLA nor CAZ/CLA
- (Not excluded presence of ESBLs)

3. ESBL + AmpC-Phenotype
- CTX or CAZ > 1 mg/L   AND
- MEM ≤ 0.12 mg/L   AND
- FOX > 8 mg/L   AND
- SYN CTX/CLA and/or CAZ/CLA

4. Carbapenemase-Phenotype
- MEM > 0.12 mg/L
- Needs confirmation
- (Not excluded presence of ESBLs or AmpC)

5. Other Phenotypes

1) IF CTX or CAZ > 1 mg/L AND
- MEM ≤ 0.12 mg/L   AND
- FOX ≤ 8 mg/L   AND
- NO SYN CTX/CLA nor CAZ/CLA
- Not excluded CPs (consult EURL)

3) IF CTX and CAZ ≤ 1 mg/L AND
- MEM ≤ 0.12 mg/L   AND
- FOX > 8 mg/L
*cAmpCs could be included here

2) IF CTX and/or CAZ ≤ 1 mg/L AND > ECOFF AND
- MEM ≤ 0.12 mg/L   AND
- FOX ≤ 8 mg/L   AND

4) IF MEM ≤ 0.12 mg/L   BUT
- ETP > ECOFF AND/OR
- IMI > ECOFF
- Not excluded CPs, needs confirmation (consult EURL)

5) Any other combinations not described in previous boxes (consult EURL)

Presumptive ESBL-producers include isolates exhibiting Phenotype 1 or 3.
Presumptive AmpC producers include isolates exhibiting Phenotype 2 or 3.

Please refer to: EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2022. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2019–2020. EFSA Journal 2022;20(3):7209, 197 pp. https://doi.org/10.2903/j.efsa.2022.7209, Figure F.1.

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2022

E. coli, Salmonella, Campylobacter and staphylococci

TEST FORMS

National Food Institute
Technical University of Denmark

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

TEST FORM – *E. coli*

Which method did you use for antimicrobial susceptibility testing of *E. coli* in this EQAS?

☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?

☐ CLSI
☐ EUCAST
☐ ISO 20776-1:2019
☐ TREK

Which incubation conditions did you use?   °C/   h

Which solvent was used for the preparation of the 0.5 McFarland solution

☐ Water
☐ Saline
☐ Mueller Hinton broth

The inoculum was prepared by adding   µl of 0.5 McFarland solution in   mL CAMHB broth

What was the expected inoculum size?   *   ^   CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

National Food Institute
Technical University of Denmark

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

TEST FORM - *Salmonella*

Which method did you use for antimicrobial susceptibility testing of *Salmonella* in this EQAS?
☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?
☐ CLSI
☐ EUCAST
☐ ISO 20776-1:2019
☐ TREK

Which incubation conditions did you use?   °C/   h

Which solvent was used for the preparation of the 0.5 McFarland solution
☐ Water
☐ Saline
☐ Mueller Hinton broth

The inoculum was prepared by adding   µl of 0.5 McFarland solution in   mL cation-adjusted Mueller Hinton broth (CAMHB).

What was the expected inoculum size?   *   ^   CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

TEST FORM - *Campylobacter*

Which method did you use for antimicrobial susceptibility testing of *Campylobacter* in this EQAS?
☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?
☐ CLSI
☐ EUCAST
☐ ISO 20776-1:2019
☐ TREK

Which incubation conditions did you use?
☐ 36-37ºC, 48 hours
☐ 42ºC, 24 hours

Which solvent was used for the preparation of the 0.5 McFarland solution
☐ Water
☐ Saline
☐ Mueller Hinton broth

The inoculum was prepared by adding   µl of 0.5 McFarland solution in   mL cation-adjusted Mueller Hinton broth supplemented with lysed horse blood (CAMHB-LHB).

What was the expected inoculum size?   *   ^   CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

TEST FORM - staphylococci

Which method did you use for antimicrobial susceptibility testing of staphylococci in this EQAS?
☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?
☐ CLSI
☐ EUCAST
☐ ISO 20776-1:2019
☐ TREK

Which incubation conditions did you use?   °C/   h

Which solvent was used for the preparation of the 0.5 McFarland solution
☐ Water
☐ Saline
☐ Mueller Hinton broth

The inoculum was prepared by adding   µl of 0.5 McFarland solution in   mL cation-adjusted Mueller Hinton broth (CAMBH)

What was the expected inoculum size?   *   ^   CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
E. coli EURL EC-17.X	Amikacin, AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

All strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem (MERO) must be included for testing in the second panel as part of confirmatory tests for ESBL-, AmpC or carbapenemase production. See further description in the protocol, section '3.3'.

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
E. coli EURL EC-17.X	Cefepime, FEP			
	Cefotaxime, FOT			
	Cefotaxime + clavulanic acid, F/C			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid, T/C			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			

Interpretation of PANEL 2 results:

☐ ESBL-phenotype   ☐ AmpC-phenotype   ☐ Other phenotype
☐ ESBL+AmpC-phenotype   ☐ Carbapenemase-phenotype   ☐ Susceptible (to panel 2 antimicrobials)

Comments:

DTU

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Page 6 of 14
G00-06-001/26.09.2022

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

TEST FORM

AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value (µg/ml)
1st panel	Amikacin, AMI	
	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* for the testing of the *E. coli* ATCC25922 reference strain, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole (CLSI M100, Table 5).

National Food Institute
Technical University of Denmark

Page 7 of 14
G00-06-001/26.09.2022

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

AST of reference strain *Acinetobacter baumannii* (2012-70-100-69)

	Antimicrobial	MIC-value (µg/ml)
1st panel	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* Sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole (CLSI M100, Table 5).

National Food Institute
Technical University of Denmark

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
Salmonella EURL S-17.X	Amikacin, AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

All strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem (MERO) must be included for testing in the second panel as part of confirmatory tests for ESBL-, AmpC or carbapenemase production. See further description in the protocol, section '3.3'.

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
Salmonella EURL S-17.X	Cefepime, FEP			
	Cefotaxime, FOT			
	Cefotaxime + clavulanic acid, F/C			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid, T/C			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			

Interpretation of PANEL 2 results:

☐ ESBL-phenotype   ☐ AmpC-phenotype   ☐ Other phenotype
☐ ESBL+AmpC-phenotype   ☐ Carbapenemase-phenotype   ☐ Susceptible (to panel 2 antimicrobials)

Comments:

TEST FORM

AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value (µg/ml)
1ˢᵗ panel	Amikacin, AMI	
	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2ⁿᵈ panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* for the testing of the *E. coli* ATCC25922 reference strain, sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole (CLSI M100, Table 3).

AST of reference strain *Acinetobacter baumannii* (2012-70-100-69)

	Antimicrobial	MIC-value (µg/ml)
1st panel	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* Sulfamethoxazole and sulfisoxazole, are regarded as comparable, i.e. the obtained MIC-value from the testing of sulfamethoxazole will be evaluated against the acceptance range listed in CLSI M100 for sulfisoxazole (CLSI M100, Table 3).

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter EURL C-17.X ☐ *C. jejuni* ☐ *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		

TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)	
		36 °C/48 hours	42 °C/24 hours
C. jejuni ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Staphylococcus EURL ST-17.X ☐ mecA/mecC positive ☐ mecA/mecC negative	Cefoxitin		
	Chloramphenicol		
	Ciprofloxacin		
	Clindamycin		
	Erythromycin		
	Fusidic acid		
	Gentamicin		
	Kanamycin		
	Linezolid		
	Mupirocin		
	Penicillin		
	Quinopristin/dalfopristin		
	Rifampin		
	Streptomycin		
	Sulfamethoxazole		
	Tetracycline		
	Tiamulin		
	Trimethoprim		
	Vancomycin		

National Food Institute
Technical University of Denmark

Susceptibility testing of *S. aureus* ATCC 29213

Strain	Antimicrobial	Interpretation	
		MIC-value (μg/ml)	S / R
S. aureus ATCC 29213	Cefoxitin		
	Chloramphenicol		
	Ciprofloxacin		
	Clindamycin		
	Erythromycin		
	Fusidic acid		
	Gentamicin		
	Kanamycin		
	Linezolid		
	Mupirocin		
	Penicillin		
	Quinopristin/dalfopristin		
	Rifampin		
	Streptomycin		
	Sulfamethoxazole		
	Tetracycline		
	Tiamulin		
	Trimethoprim		
	Vancomycin		

DTU

National Food Institute
Technical University of Denmark

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

G00-06-001/26.10.2020

EURL-AR External Quality Assurance System 2022
- E. coli, Salmonella, Campylobacter and *Staphylococcus*

Lyngby, September 2022

Dear participant in the EURL-AR AST EQAS 2022,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2022: eight *E. coli*, eight *Salmonella,* eight *Campylobacter* spp. and eight *Staphylococcus* spp.

E. coli	*Salmonella*	*Campylobacter*	*Staphylococcus*
2022 EC-17.1	2022 S-17.1	2022 C-17.1	2022 ST-17.1
2022 EC-17.2	2022 S-17.2	2022 C-17.2	2022 ST-17.2
2022 EC-17.3	2022 S-17.3	2022 C-17.3	2022 ST-17.3
2022 EC-17.4	2022 S-17.4	2022 C-17.4	2022 ST-17.4
2022 EC-17.5	2022 S-17.5	2022 C-17.5	2022 ST-17.5
2022 EC-17.6	2022 S-17.6	2022 C-17.6	2022 ST-17.6
2022 EC-17.7	2022 S-17.7	2022 C-17.7	2022 ST-17.7
2022 EC-17.8	2022 S-17.8	2022 C-17.8	2022 ST-17.8

Upon arrival to your laboratory, store the test strains in a dark place at 5-25°C until microbiological analysis. To ensure viability of the cultures, the *Campylobacter* test strains must be subcultured immediately upon arrival.

On the EURL-AR-website (www.eurl-ar.eu) the following documents relevant for this EURL-AR EQAS are available:
- Protocol for antimicrobial susceptibility testing of *E. coli*, *Salmonella, Campylobacter* and *Staphylococcus* and test forms for reporting results
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Control Strains
- Guideline for submission of results via the webtool

We ask you to test these test strains for antimicrobial susceptibility. Detailed description of the procedures to follow for antimicrobial susceptibility testing and for submitting your results via the webtool can be found in the protocol.

<u>All participants registered with an account in the submission webtool will receive a separate email presenting information related to personal username and password. The email will be sent by the time when the webtool has gone through internal quality control and has been approved for user access</u>. I will let you know when to look out for it.

	Personal username	**Personal password**
Accessing the webtool (see the EQAS protocol, item 5)	*See underlined text above*	*See underlined text above*

Results should be submitted to the database no later than **9 December 2022**.

Please acknowledge receipt of this parcel immediately upon arrival (to suska@food.dtu.dk).
Do not hesitate to contact me for further information.

Yours sincerely,

Susanne Karlsmose Pedersen
EURL-AR EQAS-Coordinator

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

DTU Food
National Food Institute

INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz.

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

a. Check the number of the culture on the label inside the ampoule

b. Make a file cut on the ampoule near the middle of the plug (see Figure 1)

c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end

d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule

e. Remove the pointed end of the ampoule into disinfectant

f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media

g. Incubate the inoculated medium at appropriate conditions for several days

h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

Notes:

▪ Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see http://www.sci.muni.cz)

▪ Cultures may need at least one subculturing before they can be optimally used in experiments

▪ Unopened ampoules should be kept in a dark and cool place!
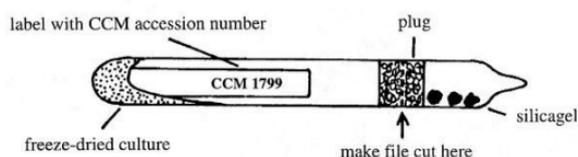
Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz

Instructions for Opening and Reviving Lyophilised Cultures   G00-06-001/01.12.2014

Page 1 of 1

The 30th EURL-AR PT for the antimicrobial susceptibility testing of E. coli, Salmonella, Campylobacter and S. aureus 2022, final version, 1. ed

SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1   PURPOSE AND REFERENCES

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test (AST) results.

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

2   DEFINITION OF TERMS

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time.

3   IMPORTANT CONSIDERATIONS

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC.
- CLSI requires that QC be performed either on the same day or weekly (after QC-validation).
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides.

- Periodically perform colony counts to check the inoculum preparation procedure.
- Ideally, test values should be in the middle of the acceptable range.
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems.

4 STORAGE OF REFERENCE STRAINS

Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen (alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

5 FREQUENCY OF TESTING

Weekly vs. daily testing

Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.

- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

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

If five acceptable QC results are available, no additional days of QC-testing are needed.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

Appendix 4: Expected MIC values and phenotype interpretation

E. coli trial

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL EC-17.1	AMI	<=	4	S	Panel1
EURL EC-17.1	AMP	>	32	R	Panel1
EURL EC-17.1	AZI	>	64	R	Panel1
EURL EC-17.1	CHL	>	64	R	Panel1
EURL EC-17.1	CIP	>	8	R	Panel1
EURL EC-17.1	COL	=	4	R	Panel1
EURL EC-17.1	ETP	=	0.12	R	Panel2
EURL EC-17.1	F/C	=	0.12/4	S	Panel2
EURL EC-17.1	FEP	=	16	R	Panel2
EURL EC-17.1	FOT	>	4	R	Panel1
EURL EC-17.1	FOT	>	64	R	Panel2
EURL EC-17.1	FOX	=	16	R	Panel2
EURL EC-17.1	GEN	>	16	R	Panel1
EURL EC-17.1	IMI	=	0.25	S	Panel2
EURL EC-17.1	MERO	<=	0.03	S	Panel1
EURL EC-17.1	MERO	=	0.03	S	Panel2
EURL EC-17.1	NAL	>	64	R	Panel1
EURL EC-17.1	SMX	>	512	R	Panel1
EURL EC-17.1	T/C	=	0.25/4	S	Panel2
EURL EC-17.1	TAZ	>	8	R	Panel1
EURL EC-17.1	TAZ	=	8	R	Panel2
EURL EC-17.1	TET	>	32	R	Panel1
EURL EC-17.1	TGC	<=	0.25	S	Panel1
EURL EC-17.1	TMP	>	16	R	Panel1
EURL EC-17.1	TRM	=	8	S	Panel2
EURL EC-17.2	AMI	<=	4	S	Panel1
EURL EC-17.2	AMP	>	32	R	Panel1
EURL EC-17.2	AZI	=	8	S	Panel1
EURL EC-17.2	CHL	>	64	R	Panel1
EURL EC-17.2	CIP	=	0.03	S	Panel1
EURL EC-17.2	COL	=	4	R	Panel1
EURL EC-17.2	ETP	=	0.03	S	Panel2
EURL EC-17.2	F/C	=	0.12/4	S	Panel2
EURL EC-17.2	FEP	=	8	R	Panel2
EURL EC-17.2	FOT	>	4	R	Panel1
EURL EC-17.2	FOT	=	64	R	Panel2
EURL EC-17.2	FOX	=	8	S	Panel2
EURL EC-17.2	GEN	=	4	R	Panel1
EURL EC-17.2	IMI	=	0.25	S	Panel2
EURL EC-17.2	MERO	<=	0.03	S	Panel1
EURL EC-17.2	MERO	=	0.03	S	Panel2
EURL EC-17.2	NAL	=	4	S	Panel1
EURL EC-17.2	SMX	>	512	R	Panel1
EURL EC-17.2	T/C	=	0.25/4	S	Panel2
EURL EC-17.2	TAZ	=	1	R	Panel1

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL EC-17.2	TAZ	=	2	R	Panel2
EURL EC-17.2	TET	>	32	R	Panel1
EURL EC-17.2	TGC	<=	0.25	S	Panel1
EURL EC-17.2	TMP	>	16	R	Panel1
EURL EC-17.2	TRM	=	8	S	Panel2
EURL EC-17.3	AMI	<=	4	S	Panel1
EURL EC-17.3	AMP	>	32	R	Panel1
EURL EC-17.3	AZI	=	8	S	Panel1
EURL EC-17.3	CHL	<=	8	S	Panel1
EURL EC-17.3	CIP	=	0.03	S	Panel1
EURL EC-17.3	COL	<=	1	S	Panel1
EURL EC-17.3	ETP	=	0.015	S	Panel2
EURL EC-17.3	F/C	=	2/4	R	Panel2
EURL EC-17.3	FEP	=	0.12	S	Panel2
EURL EC-17.3	FOT	=	2	R	Panel1
EURL EC-17.3	FOT	=	4	R	Panel2
EURL EC-17.3	FOX	>	64	R	Panel2
EURL EC-17.3	GEN	=	1	S	Panel1
EURL EC-17.3	IMI	=	0.25	S	Panel2
EURL EC-17.3	MERO	<=	0.03	S	Panel1
EURL EC-17.3	MERO	=	0.03	S	Panel2
EURL EC-17.3	NAL	<=	4	S	Panel1
EURL EC-17.3	SMX	<=	8	S	Panel1
EURL EC-17.3	T/C	=	4/4	R	Panel2
EURL EC-17.3	TAZ	=	4	R	Panel1
EURL EC-17.3	TAZ	=	4	R	Panel2
EURL EC-17.3	TET	<=	2	S	Panel1
EURL EC-17.3	TGC	<=	0.25	S	Panel1
EURL EC-17.3	TMP	=	0.5	S	Panel1
EURL EC-17.3	TRM	=	16	S	Panel2
EURL EC-17.4	AMI	<=	4	S	Panel1
EURL EC-17.4	AMP	>	32	R	Panel1
EURL EC-17.4	AZI	=	8	S	Panel1
EURL EC-17.4	CHL	>	64	R	Panel1
EURL EC-17.4	CIP	=	0.5	R	Panel1
EURL EC-17.4	COL	<=	1	S	Panel1
EURL EC-17.4	ETP	=	0.03	S	Panel2
EURL EC-17.4	F/C	=	0.06/4	S	Panel2
EURL EC-17.4	FEP	=	2	R	Panel2
EURL EC-17.4	FOT	>	4	R	Panel1
EURL EC-17.4	FOT	>	64	R	Panel2
EURL EC-17.4	FOX	=	8	S	Panel2
EURL EC-17.4	GEN	<=	0.5	S	Panel1
EURL EC-17.4	IMI	=	0.25	S	Panel2
EURL EC-17.4	MERO	<=	0.03	S	Panel1
EURL EC-17.4	MERO	=	0.03	S	Panel2
EURL EC-17.4	NAL	<=	4	S	Panel1
EURL EC-17.4	SMX	>	512	R	Panel1
EURL EC-17.4	T/C	=	0.25/4	S	Panel2

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL EC-17.4	TAZ	=	1	R	Panel1
EURL EC-17.4	TAZ	=	1	R	Panel2
EURL EC-17.4	TET	>	32	R	Panel1
EURL EC-17.4	TGC	<=	0.25	S	Panel1
EURL EC-17.4	TMP	>	16	R	Panel1
EURL EC-17.4	TRM	=	16	S	Panel2
EURL EC-17.5	AMI	<=	4	S	Panel1
EURL EC-17.5	AMP	>	32	R	Panel1
EURL EC-17.5	AZI	=	8	S	Panel1
EURL EC-17.5	CHL	<=	8	S	Panel1
EURL EC-17.5	CIP	<=	0.015	S	Panel1
EURL EC-17.5	COL	<=	1	S	Panel1
EURL EC-17.5	ETP	=	0.015	S	Panel2
EURL EC-17.5	F/C	=	0.12/4	S	Panel2
EURL EC-17.5	FEP	=	16	R	Panel2
EURL EC-17.5	FOT	>	4	R	Panel1
EURL EC-17.5	FOT	=	64	R	Panel2
EURL EC-17.5	FOX	=	4	S	Panel2
EURL EC-17.5	GEN	<=	0.5	S	Panel1
EURL EC-17.5	IMI	=	0.12	S	Panel2
EURL EC-17.5	MERO	<=	0.03	S	Panel1
EURL EC-17.5	MERO	=	0.03	S	Panel2
EURL EC-17.5	NAL	<=	4	S	Panel1
EURL EC-17.5	SMX	<=	8	S	Panel1
EURL EC-17.5	T/C	=	0.25/4	S	Panel2
EURL EC-17.5	TAZ	=	4	R	Panel1
EURL EC-17.5	TAZ	=	4	R	Panel2
EURL EC-17.5	TET	<=	2	S	Panel1
EURL EC-17.5	TGC	<=	0.25	S	Panel1
EURL EC-17.5	TMP	<=	0.25	S	Panel1
EURL EC-17.5	TRM	=	4	S	Panel2
EURL EC-17.6	AMI	<=	4	S	Panel1
EURL EC-17.6	AMP	=	2	S	Panel1
EURL EC-17.6	AZI	=	4	S	Panel1
EURL EC-17.6	CHL	<=	8	S	Panel1
EURL EC-17.6	CIP	<=	0.015	S	Panel1
EURL EC-17.6	COL	<=	1	S	Panel1
EURL EC-17.6	ETP	<=	0.015	S	Panel2
EURL EC-17.6	F/C	<=	0.06/4	S	Panel2
EURL EC-17.6	FEP	<=	0.06	S	Panel2
EURL EC-17.6	FOT	<=	0.25	S	Panel1
EURL EC-17.6	FOT	<=	0.25	S	Panel2
EURL EC-17.6	FOX	=	2	S	Panel2
EURL EC-17.6	GEN	<=	0.5	S	Panel1
EURL EC-17.6	IMI	<=	0.12	S	Panel2
EURL EC-17.6	MERO	<=	0.03	S	Panel1
EURL EC-17.6	MERO	<=	0.03	S	Panel2
EURL EC-17.6	NAL	<=	4	S	Panel1
EURL EC-17.6	SMX	<=	8	S	Panel1

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL EC-17.6	T/C	<=	0.12/4	S	Panel2
EURL EC-17.6	TAZ	<=	0.25	S	Panel1
EURL EC-17.6	TAZ	<=	0.25	S	Panel2
EURL EC-17.6	TET	<=	2	S	Panel1
EURL EC-17.6	TGC	<=	0.25	S	Panel1
EURL EC-17.6	TMP	<=	0.25	S	Panel1
EURL EC-17.6	TRM	=	8	S	Panel2
EURL EC-17.7	AMI	<=	4	S	Panel1
EURL EC-17.7	AMP	>	32	R	Panel1
EURL EC-17.7	AZI	>	64	R	Panel1
EURL EC-17.7	CHL	=	16	S	Panel1
EURL EC-17.7	CIP	=	0.5	R	Panel1
EURL EC-17.7	COL	<=	1	S	Panel1
EURL EC-17.7	ETP	=	2	R	Panel2
EURL EC-17.7	F/C	=	8/4	R	Panel2
EURL EC-17.7	FEP	=	8	R	Panel2
EURL EC-17.7	FOT	>	4	R	Panel1
EURL EC-17.7	FOT	>	64	R	Panel2
EURL EC-17.7	FOX	>	64	R	Panel2
EURL EC-17.7	GEN	<=	0.5	S	Panel1
EURL EC-17.7	IMI	=	1	R	Panel2
EURL EC-17.7	MERO	=	0.25	R	Panel1
EURL EC-17.7	MERO	=	0.5	R	Panel2
EURL EC-17.7	NAL	<=	4	S	Panel1
EURL EC-17.7	SMX	>	512	R	Panel1
EURL EC-17.7	T/C	=	32/4	R	Panel2
EURL EC-17.7	TAZ	>	8	R	Panel1
EURL EC-17.7	TAZ	=	32	R	Panel2
EURL EC-17.7	TET	>	32	R	Panel1
EURL EC-17.7	TGC	<=	0.25	S	Panel1
EURL EC-17.7	TMP	>	16	R	Panel1
EURL EC-17.7	TRM	>	128	R	Panel2
EURL EC-17.8	AMI	>	128	R	Panel1
EURL EC-17.8	AMP	>	32	R	Panel1
EURL EC-17.8	AZI	=	8	S	Panel1
EURL EC-17.8	CHL	<=	8	S	Panel1
EURL EC-17.8	CIP	>	8	R	Panel1
EURL EC-17.8	COL	<=	1	S	Panel1
EURL EC-17.8	ETP	>	2	R	Panel2
EURL EC-17.8	F/C	>	64/4	R	Panel2
EURL EC-17.8	FEP	>	32	R	Panel2
EURL EC-17.8	FOT	>	4	R	Panel1
EURL EC-17.8	FOT	>	64	R	Panel2
EURL EC-17.8	FOX	>	64	R	Panel2
EURL EC-17.8	GEN	>	16	R	Panel1
EURL EC-17.8	IMI	=	8	R	Panel2
EURL EC-17.8	MERO	=	4	R	Panel1
EURL EC-17.8	MERO	=	8	R	Panel2
EURL EC-17.8	NAL	>	64	R	Panel1

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL EC-17.8	SMX	>	512	R	Panel1
EURL EC-17.8	T/C	>	128/4	R	Panel2
EURL EC-17.8	TAZ	>	8	R	Panel1
EURL EC-17.8	TAZ	>	128	R	Panel2
EURL EC-17.8	TET	<=	2	S	Panel1
EURL EC-17.8	TGC	<=	0.25	S	Panel1
EURL EC-17.8	TMP	<=	0.25	S	Panel1
EURL EC-17.8	TRM	>	128	R	Panel2

Salmonella trial

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL S-17.1	AMI	=	4	S	Panel1
EURL S-17.1	AMP	>	32	R	Panel1
EURL S-17.1	AZI	=	16	S	Panel1
EURL S-17.1	CHL	>	64	R	Panel1
EURL S-17.1	CIP	>	8	R	Panel1
EURL S-17.1	COL	=	1	S	Panel1
EURL S-17.1	ETP	=	0.03	S	Panel2
EURL S-17.1	F/C	=	0.5/4	S	Panel2
EURL S-17.1	FEP	>	32	R	Panel2
EURL S-17.1	FOT	>	4	R	Panel1
EURL S-17.1	FOT	>	64	R	Panel2
EURL S-17.1	FOX	=	16	R	Panel2
EURL S-17.1	GEN	>	16	R	Panel1
EURL S-17.1	IMI	=	0.25	S	Panel2
EURL S-17.1	MERO	=	0.03	S	Panel1
EURL S-17.1	MERO	=	0.03	S	Panel2
EURL S-17.1	NAL	>	64	R	Panel1
EURL S-17.1	SMX	>	512	R	Panel1
EURL S-17.1	T/C	=	0.5/4	S	Panel2
EURL S-17.1	TAZ	>	8	R	Panel1
EURL S-17.1	TAZ	=	128	R	Panel2
EURL S-17.1	TET	>	32	R	Panel1
EURL S-17.1	TGC	=	2	R	Panel1
EURL S-17.1	TMP	>	16	R	Panel1
EURL S-17.1	TRM	=	32	R	Panel2
EURL S-17.2	AMI	=	4	S	Panel1
EURL S-17.2	AMP	>	32	R	Panel1
EURL S-17.2	AZI	=	4	S	Panel1
EURL S-17.2	CHL	>	64	R	Panel1
EURL S-17.2	CIP	>	8	R	Panel1
EURL S-17.2	COL	=	1	S	Panel1
EURL S-17.2	ETP	=	0.03	S	Panel2
EURL S-17.2	F/C	=	0.25/4	S	Panel2
EURL S-17.2	FEP	>	32	R	Panel2
EURL S-17.2	FOT	>	4	R	Panel1

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL S-17.2	FOT	>	64	R	Panel2
EURL S-17.2	FOX	=	8	S	Panel2
EURL S-17.2	GEN	>	16	R	Panel1
EURL S-17.2	IMI	=	0.25	S	Panel2
EURL S-17.2	MERO	=	0.03	S	Panel1
EURL S-17.2	MERO	=	0.03	S	Panel2
EURL S-17.2	NAL	>	64	R	Panel1
EURL S-17.2	SMX	>	512	R	Panel1
EURL S-17.2	T/C	=	0.5/4	S	Panel2
EURL S-17.2	TAZ	>	8	R	Panel1
EURL S-17.2	TAZ	=	128	R	Panel2
EURL S-17.2	TET	>	32	R	Panel1
EURL S-17.2	TGC	=	0.5	S	Panel1
EURL S-17.2	TMP	>	16	R	Panel1
EURL S-17.2	TRM	=	32	R	Panel2
EURL S-17.3	AMI	=	4	S	Panel1
EURL S-17.3	AMP	>	32	R	Panel1
EURL S-17.3	AZI	=	4	S	Panel1
EURL S-17.3	CHL	=	8	S	Panel1
EURL S-17.3	CIP	=	0.03	S	Panel1
EURL S-17.3	COL	=	1	S	Panel1
EURL S-17.3	ETP	=	1	R	Panel2
EURL S-17.3	F/C	=	2/4	R	Panel2
EURL S-17.3	FEP	=	0.5	R	Panel2
EURL S-17.3	FOT	=	2	R	Panel1
EURL S-17.3	FOT	=	2	R	Panel2
EURL S-17.3	FOX	=	4	S	Panel2
EURL S-17.3	GEN	=	0.5	S	Panel1
EURL S-17.3	IMI	=	1	S	Panel2
EURL S-17.3	MERO	=	0.5	R	Panel1
EURL S-17.3	MERO	=	0.5	R	Panel2
EURL S-17.3	NAL	=	4	S	Panel1
EURL S-17.3	SMX	=	8	S	Panel1
EURL S-17.3	T/C	=	0.5/4	S	Panel2
EURL S-17.3	TAZ	=	0.5	S	Panel1
EURL S-17.3	TAZ	=	0.5	S	Panel2
EURL S-17.3	TET	=	2	S	Panel1
EURL S-17.3	TGC	=	0.25	S	Panel1
EURL S-17.3	TMP	=	0.5	S	Panel1
EURL S-17.3	TRM	>	128	R	Panel2
EURL S-17.4	AMI	=	4	S	Panel1
EURL S-17.4	AMP	>	32	R	Panel1
EURL S-17.4	AZI	>	64	R	Panel1
EURL S-17.4	CHL	=	8	S	Panel1
EURL S-17.4	CIP	>	8	R	Panel1
EURL S-17.4	COL	=	1	S	Panel1
EURL S-17.4	ETP	=	1	R	Panel2
EURL S-17.4	F/C	=	2/4	R	Panel2
EURL S-17.4	FEP	=	1	R	Panel2

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL S-17.4	FOT	=	4	R	Panel1
EURL S-17.4	FOT	=	8	R	Panel2
EURL S-17.4	FOX	=	8	S	Panel2
EURL S-17.4	GEN	=	8	R	Panel1
EURL S-17.4	IMI	=	2	R	Panel2
EURL S-17.4	MERO	=	1	R	Panel1
EURL S-17.4	MERO	=	0.5	R	Panel2
EURL S-17.4	NAL	>	64	R	Panel1
EURL S-17.4	SMX	>	512	R	Panel1
EURL S-17.4	T/C	=	1/4	S	Panel2
EURL S-17.4	TAZ	=	1	S	Panel1
EURL S-17.4	TAZ	=	1	S	Panel2
EURL S-17.4	TET	>	32	R	Panel1
EURL S-17.4	TGC	=	0.5	S	Panel1
EURL S-17.4	TMP	>	16	R	Panel1
EURL S-17.4	TRM	>	128	R	Panel2
EURL S-17.5	AMI	=	4	S	Panel1
EURL S-17.5	AMP	>	32	R	Panel1
EURL S-17.5	AZI	>	64	R	Panel1
EURL S-17.5	CHL	>	64	R	Panel1
EURL S-17.5	CIP	>	8	R	Panel1
EURL S-17.5	COL	=	1	S	Panel1
EURL S-17.5	ETP	=	2	R	Panel2
EURL S-17.5	F/C	=	64/4	R	Panel2
EURL S-17.5	FEP	=	16	R	Panel2
EURL S-17.5	FOT	>	4	R	Panel1
EURL S-17.5	FOT	>	64	R	Panel2
EURL S-17.5	FOX	>	64	R	Panel2
EURL S-17.5	GEN	=	1	S	Panel1
EURL S-17.5	IMI	=	4	R	Panel2
EURL S-17.5	MERO	=	1	R	Panel1
EURL S-17.5	MERO	=	1	R	Panel2
EURL S-17.5	NAL	>	64	R	Panel1
EURL S-17.5	SMX	>	512	R	Panel1
EURL S-17.5	T/C	=	128/4	R	Panel2
EURL S-17.5	TAZ	>	8	R	Panel1
EURL S-17.5	TAZ	>	128	R	Panel2
EURL S-17.5	TET	>	32	R	Panel1
EURL S-17.5	TGC	=	0.5	S	Panel1
EURL S-17.5	TMP	>	16	R	Panel1
EURL S-17.5	TRM	>	128	R	Panel2
EURL S-17.6	AMI	=	4	S	Panel1
EURL S-17.6	AMP	=	1	S	Panel1
EURL S-17.6	AZI	=	4	S	Panel1
EURL S-17.6	CHL	=	8	S	Panel1
EURL S-17.6	CIP	=	0.015	S	Panel1
EURL S-17.6	COL	=	1	S	Panel1
EURL S-17.6	ETP	=	0.015	S	Panel2
EURL S-17.6	F/C	=	0.06/4	S	Panel2

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL S-17.6	FEP	=	0.06	S	Panel2
EURL S-17.6	FOT	=	0.25	S	Panel1
EURL S-17.6	FOT	=	0.25	S	Panel2
EURL S-17.6	FOX	=	4	S	Panel2
EURL S-17.6	GEN	=	0.5	S	Panel1
EURL S-17.6	IMI	=	0.25	S	Panel2
EURL S-17.6	MERO	=	0.03	S	Panel1
EURL S-17.6	MERO	=	0.03	S	Panel2
EURL S-17.6	NAL	=	4	S	Panel1
EURL S-17.6	SMX	=	16	S	Panel1
EURL S-17.6	T/C	=	0.5/4	S	Panel2
EURL S-17.6	TAZ	=	0.5	S	Panel1
EURL S-17.6	TAZ	=	0.5	S	Panel2
EURL S-17.6	TET	=	2	S	Panel1
EURL S-17.6	TGC	=	0.25	S	Panel1
EURL S-17.6	TMP	=	0.5	S	Panel1
EURL S-17.6	TRM	=	4	S	Panel2
EURL S-17.7	AMI	=	4	S	Panel1
EURL S-17.7	AMP	>	32	R	Panel1
EURL S-17.7	AZI	=	8	S	Panel1
EURL S-17.7	CHL	=	8	S	Panel1
EURL S-17.7	CIP	=	0.25	R	Panel1
EURL S-17.7	COL	=	1	S	Panel1
EURL S-17.7	ETP	=	0.015	S	Panel2
EURL S-17.7	F/C	=	0.12/4	S	Panel2
EURL S-17.7	FEP	=	2	R	Panel2
EURL S-17.7	FOT	>	4	R	Panel1
EURL S-17.7	FOT	=	8	R	Panel2
EURL S-17.7	FOX	=	2	S	Panel2
EURL S-17.7	GEN	=	0.5	S	Panel1
EURL S-17.7	IMI	=	0.25	S	Panel2
EURL S-17.7	MERO	=	0.03	S	Panel1
EURL S-17.7	MERO	=	0.03	S	Panel2
EURL S-17.7	NAL	>	64	R	Panel1
EURL S-17.7	SMX	=	8	S	Panel1
EURL S-17.7	T/C	=	0.25/4	S	Panel2
EURL S-17.7	TAZ	=	1	S	Panel1
EURL S-17.7	TAZ	=	1	S	Panel2
EURL S-17.7	TET	=	32	R	Panel1
EURL S-17.7	TGC	=	0.25	S	Panel1
EURL S-17.7	TMP	=	0.25	S	Panel1
EURL S-17.7	TRM	=	4	S	Panel2
EURL S-17.8	AMI	=	4	S	Panel1
EURL S-17.8	AMP	>	32	R	Panel1
EURL S-17.8	AZI	=	8	S	Panel1
EURL S-17.8	CHL	=	8	S	Panel1
EURL S-17.8	CIP	=	0.015	S	Panel1
EURL S-17.8	COL	=	2	S	Panel1
EURL S-17.8	ETP	=	0.03	S	Panel2

Strain ID	Antimicrobial	Operator	Value	Interpretation	Panel
EURL S-17.8	F/C	=	0.12/4	S	Panel2
EURL S-17.8	FEP	=	16	R	Panel2
EURL S-17.8	FOT	>	4	R	Panel1
EURL S-17.8	FOT	>	64	R	Panel2
EURL S-17.8	FOX	=	2	S	Panel2
EURL S-17.8	GEN	=	0.5	S	Panel1
EURL S-17.8	IMI	=	0.25	S	Panel2
EURL S-17.8	MERO	=	0.03	S	Panel1
EURL S-17.8	MERO	=	0.06	S	Panel2
EURL S-17.8	NAL	=	4	S	Panel1
EURL S-17.8	SMX	=	16	S	Panel1
EURL S-17.8	T/C	=	0.5/4	S	Panel2
EURL S-17.8	TAZ	>	8	R	Panel1
EURL S-17.8	TAZ	=	32	R	Panel2
EURL S-17.8	TET	=	2	S	Panel1
EURL S-17.8	TGC	=	0.25	S	Panel1
EURL S-17.8	TMP	=	0.25	S	Panel1
EURL S-17.8	TRM	=	16	S	Panel2

Campylobacter trial

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL C-17.1	CHL	=	2	S
EURL C-17.1	CIP	=	0.12	S
EURL C-17.1	ERY	=	1	S
EURL C-17.1	ETP	=	0.12	S
EURL C-17.1	GEN	=	0.5	S
EURL C-17.1	TET	=	0.5	S
EURL C-17.2	CHL	=	4	S
EURL C-17.2	CIP	=	32	R
EURL C-17.2	ERY	>	512	R
EURL C-17.2	ETP	=	1	R
EURL C-17.2	GEN	=	0.5	S
EURL C-17.2	TET	>	64	R
EURL C-17.3	CHL	=	2	S
EURL C-17.3	CIP	=	0.25	S
EURL C-17.3	ERY	=	256	R
EURL C-17.3	ETP	=	0.12	S
EURL C-17.3	GEN	=	0.5	S
EURL C-17.3	TET	=	0.5	S
EURL C-17.4	CHL	=	4	S
EURL C-17.4	CIP	=	32	R
EURL C-17.4	ERY	=	4	S
EURL C-17.4	ETP	=	0.12	S
EURL C-17.4	GEN	=	0.5	S
EURL C-17.4	TET	>	64	R
EURL C-17.5	CHL	=	2	S
EURL C-17.5	CIP	=	8	R
EURL C-17.5	ERY	=	256	R
EURL C-17.5	ETP	=	0.25	S
EURL C-17.5	GEN	>	16	R
EURL C-17.5	TET	=	64	R
EURL C-17.6	CHL	=	2	S
EURL C-17.6	CIP	=	8	R
EURL C-17.6	ERY	=	1	S
EURL C-17.6	ETP	=	0.12	S
EURL C-17.6	GEN	=	0.5	S
EURL C-17.6	TET	=	64	R
EURL C-17.7	CHL	=	2	S
EURL C-17.7	CIP	=	0.12	S
EURL C-17.7	ERY	=	128	R
EURL C-17.7	ETP	>	4	R
EURL C-17.7	GEN	=	0.25	S
EURL C-17.7	TET	>	64	R
EURL C-17.8	CHL	=	16	S
EURL C-17.8	CIP	=	16	R
EURL C-17.8	ERY	>	512	R
EURL C-17.8	ETP	=	2	R
EURL C-17.8	GEN	>	16	R
EURL C-17.8	TET	=	64	R

S. aureus trial

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL ST-17.1	CHL	=	4	S
EURL ST-17.1	CIP	=	2	R
EURL ST-17.1	CLI	=	0.12	S
EURL ST-17.1	ERY	=	0.25	S
EURL ST-17.1	FOX	=	8	R
EURL ST-17.1	FUS	=	0.25	S
EURL ST-17.1	GEN	>	16	R
EURL ST-17.1	KAN	>	32	R
EURL ST-17.1	LZD	=	1	S
EURL ST-17.1	MUP	=	0.5	S
EURL ST-17.1	PEN	>	1	R
EURL ST-17.1	RIF	>	0.5	R
EURL ST-17.1	SMX	=	512	R
EURL ST-17.1	STR	>	32	R
EURL ST-17.1	SYN	=	0.5	S
EURL ST-17.1	TET	>	16	R
EURL ST-17.1	TIA	=	0.5	S
EURL ST-17.1	TMP	=	1	S
EURL ST-17.1	VAN	=	1	S
EURL ST-17.2	CHL	=	4	S
EURL ST-17.2	CIP	=	0.25	S
EURL ST-17.2	CLI	=	0.12	S
EURL ST-17.2	ERY	=	0.5	S
EURL ST-17.2	FOX	=	16	R
EURL ST-17.2	FUS	=	0.25	S
EURL ST-17.2	GEN	=	0.5	S
EURL ST-17.2	KAN	=	4	S
EURL ST-17.2	LZD	=	1	S
EURL ST-17.2	MUP	=	0.5	S
EURL ST-17.2	PEN	>	1	R
EURL ST-17.2	RIF	=	0.015	S
EURL ST-17.2	SMX	=	64	S
EURL ST-17.2	STR	>	32	R
EURL ST-17.2	SYN	=	0.5	S
EURL ST-17.2	TET	>	16	R
EURL ST-17.2	TIA	=	0.5	S
EURL ST-17.2	TMP	=	1	S
EURL ST-17.2	VAN	=	1	S
EURL ST-17.3	CHL	=	8	S
EURL ST-17.3	CIP	=	0.25	S
EURL ST-17.3	CLI	=	0.12	S
EURL ST-17.3	ERY	=	0.5	S
EURL ST-17.3	FOX	=	4	S
EURL ST-17.3	FUS	>	4	R
EURL ST-17.3	GEN	=	0.5	S
EURL ST-17.3	KAN	=	4	S
EURL ST-17.3	LZD	=	2	S

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL ST-17.3	MUP	=	0.5	S
EURL ST-17.3	PEN	>	1	R
EURL ST-17.3	RIF	=	0.015	S
EURL ST-17.3	SMX	=	64	S
EURL ST-17.3	STR	=	4	S
EURL ST-17.3	SYN	=	0.5	S
EURL ST-17.3	TET	=	0.5	S
EURL ST-17.3	TIA	=	1	S
EURL ST-17.3	TMP	=	2	S
EURL ST-17.3	VAN	=	1	S
EURL ST-17.4	CHL	=	8	S
EURL ST-17.4	CIP	=	0.25	S
EURL ST-17.4	CLI	>	4	R
EURL ST-17.4	ERY	=	0.5	S
EURL ST-17.4	FOX	=	16	R
EURL ST-17.4	FUS	=	0.25	S
EURL ST-17.4	GEN	=	0.5	S
EURL ST-17.4	KAN	=	4	S
EURL ST-17.4	LZD	=	2	S
EURL ST-17.4	MUP	=	0.5	S
EURL ST-17.4	PEN	>	1	R
EURL ST-17.4	RIF	=	0.015	S
EURL ST-17.4	SMX	=	64	S
EURL ST-17.4	STR	=	4	S
EURL ST-17.4	SYN	=	2	R
EURL ST-17.4	TET	>	16	R
EURL ST-17.4	TIA	>	4	R
EURL ST-17.4	TMP	>	16	R
EURL ST-17.4	VAN	=	1	S
EURL ST-17.5	CHL	=	8	S
EURL ST-17.5	CIP	=	0.5	S
EURL ST-17.5	CLI	>	4	R
EURL ST-17.5	ERY	=	0.5	S
EURL ST-17.5	FOX	=	16	R
EURL ST-17.5	FUS	=	0.25	S
EURL ST-17.5	GEN	=	0.5	S
EURL ST-17.5	KAN	=	4	S
EURL ST-17.5	LZD	=	2	S
EURL ST-17.5	MUP	=	0.5	S
EURL ST-17.5	PEN	>	1	R
EURL ST-17.5	RIF	=	0.015	S
EURL ST-17.5	SMX	=	64	S
EURL ST-17.5	STR	>	32	R
EURL ST-17.5	SYN	=	2	R
EURL ST-17.5	TET	>	16	R
EURL ST-17.5	TIA	>	4	R
EURL ST-17.5	TMP	>	16	R
EURL ST-17.5	VAN	=	1	S
EURL ST-17.6	CHL	=	8	S

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL ST-17.6	CIP	=	0.5	S
EURL ST-17.6	CLI	>	4	R
EURL ST-17.6	ERY	>	8	R
EURL ST-17.6	FOX	=	4	S
EURL ST-17.6	FUS	=	0.25	S
EURL ST-17.6	GEN	>	16	R
EURL ST-17.6	KAN	>	32	R
EURL ST-17.6	LZD	=	2	S
EURL ST-17.6	MUP	=	0.5	S
EURL ST-17.6	PEN	>	1	R
EURL ST-17.6	RIF	=	0.015	S
EURL ST-17.6	SMX	=	64	S
EURL ST-17.6	STR	=	8	S
EURL ST-17.6	SYN	=	2	R
EURL ST-17.6	TET	>	16	R
EURL ST-17.6	TIA	=	1	S
EURL ST-17.6	TMP	>	16	R
EURL ST-17.6	VAN	=	1	S
EURL ST-17.7	CHL	=	8	S
EURL ST-17.7	CIP	=	0.25	S
EURL ST-17.7	CLI	=	0.12	S
EURL ST-17.7	ERY	=	0.5	S
EURL ST-17.7	FOX	=	16	R
EURL ST-17.7	FUS	=	0.25	S
EURL ST-17.7	GEN	=	0.5	S
EURL ST-17.7	KAN	=	4	S
EURL ST-17.7	LZD	=	2	S
EURL ST-17.7	MUP	=	0.5	S
EURL ST-17.7	PEN	>	1	R
EURL ST-17.7	RIF	=	0.015	S
EURL ST-17.7	SMX	=	64	S
EURL ST-17.7	STR	=	8	S
EURL ST-17.7	SYN	=	0.5	S
EURL ST-17.7	TET	=	0.5	S
EURL ST-17.7	TIA	=	0.5	S
EURL ST-17.7	TMP	=	1	S
EURL ST-17.7	VAN	=	1	S
EURL ST-17.8	CHL	>	64	R
EURL ST-17.8	CIP	=	0.5	S
EURL ST-17.8	CLI	>	4	R
EURL ST-17.8	ERY	=	0.5	S
EURL ST-17.8	FOX	=	16	R
EURL ST-17.8	FUS	=	0.25	S
EURL ST-17.8	GEN	=	0.5	S
EURL ST-17.8	KAN	=	4	S
EURL ST-17.8	LZD	=	8	R
EURL ST-17.8	MUP	=	0.5	S
EURL ST-17.8	PEN	>	1	R
EURL ST-17.8	RIF	=	0.015	S

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL ST-17.8	SMX	=	64	S
EURL ST-17.8	STR	>	32	R
EURL ST-17.8	SYN	=	4	R
EURL ST-17.8	TET	>	16	R
EURL ST-17.8	TIA	>	4	R
EURL ST-17.8	TMP	>	16	R
EURL ST-17.8	VAN	=	1	S

Lab number	Strain	Panel type	Antimicrobial	Obtained operator value	Expected operator value	Obtained mic value	Expected mic value	Obtained interpretation value	Expected interpretation value	Score interpretation
NRL-AR-004	EURL EC-17.4	Panel1	Nalidixic acid	=	<=	32	4	R	S	0
NRL-AR-004	EURL EC-17.5	Panel1	Azithromycin	=	=	32	8	R	S	0
NRL-AR-004	EURL EC-17.5	Panel1	Chloramphenicol	=	<=	32	8	R	S	0
NRL-AR-006	EURL EC-17.5	Panel2	Cefotaxime-clavulanic acid	=	=	0.12	0.12/4	R	S	0
NRL-AR-006	EURL EC-17.5	Panel2	Ceftazidime	=	=	4	4	S	R	0
NRL-AR-012	EURL EC-17.3	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-016	EURL EC-17.3	Panel1	Sulfamethoxazole	=	<=	16	8	R	S	0
NRL-AR-018	EURL EC-17.3	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-018	EURL EC-17.3	Panel2	Temocillin	=	=	32	16	R	S	0
NRL-AR-022	EURL EC-17.7	Panel1	Meropenem	=	=	0.5	0.25	S	R	0
NRL-AR-023	EURL EC-17.2	Panel1	Ceftazidime	=	=	2	1	S	R	0
NRL-AR-025	EURL EC-17.2	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-025	EURL EC-17.4	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-025	EURL EC-17.4	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-026	EURL EC-17.1	Panel2	Cefotaxime-clavulanic acid	<=	=	0.06	0.12/4	R	S	0
NRL-AR-026	EURL EC-17.1	Panel2	Ceftazidime-clavulanic acid	<=	=	0.12	0.25/4	R	S	0
NRL-AR-026	EURL EC-17.2	Panel2	Cefotaxime-clavulanic acid	=	=	0.12	0.12/4	R	S	0
NRL-AR-026	EURL EC-17.2	Panel2	Ceftazidime-clavulanic acid	<=	=	0.12	0.25/4	R	S	0
NRL-AR-026	EURL EC-17.3	Panel2	Cefepime	<=	=	0.06	0.12	R	S	0
NRL-AR-026	EURL EC-17.4	Panel2	Cefotaxime-clavulanic acid	<=	=	0.06	0.06/4	R	S	0
NRL-AR-026	EURL EC-17.4	Panel2	Ceftazidime-clavulanic acid	<=	=	0.12	0.25/4	R	S	0
NRL-AR-026	EURL EC-17.5	Panel2	Cefotaxime-clavulanic acid	<=	=	0.06	0.12/4	R	S	0
NRL-AR-026	EURL EC-17.5	Panel2	Ceftazidime-clavulanic acid	=	=	0.25	0.25/4	R	S	0
NRL-AR-029	EURL EC-17.1	Panel1	Colistin	=	=	2	4	S	R	0
NRL-AR-030	EURL EC-17.2	Panel1	Amikacin	=	<=	16	4	R	S	0
NRL-AR-034	EURL EC-17.2	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-036	EURL EC-17.3	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-037	EURL EC-17.2	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-038	EURL EC-17.3	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-038	EURL EC-17.3	Panel1	Trimethoprim	>	=	16	0.5	R	S	0
NRL-AR-039	EURL EC-17.1	Panel2	Ertapenem	=	=	0.06	0.12	S	R	0
NRL-AR-039	EURL EC-17.3	Panel2	Temocillin	=	=	32	16	R	S	0
NRL-AR-039	EURL EC-17.4	Panel1	Nalidixic acid	=	<=	16	4	R	S	0
NRL-AR-039	EURL EC-17.5	Panel2	Ertapenem	=	=	0.12	0.015	R	S	0
NRL-AR-040	EURL EC-17.3	Panel1	Sulfamethoxazole	=	<=	512	8	R	S	0
NRL-AR-045	EURL EC-17.2	Panel1	Colistin	=	=	2	4	S	R	0
NRL-AR-045	EURL EC-17.2	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-045	EURL EC-17.3	Panel2	Ertapenem	=	=	0.06	0.015	R	S	0
NRL-AR-045	EURL EC-17.4	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-045	EURL EC-17.4	Panel2	Temocillin	=	=	32	16	R	S	0
NRL-AR-056	EURL EC-17.1	Panel2	Ertapenem	=	=	0.03	0.12	S	R	0
NRL-AR-060	EURL EC-17.3	Panel1	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-060	EURL EC-17.4	Panel2	Ertapenem	=	=	0.06	0.03	R	S	0
NRL-AR-062	EURL EC-17.1	Panel2	Ertapenem	=	=	0.12	0.12	S	R	0
NRL-AR-064	EURL EC-17.2	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-064	EURL EC-17.3	Panel1	Sulfamethoxazole	=	<=	128	8	R	S	0
NRL-AR-064	EURL EC-17.5	Panel1	Chloramphenicol	=	<=	64	8	R	S	0
NRL-AR-064	EURL EC-17.5	Panel1	Ciprofloxacin	=	<=	0.25	0.015	R	S	0
NRL-AR-064	EURL EC-17.5	Panel1	Sulfamethoxazole	=	<=	512	8	R	S	0
NRL-AR-064	EURL EC-17.5	Panel1	Trimethoprim	>	<=	16	0.25	R	S	0
NRL-AR-064	EURL EC-17.5	Panel2	Cefoxitin	=	=	32	4	R	S	0
NRL-AR-064	EURL EC-17.5	Panel2	Ertapenem	=	=	2	0.015	R	S	0

Lab number	Strain	Panel type	Antimicrobial	Obtained operator value	Expected operator value	Obtained mic value	Expected mic value	Obtained interpretation value	Expected interpretation value	Score interpretation
NRL-AR-002	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-002	EURL S-17.1	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-004	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-004	EURL S-17.2	Panel1	Azithromycin	=	=	64	4	R	S	0
NRL-AR-004	EURL S-17.4	Panel1	Ceftazidime	>	=	8	1	R	S	0
NRL-AR-004	EURL S-17.4	Panel1	Chloramphenicol	>	=	64	8	R	S	0
NRL-AR-004	EURL S-17.4	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-004	EURL S-17.4	Panel2	Ceftazidime	=	=	32	1	R	S	0
NRL-AR-006	EURL S-17.1	Panel2	Cefotaxime-clavulanic	=	=	1	0.5/4	R	S	0
NRL-AR-006	EURL S-17.4	Panel1	Nalidixic acid	<=	>	4	64	S	R	0
NRL-AR-006	EURL S-17.4	Panel1	Sulfamethoxazole	=	>	16	512	S	R	0
NRL-AR-006	EURL S-17.4	Panel1	Tetracycline	<=	>	2	32	S	R	0
NRL-AR-006	EURL S-17.4	Panel1	Trimethoprim	<=	>	0.25	16	S	R	0
NRL-AR-006	EURL S-17.4	Panel2	Cefoxitin	=	=	8	8	R	S	0
NRL-AR-006	EURL S-17.7	Panel2	Cefotaxime-clavulanic	=	=	0.12	0.12/4	R	S	0
NRL-AR-006	EURL S-17.8	Panel1	Ampicillin	>	>	32	32	S	R	0
NRL-AR-009	EURL S-17.2	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-011	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-011	EURL S-17.1	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-012	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-019	EURL S-17.1	Panel1	Tigecycline	=	=	1	2	S	R	0
NRL-AR-019	EURL S-17.4	Panel2	Imipenem	=	=	1	2	S	R	0
NRL-AR-020	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-020	EURL S-17.1	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-020	EURL S-17.2	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-020	EURL S-17.4	Panel2	Imipenem	=	=	1	2	S	R	0
NRL-AR-021	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-021	EURL S-17.1	Panel2	Temocillin	=	=	32	32	S	R	0
NRL-AR-021	EURL S-17.2	Panel2	Temocillin	=	=	32	32	S	R	0
NRL-AR-021	EURL S-17.3	Panel2	Cefepime	=	=	0.5	0.5	S	R	0
NRL-AR-021	EURL S-17.4	Panel1	Tigecycline	=	=	1	0.5	R	S	0
NRL-AR-021	EURL S-17.4	Panel2	Cefepime	=	=	1	1	S	R	0
NRL-AR-021	EURL S-17.4	Panel2	Imipenem	=	=	1	2	S	R	0
NRL-AR-022	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-022	EURL S-17.2	Panel2	Cefoxitin	>	=	64	8	R	S	0
NRL-AR-025	EURL S-17.4	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-025	EURL S-17.7	Panel2	Ceftazidime	=	=	1	1	R	S	0
NRL-AR-029	EURL S-17.5	Panel2	Imipenem	=	=	1	4	S	R	0
NRL-AR-030	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-030	EURL S-17.1	Panel2	Ceftazidime-clavulanic	=	=	1	0.5/4	R	S	0
NRL-AR-033	EURL S-17.4	Panel1	Ceftazidime	=	=	2	1	R	S	0
NRL-AR-037	EURL S-17.2	Panel1	Ceftazidime	>	>	8	8	S	R	0
NRL-AR-037	EURL S-17.2	Panel1	Sulfamethoxazole	>	>	512	512	S	R	0
NRL-AR-037	EURL S-17.4	Panel2	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-038	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-038	EURL S-17.4	Panel1	Tigecycline	=	=	1	0.5	R	S	0
NRL-AR-039	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-039	EURL S-17.6	Panel1	Sulfamethoxazole	>	=	512	16	R	S	0
NRL-AR-040	EURL S-17.2	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-040	EURL S-17.4	Panel1	Ceftazidime	=	=	1	1	R	S	0
NRL-AR-040	EURL S-17.5	Panel1	Meropenem	=	=	1	1	S	R	0
NRL-AR-040	EURL S-17.6	Panel1	Sulfamethoxazole	>	=	512	16	R	S	0
NRL-AR-040	EURL S-17.8	Panel1	Sulfamethoxazole	>	=	512	16	R	S	0
NRL-AR-041	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-042	EURL S-17.2	Panel1	Amikacin	=	=	8	4	R	S	0
NRL-AR-042	EURL S-17.4	Panel2	Imipenem	=	=	1	2	S	R	0
NRL-AR-045	EURL S-17.1	Panel1	Azithromycin	=	=	32	16	R	S	0
NRL-AR-045	EURL S-17.4	Panel1	Ceftazidime	=	=	4	1	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Azithromycin	>	=	64	8	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Chloramphenicol	>	=	64	8	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Ciprofloxacin	>	=	8	0.015	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Meropenem	=	=	0.25	0.03	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Nalidixic acid	>	=	64	4	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Sulfamethoxazole	>	=	512	16	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Tetracycline	=	=	32	2	R	S	0
NRL-AR-045	EURL S-17.8	Panel1	Trimethoprim	>	=	16	0.25	R	S	0
NRL-AR-045	EURL S-17.8	Panel2	Cefoxitin	=	=	16	2	R	S	0
NRL-AR-045	EURL S-17.8	Panel2	Temocillin	=	=	32	16	R	S	0
NRL-AR-056	EURL S-17.1	Panel2	Cefoxitin	=	=	8	16	S	R	0
NRL-AR-056	EURL S-17.1	Panel2	Temocillin	=	=	16	32	S	R	0
NRL-AR-060	EURL S-17.1	Panel2	Cefotaxime-clavulanic	=	=	1	0.5/4	R	S	0
NRL-AR-062	EURL S-17.1	Panel2	Ceftazidime-clavulanic	=	=	1	0.5/4	R	S	0
NRL-AR-062	EURL S-17.2	Panel2	Cefotaxime-clavulanic	=	=	0.5	0.25/4	R	S	0
NRL-AR-062	EURL S-17.5	Panel1	Colistin	<=	=	1	1	R	S	0

Lab number	Strain	Antimicrobial	Obtained mic value	Expected mic value	Obtained interpreta tion value	Expected interpreta tion value	Score interpreta tion
NRL-AR-004	EURL C-17.5	Chloramphenicol	32	2	R	S	0
NRL-AR-004	EURL C-17.5	Ertapenem	1	0.25	R	S	0
NRL-AR-011	EURL C-17.3	Ciprofloxacin	1	0.25	R	S	0
NRL-AR-011	EURL C-17.3	Ertapenem	4	0.12	R	S	0
NRL-AR-012	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-014	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-023	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-025	EURL C-17.5	Ertapenem	2	0.25	R	S	0
NRL-AR-030	EURL C-17.3	Tetracycline	4	0.5	R	S	0
NRL-AR-033	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-037	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-039	EURL C-17.3	Ciprofloxacin	1	0.25	R	S	0
NRL-AR-040	EURL C-17.2	Ertapenem	0.5	1	S	R	0
NRL-AR-041	EURL C-17.4	Erythromycin	256	4	R	S	0

Lab number	Strain	Antimicrobial	Obtained operator value	Expected operator value	Obtained mic value	Expected mic value	Obtained interpreta tion value	Expected interpreta tion value	Score interpreta tion
NRL-AR-016	EURL ST-17.8	Linezolid	=	=	4	8	S	R	0
NRL-AR-017	EURL ST-17.8	Chloramphenicol	<=	>	4	64	S	R	0
NRL-AR-017	EURL ST-17.8	Linezolid	<=	=	1	8	S	R	0
NRL-AR-020	EURL ST-17.3	Trimethoprim	=	=	4	2	R	S	0
NRL-AR-023	EURL ST-17.2	Fusidic acid	=	=	16	0.25	R	S	0
NRL-AR-023	EURL ST-17.3	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-025	EURL ST-17.3	Kanamycin	>	=	4	4	R	S	0
NRL-AR-037	EURL ST-17.8	Chloramphenicol	<=	>	4	64	S	R	0
NRL-AR-037	EURL ST-17.8	Linezolid	=	=	2	8	S	R	0
NRL-AR-039	EURL ST-17.3	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-039	EURL ST-17.3	Trimethoprim	=	=	4	2	R	S	0
NRL-AR-039	EURL ST-17.8	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-040	EURL ST-17.1	Trimethoprim	=	=	4	1	R	S	0
NRL-AR-040	EURL ST-17.3	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-040	EURL ST-17.3	Trimethoprim	=	=	4	2	R	S	0
NRL-AR-040	EURL ST-17.6	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-040	EURL ST-17.8	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-041	EURL ST-17.3	Trimethoprim	=	=	4	2	R	S	0
NRL-AR-045	EURL ST-17.3	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-045	EURL ST-17.3	Tetracycline	=	=	2	0.5	R	S	0
NRL-AR-045	EURL ST-17.3	Trimethoprim	=	=	4	2	R	S	0
NRL-AR-045	EURL ST-17.5	Fusidic acid	=	=	2	0.25	R	S	0
NRL-AR-045	EURL ST-17.5	Sulfamethoxazole	>	=	512	64	R	S	0
NRL-AR-064	EURL ST-17.1	Sulfamethoxazole	<=	=	64	512	S	R	0
NRL-AR-064	EURL ST-17.2	Penicillin	=	>	2	1	S	R	0
NRL-AR-064	EURL ST-17.4	Quinopristin/dalfopristin	<=	=	0.5	2	S	R	0
NRL-AR-064	EURL ST-17.5	Quinopristin/dalfopristin	=	=	1	2	S	R	0
NRL-AR-064	EURL ST-17.7	Cefoxitin	=	=	2	16	S	R	0
NRL-AR-064	EURL ST-17.7	Penicillin	<=	>	0.12	1	S	R	0
NRL-AR-064	EURL ST-17.8	Quinopristin/dalfopristin	=	=	2	4	S	R	0