



The 9th EURL-AR proficiency test

Selective isolation of E. Coli
with presumptive ESBL, AMPC
or carbapenemase phenotypes
from meat or caecal samples
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1. Introduction

This report describes and summarises results from the ninth matrix-based proficiency test conducted by The National Food Institute (DTU Food) as the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR) as an External Quality Assurance System (EQAS). This proficiency test focuses on selective isolation of extended-spectrum beta-lactamase (ESBL), AmpC and carbapenemase-producing *E. coli* from meat and caecal samples of animal origin and antimicrobial susceptibility testing (AST) of the isolated *E. coli*.

Extended-spectrum beta-lactamase (ESBL) and AmpC-producing *E. coli* continue to spread in food producing animals. In 2013, the European Commission (EC) decided to include the isolation of ESBL and AmpC-producing *E. coli* as mandatory parts of the EU monitoring and this started during 2015. The screening includes matrix samples consisting of either meat or caecal samples of animal origin in the EU Member States (MS) and affiliated countries according to a common protocol defined by the EC and validated by the EURL-AR (EURL-AR, 2019). In 2016, the EQAS was extended to include carbapenemase and *bla*_{OXA-48}-producing *E. coli*, thereby including the optional isolation of these using the EURL-AR selective isolation protocol on agar plates suitable for isolation of carbapenemase-producing *E. coli* (EURL-AR, 2019). This was made mandatory with the decision from 2021 (2020/1729/EU).

Similar to the previous EURL-AR matrix-based EQAS', the aim of this specific EQAS was to i) monitor the capacity of the National Reference Laboratories (NRL-AR) for isolation, identification and AST of ESBL/AmpC or carbapenemase-producing *E. coli*; ii) identify laboratories which may need assistance to improve their performance in isolation and AST of *E. coli* from matrices; and iii) identify potential problems or focus areas for future training and research.

In reading this report, the following important considerations should be considered:

- 1) Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determination for all test strains prior to selection of strains and MICs were confirmed upon selection of strains at the Technical University of Denmark, National Food Institute (DTU Food). The genetic basis for resistance was known, as all the selected test strains had been whole-genome sequenced (WGS). The MIC determination was repeated after preparation of the matrix samples of meat or caecal.
- 2) No thresholds have been set in advance to evaluate the acceptance of performance of the participating laboratories and therefore results will not be classified as above or below a threshold but will be evaluated case by case.
- 3) Evaluation of a result as 'deviating from the expected interpretation' should be carefully analysed in a self-evaluation performed by the participant, including considerations of corrective actions in the laboratory. Note that since methods used for MIC determination has limitations, it is not considered a mistake to obtain a one level dilution difference in the MIC of a specific antimicrobial when testing the same strains. If, however, 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 categorized as susceptible and resistant. This result will be evaluated as correct in one case, but incorrect when the evaluation is based on AST interpretations. In the organization of the EQAS, we try to avoid these situations by choosing test



strains with MIC values distant from the cut-offs for resistance, which is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AR network unanimously established in 2008 that if there are less than 75% correct results for a specific strain/antimicrobial combination, the reasons for this situation must be further examined and, on selected occasions explained in detail case by case, these results may subsequently be omitted from the evaluation report.

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

This ninth matrix EQAS was organized by the EURL-AR at the National Food Institute (DTU Food), Kgs. Lyngby, Denmark. The present draft report was sent out for commenting in the network in April-May 2024, and the network was invited to a webinar with presentations of the EQAS results in June 2024. The draft report was hereafter approved.

2. Materials and Methods

2.1 Participants in EQAS 2023

A pre-notification (Appendix 1), announcing the matrix EQAS 2023, was sent out on 2 October 2023 by e-mail to the designated NRLs including all EU countries and Iceland, Norway, Switzerland, and United Kingdom. In total, 33 laboratories participated in the matrix EQAS (Appendix 2) involving one NRL from the 27 EU countries and Iceland, Norway, Switzerland, and United Kingdom. Two countries (Romania and Spain) had separate laboratories enrolled for handling meat and caecal samples and had two NRLs enrolled. Therefore, in total, 33 laboratory results from 31 countries are described. Participants from non-EU member-states and additional laboratories were charged a fee for participation, whereas participation was free of charge for EU member-states, but each laboratory was expected to cover expenses associated with the analyses.

2.2 Preparation of samples

Eight samples were prepared and dispatched for isolation of ESBL, AmpC or carbapenemase-producing *E. coli*, including identification, and antimicrobial susceptibility testing (AST) of the obtained isolates. The samples included either beef meat or swine caecal content which were either prepared by spiking with test strains or left unmodified.

The sample meat was minced beef meat of Danish origin (raised, slaughtered and packed in Denmark) acquired in local supermarkets, and four different batches (based on production date and slaughterhouse) were bought in sufficient amount for covering both the pre-tests and preparation of the samples). The meat was pretested using the official method for selective isolation of *E. coli* producing ESBL, AmpC or carbapenemases, to ensure that the batch used was negative for those, and to test the level of conflicting background bacteria. A batch fulfilling these criteria was chosen for preparation of aliquots of 25 g of meat that were spiked as follows.

The test isolates used in the spiking of meat samples within the matrix EQA 2023 were prepared in advance and sub-cultured the day before sample preparation. For the sample preparation and standardization of the spiking, suspensions equal to McFarland 0.5 were prepared in saline tubes with

the relevant isolates to contain about 10^8 CFU/mL, as confirmed by viable counts of serial dilutions on Luria Bertani (LB) agar plates. The standardized suspensions were further diluted in ten-fold dilutions and the meat samples (25 g) were spiked with 25 μ l of the chosen dilutions. The spiking dilutions were chosen based on the results obtained in the previous matrix EQAs. The final inoculum found in the samples in this EQA was expected to be approx. 10^3 CFU/g meat for samples EURL-M-9.1, M-9.2, M-9.4 and M-9.5. Sample EURL-M-9.3 was spiked as mentioned above but with a susceptible *E. coli* strain (ATCC 25922) and therefore expected to be negative for ESBL, AmpC and carbapenemase-producing *E. coli*.

One slaughterhouse provided on 27 October 2023, 15 swine caecal samples from different farms. These samples were tested using the official selective isolation protocol for ESBL, AmpC and carbapenemase-producing *E. coli*. One ESBL-negative caeca batch was chosen for preparation of the matrix caecal samples for the test strains. Thereby 1 g aliquots of pooled caecal content were used directly as blank sample or spiked with 10 μ l of a dilution containing approx. 10^6 CFU/ml, causing an expected spiking level of 10^4 CFU/g for the samples M-9.6, and M-9.8. The sample M-9.7 was not inoculated and was expected to be negative. The inoculum strains were selected for being initially isolated from swine caecal samples, as this approach has previously shown to enhance the survival of the inoculum. Otherwise, the caecal matrix samples have very poor support of the *E. coli* inoculum.

The minimal inhibitory concentrations (MIC) of selected antimicrobials were determined using broth microdilution method both for the strains used for spiking during the preparation work and for the isolates obtained in the homogeneity testing after sample preparation to generate expected results (Appendix 3).

For follow-up on the stability of the inoculum in the matrix samples after shipping, repeated testing of isolation of test strains was performed on sets the eight samples in four time points after shipment (for two weeks). In this period, the meat and caecal samples were kept at 4°C, to mimic the conditions in the shipment parcel.

2.3 Isolation and identification of ESBL, AmpC or carbapenemase producing *E. coli* from meat and caecal samples

The official protocols for selective isolation and identification of the ESBL, AmpC and/or carbapenemase-producing *E. coli* isolates contained in the samples were available on the EURL website, <http://www.eurl-ar.eu> (Appendix 4). For the confirmation of *E. coli* isolates, different methods were allowed as these are not specified in the legislation (EU Commission implementing decision on the monitoring and reporting antimicrobial resistance in zoonotic and commensal bacteria 2020/1729/EU). The description of the method used for selective isolation of presumptive ESBL, AmpC or carbapenemase-producing *E. coli* as well as species identification was requested as part of the methods sheet to be completed in the database upload system.

2.4 Antimicrobial susceptibility testing

The panels of antimicrobials recommended for AST in this proficiency test are those included in the EU Commission implementing decision on the monitoring and reporting antimicrobial resistance in zoonotic and commensal bacteria 2020/1729/EU (Table 1).

Guidelines for performing the antimicrobial susceptibility testing using dilution methods were set according to the Clinical and Laboratory Standards Institute (CLSI) document – M7 (2018) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved



Standard - 11th Edition” and whenever commercial methods were used, the guidelines of the manufacturer were followed.

MIC results were interpreted by using EUCAST epidemiological cut-off values (www.eucast.org), as included in the regulation referred above or as recommended by EFSA and described in the EQAS protocol (Appendix 4). Results of the ESBL confirmatory testing were interpreted according to the recommendations by EFSA and as referred in the regulation, using MIC testing on the second panel of antimicrobials, which is intended to be used every time a strain is found resistant to either cefotaxime, ceftazidime and/or meropenem.

2.5 Distribution

The meat samples were frozen at -80°C and kept at this temperature after preparation and until the time for shipment. The caecal samples were sent shortly after preparation, and therefore kept at 4°C until the time for shipment. At the day of shipment, the samples were tightly packed in thermos boxes with cooling elements, frozen at -80°C. The parcels contained the eight samples in tubes, and an additional tube contained a temperature logger to register the temperature at 15 min intervals during transport. Furthermore, the parcel contained a welcome letter with the laboratory ID number and a labelled envelope for returning the temperature logger to the EURL-AR.

The protocol for the EQAS and the test forms were available online on the EURL-AR website, <http://www.eurl-ar.eu> before launching this EQAS.

The thermo boxes used for the shipment of samples were enclosed in double pack containers and sent to the selected laboratories according to the [International Air Transport Association](http://www.iata.org) (IATA) regulations as “Biological Substance category B” classified UN3373. The parcels were dispatched from DTU Food on 6 November 2023.

2.6 Procedure

The laboratories were instructed to download the protocol and test forms (Appendix 4 and 5) from <http://www.eurl-ar.eu> and to process the samples following the EU protocol for selective isolation of presumptive ESBL, AmpC and/ carbapenemase-producing *E. coli* from either meat or caecal samples, precisely as they would normally do for the EFSA monitoring. For the results collection, the NRLs were instructed to upload the data in the web-based database, which was designed and prepared for this EQAS and opened after sample shipment and until the reporting deadline.

After completion of the tests, the laboratories were requested to enter the obtained results into the electronic sheet in the EURL-AR web-based database through a secured individual login. The web tool was activated on 4 December 2023 and closed on 12 December 2023.

For the first part of the results of the selective isolation procedure for ESBL/AmpC and carbapenemase-producing *E. coli*, the results obtained from the isolation procedures samples were evaluated separately by defining the samples as positive if an isolate was obtained and positively identified as *E. coli*. Additionally, the results of susceptibility testing of the obtained isolates using both MIC panels (EUVSEC3 and EUVSEC2; Table 1) were analysed separately in similar way as to the similarly to the *E. coli* AST EQAS, including the read values of MIC and their interpretations. As a conclusion of the susceptibility testing, the participants were asked to classify the isolates obtained according to the defined EFSA criteria for interpretation of ESBL/AmpC and/or carbapenemase-producing isolates.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2023

<i>Escherichia coli</i> EUVSEC3	<i>Escherichia coli</i> EUVSEC2
Ampicillin, AMP	Cefepime, FEP
Amikacin, AMK	Cefotaxime + clavulanic acid (F/C)
Azithromycin, AZI	Cefotaxime, FOT
Cefotaxime, FOT	Cefoxitin, FOX
Ceftazidime, TAZ	Ceftazidime, TAZ
Chloramphenicol, CHL	Ceftazidime+ clavulanic acid (T/C)
Ciprofloxacin, CIP	Ertapenem, ETP
Colistin, COL	Imipenem, IMI
Gentamicin, GEN	Meropenem, MERO
Meropenem, MERO	Temocillin, TRM
Nalidixic acid, NAL	
Sulfamethoxazole, SMX	
Tetracycline, TET	
Tigecycline, TGC	
Trimethoprim, TMP	

After the deadline, the qualitative results indicating if the samples were positive or negative for ESBL/AmpC, or carbapenemase-producing *E. coli* (*bla*_{OXA-48} and other), as well as the interpretations of the susceptibility tests results, and the conclusion on the observed *E. coli* phenotypes were evaluated against the expected results and scored as correct or incorrect. As no threshold is agreed, the performance was evaluated case by case and not classified into acceptable or unacceptable based on the deviation percentage.

3. Results

Upon arrival of the parcels, the participants were requested to provide more information in a small introductory questionnaire in the database, including details on sample reception (measured temperature and date/time), the monitoring activities, and the methods used in their laboratory. The registration of the temperature was extracted and read from the returned temperature loggers to provide the temperature ranges along the shipment and at sample reception/opening. All samples were expected to be in good conditions for testing at the time of opening the parcels.

3.1 Overall results of the selective isolation

The number of possible and evaluated test results for ESBL/AmpC qualitative isolation considered for this report was 264 tests, from eight samples for each of the 33 laboratories. These results are summarized in Figure 1 and further discussed in section 3.4. There were only two cases where an isolate was not recovered, Lab #037 could not recover the isolate from sample M-9.5, while Lab #040 observed no growth from sample M-9.8.

3.2 Methods used by EQAS-participants

In this trial, 28 participating NRLs reported results for all the eight samples. Two laboratories reported only results for the meat samples, two laboratories reported only results for the caecal samples, while one laboratory did not submit results for sample M-9.5. All 33 participating laboratories, which have submitted results, participated in the ESBL, AmpC and carbapenemase-producer isolation and performed the identification and susceptibility testing of the respective isolates. The number of qualitative isolation test results reported was variable, including results for three to eight samples, depending on how many samples were tested, and for the antimicrobial susceptibility tests it depended on how many isolates were isolated and further tested in the MIC panels. Information on the methods used for isolation, identification and typing was collected from the participants through the database.

All laboratories reported that the selective isolation was performed according to the exact procedures described in the provided protocols. One lab (#012) reported that they did not follow the EURL recommended protocol for isolation of carbapenem-producers. Subculturing from CARBA/OXA-48 medium was performed on the same agar (and not MacConkey) to keep the selective pressure, and thereafter, to blood agar for species determination with MALDI-TOF.

The species identification was performed using MALDI TOF (n=16), biochemical tests (n=8), chromogenic media (n=7) or PCR using published methods to confirm the ID (n=2). Additionally, three laboratories (#023, #037, #045) reported that other identification methods were used as supplements. From those labs that reported on the brand/plates used for selective isolation of carbapenem producers (n=16), CHROMID CARBA SMART Agar (n=6), CHROMID CARBA Agar (n=6), CHROMID OXA-48 (n=6) and CHROMagar mSuperCARBA (n=1) were used. One laboratory did not specify the type of the plates, only the brand (BioMérieux).

The broth microdilution testing was performed using the antimicrobials and ranges defined under the EU Commission regulation 1729/2020 for testing the isolated and identified *E. coli* isolates using panel 1 (EUVSEC3). Additional AST of the presumptive ESBL/AmpC and/or carbapenemase isolates was performed using panel 2 (EUVSEC2), if relevant, and interpretation of the results was made according to the EFSA criteria for ESBL/AmpC and carbapenemase phenotypic classification.

3.3 ESBL/AmpC and carbapenemase producing *E. coli* isolation and identification

The total amount of test results was 198 tests for the ESBL, AmpC and carbapenemase isolation qualitative results. Of them, 184 (92.9%) tests were obtained in concordance with the original strains correctly assigned ESBL, AmpC or carbapenemase phenotype (Table 2). All 66 samples expected to be negative (blank and susceptible) were correctly assigned. Regarding the 198 samples expected to be positive, all but 14 (7.1%) were correctly assigned. Lab #037 could not recover the isolate from sample M-9.5 as they did not observe any growth, while lab #040 could not grow the isolate from sample M-9.8.

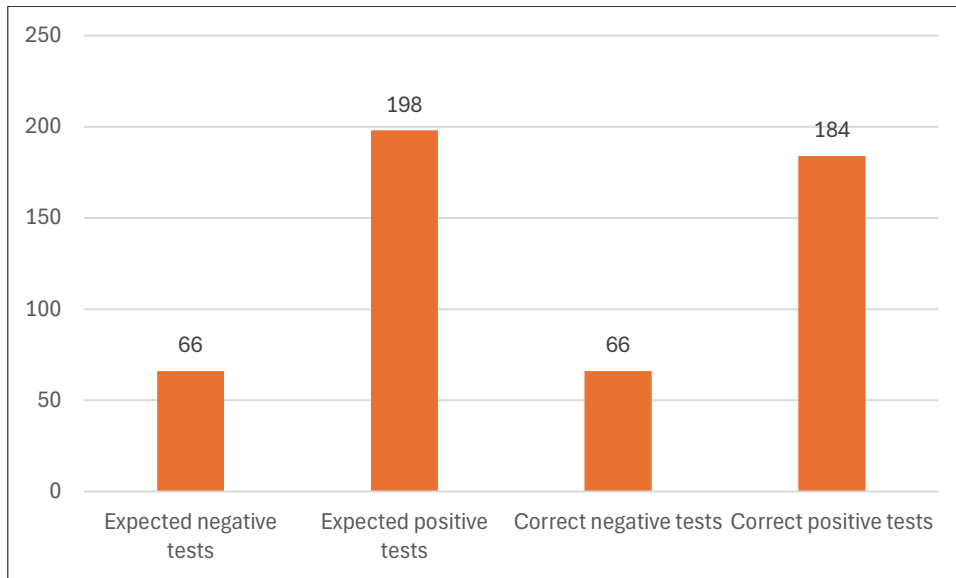


Figure 1. Overall performance of ESBL/AmpC/Carbapenemase isolation and identification, 2023

For the AmpC isolates (containing the *bla*_{CMY-2} gene) spiked in sample M-9.6, 10 labs observed in addition ertapenem resistance and hence reported it as “Other phenotype”, which was according to the EFSA guidelines, and which is often observed in *bla*_{CMY-2}-carrying isolates. For the AmpC-isolates in sample M-9.2 carrying *bla*_{DHA-1}, it was not expected to see elevated resistance to any of the carbapenems tested. Thus, the reporting of ‘Other phenotype’ has been allowed after analysing the participant data.

Table 2 Deviations in ESBL /AmpC and carbapenemase phenotype identification, 2023

Strain ID	Expected phenotype	<i>bla</i> -gene(s)/ mutation	Deviations, %	Additional phenotype approved	Deviations (%) after changing phenotype
M-9.1	Carbapenemase	<i>CTX-M-15, NDM-5</i>	0	None	0
M-9.2	AmpC	<i>DHA-1</i>	9.7	Other phenotype*	0
M-9.4	AmpC	<i>AmpC (T-32A)</i>	3.2	None	3.2
M-9.5	ESBL	<i>SHV-12</i>	0	None	0
M-9.6	AmpC + Other phenotype*	<i>CMY-2</i>	0	None	0
M-9.8	Carbapenemase	<i>OXA-48</i>	0	None	0

*According to the EFSA guidelines for reporting antimicrobial resistance, AmpC + ETP resistance is reported as “Other phenotype” and approved in this report as an additional phenotype.

The analysis of deviations in ESBL/AmpC and carbapenemase phenotype categorisation per matrix sample indicates that the highest levels of deviations were observed for sample M-9.4 (3.2%; meat) due to the report of meropenem resistance in an originally expected AmpC isolate. Samples M-9.1, M-9.5 and M-9.8 had no deviations from the original phenotypes and for the other samples the deviations ranged between 3.2 and 9.7 % (Table 2).

3.4 Antimicrobial susceptibility testing

A total of 4,615 AST results were submitted. The 33 laboratories uploaded a variable number of results, depending on the samples found positive, isolates tested in one or both panels and isolates that could not be recovered, and ranged from 50 to 165 test results per participant. The deviation percentages per laboratory ranged from 0.7% to 8.1%. However, when the one-step dilution difference at the breakpoint was not considered, the maximal deviations per laboratory decreased to 7.4% (Figure 2). As the performance on the AST depends on the isolation and identification procedures, no threshold was set for acceptance as the capacity for performing AST of *E. coli* is analysed more accurately in the *E. coli* AST EQAS. In the analysis of MIC results, it was evident that one two-fold dilution variations in the testing of both chloramphenicol and ciprofloxacin (Figure 3), and also R/S interpretation for other antimicrobials gave the highest contribution to the deviations (21.4% deviations for chloramphenicol and ciprofloxacin). Overall, there was only little agreement between the values and interpretations submitted by participating laboratories for ceftazidime for sample M-9.8. More than half of the laboratories (n=18) reported values of 8 mg/L (susceptible), while the rest of the laboratories had results of 16 mg/L or above and reported it as resistant. As this seemed to have caused an unusually high amount of deviations, the R/S interpretation for ceftazidime was blanked. This did not affect the ESBL/AmpC-type interpretation, as the isolates were additional carbapenem resistant and was successfully classified as this in all cases.

A list of deviations in ESBL phenotype interpretations and AST results is available in Appendix 7.

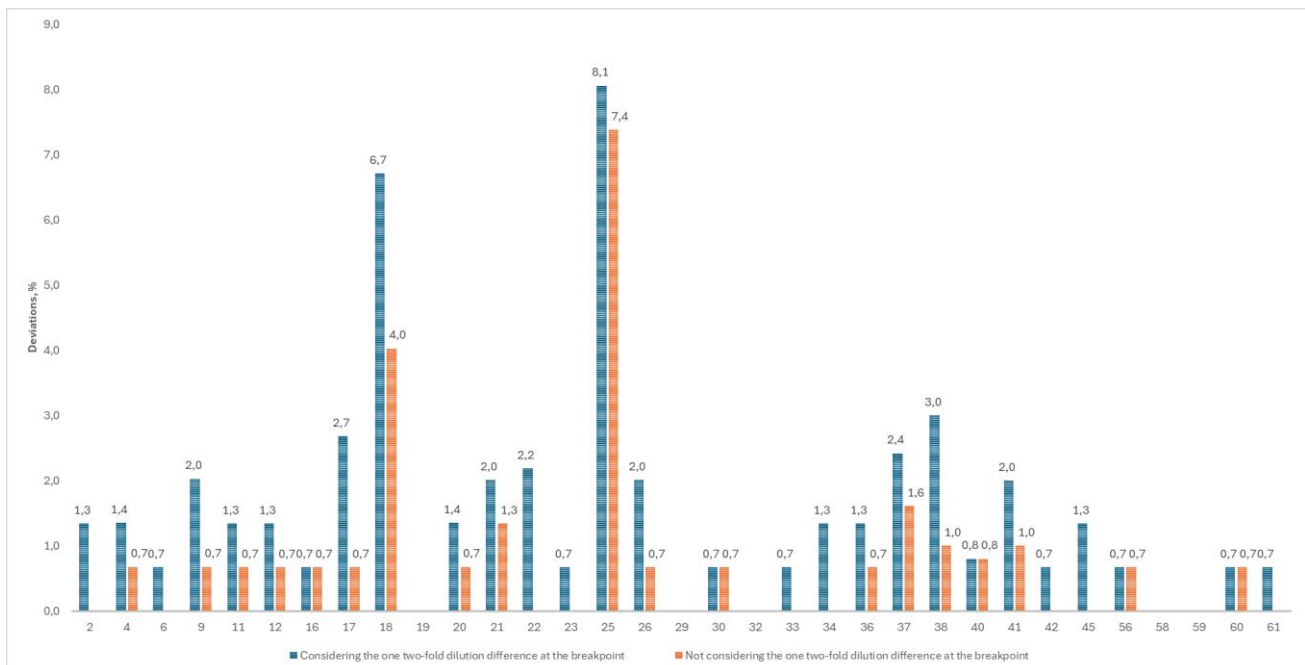


Figure 2. Percentages of deviations in AST results per participating laboratory in the EQAS Matrix 2023

In the analysis of deviations per antimicrobial, the highest deviation percentage was for chloramphenicol (7.1%), imipenem (6.6%), ciprofloxacin (4.9%) and ertapenem (3.8%) (Figure 3). However, if we do not consider the one two-fold dilution differences, which are accepted in the MIC method, the percentage deviations decrease substantially to 0.5%, 4.9%, 0.0% and 3.3%, respectively, all the remaining deviations caused by more than one two-fold dilution MIC difference or incorrect interpretations. For some antimicrobials (e.g., cefepime, ertapenem and imipenem), the deviations from the expected MIC values were caused by more than one two-fold dilution difference which completely changed the phenotype and could be explained by either acquisition of plasmids carrying genes for resistance to these antimicrobials or through isolation of background bacteria or contaminated isolates.

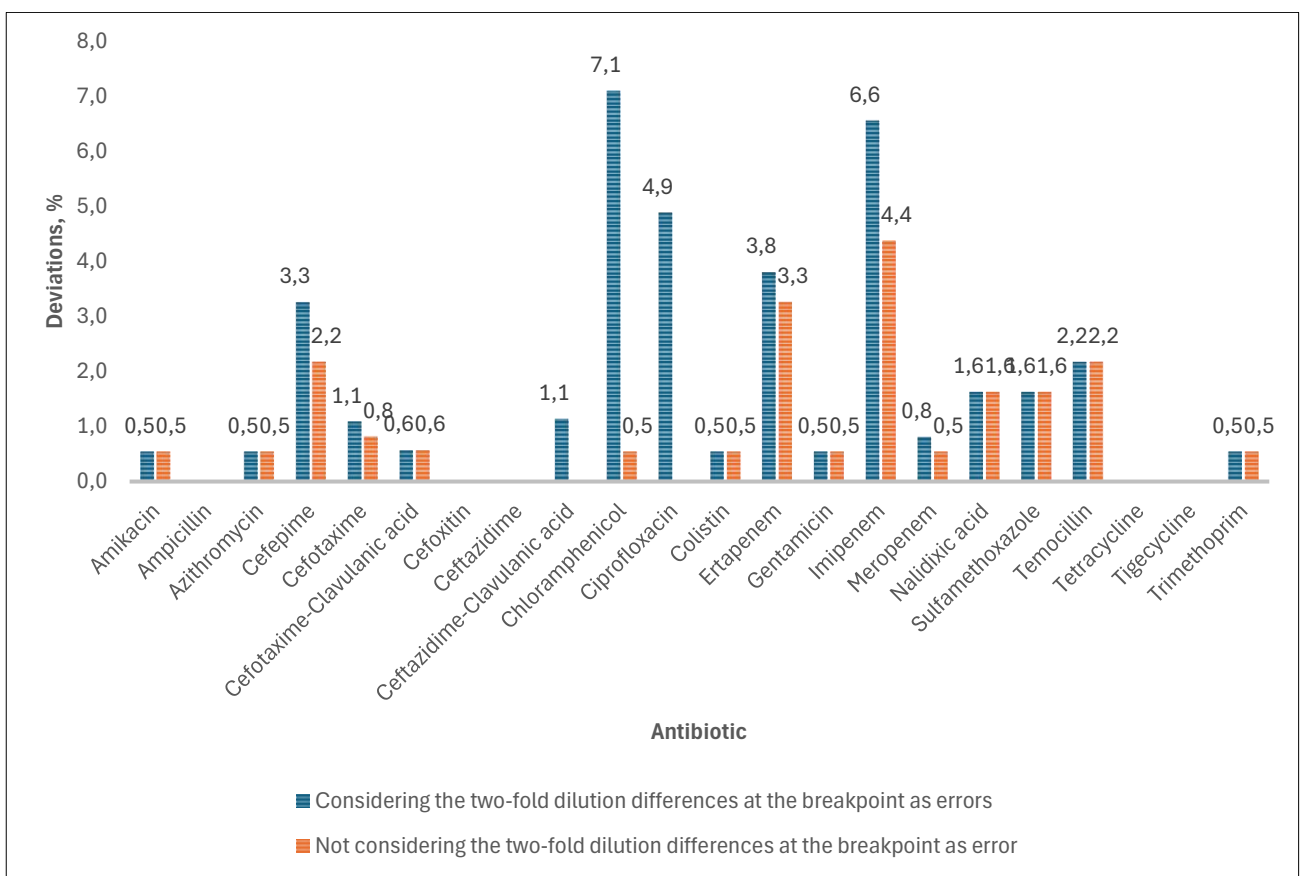


Figure 3. Percentages of deviations in AST results per antimicrobial in the Matrix EQAS 2023

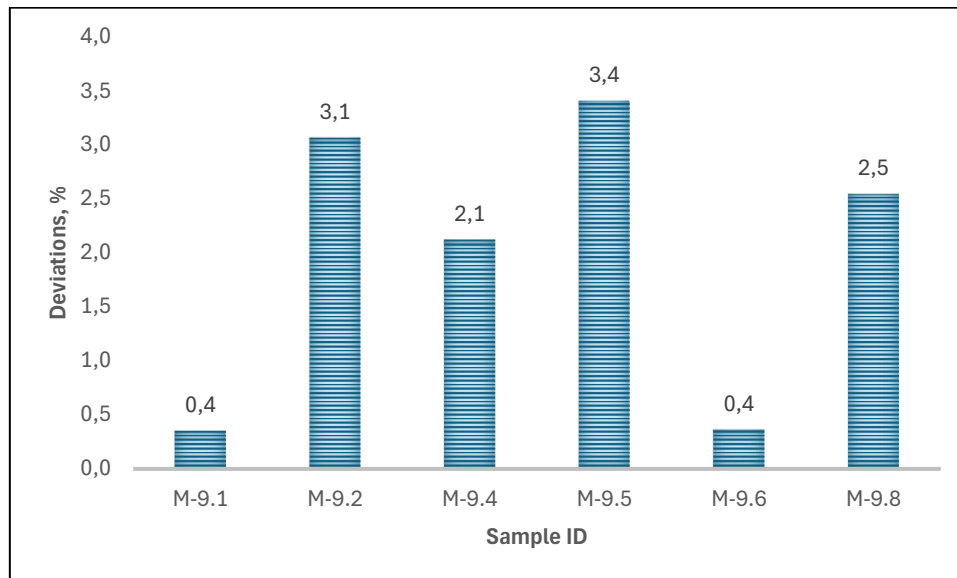


Figure 4. Percentage of deviations in MIC results per sample in the Matrix EQAS 2023

When deviations in the MIC interpretations per matrix sample were considered (Figure 4), the deviations were widely distributed and all samples have deviations of less than 4%, with least deviations found for samples M-9.1 (meat) and M-9.6 (caecal). In total, there were 31 datasets for samples M-9.1, M-9.2, M-9.4, and M-9.6, and 30 datasets for samples M-9.5 and M-9.8 (because two laboratories, #037 and #040, were not able to recover one of the two isolates). The results are also excluding the cefoxitin deviations in sample M-9.8, since they were blanked in the webtool.

3.5 ESBL/AmpC and carbapenemase phenotypic testing conclusions

Five beef meat samples (M-9.1 – M-9.5) were included in this matrix EQAS. Sample M-9.1 contained an isolate expressing a carbapenemase-producing phenotype due to the presence of a *bla*_{NDM-5} gene. The isolate harboured also an ESBL-conferring *bla*_{CTX-M-15} gene. Sample M-9.2 was inoculated with a strain carrying the AmpC gene *bla*_{DHA-1}, whereas M-9.3 was spiked with a wild-type strain. Sample M-9.4 was inoculated with an AmpC with a mutation in the promoter region (g.-32T>A) leading to overexpression. Sample M-9.5 was spiked with a *bla*_{SHV-12}-producing *E. coli* (ESBL phenotype) (Table 2). Three swine caecal samples were included in this matrix EQAS (M-9.6 – M-9.8). One of the three caecal samples (M-9.7) was not spiked (blank), while samples M-9.6 and M-9.8 contained isolates that expressed an AmpC phenotype mediated by *bla*_{CMY-2} and a carbapenemase phenotype due to the presence of a *bla*_{OXA-48}, respectively.

Overall, there were no discrepancies in differentiating between ESBL and AmpC phenotypes. 11 laboratories have indicated “Other phenotype” for the isolates from samples M-9.2 and M-9.6 (both AmpC-producers) but since according to the EFSA guidelines, AmpC + ertapenem resistance should be reported as “Other phenotype”, this was reflected in the webtool and approved in this report as an additional phenotype.

Lab #025 reported an *E. coli* isolate from sample M-9.4 with a carbapenemase-producing phenotype instead of an ESBL phenotype. This was due to the elevated MIC values reported for carbapenems, while the strain was expected to be susceptible to these.

4. Discussion

4.1 ESBL and AmpC and carbapenemase-producing *E. coli* isolation and identification

The 2023 EURL-AR Matrix EQAS trial was the ninth of its kind on samples of animal origin, since the first round of this EQAS in 2015. All laboratories recovered presumptive isolates from both the meat and caecal samples, except for two laboratories lab #037 could not recover one isolate from sample M-9.5 and lab #040 that could not grow the isolate from sample M-9.8. Additionally, one laboratory (#025) recovered a carbapenem-resistant isolate from sample M-9.4 which was expected to be an ESBL. It is expected to see deviations in the resistance phenotypes, and possibly even in the ESBL categorisations, due to sporadic interactions with bacteria and/or mobile genetic elements in the microbiota of the matrix samples, and these cannot be accounted for as they are not necessarily detected in the tests performed by the EURL-AR after the preparation and shipment of samples. As the screening of matrix material only serves to reveal possible ESBL/AmpC/carbapenemase contamination per batch and a rough estimation of the level of background bacteria, it is practically impossible to avoid having generic *Enterobacteriales* or *E. coli* and sometimes even ESBL bacteria in some parts of the meat matrix.

4.2 Antimicrobial susceptibility testing

In the 2023 iteration of the matrix EQAS, 5 laboratories had no deviations in their AST results compared to EQAS 2022 (n=2), EQAS 2021 (n=11) and EQAS 2020 (n=15). The AST deviations were caused by either i) one two-fold dilution difference, which is allowed for the broth microdilution method, around the breakpoint, which changes the phenotype interpretation, and is not necessarily due to acquisition of genes, ii) more than one two-fold dilution difference which is either caused by true errors or by variation in MIC values caused by either gene acquisition (especially to certain antimicrobials) or recovery/contamination of contaminating isolates present in the matrix; and iii) incorrect interpretation when the correct baseline value is reported which can be due to technical errors or using guidelines for interpretation different than the ones used to obtain the baseline results. There were only 8 deviations caused by wrong interpretation of the same values as the baseline. They were randomly scattered across different laboratories and antimicrobials.

Some isolates (i.e. M-9.4 and M-9.5) were reported having quite different phenotype by some laboratories (i.e. #025 and #018, respectively). This could be due to either contamination or picking up another background species from the spiked sample, and this issue gave rise to most AST deviations, but especially for chloramphenicol, ciprofloxacin, imipenem, and ertapenem in this year's set of samples. Finally, the use of different ECOFFs for cefoxitin caused additional deviations, which caused that the antimicrobial was blanked from scoring for sample M-9.8.

Thus, the challenges seen in this and previous matrix EQAS are not unexpected, as working with isolates in a matrix is likely to cause problems, such as retrieving the inoculated isolates from the samples, changes in the sample composition during enrichment which might favour isolation of one over other strain, or changes in the isolate characteristics (plasmid exchanges).

4.3 ESBL /AmpC and carbapenemase phenotypic testing conclusions

Due to the reasons mentioned above, the phenotypic interpretation of the isolated strains is not straightforward. In relation to the ESBL classification, this EQAS is dependent on the EFSA Criteria for interpretation of *Escherichia coli* (panel 2 results; see protocol), and an issue in this year's EQAS Matrix was the ertapenem resistance in *bla*_{CMY-2} carrying AmpC-type *E. coli* (M-9.6) which had an elevated MIC to ertapenem, but not to meropenem. Following the EFSA classification, the strain should be considered 'Other phenotype' if meropenem \leq 0.12 mg/L but ertapenem (or imipenem) is above the ECOFF. It is a well-known issue for *bla*_{CMY-2} isolates and therefore it is generally accepted to classify this phenotype as 'AmpC-type', although 'Other phenotype' is expected and also accepted.

4.4 Performance in AST of the quality control strains

Antimicrobial susceptibility test results for the *Escherichia coli* ATCC 25922 quality control strain were evaluated based on the CLSI quality control ranges (Appendix 6). For a third consecutive year, the *E. coli* ATCC 25922 QC strain was included in the reporting of the EQAS results. All 33 participating labs tested *E. coli* ATCC 25922 by MIC determination and reported a total of 646 out of 690 test results. Of the reported results, 99.2% (n=641) were within the acceptable range (Appendix 6). There were five deviations for cefepime, ciprofloxacin, tetracycline, tigecycline, and trimethoprim by laboratories #009, #018, #023, and #061. The laboratories should carefully consider these deviations as part of their self-assessment of the EQAS participation. Additionally, laboratories #026 and #037 did not provide data for Panel 2.

5. Conclusion

The EQAS 2023 Matrix report demonstrates that most participating labs have well established methods to isolate ESBL/AmpC and carbapenemase-carrying strains from meat or caecal samples, and in only two cases, one meat isolate and one caecal isolate were not recovered. Due to the difficult nature of the matrices and the background microflora, not all laboratories were recovering the original spiked isolates with the expected phenotypes. Additionally, ertapenem resistance was observed in one of the isolates, which changed the interpretation of 'AmpC phenotype' to "Other phenotypes".

The EURL-AR will follow up on the unexpected carbapenem resistant strain isolated from one of the meat samples, to elucidate this problem further. The susceptibility testing results were in general very satisfactory, except for several two-fold dilution differences at the breakpoint which changed the interpretation and more than one two-fold dilution differences that could be derived from genes acquisition. Also, an issue occurred by participants using the updated EUCAST ECOFFS, instead of those listed in the EQAS protocol, which are incorporated in the web-tool, and this caused additional deviations, leading to a subset of scores being blanked.

6. References

Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU (relevant as of 01.01.2021).



7. Appendices

- Appendix 1. Pre-notification EURL-AR EQAS matrix 2023
- Appendix 2. List of participants
- Appendix 3. Test strains and reference values (MIC in mg/L)
- Appendix 4. Protocol EQAS matrix 2023
- Appendix 5. Examples of Test forms EQAS matrix 2023
- Appendix 6. QC ranges *E. coli* ATCC25922
- Appendix 7. List of deviations



EURL-AR EQAS pre-notification

G00-06-001/26.10.2020

EQAS 2023 FOR SELECTIVE ISOLATION OF *E. COLI* WITH PRESUMPTIVE ESBL, AMPC PHENOTYPES OR CARBAPENEMASES FROM MEAT OR CAECAL SAMPLES

The EURL-AR announces the launch of another EQAS on matrix samples, 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 testing of eight samples for selective isolation of ESBL, AmpC or carbapenemase-presumptive *E. coli*. Additionally, quality control (QC) strains *E. coli* ATCC 25922 and *A. baumannii* 2012-70-100-69 will be included.

This EQAS is targeted NRL's on antimicrobial resistance involved in the monitoring according to the EU Commission decision 2020/1729 and specifically processing meat and/or caecal samples in the specific monitoring for ESBL. You may contact the EQAS-Coordinator if you wish to inform of changes in relation to your level of participation in compared to previous years.

Participation is free of charge for all above-mentioned designated laboratories. 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 150 EUR).

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is categorized as "UN3373, Biological Substance Category B". Eight samples which might contain ESBL, AmpC or carbapenemase-producing *E. coli* included in a matrix of pork/beef meat and/or pig/cattle caecal will be shipped. 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). 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 are expected to be shipped the second week of November 2023. The protocol for this proficiency test will be available for download from the website (<https://www.eurl-ar.eu/protocols.aspx>).

Submission of results: Results must be submitted to the National Food Institute **no later than 12 December 2023 at 16:00** via the password-protected webtool. Upon reaching the deadline, when preliminary data validation has been performed, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

EQAS report: A report summarising and comparing results from all participants will be issued. In the report, laboratories will be presented coded, which ensures full anonymity. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023



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

Sincerely,

Jette Kjeldgaard (jetk@food.dtu.dk)
EURL-AR EQAS-Coordinator

Appendix 2 List of participants

Meat	Caecal	Institute	Country
x	x	Austrian Agency for Health and Food Safety	Austria
x	x	Institute of Public Health	Belgium
x	x	National Centre of Food Safety	Bulgaria
x	x	Croatian Veterinary Institut	Croatia
x	x	Veterinary Services	Cyprus
x	x	State Veterinary Institute Praha	Czechia
x	x	Danish Veterinary and Food Administration, DVFA	Denmark
x	x	Estonian Veterinary and Food Laboratory	Estonia
x	x	Finnish Food Safety Authority EVIRA	Finland
x	x	Agence nationale de sécurité sanitaire alimentation, environnement, travail (ANSES) - Laboratoire de Fougères LERMVD	France
x	x	Federal Institute for Risk Assessment	Germany
x	x	Veterinary Laboratory of Chalkida	Greece
x	x	Central Agricultural Office Veterinary Diagnostic Directorate	Hungary
x	x	Institute For Experimental Pathology, University of Iceland, KELDUR	Iceland
x	x	Central Veterinary Research Laboratory	Ireland
x	x	Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Italy
x	x	Institute of Food Safety, Animal Health and Environment BIOR	Latvia
x	x	National Food and Veterinary Risk Assessment Institute	Lithuania
x	x	Laboratoire de Medecine Vétérinaire	Luxembourg
x	x	Public Health Laboratory/National Veterinary Laboratory	Malta
x	x	Wageningen Bioveterinary Research (WBVR)	Netherlands
x	x	Veterinærinstituttet	Norway
x	x	National Veterinary Research Institute	Poland
x	x	Instituto Nacional de Investigação Agrária e Veterinária	Portugal
x	x	Institute for Diagnosis and Animal Health	Romania
x	x	Institute for Hygiene and Veterinary Public Health	Romania
x	x	State Veterinary and Food Institute (SVFI)	Slovakia
x	x	National Veterinary Institute	Slovenia
x	x	Laboratorio Central de Veterinaria	Spain
x	x	Centro Nacional de Alimentación (AECOSAN)	Spain
x	x	National Veterinary Institute, SVA	Sweden
x	x	Institute of Veterinary Bacteriology, Vetsuisse Faculty Bern	Switzerland
x	x	Animal & Plant Health Agency (APHA)	United Kingdom

Designated NRL-AR by the competent authority of the member state

Non-NRL-AR enrolled by the EURL-AR

Non-MS NRL-AR

**Appendix 3 Test strain
reference values p. 1/2**

Panel 1 =EUVSEC3

Strain ID	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Gene	Prediction
EURL-M 9.1	>32	32	≤4	≤0.5	≤0.25	>8	>4	≤1	>64	>32	>16	>512	≤8	16	>8	CTX-M-15, NDM-5	Carbapenemase
EURL-M 9.2	>32	64	≤4	≤0.5	≤0.25	4	1	≤1	≤4	>32	>16	>512	≤8	≤0.03	0.25	DHA-1	AmpC
EURL-M 9.3																	Susceptible
EURL-M 9.4	>32	4	≤4	≤0.5	≤0.25	4	1	≤1	≤4	>32	≤0.25	≤8	≤8	≤0.03	≤0.015	AmpC (g.-32T>A)	AmpC
EURL-M 9.5	>32	>64	≤4	≤0.5	≤0.25	>8	2	≤1	≤4	>32	>16	>512	≤8	≤0.03	≤0.015	SHV-12	ESBL
EURL-M 9.6	>32	4	≤4	≤0.5	≤0.25	>8	>4	4	≤4	>32	≤0.25	>512	0.06	0.06	0.03	CMY-2	AmpC*
EURL-M 9.7																	
EURL-M 9.8	>32	32	≤4	>16	≤0.25	≤0.25	1	≤1	16	>32	>16	>512	≤8	0.5	0.06	OXA-48	Carbapenemase

Strain ID	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Gene	Prediction
EURL-M 9.1	R	R	S	S	S	R	R	S	R	R	R	R	S	R	R	CTX-M-15, NDM-5	Carbapenemase
EURL-M 9.2	R	R	S	S	S	R	R	S	S	R	R	R	S	S	R	DHA-1	AmpC
EURL-M 9.3																	Susceptible
EURL-M 9.4	R	S	S	S	S	R	R	S	S	R	S	S	S	S	S	AmpC (g.-32T>A)	AmpC
EURL-M 9.5	R	R	S	S	S	R	R	S	S	R	R	R	S	S	S	SHV-12	ESBL
EURL-M 9.6	R	S	S	S	S	R	R	R	S	R	S	R	R	S	S	CMY-2	AmpC*
EURL-M 9.7																	
EURL-M 9.8	R	R	S	R	S	S	R	S	R	R	R	R	S	R	S	OXA-48	Carbapenemase

* For sample 9.6 'Other phenotype' is additionally accepted

Appendix 3 Test strain reference values p. 2/2

Panel 2=EUVSEC2

Strain ID	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	ESBL conclusion	Gene
EURL-M 9.1	>64	>64	>2	8	16	>128	>32	>64	>128	128	CTX-M-15, NDM-5	Carbapenemase
EURL-M 9.2	64	1	≤0.015	0.25	≤0.03	4	≤0.06	1	4	4	DHA-1	AmpC
EURL-M 9.3												Susceptible
EURL-M 9.4	16	1	≤0.015	0.25	≤0.03	4	≤0.06	0.5	2	2	ampC(g.-32T>A)	AmpC
EURL-M 9.5	4	2	≤0.015	≤0.12	≤0.03	16	0,5	≤0.06	≤0.12	4	SHV-12	ESBL
EURL-M 9.6	64	32	0.12	0.25	0.06	32	1	16	16	8	CMY-2	AmpC*
EURL-M 9.7												
EURL-M 9.8	8	1	2	1	0,5	≤0.25	0.25	0.5	≤0.12	128	OXA-48	Carbapenemase

Strain ID	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	Gene	Prediction
EURL-M 9.1	R	R	R	R	R	R	R	R	R	R	CTX-M-15, NDM-5	Carbapenemase
EURL-M 9.2	R	R	S	S	S	R	S	R	R	S	DHA-1	AmpC
EURL-M 9.3												Susceptible
EURL-M 9.4	R	R	S	S	S	R	S	R	R	S	AmpC (g.-32T>A)	AmpC
EURL-M 9.5	S	R	S	S	S	R	R	S	S	S	SHV-12	ESBL
EURL-M 9.6	R	R	R	S	S	R	R	R	R	S	CMY-2	AmpC*
EURL-M 9.7												
EURL-M 9.8	S	R	R	R	R	S	R	R	S	R	OXA-48	Carbapenemase

* For sample 9.6 'Other phenotype' is additionally accepted



PROTOCOL

for selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from meat and caecal samples (Matrix EQAS)

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1 INTRODUCTION

The organisation and implementation of an External Quality Assurance System (EQAS) on selective isolation of presumptive extended spectrum beta-lactamase (ESBL)-, AmpC- or carbapenemase-producing *E. coli* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), and will include the selective isolation procedures and antimicrobial susceptibility testing (AST) of obtained isolates of eight samples of either meat or caecal content. In 2023, these eight samples will include five 25-g samples of beef meat and three 1-g samples of swine caecal content. These samples may contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes or point mutations leading to resistance phenotypes.



It is expected that the participating laboratories apply the same analysis procedures used in the monitoring, described by the regulation 2020/1729/EU, and perform the selective isolation following the by EU recommended methods, published on the EURL-AR website www.eurl-ar.eu.

2 OBJECTIVES

This EQAS aims to assess and, if necessary, to improve the quality of results obtained in the selective isolation of presumptive ESBL-, AmpC- or carbapenemase-producing isolates from meat and caecal samples. Further objectives are to evaluate and improve the comparability of surveillance data on ESBL-, AmpC- or carbapenemase -producing *E. coli* reported to EFSA by different laboratories.

3 OUTLINE OF THE MATRIX EQAS 2023

3.1 Shipping, receipt and storage of samples

In November 2023, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight samples from the National Food Institute. All strains used in the spiking of samples belong to UN3373, Biological substance, category B. Participants should expect that ESBL-, AmpC- and/or carbapenemase-enzymes producing strains will be included in (some of) the sample matrices.

The samples will be spiked matrices of either beef meat or swine caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 9.1 to 9.8. Hereof 9.1 to 9.5 being samples of meat (each 25 g) and 9.6 to 9.8 being samples of caecal content (each 1 g).

The matrix samples will be shipped on 6 November in frozen/chilled state in separate tubes and contained in a cooling box with a temperature logging device and freezing elements.

Upon arrival, it is very important to open the parcel as soon as possible and proceed to the analysis (following the normal procedures for sample testing in the monitoring).

It is required that participants

- **when opening the parcel, note the date and exact time at opening (this data is very important to follow the temperature data checks)**
- **proceed to sample analysis immediately after opening the parcel**
- **register the date for start of analysis for each sample**
- **collect the temperature logging device from the parcel (small discoid device located in a bag inserted in a labelled tube);** open the tube and take out the bag with the device inside. Place this bag with the device in the labelled bubble envelope provided and return it to the EURL-AR as soon as possible. Please note that you will have to arrange for stamps/postage (the post systems differ from country to country, why this cannot be arranged and paid from the EURL-AR in advance).



3.2 QC reference strains

Include the *E. coli* ATCC25922 and *Acinetobacter baumannii* (2012-70-100-69) reference strains in the MIC testing, and report results of these together with the isolates obtained from the EQAS samples. 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.

3.3 Selective isolation of ESBL, AmpC or carbapenemase producing *E. coli* from the samples

The samples provided in each parcel are weighed beforehand and therefore no further weighing is required. Proceed immediately to the first enrichment step by adding the sample to the necessary volume of media (225 ml of Buffered Peptone water for the meat samples and 9 ml for the caecal samples) as referred in the official EURL-AR protocols. **Results should be produced according to the laboratory's routine procedures for antimicrobial susceptibility testing by MIC determination.** All the following procedures should follow the methods used in the monitoring for ESBL and AmpC *E. coli* according to the 2020/1729/EU Decision. If any changes are introduced to the official protocols, these changes should be described with details in the online database on the methods upload page. The participants are responsible for assuring the validity of the plates and therefore the protocol for "Validation of selective MacConkey agar plates supplemented with 1 mg/L cefotaxime for monitoring of ESBL and AmpC producing *E. coli* in meat and animals" should be run beforehand, as stated on the EURL-AR webpage (see <https://www.eurl-ar.eu/protocols.aspx>).

According to the 2020/1729/EU Decision, **the monitoring of carbapenemase-producing *E. coli* from the samples is now mandatory**, and should be performed following the official protocols and plating on suitable agar plates. Similarly, the agar plates used for the carbapenemase isolation should be validated using the protocol for "Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*".

The officially recommended protocols are found on the EURL-AR webpage (<http://eurl-ar.eu/233-protocols.htm>):

- Follow the protocol for meat when testing samples 9.1 to 9.5
- Follow the protocol for caecal content when testing samples 9.6 to 9.8

As referred in these protocols, the isolates obtained from isolation procedure should be identified as *E. coli* using the procedures for *E. coli* species identification applied at the participant's laboratory for the specific monitoring of ESBL-, AmpC-, and carbapenemase producing *E. coli*.

Please store the isolates obtained in the isolation procedure and document the whole process as well as all the findings in each step.

As part of the results submission, you will be requested to describe the findings along the enrichment process and selective isolation including growth in the media, isolation of suspected colonies, species



identification results and finally regarding the finding (or not) of presumptive *E. coli* isolates harbouring one of the selected resistances (this result will be evaluated in relation to the expected result as a qualitative result) (see details in the Test Form).

3.4 Antimicrobial susceptibility testing

If the sample is deemed positive for ESBL-, AmpC- or carbapenemase -producing *E. coli*, one *E. coli* isolate per sample should be taken further and tested for susceptibility to antimicrobials as stated in the EU regulation (antimicrobials listed in Tables 1 and 2 in this document). Only one *E. coli* isolate is expected to be tested for AST and these results will be evaluated in the database comparing to expected results.

AST results to be reported should be from:

- A presumptive carbapenemase positive isolate (from the CARBA or OXA-48 selective plates), if a presumptive carbapenemase positive *E. coli* isolate was detected.
- An ESBL- or AmpC-presumptive isolate (if you do not have a carbapenemase positive isolate) if an ESBL- or AmpC-presumptive isolate was detected.

The testing should be performed using the same method as implemented in your laboratory for performing AST when monitoring for EFSA according to the Decision 2020/1729/EU (using the two-step approach, i.e. both testing panels) and applying the interpretative criteria listed below.

Table 1: Panel 1 antimicrobials recommended for AST of *E. coli* spp. and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (accessed on 20.09.2023) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	8
Ampicillin (AMP)	8
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.25
Ceftazidime (TAZ or CAZ)	1
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.06
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)

Beta-lactam resistance

Confirmatory testing for ESBL and carbapenemase production is mandatory on all



strains resistant to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) and should be performed by testing the second panel of antimicrobials (Table 2).

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 ((T)ECOFFs) according to latest updates from EUCAST (20.09.2023)

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.125
Cefotaxime (FOT or CTX)	0.25
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.25
Cefoxitin (FOX)	8*
Ceftazidime (TAZ or CAZ)	1
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	1
Ertapenem (ETP)	0.03
Imipenem (IMI)	0.5
Meropenem (MERO or MEM)	0.06
Temocillin (TRM)	16

*For the purpose of this EQAS and to be in alignment with the ESBL-phenotype categorization scheme (see Appendix), the MIC-value used for interpretation of cefoxitin for *E. coli* is set at 8 mg/L, and is therefore not according to the latest EUCAST update

Confirmatory test for ESBL production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a β -lactamase inhibitor (clavulanic acid). Synergy is defined either as i) a ≥ 3 twofold concentration decrease in a 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 results should be based on the most recent EFSA recommendations (See the appendix to this protocol). Importantly: Note that for *E. coli*, two cut-off values apply for cefotaxime and ceftazidime: the EUCAST cut-off values, those that define R/S (see Tables 1 and 2), 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.



4 REPORTING OF RESULTS AND EVALUATION

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

4.1 General recommendations for data upload

We recommend reading carefully the description reported in paragraph 5 before entering your results in the web database. Results must be submitted **no later than 12 December 2023 at 16:00**. After the deadline when all participants have uploaded results, you will be able to login to the database once again, and to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as ‘correct’, while results deviating from the expected interpretation are categorised as ‘incorrect’.

If you experience difficulties in entering your results, please contact us directly.

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

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

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5 HOW TO ENTER RESULTS IN THE INTERACTIVE DATABASE

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 please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once!** '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 jetk@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 jetk@food.dtu.dk.

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

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APPENDIX

Criteria for interpretation of *Escherichia coli*, panel 2 results

SUSCEPTIBLE
CTX - CAZ – FOX- MEM ≤ ECOFF

1. ESBL-Phenotype

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

2. AmpC-Phenotype

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

3. ESBL + AmpC-Phenotype

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

4. Carbapenemase-Phenotype

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

5. Other Phenotypes

1) IF CTX or CAZ > 1 mg/L AND

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

3) IF CTX and CAZ ≤ 1 mg/L AND

- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L
- *cAmpCs could be included here

2) IF CTX and/or CAZ ≤ 1 mg/L AND > ECOFF AND

- MEM ≤ 0.12 mg/L AND
- FOX ≤ 8 mg/L AND

4) IF MEM ≤ 0.12 mg/L BUT

- ETP > ECOFF AND/OR
- IMI > ECOFF
- Not excluded CPs, needs confirmation (consult EURL)

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

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

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



Test forms, Isolation of ESBL/AmpC- and carbapenemase-producers from matrices

Username:

Contact person:

Country:

Date for filling in test forms:

SAMPLES

Reception date and exact time of opening the parcel of the proficiency test samples at the laboratory: (date and time is required)

Temperature of the contents of the parcel at arrival: °C

How many samples did your laboratory process in 2023 for monitoring of ESBL/AmpC detection in relation to 2020/1729/EU? (Choose only one option)

- none
- less than 100
- 101-200
- 201-300
- 301-400
- 401- 1000
- more than 1000

Which kind of samples did your laboratory process in 2023 for monitoring of ESBL/AmpC detection in relation to 2020/1729/EU? (You may choose more than one option)

- caecal, swine and cattle
- meat, pork and beef
- none
- other matrices, please specify:



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How many samples did your laboratory process in 2023 for monitoring of carbapenemases in relation to 2020/1729/EU? (Choose only one option)

- none
- less than 100
- 101-200
- 201-300
- 301-400
- 401- 1000
- more than 1000

Which kind of samples did your laboratory process in 2023 for monitoring of carbapenemase-production in relation to 2020/1729/EU? (you may choose more than one option)

- caecal, swine and cattle
- meat, pork and beef
- none
- other matrices, please specify:

Any other comments:



METHODS

1- Method used for selective isolation of ESBL/AmpC in this EQAS:

Regarding the methods used for selective isolation of ESBL/AmpC in this EQAS, please indicate if you had any modifications to the selective isolation procedure using the EURL recommended protocols that refer to the EU decision 2020/1729/EU:

- The protocol was used without modifications (please jump to question 2)
- The protocol was used, however, the pre-enrichment was modified (please respond to question 1.1)
- The protocol was used, however, the selective isolation procedures were modified (please respond to question 1.2)
- The protocol was used, however, the incubation conditions in the selective plating were modified (please respond to question 1.3)

1.1- If you modified the pre-enrichment, please indicate the differences introduced:

Different sample amount (weight) used for the enrichment procedure:

g in meat samples

g for caecal samples

Different volume of enrichment in the isolation step:

ml for meat samples

ml for caecal samples

Different pre-enrichment medium:

Different incubation conditions in pre-enrichment °C/ h;

Please justify these changes:

1.2- Did you make changes in the selective isolation procedure:

Different sample amount (weight) used for the enrichment procedure

for meat samples

for caecal samples

Different concentration of cefotaxime: mg/L

Different antimicrobial

Different medium

Please justify these changes:



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1.3- If you used different incubation conditions in the selective plating, please indicate the conditions used: °C/ h;

Please justify these changes:

2- Method used for selective isolation of carbapenemase-producers in this EQAS:

Selective isolation procedure using the EURL recommended protocols for isolation of carbapenemase-producers:

- The protocol was used without modifications
- The protocol was modified

Plates used (brand/type)

Volume of sample plated

Please justify any changes:

Comments:

3- Method used for confirmation of *E. coli* species identification. Please indicate the primary *E. coli* identification method used (choose only one option; if you used more than one method, please explain in the comments field)

- PCR using published methods
- PCR using in-house method
- Biochemical tests
- MALDI-ToF
- DNA Sequencing
- Chromogenic media

Comments:

4- Method used for general antimicrobial susceptibility testing of the strains (choose only one option)

- Microbroth dilution test on EUVSEC3 panel
- Microbroth dilution test on another panel
- Agar dilution method
- E-test
- Disk diffusion test

5- Method used for phenotypic confirmatory testing of ESBL/AmpC/Carbapenemase presumptive strains (choose only one option)

- Microbroth dilution test on EUVSEC2 panel
- Microbroth dilution test on another panel



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- Agar dilution method
- E-test
- Disk diffusion test

6- Additional comments. Please include here description and justification of your choice if you modified something in relation to the method defined in the EU regulation 2020/1729/EU:



TEST FORM – SAMPLE ‘EURL M-9.1’

Date the isolation procedure was started:

Please describe the results you have observed regarding this sample:

Visible growth in pre-enrichment:

Yes / No

Growth on ESBL/AmpC-selective plates:

Yes / No

Please describe the growth observed on ESBL/AmpC-selective plates? (choose only one option)

- Mixed culture containing typical *E. coli* colonies
- Mixed culture without typical *E. coli* colonies
- Pure culture of typical *E. coli* colonies
- Pure culture without typical *E. coli* colonies
- No growth

Results of species identification: (choose only one option)

- No isolates tested (sample negative)
- Presumptive ESBL/AmpC isolate identified as *E. coli* (sample considered positive)

Comments:

Growth on CARBA-selective plates:

Yes / No

Growth on OXA-48 selective plates:

Yes / No

Results of species identification (isolates from carbapenemase selective plating): (choose only one option)

- No isolates tested (sample negative)
- Presumptive other carbapenemase isolate identified as *E. coli* (sample considered positive)
- Presumptive OXA-48 isolate identified as *E. coli* (sample considered positive)

Comments:



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If you have found a presumptive carbapenemase positive isolate, please insert the results of antimicrobial susceptibility testing for the selected *E. coli* isolate, if you do not have a carbapenemase positive isolate and you have an ESBL presumptive isolate, please insert the results for this isolate (only one *E.coli* isolate is expected to be tested and these results will be evaluated in our database against the expected results).

Please confirm where the isolate tested for antimicrobial susceptibility originated from (compulsory):

- ESBL/ampC isolation on MacConkey with cefotaxime
- CARBA plate
- OXA-48 plate

Based on the results from the first AST panel, was the isolate found resistant to cefotaxime, ceftazidime or meropenem so that the second panel was tested?

Yes / No



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AST results

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (mg/L)	S / R
<i>E. coli</i> EURL M-9.1	Amikacin AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT or CTX			
	Ceftazidime, TAZ or CAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO or MEM			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
Trimethoprim, TMP				

Second *E. coli* AST panel (confirmatory testing for ESBL/AmpC/carbapenemase-production)

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (mg/L)	S / R
<i>E. coli</i> EURL M-9.1	Cefepime, FEP			
	Cefotaxime + clavulanic acid F/C or CTX/CLA			
	Cefotaxime, FOT or CTX			
	Cefoxitin, FOX			
	Ceftazidime, TAZ or CAZ			
	Ceftazidime+ clavulanic acid T/C or CAZ/CLA			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO or MEM			
	Temocillin, TRM			

Conclusions of confirmatory phenotypic testing: (choose only one option and please note that the final result will be evaluated by the database)

Interpretation of PANEL 2 results:

<input type="checkbox"/> Presumptive ESBL	<input type="checkbox"/> Presumptive AmpC	<input type="checkbox"/> Other phenotype
<input type="checkbox"/> Presumptive ESBL+ AmpC	<input type="checkbox"/> Presumptive carbapenemase	<input type="checkbox"/> Susceptible

Comments (include optional genotype or other results):

Appendix 6 - QC ranges

Escherichia coli ATCC 25922

Panel	Antimicrobial	Abbreviation	Acceptable range	
			Min	Max
Panel 1	Ampicillin	AMP	2	8
Panel 1	Amikacin	AMI	NA	NA
Panel 1	Azithromycin	AZI	NA	NA
Panel 1	Cefotaxime	FOT	0.03	0.12
Panel 1	Ceftazidime	TAZ	0.06	0.5
Panel 1	Chloramphenicol	CHL	2	8
Panel 1	Ciprofloxacin	CIP	0.004	0.016
Panel 1	Colistin	COL	0.25	2
Panel 1	Gentamicin	GEN	0.25	1
Panel 1	Meropenem	MER	0.008	0.06
Panel 1	Nalidixic acid	NAL	1	4
Panel 1	Sulfamethoxazole	SMX	8	32
Panel 1	Tetracycline	TET	0.5	2
Panel 1	Tigecycline	TGC	0.03	0.25
Panel 1	Trimethoprim	TMP	0.5	2

Panel 2	Cefepime	FEP	0.016	0.12
Panel 2	Cefotaxime/clavulanic	F/C	NA	NA
Panel 2	Cefotaxime	FOT	0.03	0.12
Panel 2	Cefoxitin	FOX	2	8
Panel 2	Ceftazidime	TAZ	0.06	0.5
Panel 2	Ceftazidime/clavulanic	T/C	NA	NA
Panel 2	Ertapenem	ETP	0.004	0.016
Panel 2	Imipenem	IMI	0.06	0.25
Panel 2	Meropenem	MER	0.008	0.06
Panel 2	Temocillin	TRM	NA	NA

NA: Not available

Appendix 7 List of deviations 3 pp

Deviations in ESBL categorisation - full list

Lab number	Strain	Obtained ESBL-phenotype value	Expected ESBL-phenotype value	Score ESBL-phenotype
NRL-AR-025	EURL M-9.4	Carbapenemase-phenotype	AmpC-phenotype	0

Deviations in AST results - full list

Grey markings refer to 1 two-fold dilution differences

Lab number	Strain	Antimicrobial	Obtained operator	Expected operator	Obtained MIC value	Expected MIC value	Obtained interpretation	Expected interpretation	Score interpretation
NRL-AR-002	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-002	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-004	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-004	EURL M-9.8	Cefepime	=	=	0.25	0.25	S	R	0
NRL-AR-006	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-009	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-009	EURL M-9.6	Meropenem	<=	=	0.03	0.06	R	S	0
NRL-AR-009	EURL M-9.1	Cefotaxime	>	>	64	64	S	R	0
NRL-AR-011	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-011	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-012	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-012	EURL M-9.2	Ertapenem	=	<=	0.12	0.015	R	S	0
NRL-AR-016	EURL M-9.2	Ertapenem	=	<=	0.12	0.015	R	S	0
NRL-AR-017	EURL M-9.5	Chloramphenicol	=	<=	16	16	R	S	0
NRL-AR-017	EURL M-9.2	Ertapenem	=	<=	0.25	0.015	R	S	0
NRL-AR-017	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-017	EURL M-9.1	Temocillin	=	=	128	128	S	R	0
NRL-AR-018	EURL M-9.4	Cefotaxime	=	=	2	1	S	R	0
NRL-AR-018	EURL M-9.4	Ceftazidime-Clavulanic acid	=	=	4	2	S	R	0
NRL-AR-018	EURL M-9.1	Chloramphenicol	=	<=	16	8	R	S	0
NRL-AR-018	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0

NRL-AR-018	EURL M-9.5	Amikacin	=	<=	16	4	R	S	0
NRL-AR-018	EURL M-9.5	Cefotaxime	<=	=	0.06	2	S	R	0
NRL-AR-018	EURL M-9.5	Cefotaxime-Clavulanic acid	=	<=	16	0.06	R	S	0
NRL-AR-018	EURL M-9.5	Gentamicin	=	<=	4	0,5	R	S	0
NRL-AR-018	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-018	EURL M-9.2	Temocillin	=	=	32	4	R	S	0
NRL-AR-020	EURL M-9.8	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-020	EURL M-9.8	Sulfamethoxazole	>	>	512	512	S	R	0
NRL-AR-021	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-021	EURL M-9.8	Colistin	=	<=	1	1	R	S	0
NRL-AR-021	EURL M-9.2	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-022	EURL M-9.8	Cefepime	=	=	0.12	0.25	S	R	0
NRL-AR-022	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-022	EURL M-9.6	Ertapenem	=	=	0.06	0.12	S	R	0
NRL-AR-023	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-025	EURL M-9.4	Azithromycin	=	=	64	4	R	S	0
NRL-AR-025	EURL M-9.4	Cefepime	>	<=	32	0.06	R	S	0
NRL-AR-025	EURL M-9.4	Ciprofloxacin	>	<=	8	0.015	R	S	0
NRL-AR-025	EURL M-9.4	Ertapenem	>	<=	2	0.015	R	S	0
NRL-AR-025	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-025	EURL M-9.4	Imipenem	=	=	16	0.25	R	S	0
NRL-AR-025	EURL M-9.4	Meropenem	>	<=	16	0.03	R	S	0
NRL-AR-025	EURL M-9.4	Meropenem	>	<=	16	0.03	R	S	0
NRL-AR-025	EURL M-9.4	Nalidixic acid	>	<=	64	4	R	S	0
NRL-AR-025	EURL M-9.4	Sulfamethoxazole	>	<=	512	8	R	S	0
NRL-AR-025	EURL M-9.4	Temocillin	>	=	128	2	R	S	0
NRL-AR-025	EURL M-9.4	Trimethoprim	>	<=	16	0.25	R	S	0
NRL-AR-026	EURL M-9.8	Cefepime	=	=	0.12	0.25	S	R	0
NRL-AR-026	EURL M-9.8	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-026	EURL M-9.2	Cefepime	=	<=	0.25	0.06	R	S	0
NRL-AR-030	EURL M-9.4	Cefotaxime	=	=	1	1	S	R	0
NRL-AR-033	EURL M-9.8	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-034	EURL M-9.4	Ceftazidime-Clavulanic acid	=	=	1	2	S	R	0

NRL-AR-034	EURL M-9.8	Imipenem	=	=	0.5	1	S	R	0
NRL-AR-036	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-036	EURL M-9.6	Ertapenem	=	=	0.125	0.12	S	R	0
NRL-AR-037	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-037	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-037	EURL M-9.2	Temocillin	=	=	32	4	R	S	0
NRL-AR-038	EURL M-9.5	Cefepime	=	=	0.25	0.5	S	R	0
NRL-AR-038	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-038	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-040	EURL M-9.4	Sulfamethoxazole	=	<=	128	8	R	S	0
NRL-AR-041	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-041	EURL M-9.2	Ertapenem	=	<=	0.064	0.015	R	S	0
NRL-AR-042	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-045	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0
NRL-AR-045	EURL M-9.8	Ciprofloxacin	=	=	0.12	0.06	R	S	0
NRL-AR-056	EURL M-9.2	Nalidixic acid	=	<=	16	4	R	S	0
NRL-AR-060	EURL M-9.2	Imipenem	=	=	1	0.25	R	S	0
NRL-AR-061	EURL M-9.5	Chloramphenicol	=	<=	32	16	R	S	0



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