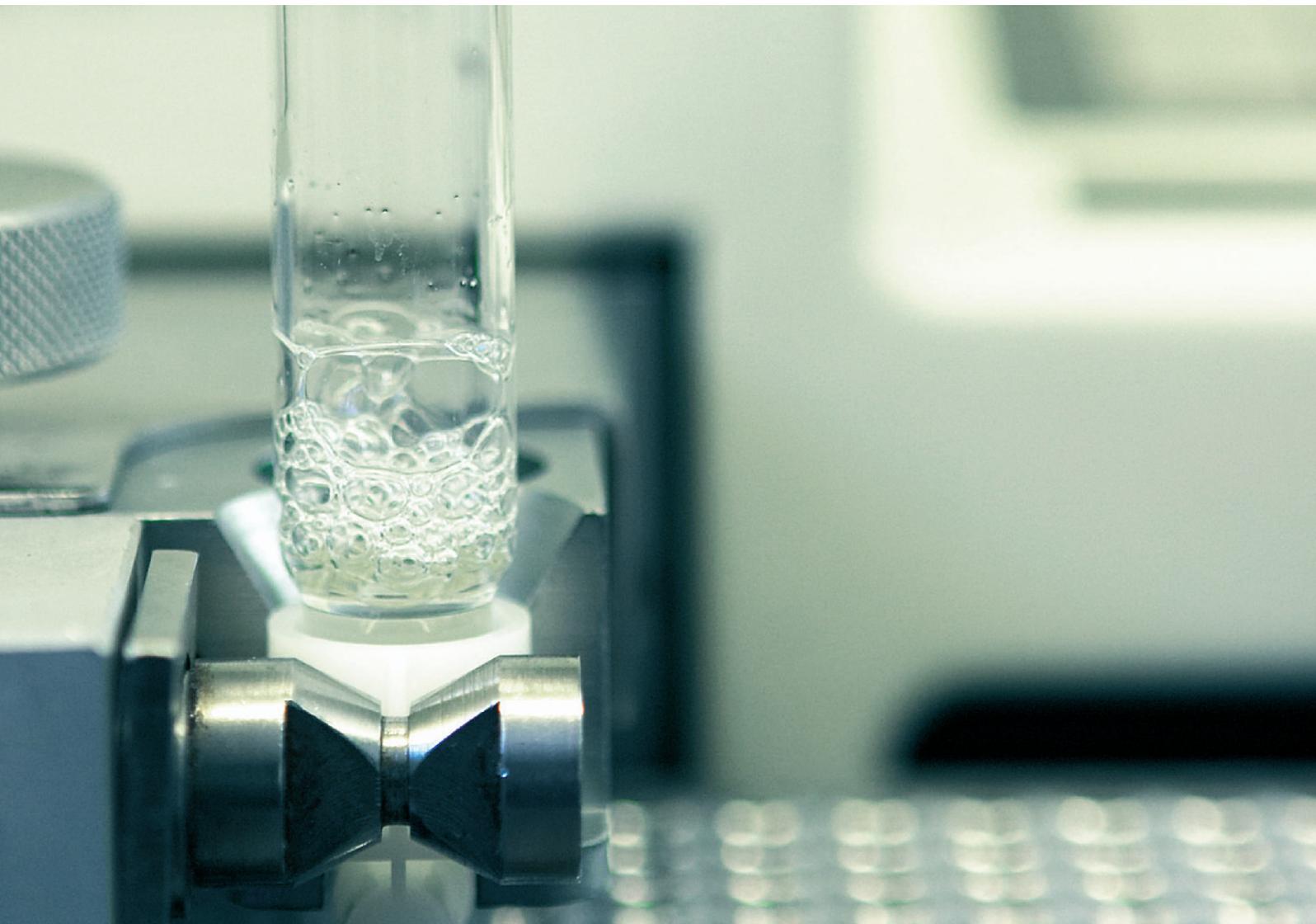


The 29th EURL-AR Proficiency Test

Escherichia coli, Salmonella, Campylobacter and Enterococcus (2021)



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1. Introduction

This report describes and summarizes the results of the 29th 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 *Enterococcus*. For *E. coli* this is the 14th PT, for *Salmonella* and *Campylobacter* it is the 15th, whereas for *Enterococcus*, it is the 13th. The PT includes categorisation of the relevant *E. coli* and *Salmonella* strains as ESBL-, AmpC- and carbapenemase-phenotypes, as well as the identification of the *Campylobacter* and *Enterococcus* species as *C. jejuni* or *C. coli* and as *E. faecium* or *E. faecalis*, respectively.

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 *Enterococcus* 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 *Enterococcus*).
- 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 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 2021

A pre-notification (Appendix 1) to announce the EURL-AR PT on AST of *E. coli*, *Salmonella*, *Campylobacter* and *Enterococcus* was distributed on 16 June 2021 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 29th EURL-AR PT (n=33) that performed antimicrobial susceptibility testing of *E. coli* (EC) *Salmonella* (Salm), *Campylobacter* (Camp) and *Enterococcus* (Ent). The map was created using 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 *Enterococcus* 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 signed up for all organisms included in the present PT, except for one (Serbia), which did not sign up for *Campylobacter* and six countries (Croatia, Cyprus, Greece, Hungary, Italy and Serbia) which did not sign up for *Enterococcus*. The level of participation per country is presented in Figure 1. In total, this report evaluates 33, 33, 32 and 27 sets of results from the *E. coli*, *Salmonella*, *Campylobacter* and *Enterococcus* trials respectively.

1.2. Strains

Eight strains for each organism (*E. coli*, *Salmonella*, *Campylobacter* and *Enterococcus*) 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. 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 *E. faecalis* CCM 4224 (ATCC 29212) 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), *C. coli* (2012-70-443-2) as well as *E. faecalis* (2012-70-103-3) and *E. faecium* (2012-70-76-8) for the purpose of performing internal method QC when performing ASTs for *E. coli*, *Salmonella*, *Campylobacter* or *Enterococcus* 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, 30th ed. (2020) "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) from Trek Diagnostic



Systems Ltd, UK. MIC determination for the *Campylobacter* and *Enterococcus* testing was performed using the Sensititre systems (EUCAMP3 and EUVENC respectively) from Trek Diagnostic Systems Ltd, UK. Participants of the *Campylobacter* and *Enterococcus* PT were additionally requested to identify the species of the *Campylobacter* spp. strains as *C. jejuni* or *C. coli*, as well as of the *Enterococcus* spp. strains, as *E. faecalis* or *E. faecium*. The panels of antimicrobials for included in EURL-AR PT 2021 are presented in Table 1.

1.4. Distribution

In September 2021, bacterial strains in agar stab cultures (*E. coli*, *Salmonella* and *Enterococcus*) 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 2021.

<i>E. coli</i> & <i>Salmonella</i>		<i>Campylobacter</i>	<i>Enterococcus</i>
Panel 1 (EUVSEC3)	Panel 2 (EUVSEC2)	Panel 1 (EUCAMP3)	Panel 1 (EUVENC)
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 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)	Ampicillin (AMP) Chloramphenicol (CHL) Ciprofloxacin (CIP) Daptomycin (DAP) Erythromycin (ERY) Gentamicin (GEN) Linezolid (LZD) Quinopristin/Dalfopristin Teicoplanin (TEI) Tetracycline (TET) Tigecycline (TGC) Vancomycin (VAN)

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 4°C (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. 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 *E. faecalis* (ATCC 29212). The results were evaluated towards the quality control ranges according to the relevant guidelines, *i.e.*, the CLSI documents VET06 (2017) or M100, 30th ed. (2020).

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 5 May 2022. 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 and deviations in relation to the species detection of *Campylobacter* and *Enterococcus*.

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 2021 reflect a high level of performance, with the overall deviation level for each trial ranging from 0.4% (*Enterococcus* trial) to 1.0% (*Campylobacter* trial), and for the internal control strains ranging from 0.0% (*Campylobacter* trial) to 1.2% (*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 *Enterococcus*).

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



Trial	Strain/antimicrobial combination omitted from the evaluation (Scores blanked)	%Deviation level
<i>E. coli</i>	EURL EC-16.2/Imipenem	75.8
<i>E. coli</i>	EURL EC-16.2/Trimethoprim	60.6
<i>E. coli</i>	EURL EC-16.2/Cefoxitin	54.5
<i>E. coli</i>	EURL EC-16.2/Cefepime	48.5
<i>E. coli</i>	EURL EC-16.8/Cefoxitin	27.3
<i>Salmonella</i>	EURL S-16.2/Tigecycline	43.8
<i>Salmonella</i>	EURL S-16.4/Tigecycline	70.0
<i>Salmonella</i>	EURL S-16.6/Tigecycline	28.1
<i>Enterococcus</i>	EURL ENT-16.1/Quinupristin-dalfopristin	25.0
<i>Enterococcus</i>	EURL ENT-16.8/Chloramphenicol	38.5

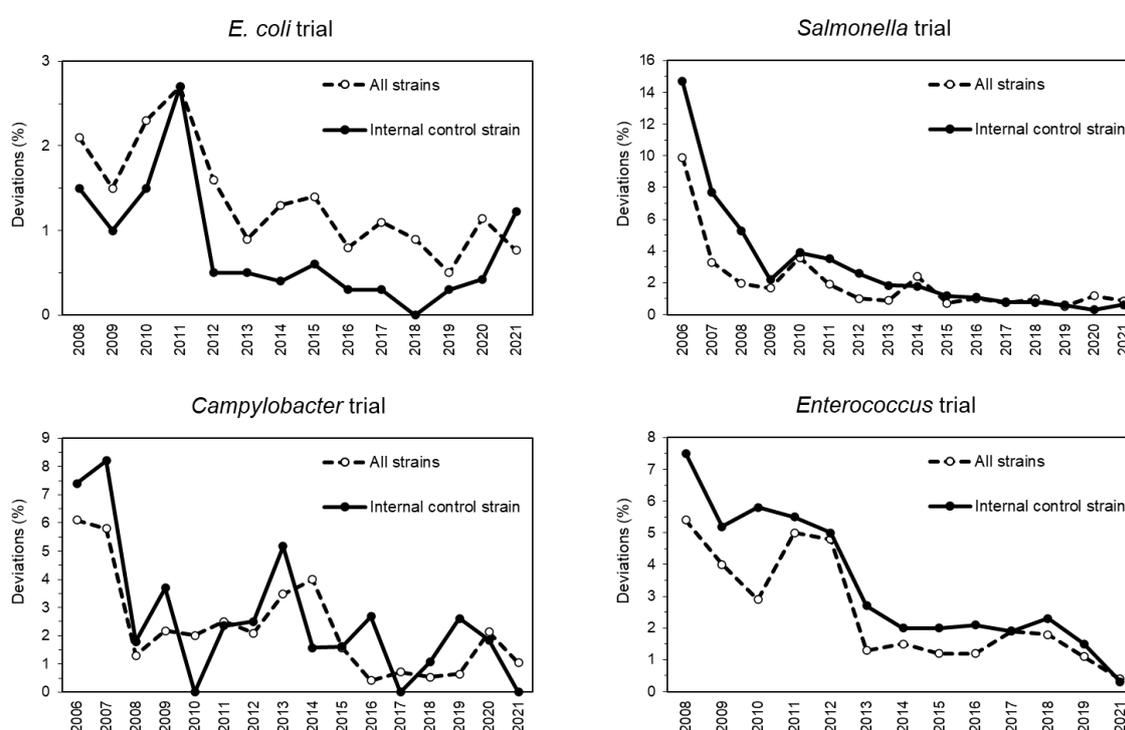


Figure 2. A comparison between the EURL-AR proficiency tests since 2006, showing the total percentage of deviations for antimicrobial susceptibility testing for each trial (Top Left: *E. coli*, Top Right: *Salmonella*, Bottom Left: *Campylobacter*, Bottom Right: *Enterococcus*).

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 was generally low, with the majority of participants reporting all result correctly (Figure 3). Laboratories #37 and #32 obtained a deviation level slightly over the 5% limit (5.7 and 5.2% respectively). Similarly, the deviation level for each strain-antimicrobial combination was generally very low (Figure 4).

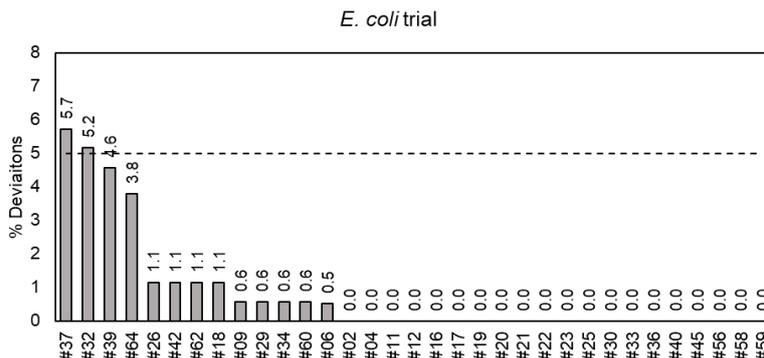


Figure 3. Percent deviation level for each laboratory, *E. coli* trial.

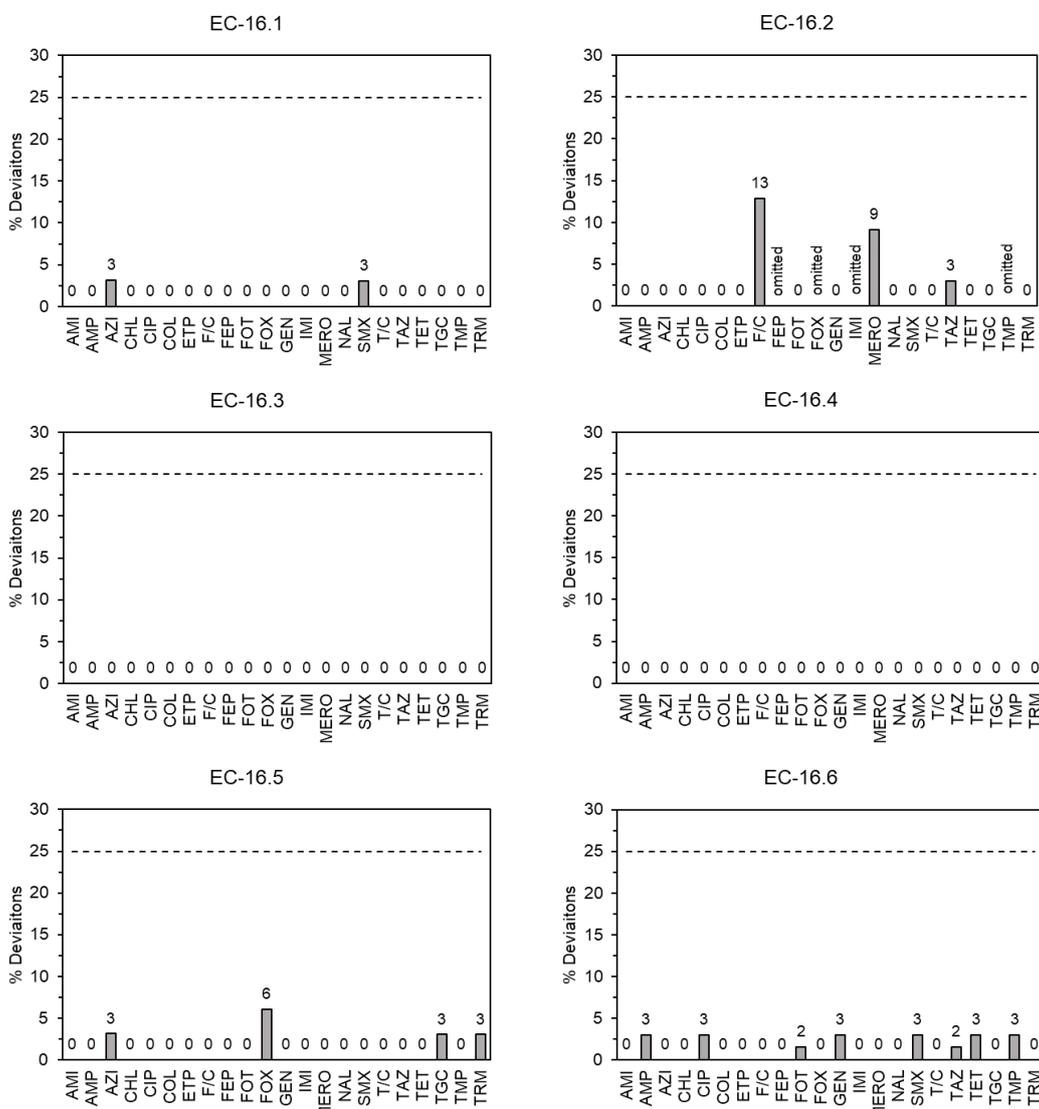


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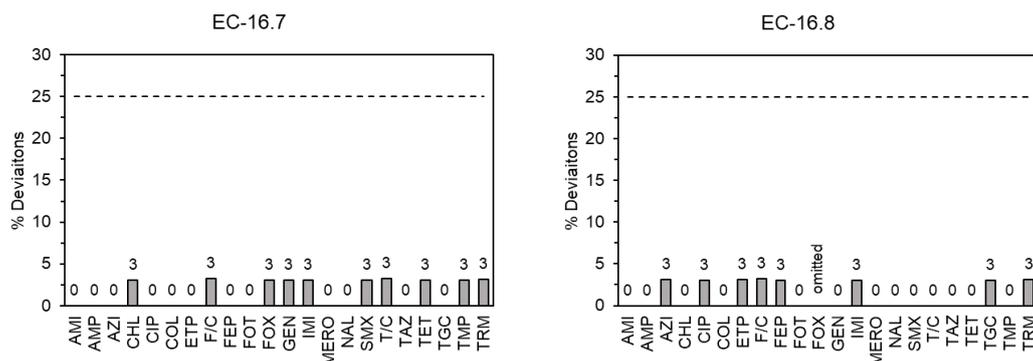


Figure 4. Percent deviation level for each strain-antimicrobial combination, *E. coli* trial.

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, except for one, tested the ESBL-, carbapenemase- and AmpC-phenotype strains to panel 2. Lab #32 did not proceed to testing strain EC-16.6 to panel 2, and this was because this laboratory reported a susceptible phenotype for FOT and TAZ, while resistant phenotypes were expected for both antimicrobials for this strain (the strain was susceptible to MERO). All participants identified the correct ESBL phenotype for the susceptible strains to FOT, TAZ and MERO (EC-16.3 and EC-16.4). Moreover, 100% correct results were obtained for strains EC-16.1 and EC-16.6, both categorised as ESBL-phenotype.

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

Strain	Criteria	Expected ESBL phenotype	Genotype
EC-16.1	<ul style="list-style-type: none"> ▪ FOT/TAZ > 1 mg/L ▪ MERO ≤ 0.12 mg/L ▪ FOX ≤ 8 mg/L ▪ SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{SHV-12}
EC-16.2	MERO > 0.12 mg/L	Carbapenemase-phenotype	<i>bla</i> _{OXA-162}
EC-16.3	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
EC-16.4	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
EC-16.5	<ul style="list-style-type: none"> ▪ FOT/TAZ > 1 mg/L ▪ MERO ≤ 0.12 mg/L ▪ FOX ≤ 8 mg/L ▪ SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-15}
EC-16.6	<ul style="list-style-type: none"> ▪ FOT/TAZ > 1 mg/L ▪ MERO ≤ 0.12 mg/L ▪ FOX ≤ 8 mg/L ▪ SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-15}
EC-16.7	<ul style="list-style-type: none"> ▪ FOT/TAZ > 1 mg/L ▪ MERO ≤ 0.12 mg/L ▪ FOX ≤ 8 mg/L ▪ SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-1}
EC-16.8	<ul style="list-style-type: none"> ▪ TAZ > 1 mg/L ▪ MERO ≤ 0.12 mg/L ▪ FOX > 8 mg/L 	AmpC-phenotype	ampC-promoter (g.-42C>T)

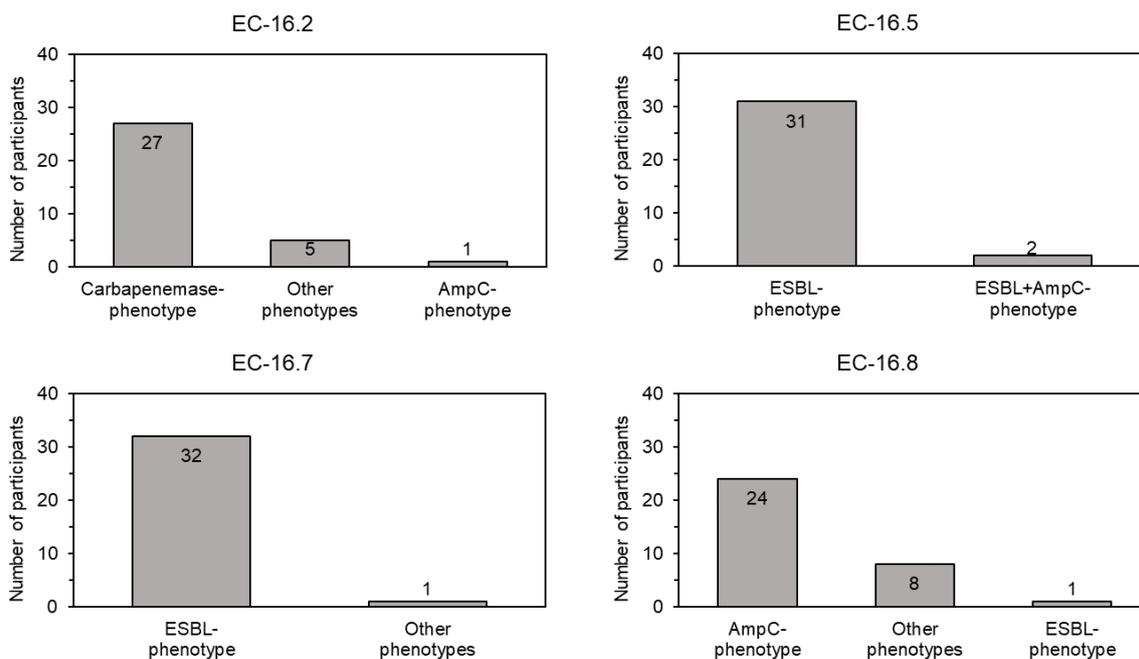


Figure 5. Obtained ESBL phenotypes for strains EC-16.2, EC-16.5, EC-16.7 and EC-16.8.

Unexpected ESBL phenotypes were reported for strains EC-16.2, EC-16.5, EC-16.7 and EC-16.8— see Figure 5. For strain EC-16.2, five participants (#18, #19, #26, #33 and #58) categorised the strain as “other phenotypes” and one participant (#64) as “AmpC phenotype”. The above six labs reported $MIC_{(MERO)}=0.12$, which is one two-fold dilution lower than the expected value ($MIC_{(MERO)}=0.25$, confirmed in four independent tests by the EURL-AR and SSI), and therefore within the method limitation.

For strain EC-16.5, two laboratories (#06 and #32) identified it as “ESBL+AmpC-phenotype”, while “ESBL-phenotype” was expected. This is because these laboratories submitted $MIC_{(FOX)}=16$ in panel 2, which is one two-fold dilution higher than the expected value $MIC_{(FOX)}=8$. Concerning FOX, the criterion to meet the ESBL+AmpC-phenotype is $MIC(FOX)>8$, while when $MIC(FOX)\leq 8$ the strain is categorised as ESBL-phenotype.

For strain EC-16.7, one laboratory identified it as “Other phenotypes”, further commenting that “*It was considered "Other Phenotype" as it was resistant to IMI. Based on the submitted results for panel 2 antimicrobials ($MIC_{(FOT)}>64$, $MIC_{(TAZ)}>128$, $MIC_{(MERO)}=0.06$ and $MIC_{(FOX)}=16$) and because there is F/C and T/C synergy based on the submitted data, the strain qualified to be identified as “ESBL+AmpC-phenotype”.* However $MIC_{(FOX)}=4$ was expected for this strain; which justified for the expected categorisation as “ESBL-phenotype”.

The expected ESBL phenotype for strain EC-16.8 was “AmpC-phenotype”; however, eight laboratories (#04, #19, #20, #26, #34, #45, #62 and #64) identified the strain as “Other-phenotype” and one (#59) as “ESBL-phenotype”. All eight laboratories that submitted “Other phenotype” reported $MIC_{(FOX)}=8$, which is one two-fold dilution lower compared to the expected value. Because this was handled as a breakpoint issue and the EC-16.8/Cefoxitin combination was omitted from the evaluation (Table 2), “Other phenotype” is also accepted for this strain. Laboratory #59, reported $MIC_{(FOX)}=8$, and therefore categorised the strain as “AmpC-phenotype”, even though all the other conditions for this phenotype were met. Based on the MIC data submitted by laboratory #59, there is no synergy for F/C or T/C; therefore, the strain does not qualify to be categorised as “ESBL-phenotype”.



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 30th edition. No QC range was available for AZI, F/C, T/C and TRM. For the *E. coli* trial all participating laboratories (n=33) submitted data for panel 1 antimicrobials, while 30 laboratories submitted data for both panel 1 and panel 2 antimicrobials. Laboratories #26, #37 and #42 did not test panel 2. Moreover, data were not submitted for the following cases: a) laboratory #29 did not submit data for AMI and AZI from panel 1, b) laboratory #17 did not submit data for F/C and T/C from panel 2 and c) laboratory #29 did not submit data for F/C, T/C and TRM, from panel 2. The deviations for the reference strain results are presented in Figure 6. Laboratory #11 reported MIC_(ETP)≤0.06, which is outside the QC range. Similarly, laboratories #22 and #39 reported MIC(AMI)=8 and MIC(SMX)>512, respectively, which are both outside the QC range. Almost all participants (31/33) reported MIC_(FOT)≤0.25, which is one two-fold dilution higher 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
AMI	0.5-4
AMP	2-8
AZI	none
FEP	0.016-0.12
FOT	0.03-0.12
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

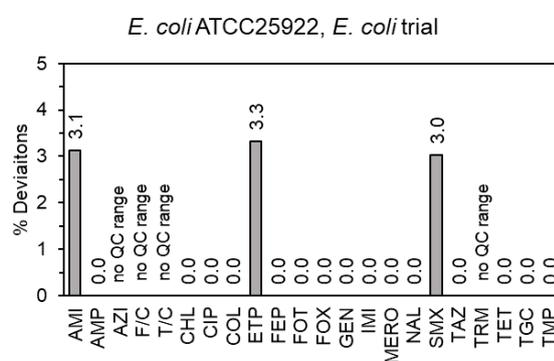


Figure 6. Percent deviation level for each antimicrobial for the *E. coli* ATCC25922 reference strain, *E. coli* trial.

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 was generally low, with the majority of participants obtaining less than 2% deviation level (Figure 7). Similarly the deviation level for each strain-antimicrobial combination was generally low (Figure 8), with few exceptions.



Salmonella trial

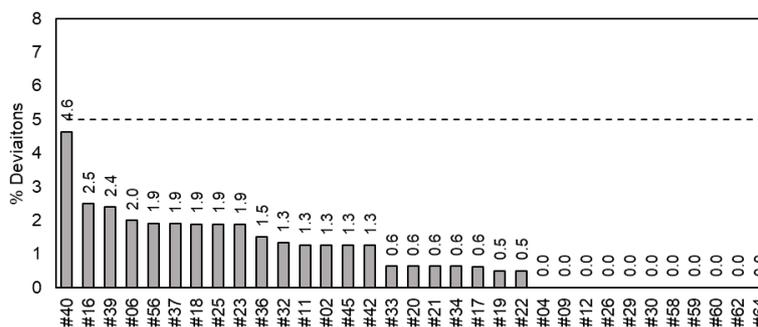


Figure 7. Percent deviation level for each laboratory, Salmonella trial.

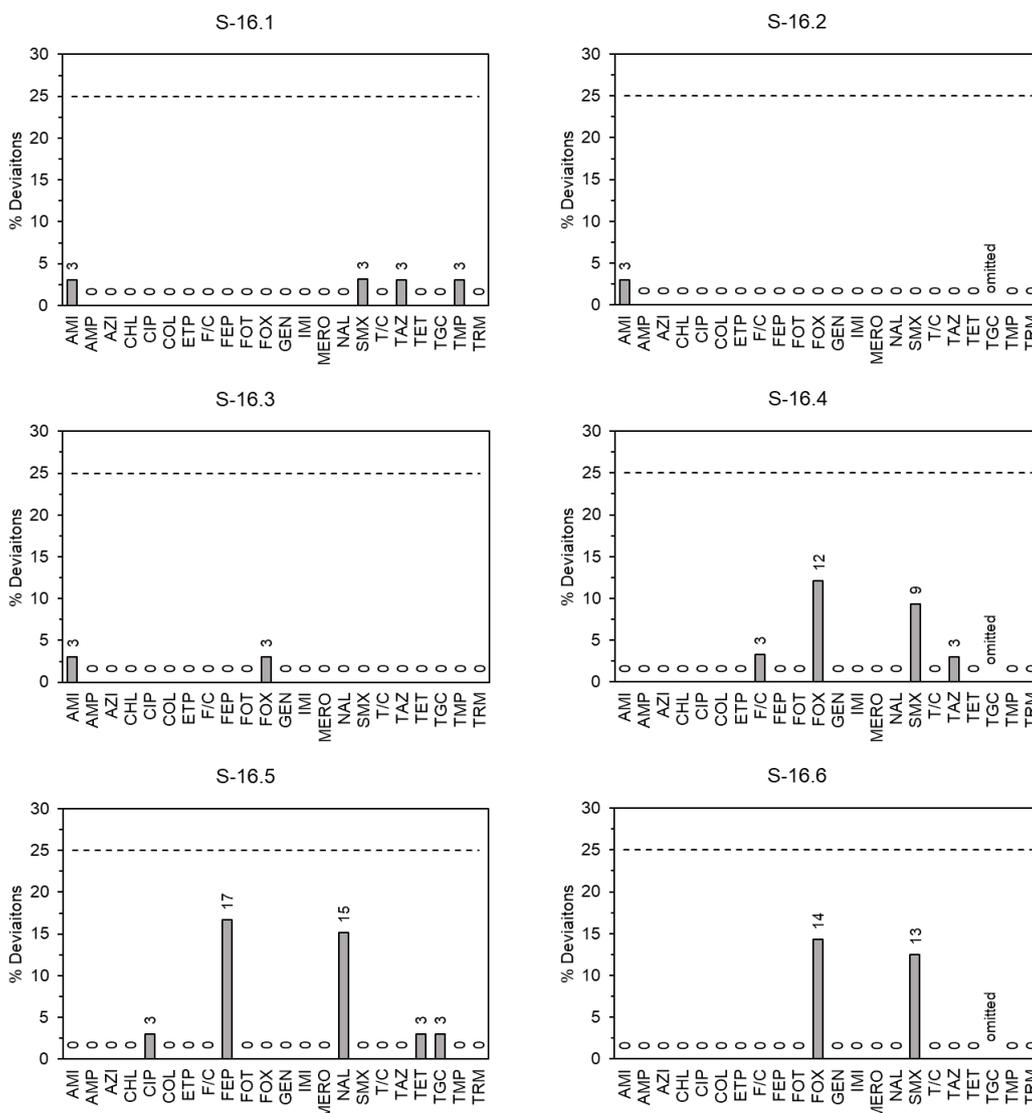
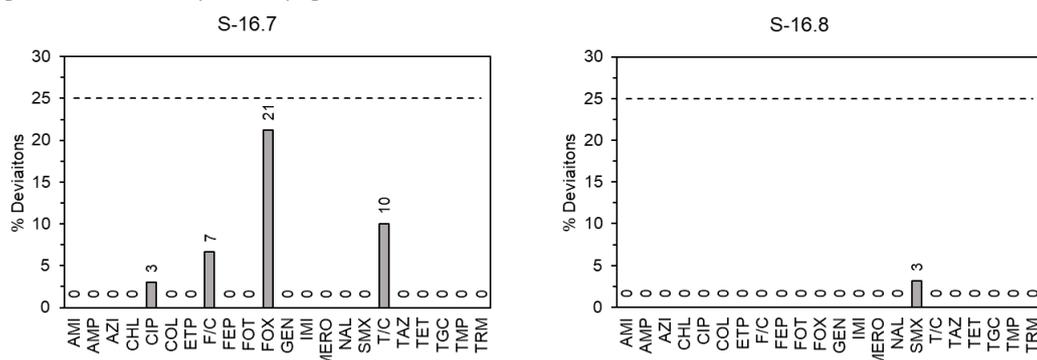


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Figure 8. Percent deviation level for each strain-antimicrobial combination, *Salmonella* trial.

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 identified the correct ESBL phenotype for the strains susceptible to FOT, TAZ and MERO (S-16.2, S-16.5, S-16.6 and S-16.8). All participants except for one, reported susceptible phenotypes to FOT, TAZ, FOX and MERO for the four susceptible strains. Participant #39 reported a phenotype resistant to FOX for strain S-16.6, as the obtained MIC value (=16) was one two-fold dilution higher than the expected MIC value (=8) and the ECOFF is 8 mg/L. This participant categorised the strain as “Other phenotypes”. Moreover, 100% correct results were obtained for the ESBL-phenotype strain S-16.1, while for the ESBL-phenotype strains S-16.3, S-16.4 and S-16.7 a few participants submitted unexpected phenotypes (Figure 9). One laboratory (#25) identified strain S-16.3 as ESBL+AmpC-phenotype, while an ESBL-phenotype was expected. For strain S-16.4, the same was observed for three laboratories (#6, #16 and #45), while for S-16.7, the same incident occurred for seven laboratories (#6, #11, #16, #18, #23, #25 and #37). The cause of this discrepancy was a reported MIC_(FOX)=16 mg/L, which is one two-fold dilution higher compared to the expected MIC value (same expected MIC value for all three strains, =8 mg/L). Even though this within the method limitations, a MIC_(FOX)=16 qualifies the strains to be classified as ESBL+AmpC-phenotype, as all criteria are met.

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

Strain	Criteria	Expected ESBL phenotype	Genotype
S-16.1	<ul style="list-style-type: none"> ▪FOT > 1 mg/L ▪MERO ≤ 0.12 mg/L ▪FOX ≤ 8 mg/L ▪SYN F/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-9}
S-16.2	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
S-16.3	<ul style="list-style-type: none"> ▪FOT/TAZ > 1 mg/L ▪MERO ≤ 0.12 mg/L ▪FOX ≤ 8 mg/L ▪SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-1}
S-16.4	<ul style="list-style-type: none"> ▪FOT/TAZ > 1 mg/L ▪MERO ≤ 0.12 mg/L ▪FOX ≤ 8 mg/L ▪SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-1}
S-16.5	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
S-16.6	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes
S-16.7	<ul style="list-style-type: none"> ▪FOT/TAZ > 1 mg/L ▪MERO ≤ 0.12 mg/L ▪FOX ≤ 8 mg/L ▪SYN F/C, T/C 	ESBL-phenotype	<i>bla</i> _{CTX-M-15} <i>bla</i> _{SHV-12}
S-16.8	FOT/TAZ/FOX/MERO ≤ ECOFF	Susceptible phenotype	No ESBL genes

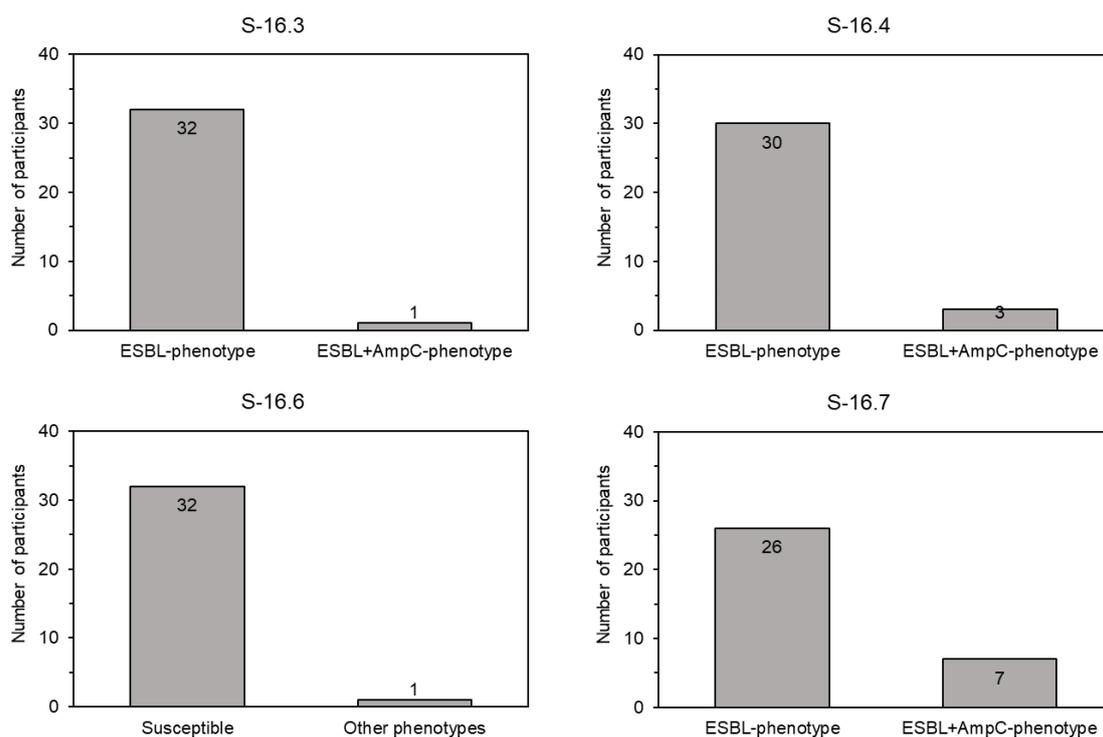


Figure 9. Obtained ESBL phenotypes for strains S-16.3, S-16.4, S-16.6 and S-16.7.

2.2.3. Reference strain results

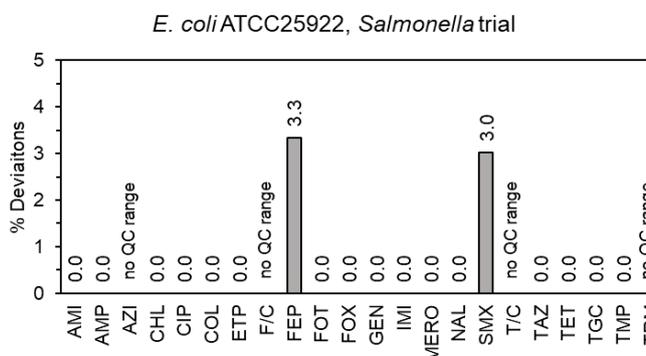
For the reference strain *E. coli* ATCC25922 results, deviations were defined as submitted MIC values that were outside the quality control (QC) acceptance intervals (Table 6) as defined in the CLSI M100 30th edition. No QC range is available for AZI, F/C, T/C and TRM. Deviations were defined as submitted MIC values that are outside the QC acceptance intervals. For the *Salmonella* trial all participating laboratories (n=33) submitted data for panel 1 antimicrobials, while 30 laboratories submitted data for both panel 1 and panel 2 antimicrobials. Similarly to the results in the *E. coli* trial, laboratories #26, #37 and #42 did not test panel 2. Moreover, data were not submitted for the following cases: a) laboratory #29 did not submit data for AMI and AZI from panel 1, b) laboratory #17 did not submit data for F/C and T/C from panel 2 and c) laboratory #29 did not submit data for F/C, T/C and TRM, from panel 2. The deviations for the reference strain are presented in Figure 10. Laboratory #39 reported MIC_(SMX)=128 mg/L, which is 4 times higher than the upper limit of the accepted QC range (8-32 mg/L). The respective MIC value for SMX reported by the same laboratory in the *E. coli* trial was off the QC limits too. Moreover, laboratory #11 reported MIC_(FEP)=0.6 mg/L, which is five times higher than the upper QC limit (0.125 mg/L). Finally, almost all participants (32/33 for panel 1 and 29/30 for panel 2) reported MIC_(FOT)≤0.25 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.



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

Antimicrobial	MIC
AMI	0.5-4
AMP	2-8
AZI	-
FEP	0.016-0.12
FOT	0.03-0.12
F/C	-
FOX	2-8
TAZ	0.06-0.5
T/C	-
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	-
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 majority of participants (n=21/32) ran the assay at 36-37°C/48h, and the rest (n=11/32) at 42°C/24h. The percent deviation level for each laboratory in the *Campylobacter* trial is presented in Figure 11 and was generally low, with the majority of participant reporting 100% correct results. However, two laboratories obtained an overall deviation level greater than 5%: laboratory #22 14.6% and laboratory #64 7.5%. Laboratory #22 reported MIC values that were higher (8- to 512- fold difference) compared to the expected values, for all panel antimicrobials which was due to contamination issues (personal communication with the laboratory). Laboratory #64 reported MIC values 16-32 times higher compared to the expected values, for ERY, CHL and GEN, which were attributed to difficulties in culturing the strains (personal communication with the laboratory). The overall performance for each strain/antimicrobial combination included in the *Campylobacter* trial is presented in Figure 12.

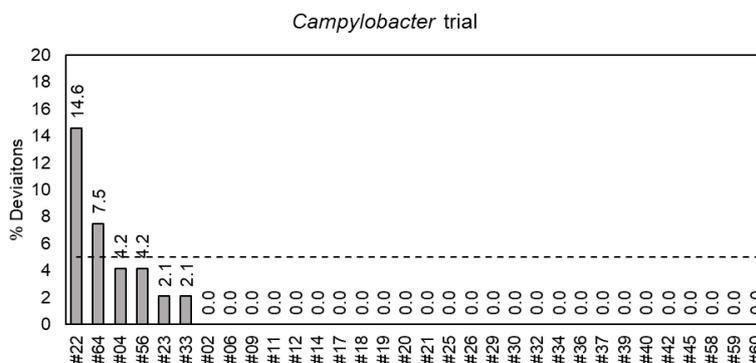


Figure 11. Percent deviation level for each laboratory, *Campylobacter* trial.

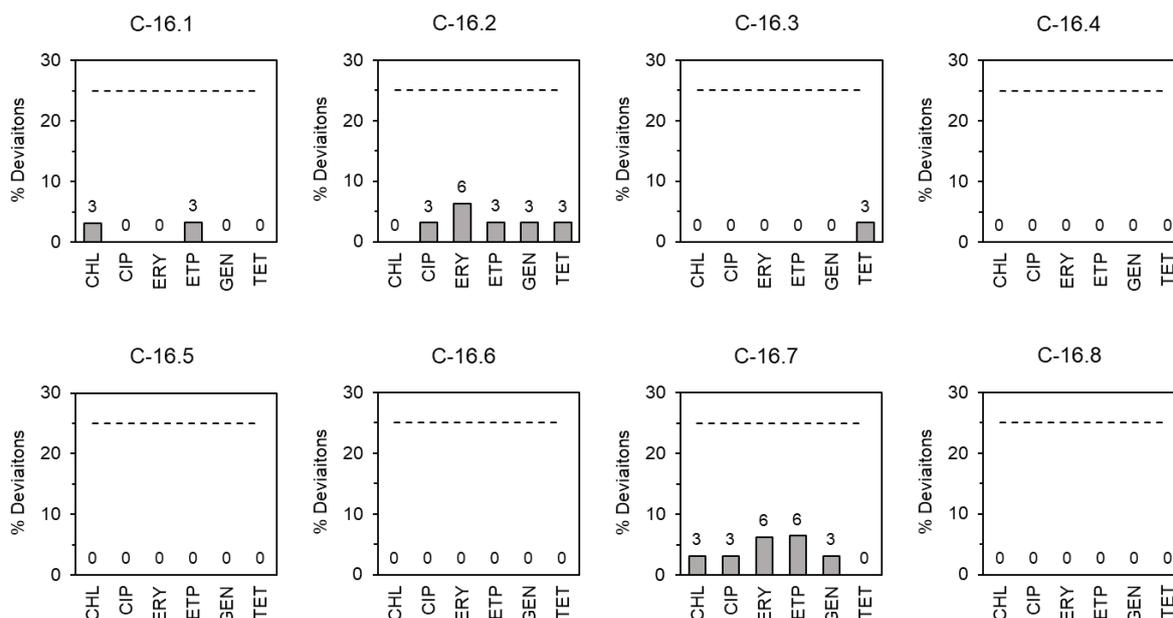


Figure 12. Percent deviation level for each strain-antimicrobial combination, *Campylobacter* trial.

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 four, achieved 100% correct results for species identification (Figure 13). Laboratories #30, #34 and #40 obtained a 12.5% deviation level, since they did not identify the correct species for one strain. Laboratory #64 had the lowest performance in *Campylobacter* species identification, with 50% correct results. All participants identified the correct species for strains C-16.1, C-16.5 and C-16.6.

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

<i>Campylobacter</i>	Species
C-16.1	<i>C. coli</i>
C-16.2	<i>C. jejuni</i>
C-16.3	<i>C. jejuni</i>
C-16.4	<i>C. jejuni</i>
C-16.5	<i>C. coli</i>
C-16.6	<i>C. jejuni</i>
C-16.7	<i>C. jejuni</i>
C-16.8	<i>C. coli</i>

Species identification, *Campylobacter* trial

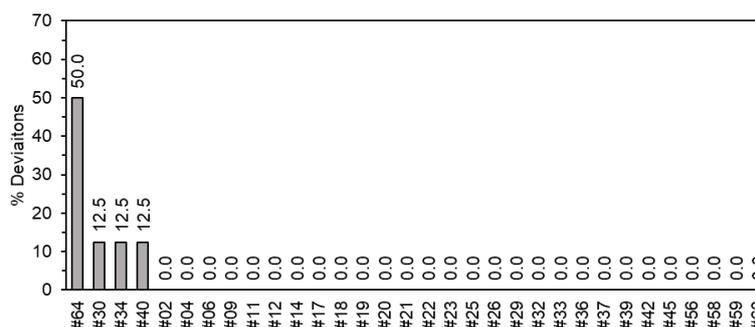


Figure 13. Percent deviation per laboratory, species identification, *Campylobacter* trial.

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). One (#4) did not submit data

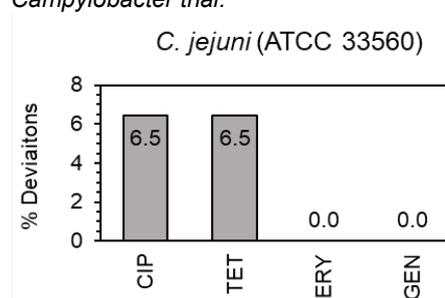


for the reference strain, and four laboratories did not submit data for two or more antimicrobials regarding the reference strain testing (#9 and #29 did not submit data for CHL and ETP, #23 for CHL and ERY and #64 for CHL, ETP, ERY and GEN). For the laboratories that used 36-37°C/48h for the plate incubation, all MIC values reported were within the accepted QC range. For the laboratories that used 42°C/24h, four deviations occurred (Figure 14). Laboratories #56 and #64 reported MIC_(CIP)=0.25 and MIC_(CIP)=4 mg/L, which are 2 and 32 times respectively higher than the upper QC value (0.125 mg/L). For TET, two laboratories (#18, #56) reported an MIC value of 2, which is one two-fold dilution step higher than the upper QC value (1 mg/L).

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	0.03-0.12
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. *Enterococcus* trial

2.4.1. AST data

Expected MIC values and phenotype interpretation (R/S) for the *Enterococcus* 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 2.3%, thus all laboratories complied to the 5% maximum deviation limit. The overall performance for each strain/antimicrobial included in the *Enterococcus* trial is presented in Figure 16. The deviation level was generally low and ranged from 0% to 4%, except for ENT-16.2/ERY which had a deviation level of 8%.

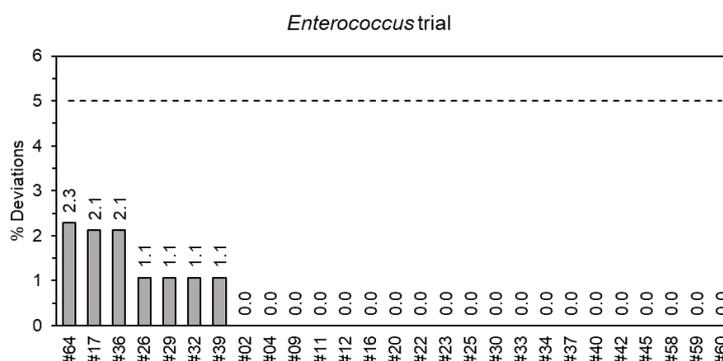


Figure 15. Percent deviation level for each laboratory, *Enterococcus* trial.

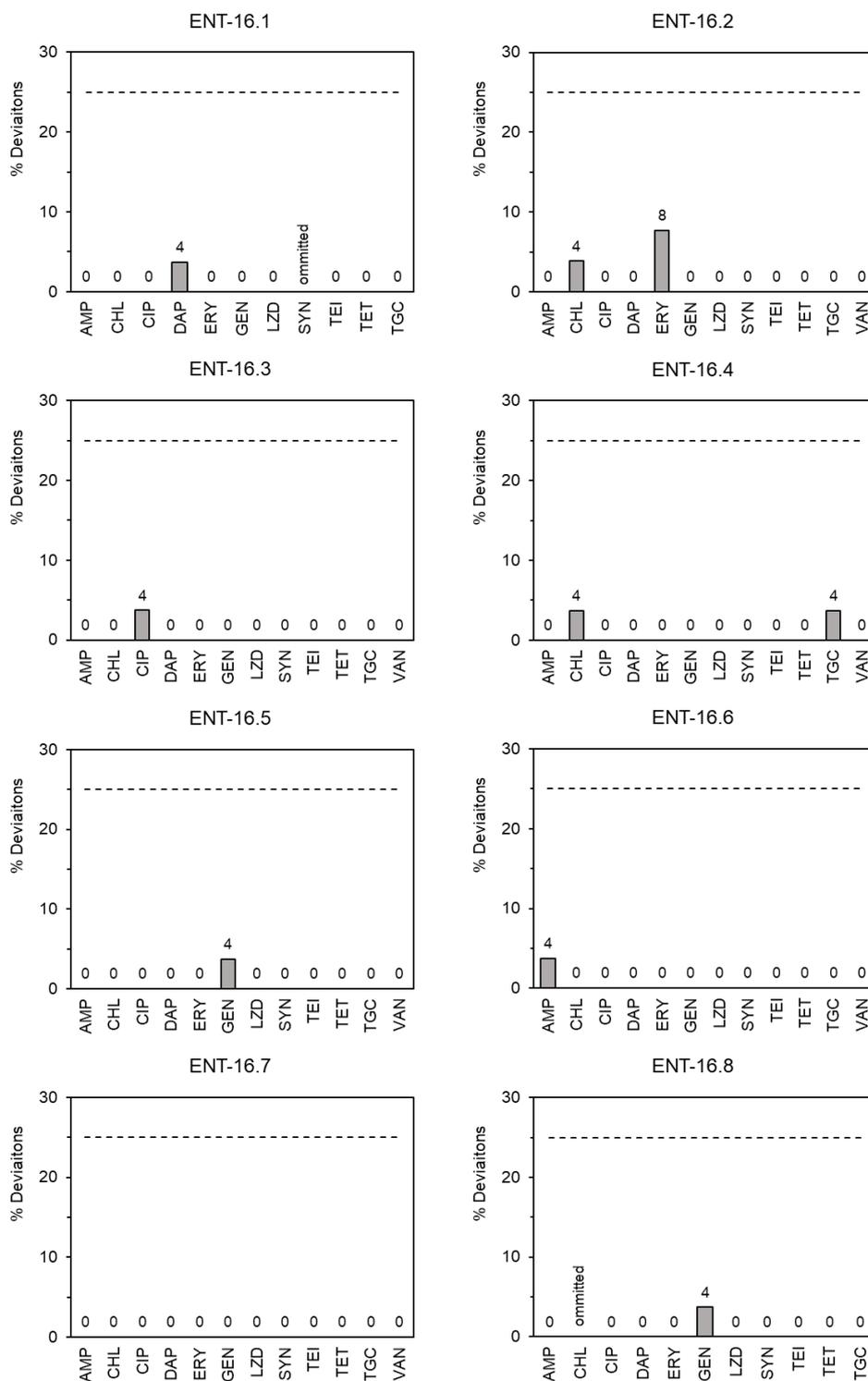


Figure 16. Percent deviation level for each strain-antimicrobial combination, *Enterococcus* trial.

2.4.2. Species identification results

The participants were requested to perform species identification of the *Enterococcus* test strains, as *E. faecium* or *E. faecalis*, using in-house methods or adopting the protocol available on the EURL-AR



website. From the 27 participants of the *Enterococcus* trial, all identified the correct species for all strains: *E. faecium* for ENT-16.1, ENT-16.2, ENT-16.3, ENT-16.6 and *E. faecalis* for ENT-16.4, ENT-16.5, ENT-16.7 and ENT-16.8; however, laboratory #16 did not test strain ENT-16.2.

2.4.3. Reference strain results

For the reference strain *E. faecalis* ATCC 29212 results, deviations were defined as submitted MIC values that are outside the quality control (QC) acceptance intervals (Table 9). For the *Enterococcus* trial, among the participating laboratories (n=27) all, except for one (#64), submitted data for the reference strain. All submitted results were correct, apart from one deviation: laboratory #36 reported MIC_(AMP)=4 mg/L while the QC range for ampicillin is 0.5 to 2 mg/L.

Antimicrobial	MIC
AMP	0.5-2
CHL	4-16
CIP	0.25-2
DAP	1-4
ERY	1-4
GEN	4-16
LZD	1-4
SYN	2-8
TEI	0.25-1
TET	8-32
TGC	0.03-0.12
VAN	1-4

Table 9. Quality control (QC) acceptance intervals for the reference strain *E. faecalis* (ATCC 29212).

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 four cases: laboratories #37 and #32 obtained deviation levels of 5.7 and 5.2% respectively in the *E. coli* trial, and laboratories #22 and #64 obtained deviation levels of 14.6 and 7.5 % respectively in the *Campylobacter* trial. The test covering the identification of the phenotype of *E. coli* and *Salmonella* strains producing betalactamases of the ESBL-, AmpC, and carbapenemase type rendered 18 deviations (90.9% correct categorisations) and 11 deviations (91.7% correct categorisations), respectively. 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 2021 for *E. coli*, *Salmonella*, *Campylobacter* and enterococci

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 enterococci isolates. Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954), *C. jejuni* ATCC 33560 (CCM 6214), and *E. faecalis* ATCC 29212 (CCM 4224) 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 enterococci 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 at the beginning of September 2021. 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 10 December 2021** 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.

Next EQAS: The next EQAS provided by the EURL-AR will be on selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from meat and caecal samples (Matrix EQAS) and is planned to be carried out late September 2021.

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

Sincerely,

Susanne Karlsdose Pedersen (suska@food.dtu.dk)
EURL-AR EQAS-Coordinator

PROTOCOL

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

1	INTRODUCTION	1
2	OBJECTIVES	2
3	OUTLINE OF THE EC/SALM/CAMP/ENT EQAS 2021	2
	3.1 Shipping, receipt and storage of strains	2
	3.2 QC reference strains	3
	3.3 Antimicrobial susceptibility testing	3
4	REPORTING OF RESULTS AND EVALUATION	9
5	HOW TO SUBMIT RESULTS VIA THE WEBTOOL	10
	APPENDIX	11

HISTORY OF CHANGES:

Version 2 (13 September 2021): Under '3.3.4 Enterococci', paragraph referring to different incubation conditions in CLSI document VET06 for enterococci deleted.

1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *Escherichia coli*, *Salmonella*, *Campylobacter* and *Enterococcus* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). The current EQAS 2021 will include AST of eight *E. coli*, *Salmonella*, *Campylobacter* and *Enterococcus* strains and AST of reference strains *E. coli* ATCC 25922 (CCM

3954), *Campylobacter jejuni* ATCC 33560 (CCM 6214) and *Enterococcus faecalis* ATCC 29212 (CCM 4224) together with AST of the internal EURL reference strains, *Acinetobacter baumannii* (2012-70-100-69), *Campylobacter coli* (2012-70-443-2), *E. faecalis* (2012-70-103-3) and *E. faecium* (2012-70-76-8).

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 QC Strains’ available on the EURL-AR website (see www.eurl-ar.eu).

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

3 OUTLINE OF THE EC/SALM/CAMP/ENT EQAS 2021

3.1 Shipping, receipt and storage of strains

In September 2021, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, *Salmonella*, *Campylobacter* and enterococci strains, respectively from the National Food Institute. For participants who did not receive them previously, this parcel will also contain reference strains.

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.

The *E. coli*, *Salmonella* and enterococci test strains are shipped as stab cultures, the *Campylobacter* test strains are shipped as a charcoal swabs and the reference strains are shipped lyophilised. Upon arrival to your laboratory, the strains should be stored in a dark place at 4°C for stabs and charcoal swabs, and in a dark and cool place for freeze-dried strains. Charcoal swabs 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 strains 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), for the *Campylobacter* testing, include *Campylobacter jejuni* ATCC 33560 (CCM 6214) together with *Campylobacter coli* (2012-70-443-2) and for the *Enterococcus* testing, include *Enterococcus faecalis* ATCC 29212 (CCM 4224) together with *E. faecalis* (2012-70-103-3) and *E. faecium* (2012-70-76-8).

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 www.eurl-ar.eu).

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 1, 2, 3, 4, 5 and 6 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.

Beta-lactam and carbapenem resistance

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 2 and Table 4 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 1, 2, 3 and 4), 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.

3.3.1 *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 1 and 2.

Table 1: Panel 1 antimicrobials recommended for AST of *E. coli* spp. and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC ($\mu\text{g/mL}$) (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 2: Panel 2 antimicrobials recommended for AST of *E. coli* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC ($\mu\text{g/mL}$) (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)	0.06*
Imipenem (IMI)	0.5
Meropenem (MERO or MEM)	0.06
Temocillin (TRM)	16

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

3.3.2 Salmonella

The interpretative criteria that should be applied for categorizing the *Salmonella* test strain as resistant or susceptible are those listed in Tables 3 and 4.

Table 3: Panel 1 antimicrobials recommended for AST of *Salmonella* spp. and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC ($\mu\text{g/mL}$) (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 4: : Panel 2 antimicrobials recommended for AST of *Salmonella* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC ($\mu\text{g/mL}$) (R>)
Cefepime (FEP)	0.125*
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)

3.3.3 Campylobacter

The interpretative criteria to be applied for categorizing the *Campylobacter* test strain as resistant or susceptible are those listed in Table 5.

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 36-37°C for 48 hours or 42°C for 24 hours.

Table 5: Antimicrobials recommended for AST of *Campylobacter jejuni* and *C. coli* and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.2

Antimicrobial	<i>C. jejuni</i>	<i>C. coli</i>
	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)	1	1
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>.

3.3.4 Enterococci

The interpretative criteria that should be applied for categorizing the Enterococci test strain as resistant or susceptible are those listed in Table 6.

Table 6: Antimicrobials recommended for AST of *Enterococcus faecalis* and *E. faecium* and interpretative criteria (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.3

Antimicrobial	<i>E. faecalis</i>	<i>E. faecium</i>
	MIC (µg/mL) (R>)	MIC (µg/mL) (R>)
Ampicillin (AMP)	4	8
Chloramphenicol (CHL)	32	32
Ciprofloxacin (CIP)	4	8
Daptomycin (DAP)	4	8
Erythromycin (ERY)	4	4
Gentamicin (GEN)	64	32
Linezolid (LZD)	4	4
Quinopristin/Dalfopristin (Synercid) (SYN)	0.5*	1*
Teicoplanin (TEI)	2	2
Tetracycline (TET)	4	4
Tigecycline (TGC)	0.25	0.25
Vancomycin (VAN)	4	4

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

Identification of Enterococci species

Species identification of the Enterococci 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>.

4 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 10th 2021.

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:

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

5 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 enterococci, 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.

⇒ About login to the webtool:

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:

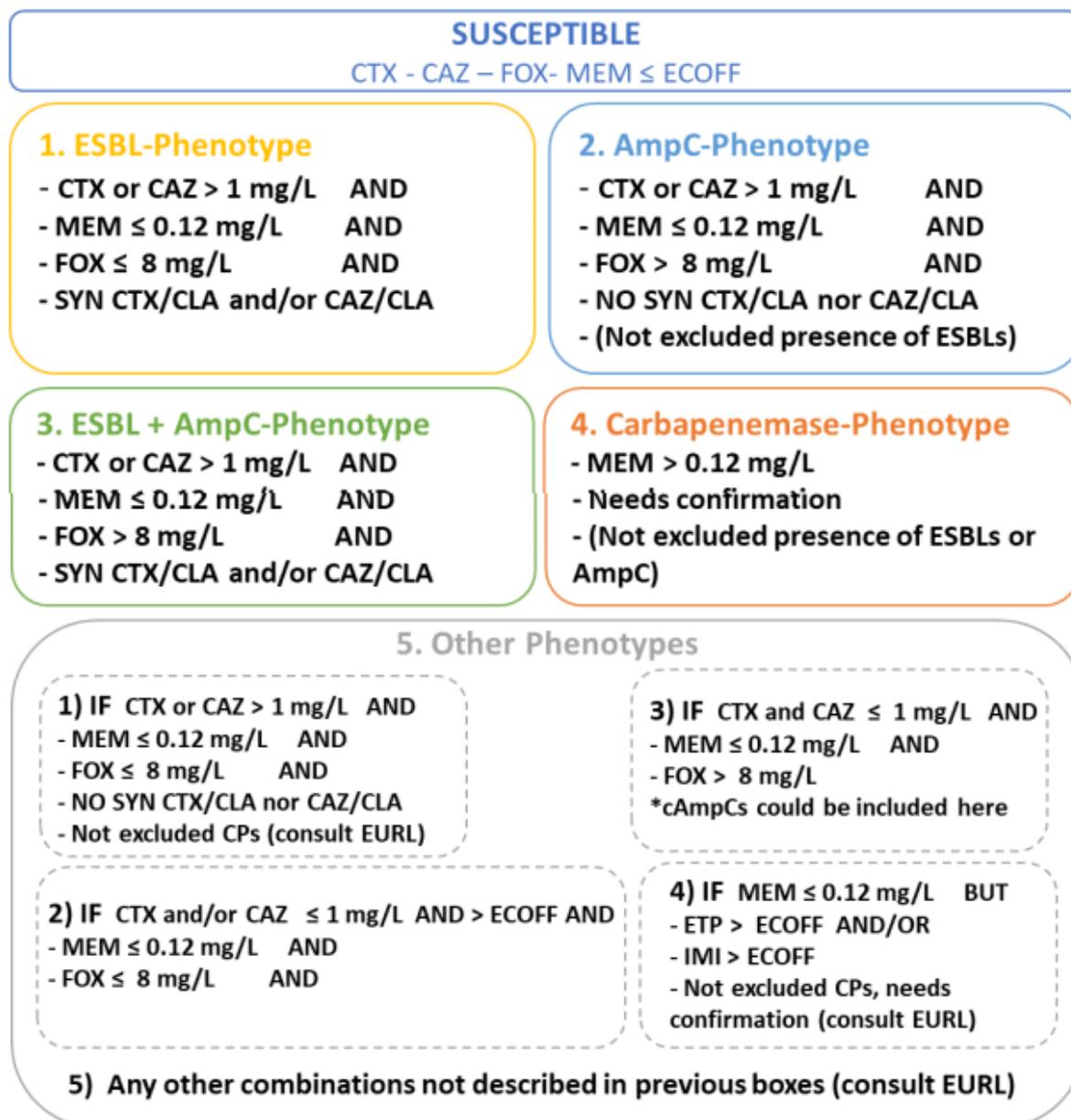
- a) If the EURL-AR has only one contact person for an NRL, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact suska@food.dtu.dk
- b) If your laboratory has two or more contact points on the EURL-AR contact list, 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.

--- --- ---

APPENDIX

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



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), 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal EFSA Journal 2021;19(4):6490. <https://doi.org/10.2903/j.efsa.2021.6490> (Annex A).

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E. coli, Salmonella, Campylobacter and enterococci

TEST FORMS

HISTORY OF CHANGES:

Version 2 (17 September 2021): Question related to incubation conditions for enterococci corrected to: Which incubation conditions did you use? °C/ h



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TEST FORM – *E. coli*

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

- MIC - Broth microdilution
 MIC – Agar dilution (note: not evaluated in the final report)

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:



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TEST FORM - *Salmonella*

Which method did you use for antimicrobial susceptibility testing of *Salmonella* in this EQAS?

- MIC - Broth microdilution
 MIC – Agar dilution (note: not evaluated in the final report)

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:



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TEST FORM - *Campylobacter*

Which method did you use for antimicrobial susceptibility testing of *Campylobacter* in this EQAS?

- MIC - Broth microdilution
 MIC – Agar dilution (note: not evaluated in the final report)

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

- CLSI
 EUCAST
 ISO 20776-1:2019
 TREK

Which incubation conditions did you use?

- 36-37°C, 48 hours
 42°C, 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 _____ μ l 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:



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TEST FORM - *Enterococci*

Which method did you use for antimicrobial susceptibility testing of *Enterococci* in this EQAS?

- MIC - Broth microdilution
 MIC – Agar dilution (note: not evaluated in the final report)

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 μ l 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:



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TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
<i>E. coli</i> EURL EC-16.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
<i>E. coli</i> EURL EC-16.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:

- | | | |
|--|--|--|
| <input type="checkbox"/> ESBL-phenotype | <input type="checkbox"/> AmpC-phenotype | <input type="checkbox"/> Other phenotype |
| <input type="checkbox"/> ESBL+AmpC-phenotype | <input type="checkbox"/> Carbapenemase-phenotype | <input type="checkbox"/> Susceptible (to panel 2 antimicrobials) |

Comments:



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TEST FORM



AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value (µg/ml)
1 st 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		
2 nd 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).



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

	Antimicrobial	MIC-value (µg/ml)
1 st 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		
2 nd 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).



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TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
<i>Salmonella</i> EURL S-16.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
<i>Salmonella</i> EURL S-16.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:

- | | | |
|--|--|--|
| <input type="checkbox"/> ESBL-phenotype | <input type="checkbox"/> AmpC-phenotype | <input type="checkbox"/> Other phenotype |
| <input type="checkbox"/> ESBL+AmpC-phenotype | <input type="checkbox"/> Carbapenemase-phenotype | <input type="checkbox"/> Susceptible (to panel 2 antimicrobials) |

Comments:



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TEST FORM



AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value ($\mu\text{g/ml}$)
1 st 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		
2 nd 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).



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

	Antimicrobial	MIC-value (µg/ml)
1 st 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		
2 nd 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).



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TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
<i>Campylobacter</i> EURL C-16.X <input type="checkbox"/> <i>C. jejuni</i> <input type="checkbox"/> <i>C. coli</i>	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		
<i>Campylobacter</i> EURL C-16.X <input type="checkbox"/> <i>C. jejuni</i> <input type="checkbox"/> <i>C. coli</i>	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		
<i>Campylobacter</i> EURL C-16.X <input type="checkbox"/> <i>C. jejuni</i> <input type="checkbox"/> <i>C. coli</i>	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		
<i>Campylobacter</i> EURL C-16.X <input type="checkbox"/> <i>C. jejuni</i> <input type="checkbox"/> <i>C. coli</i>	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		



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TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value ($\mu\text{g/ml}$)	
		36 °C/48 hours	42 °C/24 hours
<i>C. jejuni</i> ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		

Susceptibility testing of *Campylobacter coli* reference strain (2012-70-443-2)

Strain	Antimicrobial	MIC-value ($\mu\text{g/ml}$)
<i>C. coli</i> (2012-70-443-2)	Chloramphenicol	
	Ciprofloxacin	
	Ertapenem	
	Erythromycin	
	Gentamicin	
	Tetracycline	



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TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value ($\mu\text{g/ml}$)	S / R
<i>Enterococcus</i> EURL ENT-16.X <input type="checkbox"/> <i>E. faecium</i> <input type="checkbox"/> <i>E. faecalis</i>	Ampicillin		
	Chloramphenicol		
	Ciprofloxacin		
	Daptomycin		
	Erythromycin		
	Gentamicin		
	Linezolid		
	Quinopristin/Dalfopristin		
	Teicoplanin		
	Tetracycline		
	Tigecycline		
	Vancomycin		
<i>Enterococcus</i> EURL ENT-16.X <input type="checkbox"/> <i>E. faecium</i> <input type="checkbox"/> <i>E. faecalis</i>	Ampicillin		
	Chloramphenicol		
	Ciprofloxacin		
	Daptomycin		
	Erythromycin		
	Gentamicin		
	Linezolid		
	Quinopristin/Dalfopristin		
	Teicoplanin		
	Tetracycline		
	Tigecycline		
	Vancomycin		



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Susceptibility testing of *E. faecalis* ATCC 29212 (CCM 4224)

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
<i>E. faecalis</i> ATCC 29212 (CCM 4224)	Ampicillin		
	Chloramphenicol		
	Ciprofloxacin		
	Daptomycin		
	Erythromycin		
	Gentamicin		
	Linezolid		
	Quinopristin/Dalfopristin		
	Teicoplanin		
	Tetracycline		
	Tigecycline		
	Vancomycin		

Susceptibility testing of *E. faecalis* 2012-70-103-3

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
<i>E. faecalis</i> 2012-70-103-3	Ampicillin		
	Chloramphenicol		
	Ciprofloxacin		
	Daptomycin		
	Erythromycin		
	Gentamicin		
	Linezolid		
	Quinopristin/Dalfopristin		
	Teicoplanin		
	Tetracycline		
	Tigecycline		
	Vancomycin		



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Version 2



Susceptibility testing of *E. faecium* 2012-70-76-8

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
<i>E. faecium</i> 2012-70-76-8	Ampicillin		
	Chloramphenicol		
	Ciprofloxacin		
	Daptomycin		
	Erythromycin		
	Gentamicin		
	Linezolid		
	Quinopristin/Dalfopristin		
	Teicoplanin		
	Tetracycline		
	Tigecycline		
	Vancomycin		



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G00-06-001/26.10.2020

EURL-AR External Quality Assurance System 2021

- *E. coli*, *Salmonella*, *Campylobacter* and enterococcus

Lyngby, September 2021

Dear participant in the EURL-AR AST EQAS 2021,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2021: eight *E. coli*, eight *Salmonella*, eight *Campylobacter* spp. and eight *Enterococcus* spp. Upon arrival to your laboratory, the strains should be stored in a dark place at 4°C for stabs, and in a dark and cool place for freeze-dried strains. Charcoal swabs must be subcultured straight away.

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 *Enterococcus* 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.

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. I will let you know when to look out for it.

	Personal username	Personal password
Accessing the webtool (see the PT protocol, item 5)	<u>See underlined text above</u>	<u>See underlined text above</u>

Results should be submitted to the database no later than **10 December 2021**.

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 Karlsmosse Pedersen
EURL-AR EQAS-Coordinator



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!

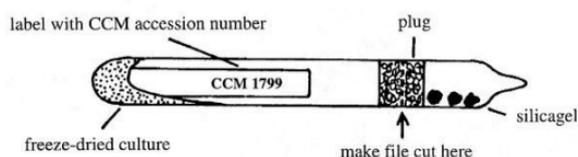


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on <http://www.sci.muni.cz>



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

Resistant phenotypes are highlighted in bold.

E. coli trial

Strain	EC-16.1	EC-16.2	EC-16.3	EC-16.4	EC-16.5	EC-16.6	EC-16.7	EC-16.8
AMI	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S
AMP	> 32 R	> 32 R	= 2 S	> 32 R	> 32 R	> 32 R	> 32 R	> 32 R
AZI	= 8 S	= 8 S	= 8 S	> 64 R	= 8 S	= 4 S	= 16 S	= 8 S
FEP	= 0.5 R	= 0.25 S	<= 0.06 S	<= 0.06 S	= 32 R	= 16 R	> 32 R	= 0.12 S
FOT	= 4 R	= 0.5 R	<= 0.25 S	<= 0.25 S	> 64 R	> 64 R	> 64 R	= 1 R
F/C ratio	<= 0.06 S >=8	= 0.5 R <8	<= 0.06 S <8	<= 0.06 S <8	<= 0.06 S >=8	<= 0.06 S >=8	= 0.12 S >=8	= 1 R <8
FOX	= 4 S	= 16 R	= 4 S	= 4 S	= 8 S	= 4 S	= 4 S	= 16 R
TAZ	= 16 R	= 0.5 S	<= 0.25 S	<= 0.25 S	= 16 R	= 8 R	= 4 R	= 4 R
T/C ratio	= 0.25 S >=8	= 0.25 S <8	<= 0.12 S <8	<= 0.12 S <8	= 0.25 S >=8	<= 0.12 S >=8	= 0.25 S >=8	= 2 R <8
CHL	<= 8 S	> 64 R	<= 8 S	<= 8 S	> 64 R	<= 8 S	<= 8 S	<= 8 S
CIP	= 2 R	> 8 R	<= 0.015 S	<= 0.015 S	> 8 R	= 0.25 R	<= 0.015 S	<= 0.015 S
COL	= 2 S	<= 1 S	<= 1 S	<= 1 S	= 8 R	<= 1 S	<= 1 S	<= 1 S
ETP	<= 0.015 S	= 1 R	<= 0.015 S	<= 0.015 S	= 0.03 S	<= 0.015 S	<= 0.015 S	<= 0.015 S
GEN	= 1 S	= 1 S	<= 0.5 S	= 16 R	> 16 R	= 1 S	= 1 S	= 1 S
IMI	<= 0.125 S	= 1 R	<= 0.125 S	= 0.25 S	= 0.25 S	<= 0.125 S	<= 0.125 S	= 0.25 S
MERO	<= 0.03 S	= 0.25 R	<= 0.03 S	<= 0.03 S	<= 0.03 S	<= 0.03 S	<= 0.03 S	<= 0.03 S
NAL	> 64 R	> 64 R	<= 4 S	<= 4 S	> 64 R	= 8 S	<= 4 S	<= 4 S
SMX	<= 8 S	> 512 R	<= 8 S	> 512 R	> 512 R	> 512 R	<= 8 S	> 512 R
TRM	= 8 S	> 128 R	= 8 S	= 8 S	= 8 S	= 8 S	= 4 S	= 8 S
TET	<= 2 S	> 32 R	<= 2 S	> 32 R	> 32 R	> 32 R	<= 2 S	> 32 R
TGC	<= 0.25 S	= 0.5 S	<= 0.25 S	= 0.5 S	<= 0.25 S	<= 0.25 S	<= 0.25 S	= 0.5 S
TMP	<= 0.25 S	= 2 S	<= 0.25 S	> 16 R	> 16 R	> 16 R	<= 0.25 S	> 16 R

Salmonella trial

Strain	S-16.1	S-16.2	S-16.3	S-16.4	S-16.5	S-16.6	S-16.7	S-16.8
AMI	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S	<= 4 S
AMP	> 32 R	= 2 S	> 32 R	> 32 R	> 32 R	> 32 R	> 32 R	= 1 S
AZI	= 8 S	= 8 S	= 8 S	= 8 S	= 4 S	> 64 R	= 8 S	= 4 S
FEP	= 2 R	<= 0.06 S	> 32 R	= 16 R	= 0.12 S	= 0.12 S	> 32 R	= 0.06 S
FOT	= 8 R	<= 0.25 S	> 64 R	> 64 R	<= 0.25 S	<= 0.25 S	> 64 R	<= 0.25 S
F/C ratio	= 0.12 S >=8	= 0.12 S <8	= 0.12 S >=8	= 0.25 S >=8	<= 0.06 S <8	= 0.12 S <8	= 0.5 S >=8	= 0.12 S <8
FOX	= 4 S	= 4 S	= 8 S	= 8 S	= 4 S	= 8 S	= 8 S	= 8 S
TAZ	= 1 S	= 0.5 S	= 16 R	= 4 R	= 0.5 S	= 0.5 S	> 128 R	= 0.5 S
T/C ratio	= 0.5 S <8	= 0.5 S <8	= 1 S >=8	= 0.5 S >=8	= 0.5 S <8	= 0.5 S <8	= 2 S >=8	= 0.5 S <8
CHL	<= 8 S	<= 8 S	<= 8 S	<= 8 S	<= 8 S	> 64 R	> 64 R	<= 8 S
CIP	= 0.5 R	= 0.03 S	= 0.03 S	= 0.25 R	= 0.5 R	= 0.03 S	= 0.03 S	<= 0.015 S
COL	= 2 S	= 2 S	= 2 S	= 2 S	= 2 S	= 2 S	= 2 S	= 2 S
ETP	<= 0.015 S	<= 0.015 S	<= 0.015 S	<= 0.015 S	<= 0.015 S	<= 0.015 S	= 0.06 S	<= 0.015 S
GEN	<= 0.5 S	<= 0.5 S	<= 0.5 S	<= 0.5 S	= 1 S	<= 0.5 S	> 16 R	<= 0.5 S
IMI	= 0.25 S	= 0.25 S	= 0.5 S	= 0.25 S	= 0.25 S	= 0.25 S	= 0.5 S	= 0.25 S
MERO	<= 0.03 S	= 0.06 S	= 0.06 S	<= 0.03 S	<= 0.03 S	<= 0.03 S	= 0.06 S	<= 0.03 S
NAL	> 64 R	<= 4 S	<= 4 S	> 64 R	= 8 S	<= 4 S	<= 4 S	<= 4 S
SMX	= 16 S	= 16 S	= 16 S	> 512 R	> 512 R	> 512 R	> 512 R	= 16 S
TRM	= 4 S	= 8 S	= 16 S	= 8 S	= 8 S	= 8 S	= 64 R	= 8 S
TET	= 32 R	> 32 R	<= 2 S	> 32 R	<= 2 S	> 32 R	> 32 R	<= 2 S
TGC	<= 0.25 S	= 1 R	<= 0.25 S	= 1 R	<= 0.25 S	= 1 R	= 0.5 S	<= 0.25 S
TMP	<= 0.25 S	<= 0.25 S	<= 0.25 S	> 16 R	> 16 R	> 16 R	> 16 R	<= 0.25 S

Campylobacter trial

Strain	C-16.1	C-16.2	C-16.3	C-16.4	C-16.5	C-16.6	C-16.7	C-16.8
CIP	= 32 R	<= 0,12 S	<= 0,12 S	= 32 R	<= 0,12 S	= 8 R	= 8 R	<= 0,12 S
ERY	> 512 R	<= 1 S	<= 1 S	> 512 R	> 512 R	<= 1 S	<= 1 S	= 512 R
GEN	= 16 R	<= 0,25 S	<= 0,25 S	> 16 R	<= 0,25 S	<= 0,25 S	<= 0,25 S	<= 0,25 S
CHL	= 32 R	= 4 S	= 4 S	= 4 S	= 8 S	<= 2 S	= 4 S	= 4 S
ETP	= 2 R	= 0,25 S	= 0,25 S	= 0,25 S	<= 0,12 S	<= 0,12 S	= 0,5 S	> 4 R
TET	= 64 R	<= 0,5 S	= 8 R	= 64 R	<= 0,5 S	<= 0,5 S	= 64 R	> 64 R

Enterococcus trial

Strain	ENT-16.1	ENT-16.2	ENT-16.3	ENT-16.4	ENT-16.5	ENT-16.6	ENT-16.7	ENT-16.8
AMP	> 64 R	> 64 R	= 64 R	= 2 S	= 1 S	= 4 S	= 1 S	= 1 S
CHL	= 16 S	= 4 S	= 32 S	= 64 R	= 8 S	= 4 S	= 8 S	= 64 R
CIP	> 16 R	> 16 R	= 4 S	> 16 R	= 0,5 S	= 0,5 S	= 1 S	= 1 S
DAP	= 4 S	= 4 S	= 2 S	= 1 S	= 2 S	= 1 S	= 2 S	= 1 S
ERY	> 128 R	= 4 S	> 128 R	> 128 R	> 128 R	= 2 S	<= 1 S	> 128 R
GEN	<= 8 S	<= 8 S	= 1024 R	> 1024 R	<= 8 S	<= 8 S	= 8 S	= 16 S
LZD	= 1 S	= 2 S	= 2 S	= 1 S	= 1 S	= 2 S	= 2 S	= 8 R
SYN	= 1 S	= 4 R	= 8 R	= 16 R	= 16 R	= 4 R	= 8 R	= 16 R
TEI	= 64 R	= 1 S	<= 0,5 S	<= 0,5 S	<= 0,5 S	= 64 R	<= 0,5 S	<= 0,5 S
TET	= 32 R	= 32 R	= 64 R	= 64 R	<= 1 S	= 64 R	<= 1 S	= 128 R
TGC	= 0,12 S	= 0,12 S	= 0,25 S	= 0,25 S	= 0,12 S	= 0,12 S	= 0,25 S	= 0,25 S
VAN	> 128 R	= 32 R	= 4 S	= 2 S	= 2 S	> 128 R	= 2 S	= 2 S

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