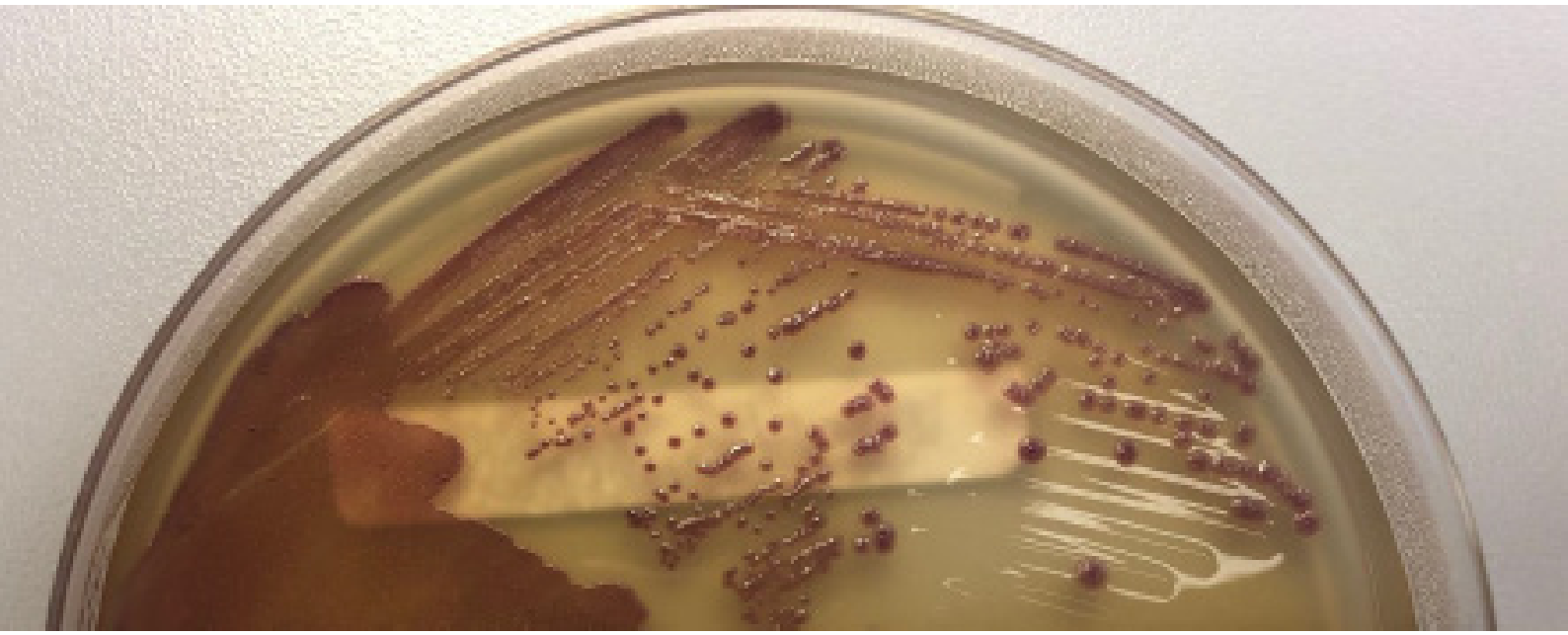
DTU National Food Institute

The 7th EURL-AR PROFICIENCY TEST ON SELECTIVE ISOLATION OF E. COLI WITH PRESUMPTIVE ESBL, AMPC OR CARBAPENEMASE PHENOTYPES FROM MEAT OR CAECAL SAMPLES - 2021



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The 7th EURL-AR Proficiency Test on selective isolation of Escherichia coli with presumptive ESBL, AmpC or carbapenemase phenotypes from meat and caecal samples – 2021

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1. Introduction

This report describes and summarises results from the seventh 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 *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 new 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 taken into account:

1) Expected results were generated by performing Minimum Inhibitory Concentration (MIC) determination for all test strains prior to selection of strains and MIC's 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 and caecal, which revealed a risk for deviating phenotypic results and unclear phenotypes (See section 3.1).

2) No thresholds have been set in advance to evaluate the acceptance of the performance of the participating laboratories and therefore the 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 details 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 seventh matrix EQAS was organized by the EURL-AR at the National Food Institute (DTU Food), Kgs. Lyngby, Denmark. The report was approved in its draft version by the network, as it was send around for commenting in a three weeks period from March 2023 and no substantial changes were made in this final report.

2. Materials and Methods

2.1 Participants in EQAS 2021

A pre-notification (App. 1), announcing the matrix EQAS 2021, was distributed on 27th September 2021 by e-mail to the designated NRLs including all EU countries and Iceland, Norway, Switzerland and United Kingdom. In total, 37 laboratories participated in the matrix EQAS (App. 2) involving one NRL from the 27 EU countries and Iceland, Norway, Switzerland and United Kingdom. Three countries (the Netherlands, Spain and Malta) had additional laboratories participating in the EQAS. These were invited based on their participation in previous EQAS iterations and/or affiliation to the EU network and provided results but were not included further in the report. Two countries (Romania and Spain) had separate laboratories enrolled for handling meat and caecal samples and had two NRLs enrolled. One EU candidate country (Lab 064) participated for the first time in this EQA, but the results were not considered in the report. Therefore, in total, 33 laboratory results from 31 countries are described. Participants from non-EU member states 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 five beef meat and three pig caecal samples and were prepared either by spiking with test strains or unmodified. It is the first time in a EURL-AR EQAS to use this combination of matrices.

The meat used to prepare the samples was minced beef meat of Danish origin (raised, slaughtered and packed in Denmark) acquired in local supermarkets (four different batches 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 contained some background microflora. 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 EQAS matrix 2021 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 µl 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 EQAS was expected to be approx. 10^3 CFU/g meat for samples EURL-M-7.1, M-7.2, M-7.4 and M-7.5. Sample EURL-M-7.3 was spiked as mentioned above but with a susceptible *E. coli* strain (ATCC 25922) and therefore expected to be negative for ESBL, AmpC or carbapenemase-producing *E. coli*.

One slaughterhouse provided on 17th September 15 pig caecal samples from different herds. 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 EQAS strains. Thereby 1 g aliquots of caecal content were used directly as blank sample or spiked with 10 µl of a dilution containing 10^6 CFU/ml, causing an expected spiking level of 10^4 CFU/g for the samples M-7.6, and M-7.8. The sample M-7.7 was uninoculated and expected to be negative. Unlike previous years, meat samples were inoculated with both carbapenemase-producing *E. coli*, whereas the caecal samples were inoculated with one ESBL and one AmpC strain, as this time it was not possible to find a relevant carbapenem resistant *E. coli* strain isolated from pig caecal samples. Selecting strains initially isolated from caecal samples will enhance the survival of the inoculum, especially in pig caecal samples, which otherwise have a 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 (App. 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 (during two weeks). In this period, the meat and caeca 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 (App. 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 (App. 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 or meropenem.

Table 1. Panel of antimicrobials recommended for susceptibility testing of bacteria included in this EQAS 2021 component

Escherichia coli EUVSEC3	*Escherichia coli* 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	

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 (IATA) regulations as "Biological Substance category B" classified UN3373. The parcels were dispatched from DTU Food 27 September 2021.

2.6 Procedure

The laboratories were instructed to download the protocol and test forms (App. 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 (App. 4). The database was delayed due to unexpected events hindering the final development and opening of the web tool. Eventually, the web tool was activated on the 31st of March 2022 and closed April 11th 2022.

For the first part of the results of the selective isolation procedure for ESBL/AmpC and for carbapenemases, 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 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.

After the deadline, the qualitative results indicating if the samples were positive or negative for ESBL/AmpC, or carbapenemase-producing *E. coli* (*bla*$_{\text{OXA-48}}$ 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 on 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/Carbapenemase qualitative isolation considered for this report was 248 tests, eight samples from each of the 31 countries. These results are summarized in Figure 1 and further discussed in section 3.4.
A few deviations in the results occurred, which were not considered errors as the countries provided correct evaluation of the phenotype of the isolated strains. Specifically, Lab 026 recovered an *E. coli* isolate from sample M-7.1 with an ESBL + AmpC phenotype, originally inoculated with an AmpC strain. Labs 060 and 018 isolated a strain with ertapenem MIC = 0.12 mg/L instead of MIC = 0.064 mg/L from sample M-7.6 (originally inoculated with AmpC-positive *E. coli*), hence reported it as "Other phenotype". Additionally, lab 004 recovered an isolate from sample M-7.8 with a completely different beta-lactam profile from the originally inoculated strain. Instead of an ESBL phenotype, the lab isolated a strain with resistance to cefoxitin (FOX = 64 mg/L) with no synergy and accurately reported it as an AmpC phenotype. These results were accepted as correct, and laboratories had been given a positive score.

3.2 Methods used by EQAS-participants

In this trial, 33 participating NRL's reported results for all the eight samples sent. Two laboratories reported only results for the meat samples (Labs 038 and 041) and two laboratories reported only results for the caecal samples (Labs 032 and 058). 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. One laboratory (Lab 039) did not perform the carbapenemase selective isolation due to a delay in the delivery of the culture media. 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 isolation had been performed following the exact procedures described in the protocols provided. The species identification was performed using MALDI TOF (n=12), biochemical tests (n=10), or chromogenic media (n=10), and PCR using specific targets to confirm the ID (n=5). Additionally, four laboratories reported using second and third identification methods as supplement.

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 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 186 tests for the ESBL, AmpC and carbapenemase isolation qualitative results. In total, 181 (97%) tests were assigned the correct ESBL, AmpC or carbapenemase phenotype (Figure 1). All 62 samples expected to be negative (blank and susceptible) were correctly assigned. Regarding the 186 samples expected to be positive, all but five were correctly assigned (97%). Some laboratories could not isolate *E. coli* from samples M-7.1 (Lab 004) and M-7.6 (Lab 021), both inoculated with an AmpC positive *E. coli*, and sample M-7.4 (Lab 039) with a carbapenemase-positive *E. coli*. The other two errors were observed in Lab 036 for strain M-7.4 and Lab 059 for strain M-7.6. Lab 036 wrongly reported "Other phenotype" to the carbapenemase phenotype of sample M-7.4 due to an isolation of two strains - one from MacConkey and one from CARBA selective media, which only differed by an additional *mph(A)* gene in one of the isolates. Lab 059 incorrectly assigned a carbapenem resistance phenotype to the strain M-7.6 with an original AmpC phenotype.

The specific isolation of presumptive carbapenemase producing *E. coli* was performed by extending the protocol to include isolation on CARBA selective agar plates as described in the EURL-AR protocols. Lab 039 did not perform the carbapenemase selective isolation due to a delay in the media delivery, but defined results based on the findings in the ESBL/AmpC isolation on MacConkey with cefotaxime.

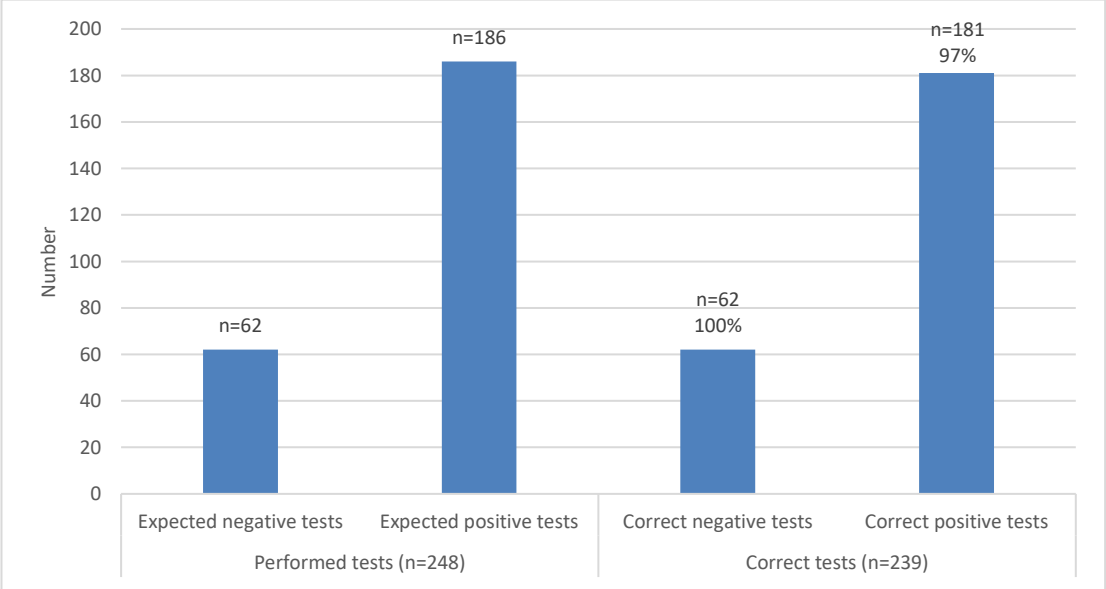

Figure 1. Overall performance of ESBL/AmpC/Carbapenemase isolation and identification, 2021

Table 2 Deviations in ESBL /AmpC and carbapenemase phenotype identification, 2021

Strain ID	Phenotype*	*bla* genes	Deviations, %
M-7.1	AmpC	ampC-promoter (g.-42C>T)	3.2
M-7.2	ESBL	SHV-12	0
M-7.3	Susceptible	None	0
M-7.4	Carbapenemase	NDM-5, CTX-M-15	3.2
M-7.5	Carbapenemase	OXA-181, TEM-35	3.2
M-7.6	AmpC/Other phenotype	CMY-2	6.5
M-7.7	Blank	None	0
M-7.8	ESBL	CTX-M-32	0

*Expected phenotype based on both susceptibility testing and genomic analysis

There were no difficulties in isolating the carbapenemase producing *E. coli*, since the selected strains were able to grow on the ESBL selective media. According to the protocols, laboratories can chose any suitable plates for selective isolation of carbapenemase- and *bla*$_{\text{OXA-48}}$-producing *E. coli*. Eighteen laboratories (55%) declared the use of chromogenic agar, ChromID CARBA or ChromID CARBA Smart combination plates and ChromID OXA48. Lab 037 used the BD CHROMagar CPE. Fifteen laboratories (45%) did not report the brand or specific type of plates being used for this purpose and 14 of them reported that the EURL-AR protocol was followed. Lab 039 did not test for carbapenemase-producing *E. coli* due to a delay in the media delivery.

3.4 Antimicrobial susceptibility testing

A total of 4,650 AST results were submitted. Of them, 101 tests will not be considered because of either i) strain was not isolated or ii) no score was given to the laboratory due to obtained MIC values consistently different from the expected which can suggest either isolation of a strain different from the expected (background bacteria) or that the original strain acquired a plasmid that changed the phenotype. Therefore, 4,549 tests were considered in the analysis. Of them, 4,501 (98.9%) were correct. 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 44 to 150 test results per participant.

Of the 48 deviations detected, 38 were caused by more than one-step dilution difference in the MIC, which resulted in a different susceptible/resistant interpretation. (Appendix 7). The other ten deviations were caused by an incorrect susceptible/resistant interpretation of otherwise accurately obtained MIC values.

The analysis per laboratory identified 11 laboratories with no deviations, while the others had deviation percentages ranging from 0.7% to 3.4 %. (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 deviations per antimicrobial, it was observed that the highest deviation percentage was found for trimethoprim (6.6%) (Figure 3), all of them caused by more than one two-fold dilution MIC difference. Further, some deviations were observed for cefepime (5.5%), cefoxitin (4.4%),

ceftazidime-clavulanic acid (2.3%), cefotaxime-clavulanic acid (1.1%), and ciprofloxacin (1.1%). These were either due to more than one-step dilution difference or wrong phenotype interpretation. Otherwise, majority of the deviations were <1% per antimicrobial (Figure 3).

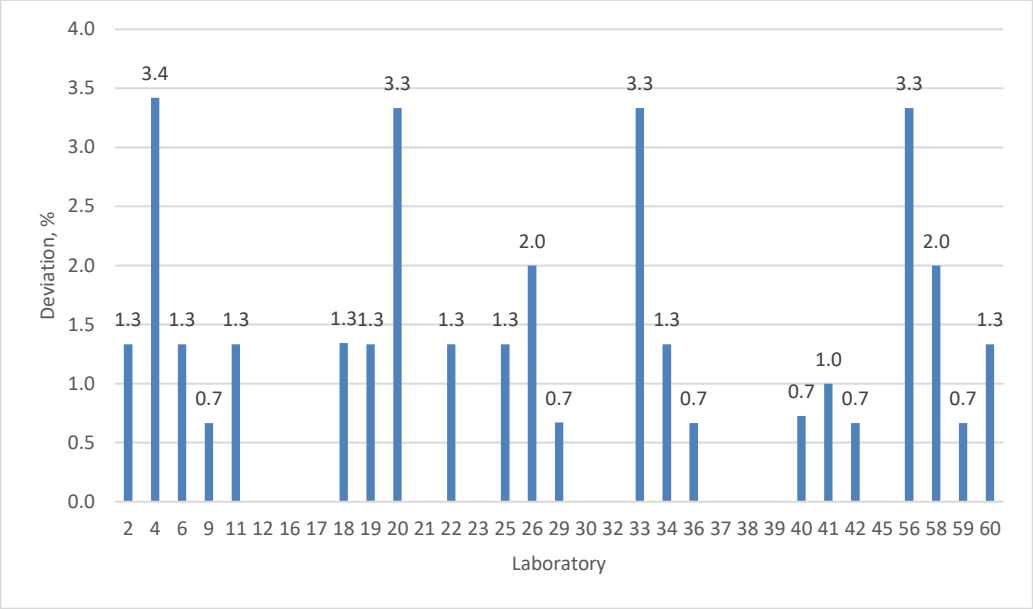

Figure 2. Percentages of deviations in AST per participating laboratory in the EQAS Matrix 2021

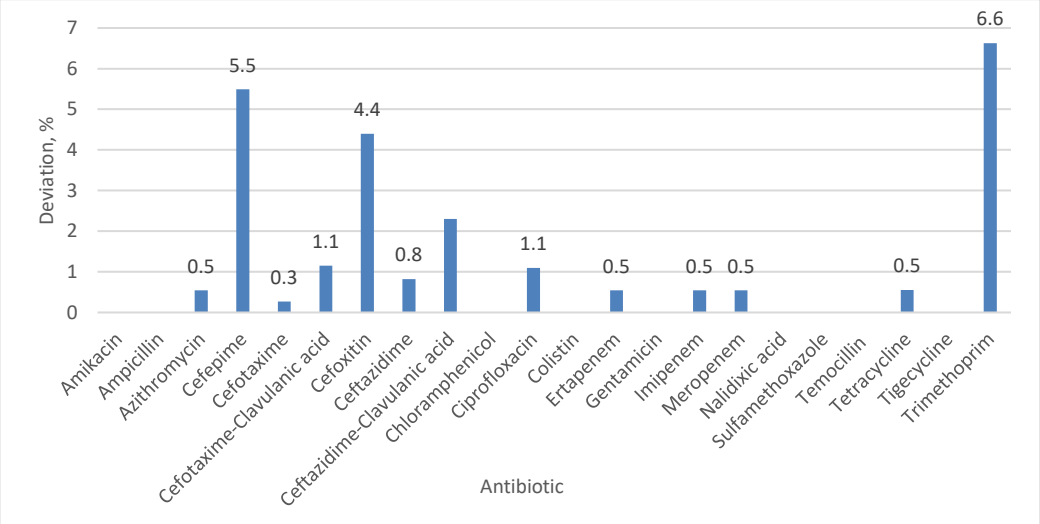

Figure 3. Percentages of deviations of AST results per antimicrobial in the EQAS Matrix 2021

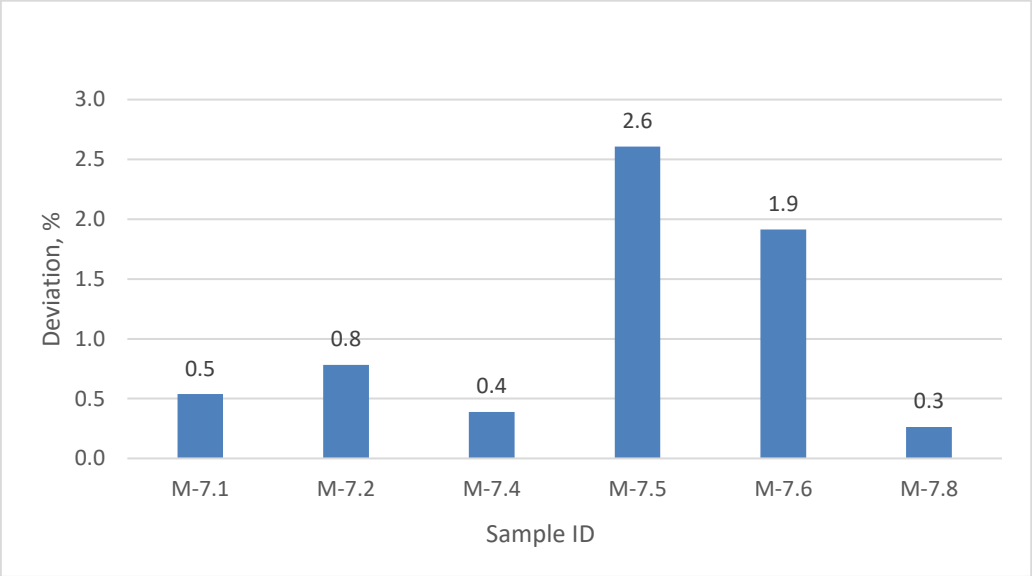

Figure 4. Percentages of AST deviations per sample in the EQAS matrix 2021

The analysis of deviations per matrix sample indicates that the highest levels of deviations were observed for sample M-7.5 (2.6%; caecal) and M-7.6 (1.9%; caecal). All other samples had deviation levels < 1% (Figure 4).

3.5 ESBL/AmpC and carbapenemase phenotypic testing conclusions

Five beef meat samples (M-7.1 – M-7.5) were included in this matrix EQAS. The sample M-7.1 contained an isolate expressing AmpC phenotype due to a mutation in the *ampC* promoter region (g.-42C>T); sample M-7.2 was inoculated with a strain carrying the ESBL gene *bla*$_{\text{SHV-12}}$, whereas M-7.4 and 7.5 contained *bla*$_{\text{NDM-4}}$ and *bla*$_{\text{OXA-48}}$-like genes, respectively, expressing carbapenemase phenotype. Sample M-7.3 was spiked with a susceptible strain of *E. coli* (Table 3).
One of the three pig caecal samples (M-7.7) was not spiked with *E. coli* (blank), while samples M-7.6 and M-7.8 contained isolates that express AmpC and an ESBL mediated by *bla*$_{\text{CMY-2}}$ and $_{\text{blaCTX-M-32}}$ genes, respectively. Overall, there were very few discrepancies in differentiating between ESBL and AmpC phenotypes. Lab 026 recovered an *E. coli* isolate from sample M-7.1 with an ESBL + AmpC phenotype instead of an AmpC phenotype due to an observed synergy of cefotaxime and ceftazidime with clavulanic acid. Labs 060 and 018 isolated a strain with resistance to ertapenem (MIC = 0.12 mg/L) from sample M-7.6 and reported it as “Other phenotype”. Lab 004 recovered an isolate with an AmpC phenotype from sample M-7.8 with resistance to cefoxitin (FOX = 64 mg/L) without synergy. Lab 036 reported “Other phenotype” to the carbapenemase phenotype of sample M-7.4 due to an isolation of two strains - one from MacConkey and one from CARBA selective media, which only differed by an additional *mph*(A) gene in one of the isolates. Additionally, strains M-7.1, M-7.5 and M-7.6 could not be recovered from Labs 004, 039 and 021, respectively.

4. Discussion

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

The 2021 EURL-AR matrix EQAS trial was the seventh of its kind on samples of animal origin since the first round of this EQAS in 2015. The challenges with the inability of strains to survive in caecal samples were negligible this year and only one laboratory could not recover a strain from a caecal sample (M-7.6). On the other hand, two test strains (M-7.1 and M-7.5) were not recovered by two laboratories from the meat matrices. Additionally, in this round, three of the six inoculated resistant *E. coli* strains did not completely maintain the expected phenotype after recovery from meat or caecal matrix samples in four laboratories. It is expected to see some 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 *Enterobacteriaceae* or *E. coli* and sometimes even ESBL bacteria in some parts of the meat matrix. The issue with inconsistent phenotypes hindering the clear differentiation between the ESBL/AmpC/carbapenemase phenotypes, was less pronounced as compared to the previous EQAS. One sample was omitted from the analyses; one ESBL-producing strain (M-7.8), as the obtained AST results reflected a completely different phenotype than expected. It cannot be ruled out that other, more sporadic, AST deviations can be caused by interactions with the matrix content, but this can generally not be accounted for.
In this EQAS, all except one laboratory were able to selectively isolate carbapenemase-producing *E. coli*. A list of deviations in ESBL phenotype interpretations is available in App. 7.

4.2 Antimicrobial susceptibility testing

In the 2021 iteration of the matrix EQAS, 11 laboratories had no deviations in their AST results, which is comparable but decreased from the EQAS 2020 (n = 15). The majority of AST deviations were caused by more than one-fold dilution difference, either explained by acquiring genes from the background bacteria in the matrices or point mutations. The other most common deviation was due to a one-step dilution difference. It is in general a problem when the expected MIC values are close to the breakpoint between susceptible and resistant phenotypes. Although one two-fold MIC level deviation is accepted, it could be problematic when it changes the susceptible/resistant interpretation. As it can be difficult to select test strains with clear phenotypes for all antibiotics and expected to survive in the matrix, this issue gave rise to the majority of AST deviations, but especially for ceftazidime, ceftazidime-clavulanic acid and ertapenem. Some deviations came also from a wrong interpretation of the phenotype based on the obtained MIC values, which could be in relation to data handling.

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 one issue which two laboratories encountered, was a *bla*$_{CMY-2}$ carrying AmpC-type *E. coli* (M-7.6) which had an elevated MIC to ertapenem, but not to meropenem. Following the EFSA classification, the strain should be considered 'Other phenotype' if meropenem ≤ 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. The other discrepancy in the interpretation came from changes in the synergy with clavulanic acid which led to changes in the AmpC and ESBL phenotype interpretation, or isolation of an isolate with an overall different phenotype compared to the original strain. This could be explained by isolation of strains with other characteristics or acquisition of plasmids or mutations during enrichment.

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 (App. 6). For a second year, the *E. coli* ATCC 25922 QC strain was included in the reporting of the EQAS results, while *Acinetobacter baumannii* 2012-70-100-69 was included as a QC quality control strain for the first time in 2021, however, no acceptable MIC ranges are available yet. All 33 participating labs tested *E. coli* ATCC 25922 by MIC determination and reported a total of 865 out of 900 test results. Of the reported results, 99.7% were within the acceptable range (App. 6). There were two deviations, by Labs 039 and 042 for sulfamethoxazole. Additionally, Labs 026, 029, 037 and 060 did not provide data for amikacin, azithromycin for Panel 1 and cefepime, cefotaxime, cefoxitin, ceftazidime, ertapenem imipenem, meropenem, temocillin and F/C and T/C for Panel 2 and were not scored.

5. Conclusion

In general, the results of this matrix EQAS demonstrate that most participating labs have well established methods to isolate ESBL/ AmpC and carbapenemase-carrying *E. coli* strains from meat or caecal samples, despite the difficult nature of the matrices.

The susceptibility testing results were in general very satisfactory, with only few deviations, typically derived from matrix-associated issues rather than problems in the AST methodology. Thus, there are still some preventable deviations, including wrong interpretation of susceptible or resistant phenotypes, which are less prevalent this year in comparison to the previous rounds of the EQAS.

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 2020
Appendix 2. List of participants
Appendix 3. Test strains and reference values (MIC in mg/L)
Appendix 4. Protocol EQAS matrix 2020
Appendix 5. Examples of Test forms EQAS matrix 2020
Appendix 6. QC ranges *E. coli* ATCC25922
Appendix 7. List of deviations

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2021

DTU Food
National Food Institute

EURL-AR EQAS pre-notification

G00-06-001/26.10.2020

EQAS 2021 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, and these will be distributed to participants on request.
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 beef meat and/or pig caecal will be shipped. Please provide the EQAS coordinator with documents or other information that can simplify customs procedures. 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 in last week of September 2021. 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 10 December 2021** via the password-protected webtool. Upon reaching the deadline, each participating laboratory is kindly asked to enter the password-protected website once again to download an automatically generated evaluation report.

EQAS report: A report summarising and comparing results 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.

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

Sincerely,

Jette Kjeldgaard, 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 Diagnostic and Research Veterinary Institute	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	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	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	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	Latvia
x	x	National Food and Veterinary Risk Assessment Institute	Lithuania
x	x	Laboratoire de Medecine Vétérinaire	Luxembourg
x	x	Public Health Laboratory	Malta
x	x	Public Health Laboratory/National Veterinary Laboratory*	Malta
x	x	Wageningen Bioveterinary Research	Netherlands
x	x	The Netherlands Food and Consumer Product Safety Authority*	Netherlands
x	x	Faculty of Veterinary Medicine*	North Macedonia
x	x	Veterinærinstituttet	Norway
x	x	National Veterinary Research Institute	Poland
x	x	Instituto Nacional de Investigação Agrária e Veterinária	Portugal
	x	Institute for Diagnosis and Animal Health	Romania
x		Institute for Hygiene and Veterinary Public Health	Romania
x	x	State Veterinary and Food Institute	Slovakia
x	x	National Veterinary Institute	Slovenia
	x	Laboratorio Central de Veterinaria	Spain
x		Centro Nacional de Alimentación	Spain
x	x	Foodborne Zoonoses and Antimicrobial Resistance Unit*	Spain
x	x	National Veterinary Institute	Sweden
x	x	Institute of Veterinary Bacteriology	Switzerland
x	x	Animal & Plant Health Agency	United Kingdom

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

Non-NRL-AR enrolled by the EURL-AR

Non-MS NRL-AR

* Submitted results were not included in the current report (one dataset per country, only)

Panel 1

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Prediction
EURL-M-7.1	>32	8	≤4	1	≤0.25	>8	4	≤1	>64	≤2	≤0.25	>512	>64	≤0.03	4	AmpC
EURL-M-7.2	>32	8	≤4	≤0.5	0.5	8	>4	≤1	>64	>32	>16	>512	≤8	≤0.03	>8	ESBL
EURL-M-7.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Susceptible
EURL-M-7.4	>32	64	≤4	≤0.5	≤0.25	>8	>4	≤1	>64	>32	>16	>512	16	>16	>8	Carbapenemase
EURL-M-7.5	>32	8	≤4	≤0.5	≤0.25	1	4	≤1	16	>32	>16	>512	≤8	2	1	Carbapenemase
EURL-M-7.6	>32	8	≤4	≤0.5	≤0.25	>8	>4	≤1	>64	≤2	1	≤8	≤8	≤0.03	1	AmpC
EURL-M-7.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	blank
EURL-M-7.8	>32	8	≤4	≤0.5	≤0.25	>8	>4	≤1	≤4	>32	>16	>512	≤8	≤0.03	0.03	ESBL

Interpretation

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	
EURL-M-7.1	R	S	S	S	S	R	R	S	R	S	S	R	R	S	R	AmpC
EURL-M-7.2	R	S	S	S	S	R	R	S	R	R	R	R	S	S	R	ESBL
EURL-M-7.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Susceptible
EURL-M-7.4	R	R	S	S	S	R	R	S	R	R	R	R	S	R	R	Carbapenemase
EURL-M-7.5	R	S	S	S	S	R	R	S	R	R	R	R	S	R	R	Carbapenemase
EURL-M-7.6	R	S	S	S	S	R	R	S	R	S	S	S	S	S	R	AmpC
EURL-M-7.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	blank
EURL-M-7.8	R	S	S	S	S	R	R	S	S	R	R	R	S	S	S	ESBL

Panel 2

Strain	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	ESBL conclusion	Genotype
EURL-M-7.1	>64	4	0.03	0.25	≤0.03	16	0.25	4/4	8/4	16	AmpC	ampC-promoter
EURL-M-7.2	4	8	≤0.015	≤0.12	≤0.03	8	0.5	≤0.06/4	≤0.12/4	4	ESBL	blaSHV-12
EURL-M-7.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Susceptible	none
EURL-M-7.4	>64	>64	>2	8	>16	>128	>32	>64/4	>128/4	>128	Carbapenemase	NDM-5, CTX-M-15, EC-8
EURL-M-7.5	32	4	>2	4	2	1	2	4/4	1/4	>128	Carbapenemase	OXA-181, EC-18, TEM-35
EURL-M-7.6	64	8	0.06	≤0.12	≤0.03	16	0.25	4/4	8/4	16	AmpC	blaCMY-2
EURL-M-7.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	blank	blank
EURL-M-7.8	8	>64	0.03	≤0.12	≤0.03	16	16	≤0.06/4	0.25/4	16	ESBL	blaCTX-M-32

Interpretation

Strain	FOX	FOT	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	TRM	ESBL conclusion	Genotype
EURL-M-7.1	R	R	S	S	S	R	S	No synergy	No synergy	S	AmpC	ampC-promoter
EURL-M-7.2	S	R	S	S	S	R	R	Synergy	Synergy	S	ESBL	blaSHV-12
EURL-M-7.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Susceptible	none
EURL-M-7.4	R	R	R	R	R	R	R	No synergy	No synergy	R	Carbapenemase	NDM-5, CTX-M-15, EC-8
EURL-M-7.5	R	R	R	R	R	R	R	No synergy	No synergy	R	Carbapenemase	OXA-181, EC-18, TEM-35
EURL-M-7.6	R	R	S	S	S	R	S	No synergy	No synergy	S	AmpC	blaCMY-2
EURL-M-7.7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	blank	blank
EURL-M-7.8	S	R	S	S	S	R	R	Synergy	Synergy	S	ESBL	blaCTX-M-32

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2021

DTU Food
National Food Institute

PROTOCOL

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

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APPENDIX 8

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 2021, these eight samples will include five 25-g samples of beef meat and three 1-g samples of pig caecal content. These samples may contain *E. coli* presumptive of producing either ESBL-, AmpC- or carbapenemase-enzymes.

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 2021

3.1 Shipping, receipt and storage of samples

In September 2021, 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 bovine meat or pig caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 7.1 to 7.8. Hereof 7.1 to 7.5 being samples of meat (each 25 g) and 7.6 to 7.8 being samples of caecal content (each 1 g).

The matrix samples will be shipped on September 27th 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 7.1 to 7.5
- Follow the protocol for caecal content when testing samples 7.6 to 7.8

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 (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

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

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

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 (ECOFFs) according to latest updates from EUCAST (01.09.2021) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.25
Cefotaxime (FOT or CTX)	0.25
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)

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 EFSA Journal 2021;19(4):6490, doi:10.2903/j.efsa.2021.6490 (Annex A), and the appendix to this protocol).

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 10th December, 2021.** 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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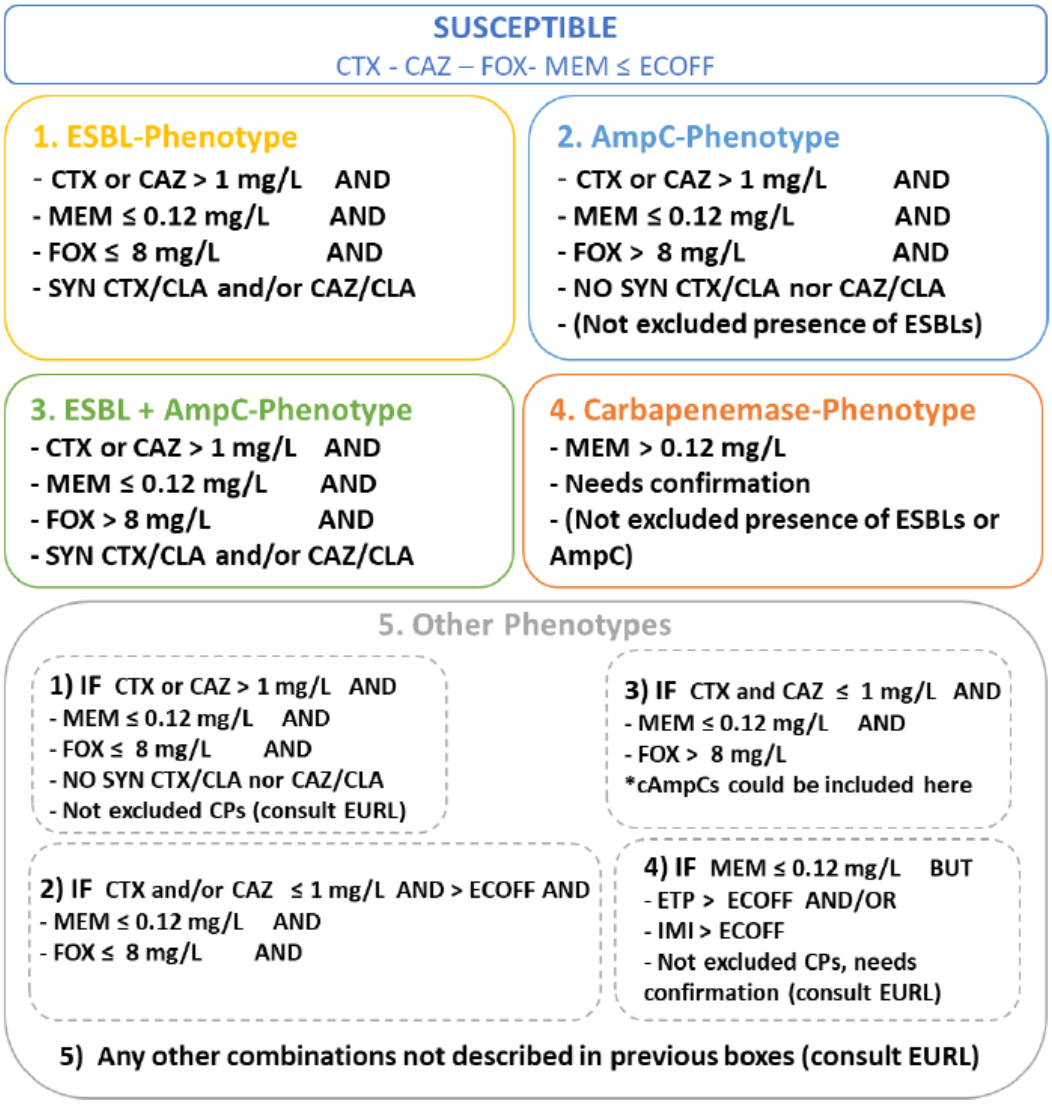
APPENDIX

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



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

Figure 1: Phenotypes inferred based on the resistance to the β-lactams included in Panel 2

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 2021;19(4):6490, doi:10.2903/j.efsa.2021.6490 (Annex A).

EU Reference Laboratory for Antimicrobial Resistance
Isolation of ESBL/AmpC- and carbapenemase-producers
External Quality Assurance System (Matrix EQAS) 2021

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 2021 for monitoring of ESBL/AmpC-detection in relation to 2020/1729/EU? (Choose only one option)

- ☐ less than 100
- ☐ 101-200
- ☐ 201-300
- ☐ 301-400
- ☐ 401- 1000
- ☐ more than 1000

Which kind of samples did your laboratory process in 2021 for monitoring of ESBL/AmpC-detection in relation to 2020/1729/EU? (You may choose more than one option)

- ☐ caecal, pig/cattle
- ☐ meat, pork/beef
- ☐ other matrices, please specify:

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How many samples did your laboratory process in 2021 for monitoring of carbapenemases in relation to 2020/1729/EU? (Choose only one option)

- ☐ less than 100
- ☐ 101-200
- ☐ 201-300
- ☐ 301-400
- ☐ 401- 1000
- ☐ more than 1000

Which kind of samples did your laboratory process in 2021 for monitoring of carbapenemase-production in relation to 2020/1729/EU? (you may choose more than one option)

- ☐ caecal, pig/cattle
- ☐ meat, pork/beef
- ☐ other matrices, please specify:

Any other comments:

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METHODS

1- Method used for selective isolation of ESBL/AmpC in this EQAS:
Selective isolation procedure using the EURL recommended protocols that refer to the EU regulation 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- If you made changes in the selective isolation procedure:
Different sample amount (weight) used for the enrichment procedure:

g in meat samples

g for caecal samples

Different concentration of cefotaxime**:** mg/L

Different antimicrobial

Different medium

Please justify these changes:

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

Please justify these changes:

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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)

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
☐ 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-7.1'

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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External Quality Assurance System (Matrix EQAS) 2021

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
E. coli EURL M-7.1	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
E. coli EURL M-7.1	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:

☐ Presumptive ESBL	☐ Presumptive AmpC	☐ Other phenotype
☐ Presumptive ESBL+ AmpC	☐ Presumptive carbapenemase	☐ Susceptible

Comments (include optional genotype or other results):

***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

Grey markings refer to 1-dilution differences

Lab number	Strain	Panel type	Antimicrobial	Obtained operator	Expected operator	Obtained MIC value	Expected MIC value	Obtained interpretation	Expected interpretation	Score interpretation
NRL-AR-002	EURL M-7.2	Panel2	Cefepime	=	=	0.25	0.5	S	R	0
NRL-AR-002	EURL M-7.6	Panel1	Trimethoprim	=	=	8	1	R	S	Blanked
NRL-AR-004	EURL M-7.4	Panel1	Azithromycin	=	=	8	64	S	R	0
NRL-AR-004	EURL M-7.4	Panel1	Meropenem	<=	>	0.03	16	S	R	0
NRL-AR-004	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-004	EURL M-7.6	Panel1	Ciprofloxacin	=	=	1	1	S	R	0
NRL-AR-004	EURL M-7.6	Panel2	Meropenem	<=	<=	0.03	0.03	R	S	0
NRL-AR-004	EURL M-7.8	Panel1	Nalidixic acid	>	<=	64	4	R	S	Blanked
NRL-AR-004	EURL M-7.8	Panel1	Sulfamethoxazole	=	>	64	512	S	R	Blanked
NRL-AR-004	EURL M-7.8	Panel1	Tetracycline	<=	>	2	32	S	R	Blanked
NRL-AR-004	EURL M-7.8	Panel1	Trimethoprim	=	>	4	16	S	R	Blanked
NRL-AR-004	EURL M-7.8	Panel2	Cefepime	=	=	0.12	16	S	R	Blanked
NRL-AR-004	EURL M-7.8	Panel2	Cefotaxime-Clavulanic acid	=	<=	4	0.06	R	S	Blanked
NRL-AR-004	EURL M-7.8	Panel2	Cefoxitin	=	=	64	8	R	S	Blanked
NRL-AR-004	EURL M-7.8	Panel2	Ceftazidime-Clavulanic acid	=	=	8	0.25	R	S	Blanked
NRL-AR-006	EURL M-7.2	Panel2	Cefotaxime-Clavulanic acid	=	<=	1	0.06	R	S	0
NRL-AR-006	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-006	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-006	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-006	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-009	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-009	EURL M-7.6	Panel2	Ertapenem	=	=	0.12	0.06	R	S	0
NRL-AR-011	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-011	EURL M-7.5	Panel2	Cefepime	=	=	0.25	2	S	R	0
NRL-AR-011	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-011	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-011	EURL M-7.8	Panel1	Tetracycline	<=	>	2	32	S	R	0
NRL-AR-012	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-012	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-012	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-018	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-018	EURL M-7.5	Panel2	Cefepime	=	=	0.25	2	S	R	0
NRL-AR-018	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-018	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0

NRL-AR-018	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-018	EURL M-7.6	Panel2	Ertapenem	=	=	0.12	0.06	R	S	0
NRL-AR-019	EURL M-7.2	Panel2	Cefepime	=	=	0.25	0.5	S	R	0
NRL-AR-019	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-020	EURL M-7.2	Panel2	Cefepime	=	=	0.25	0.5	S	R	0
NRL-AR-020	EURL M-7.5	Panel1	Ceftazidime	<=	=	0.25	1	S	R	0
NRL-AR-020	EURL M-7.5	Panel2	Cefepime	=	=	0.25	2	S	R	0
NRL-AR-020	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-020	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-020	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.25	1	S	R	0
NRL-AR-022	EURL M-7.1	Panel2	Cefotaxime	=	=	4	4	S	R	0
NRL-AR-022	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-022	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-022	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-025	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-025	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-025	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-025	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-025	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-026	EURL M-7.1	Panel2	Cefotaxime-Clavulanic acid	<=	=	0.06	4	S	R	0
NRL-AR-026	EURL M-7.1	Panel2	Ceftazidime-Clavulanic acid	<=	=	0.12	8	S	R	0
NRL-AR-026	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-029	EURL M-7.8	Panel2	Ceftazidime-Clavulanic acid	=	=	0.25	0.25	R	S	0
NRL-AR-033	EURL M-7.2	Panel2	Cefepime	=	=	0.25	0.5	S	R	0
NRL-AR-033	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-033	EURL M-7.5	Panel2	Cefepime	=	=	0.25	2	S	R	0
NRL-AR-033	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-033	EURL M-7.5	Panel2	Ceftazidime	<=	=	0.25	1	S	R	0
NRL-AR-033	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-033	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-034	EURL M-7.2	Panel2	Cefepime	=	=	0.12	0.5	S	R	0
NRL-AR-034	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-036	EURL M-7.1	Panel2	Ertapenem	=	=	0.03	0.03	R	S	0
NRL-AR-037	EURL M-7.6	Panel2	Ertapenem	=	=	0.12	0.06	R	S	0
NRL-AR-039	EURL M-7.6	Panel2	Ertapenem	=	=	0.12	0.06	R	S	0
NRL-AR-040	EURL M-7.4	Panel1	Ciprofloxacin	>	>	8	8	S	R	0
NRL-AR-041	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-041	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-041	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0

NRL-AR-041	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-042	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-042	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-042	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-042	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-045	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-045	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-056	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-056	EURL M-7.5	Panel2	Cefepime	=	=	0.25	2	S	R	0
NRL-AR-056	EURL M-7.5	Panel2	Cefoxitin	=	=	4	32	S	R	0
NRL-AR-056	EURL M-7.5	Panel2	Ceftazidime	<=	=	0.25	1	S	R	0
NRL-AR-056	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.25	1	S	R	0
NRL-AR-056	EURL M-7.5	Panel2	Imipenem	=	=	0.5	4	S	R	0
NRL-AR-058	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-059	EURL M-7.5	Panel1	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-059	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-059	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-060	EURL M-7.5	Panel2	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-060	EURL M-7.5	Panel2	Ceftazidime	=	=	0.5	1	S	R	0
NRL-AR-060	EURL M-7.5	Panel2	Ceftazidime-Clavulanic acid	=	=	0.5	1	S	R	0
NRL-AR-060	EURL M-7.6	Panel1	Trimethoprim	=	=	4	1	R	S	Blanked
NRL-AR-060	EURL M-7.6	Panel2	Ertapenem	=	=	0.12	0.06	R	S	0

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