DTU Fødevareinstituttet
DTU National Food Institute

The 10th EURL-AMR Proficiency Test

Selective isolation of E. coli with presumptive ESBL, AmpC or carbapenemase phenotypes from meat or caecal samples 2024

European Union Reference Laboratory
Antimicrobial Resistance

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Report by:
Dafni Paspaliari
Jette Kjeldgaard,
Susanne Karlsmose Pedersen,
Rene S. Hendriksen

PT coordinator:
Jette Kjeldgaard

Photo:
DTU National Food Institute

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Index

1. Introduction

This report describes and summarises the results from the tenth round of matrix-based proficiency testing (PT), conducted by The National Food Institute (DTU Food) in its capacity as the EU Reference Laboratory for Antimicrobial Resistance in Food, Feed & Animal Health (EURL-AMR), for the purposes of External Quality Assessment (EQA). The PT focused on the selective isolation of extended-spectrum beta-lactamase (ESBL)-, AmpC- and carbapenemase (CP)-producing *Escherichia coli* from meat and caecal samples of animal origin as well as on antimicrobial susceptibility testing (AST) of the isolated *E. coli*.

In the European Union (EU), the mandatory, harmonized AMR monitoring includes ESBL-/AmpC- and CP-producing indicator *E. coli* in food-producing animals. To support EU Member States (MS) and affiliated countries in their capacity to selectively isolate and characterize ESBL-/AmpC- and CP-producing indicator *E. coli*, the EURL-AMR offers matrix-based PT schemes on an annual basis. These schemes were initiated in 2015 and originally targeted only ESBL- and AmpC-producing *E. coli* but were later expanded to also include CP-producing *E. coli*. The PT schemes consist of screening of either meat or caecal matrix samples of animal origin, according to common protocols defined by the European Commission (EC) and validated by the EURL-AMR].

Similar to previous EURL-AMR matrix-based PT rounds, the aim of this specific PT was to: i) monitor the capacity of the National Reference Laboratories (NRL-AMR) for isolation, identification and AST of ESBL-/AmpC- or CP-producing *E. coli*; ii) identify laboratories that may require assistance to improve their performance in the isolation and AST of *E. coli* from meat and caecal matrices; and iii) identify potential problems or focus areas for future training and research.

When reading this report, the following should be taken under consideration:

1) The selection of test strains was based on phenotypes determined through Minimum Inhibitory Concentration (MIC) results obtained at the EURL-AMR lab at the Technical University of Denmark, National Food Institute (DTU Food). After strain selection, the MIC testing was repeated, to confirm the MIC values and generate the table of expected AST results. The genetic basis for resistance was known, as all the selected test strains had been whole-genome sequenced (WGS). After preparation of the matrix meat and caecal samples, the MIC values were re-confirmed by isolating the test strains from the samples and performing AST once more.

2) No thresholds were set in advance for the evaluation of the performance of the participating laboratories. Therefore, results were not classified as above or below a certain threshold but were, instead, evaluated case by case.

3) When receiving results 'deviating from the expected interpretation', participants should carefully analyse those in a self-evaluation, including considerations for corrective actions. Note that, since the methods used for MIC determination have 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 strain. However, if the expected MIC is close to the breakpoint value for categorising the strain as susceptible or resistant, that two-fold dilution difference (which is acceptable) may result in two different interpretations, i.e. the same strain may be categorized as susceptible and resistant. This result will be evaluated as correct in terms of determined MIC value but incorrect in terms of ascribed resistance phenotype. When organizing PTs, we try to avoid these situations by choosing test strains with MIC

values distant from the cut-offs for resistance. However, that is not always feasible for all strains and all antimicrobials. Therefore, the EURL-AMR network unanimously agreed in 2008 that if there is a high proportion of incorrect results received for a specific strain/antimicrobial combination (over 25%), then the reasons behind this discordance must be further examined and, on selected occasions, explained in detail case by case, these results may subsequently be omitted from the evaluation report.

For reasons of anonymity, laboratory names have been replaced with laboratory codes in this report. Each laboratory code is only known by its corresponding laboratory, and the entire list of laboratories and their codes is confidential and known only by relevant representatives of the EURL-AMR and the EU Commission. All conclusions are public.

The draft PT report was distributed to the network for review in September 2025. A webinar presenting the PT results was held on September 23, 2025. Following the deadline for submitting comments, the report underwent minor editorial revisions and was subsequently approved.

2. Materials and Methods

2.1 Participants in PT 2024

A pre-notification (Appendix 1), announcing the matrix PT 2024, was sent out on 2 October 2024 by e-mail to the designated NRLs of all EU countries and Iceland, Norway, Switzerland, and the United Kingdom. In total, 33 laboratories participated in the matrix PT, involving one NRL from each of the 27 EU countries and Iceland, Norway, Switzerland, and the 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 sets of laboratory results from 31 countries are included in this report. 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-/CP-producing *E. coli*, as well as identification and antimicrobial susceptibility testing (AST) of the obtained isolates. The samples included either chicken meat or pooled chicken caecal content and were either spiked with test strains or left unmodified.

The meat used for the meat samples was minced chicken of Danish origin (raised, slaughtered and packed in Denmark) acquired in local supermarkets on 28 October 2024. The meat came from four different batches (based on production date and slaughterhouse), each bought in sufficient amount to cover both the pre-testing and preparation of the samples. The meat was pretested using the official method for selective isolation of *E. coli* producing ESBL, AmpC or carbapenemases from meat (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols). The aim of pretesting was to ensure the absence of ESBL-, AmpC- or CP-producing *E. coli* in the batch of meat selected for sample preparation, as well as to ascertain the level of background bacteria present in the meat matrix. A batch fulfilling pretesting requirements was chosen for preparation of aliquots of 25 g of meat, which were then spiked with the test isolates, as follows.

The test isolates selected for the matrix PT 2024 were prepared in advance of spiking and sub-cultured the day before sample preparation. For sample preparation and standardization of the spiking, suspensions of the relevant isolates equal to McFarland 0.5 were prepared in saline tubes. This corresponded to approximately10^8 CFU/mL, as confirmed by viable counts of serial dilutions on Luria Bertani (LB) agar plates. The standardized suspensions were further diluted to obtain the desired concentration per test isolate, selected in advance based on the results of previous matrix PTs. 25 µl of each test isolate at the appropriate dilution was used to spike 25 g of meat sample, thus yielding the final matrix samples for the PT. Sample EURL-M-10.2 was spiked in the same way as the test samples but with a susceptible *E. coli* strain (ATCC 25922), and was, therefore, expected to be negative for ESBL-, AmpC- and CP-producing *E. coli*. The final inoculum in samples EURL-M-10.1, M-10.2, M-10.3 and M-10.4 of this PT was expected to be approx. 10^3 CFU/g meat.

One slaughterhouse provided on 29 October 2024 500 chicken caecal samples from four different farms (125 samples from each farm). The caecal samples were pooled per farm, mixed thoroughly, and tested using the official selective isolation protocol for ESBL-, AmpC- and CP-producing *E. coli* from caeca (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols). One ESBL-negative pooled caeca batch was chosen for preparation of the matrix caecal samples for the test strains. 1 g aliquots of pooled caecal content were either used directly as blank sample or spiked with 10 µl of a dilution containing approx. 10^6 CFU/ml, causing an expected spiking level of 10^4 CFU/g for samples EURL-M-10.6, M-10.7 and M-10.8. Sample EURL-M-10.5 was not inoculated and was expected to be negative. The inoculum strains were selected on the basis of having been initially isolated from poultry caecal samples, as this approach has previously been shown to enhance the survival of the inoculum. Otherwise, the caecal matrix samples provide poor support for the *E. coli* inoculum.

The minimal inhibitory concentrations (MIC) for the targeted antimicrobials were determined using the broth microdilution method both when testing the strains used for spiking in the preparatory phase as well as when testing the isolates after sample preparation to generate the expected results (Appendix 2).

To follow up on the stability of the inoculum in the matrix samples after shipping, sets of the eight samples were tested at four time points after shipment (for two weeks) using the official selective *E. coli* isolation protocols. 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 the selective isolation and identification of the ESBL-, AmpC- and/or CP-producing *E. coli* isolates contained in the samples were available on the EURL website, https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/ (Appendix 3). For confirmation of presumptive *E. coli* isolates, various methods were permitted, as these are not specified in the legislation (EU Commission implementing decision on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria, 2020/1729/EU). Laboratories were requested to describe the methods they used for the selective isolation and identification of presumptive ESBL-, AmpC- or CP-producing *E. coli* using a dedicated methods sheet to be completed in the database upload system.

2.4 Antimicrobial susceptibility testing

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

Guidelines for performing the antimicrobial susceptibility testing using dilution methods were set according to ISO 20776-1:2019 and whenever commercial methods were used, the guidelines of the manufacturer were followed.

MIC results were interpreted using the EUCAST epidemiological cut-off values (www.eucast.org), as per the regulation referred to above, or as recommended by EFSA and described in the PT protocol (Appendix 3). The results of the ESBL confirmatory testing were interpreted according to the recommendations by EFSA and as described in the regulation, based on MIC testing of 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. On 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 containing 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-AMR.

The protocol for the PT and the test forms was made available online on the EURL-AMR website, http://www.eurl-ar.eu (new link available now, see section 2.3) before launching this PT.

The thermos boxes used for the shipment of the samples were enclosed in double-pack containers and sent to the selected laboratories as "Biological Substance category B" classified UN3373, according to the International Air Transport Association (IATA) regulations. The parcels were dispatched from DTU Food on 11 November 2024.

2.6 Procedure

The laboratories were instructed to download the protocol and test forms (Appendices 3 and 4) from http://www.eurl-ar.eu and to process the samples following the EU protocol for selective isolation of presumptive ESBL-, AmpC- and CP-producing *E. coli* from meat or caecal samples, precisely as they would normally do for the EFSA monitoring. For results collection, the NRLs were instructed to upload the data into the web-based database, which was designed and prepared for this PT. The database was opened after sample shipment and kept open until the reporting deadline.
Upon arrival of the parcels, the participants were requested to fill in a small introductory questionnaire available in the database, which included details on sample reception (measured temperature and date/time), the laboratory's monitoring activities, and the methods used in the 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.

After completion of the tests, the laboratories were requested to enter the obtained results into an electronic sheet in the EURL-AMR web-based database through a secure individual login. The web tool was activated on January 10th, 2025, but unfortunately a critical error was later found in the web tool setup. On January 30th, the web tool was temporarily closed, and all participants were asked to check and, if necessary, re-submit their results. The deadline for data collection was extended until February 21st, 2025.

The results from the selective isolation of ESBL/AmpC/CP-producing *E. coli* were evaluated separately from the AST results. In terms of the isolation results, samples were defined as positive if an isolate was obtained and positively identified as *E. coli*. In terms of AST testing, the results of susceptibility testing of the obtained isolates using both MIC panels (EUVSEC3 and EUVSEC2; Table 1) were analysed separately in a similar way as for the *E. coli* AST PTs, 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/CP- producing isolates.

After the deadline, the qualitative results indicating if the samples were positive or negative for ESBL/AmpC, or CP-producing *E. coli*, 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.

Table 1. Panels of antimicrobials required for susceptibility testing of bacteria included in PT 2024

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	
Trimethoprim, TMP	

3. Results

Sample shipment and delivery took place within the expected timeframe and all temperature measurements recorded by the loggers during parcel shipment and by the participants at the time of parcel opening were within the range considered acceptable for sample integrity. A few loggers could not readily be returned to DTU from non-EU countries, due to complicated customs procedures. Nonetheless, the respective samples appeared intact at the point of receipt and there was no indication to suggest that their quality was compromised during transport. All samples received have thus been considered acceptable for testing for this PT round.

All 33 participating laboratories processed and tested the samples as instructed and submitted results. For the eight PT samples (four meat and four caecal), laboratories were expected to enter on the webtool a total of 6200 MIC results together with the respective susceptible/resistant phenotype assignment. Those correspond to 4650 results for the six test strains, if the control (M-10.2) and the blank sample (M-10.5) are excluded. Laboratories were also asked to enter MIC results for the control strain *E. coli* ATCC 25922. Additionally, for each of the eight samples, participants were asked to submit their categorization of the resistance phenotype (ESBL, AmpC, ESBL+AmpC, carbapenemase, or other) corresponding to 248 interpretation results evaluated in this report. It should be noted that the two laboratories that test only meat and the two laboratories that test only caeca were only evaluated on the results they submitted for the four meat samples or the four caecal samples, respectively.

3.1 Overall results of the qualitative, selective isolation

This iteration of the Matrix PT showed an unusual number of samples from which no spiked *E. coli* isolate was recovered. In total, there were 13 instances of unsuccessful isolation of spiked *E. coli* test strains, distributed as five from sample M-10.4, six from sample M-10.6 and two from sample M-10.7 (see Figure 1).

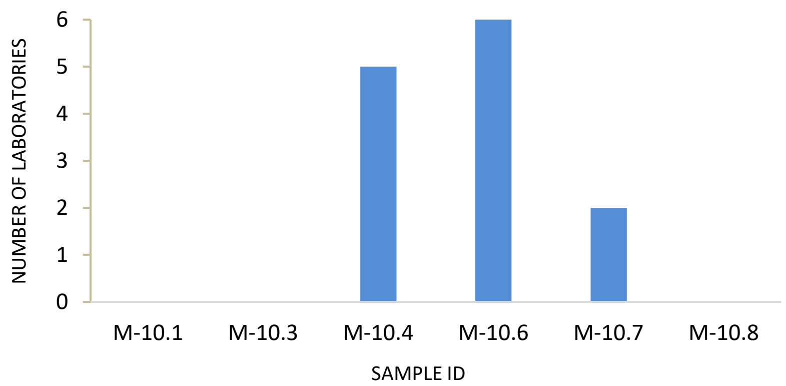

Figure 1. Number of laboratories that failed to isolate the inoculated *E. coli* per sample ID'

The EURL-AMR had expected some difficulties for the strain spiked in sample M-10.4, as the test strain was a non-fermentative *E. coli,* which does not show the *E. coli*-typical pink/red coloration on

MacConkey agar, although still growing well on this agar, as well as on several selective carbapenemase agars (see Figure 2).

Furthermore, difficulties were expected for the sample M-10.6, as the inoculum's stability seemed lower than expected, compared to other samples. During the preparation of the samples, a lower number of bacteria were successfully recovered from serial dilutions of the caecal samples after spiking. Because of this, a second portion (10 µl) of inoculum had been added to the caecal sample set M-10.6 before samples were shipped. Still, six laboratories could not recover any ESBL or carbapenem-resistant *E. coli* from this sample.

Figure 2 Growth of the strain M-10.4 on different agars after isolation from meat in the EURL-AMR lab. Top left: mSuperCarba agar, top right: MacConkey + CTX, bottom left: CHROMID Carba, bottom right: CHROMID OXA-48.

One laboratory (#006) reported a positive finding of an AmpC-producing *E. coli* isolate from sample M-10.2, which had been inoculated with a susceptible strain only. Except for this instance, no other laboratory reported any results for the two negative control samples M-10.2 and M-10.5 (blank control).

3.2 Methods used by participants

Information on the methods used for the selective isolation, identification and typing was collected from the participants through the webtool. All 33 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 carbapenemase 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. Another lab (#33) reported that the protocol was modified by adding 0,12 mg/L of meropenem to the MacConkey medium.

The species identification (ID) was performed using MALDI TOF (n=16), biochemical tests (n=8), chromogenic media (n=5) or PCR using published methods (n=4). 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 the selective isolation of carbapenem producers (n=13), CHROMID CARBA SMART Agar (n=6), CHROMID CARBA Agar (n=7), CHROMID OXA-48 (n=6) and CHROMagar mSuperCARBA (n=1) were used.

All laboratories performed the broth microdilution testing using panel 1 (EUVSEC3), with the antimicrobials and ranges defined for *E. coli* isolates under the EU Commission regulation 1729/2020. All laboratories reported that they applied the microbroth dilution method using panel 2 (EUVSEC2) to test presumptive ESBL/AmpC and/or carbapenemase isolates, when relevant, but one lab failed to report the results for that panel (#021). Results were interpreted according to the EFSA criteria for ESBL, AmpC and carbapenemase phenotypic classification. Additionally, one lab confirmed the phenotypes with NGS and one with a Bluecarba-test.

3.3 Antimicrobial susceptibility testing

Out of the expected 4,650 AST results, laboratories submitted on the webtool a total of 4,290 MIC results together with 4,283 corresponding interpretations (susceptible vs resistant), excluding empty fields. The number of results submitted per laboratory varied and ranged between 90 and 150 MIC test results per participant for laboratories reporting on both meat and caecal samples; the rest of the laboratories submitted 75 results each, as expected. Figure 2 shows the percentage of submitted versus expected number of MIC interpretation results per participating laboratory. For several laboratories, the percentage of submitted results was low, with 11 laboratories having submitted less than 85% of expected results, including four laboratories (i.e. #006, 021, 026 and 060) that submitted less than 70%. In addition, in seven instances, laboratories submitted MIC values without corresponding susceptibility interpretations. The variability in the number of submitted results has probably largely resulted from failure to isolate some of the test strains (see missing isolates per laboratory, Fig. 3). In addition, one laboratory reported on only one instead of both panels.

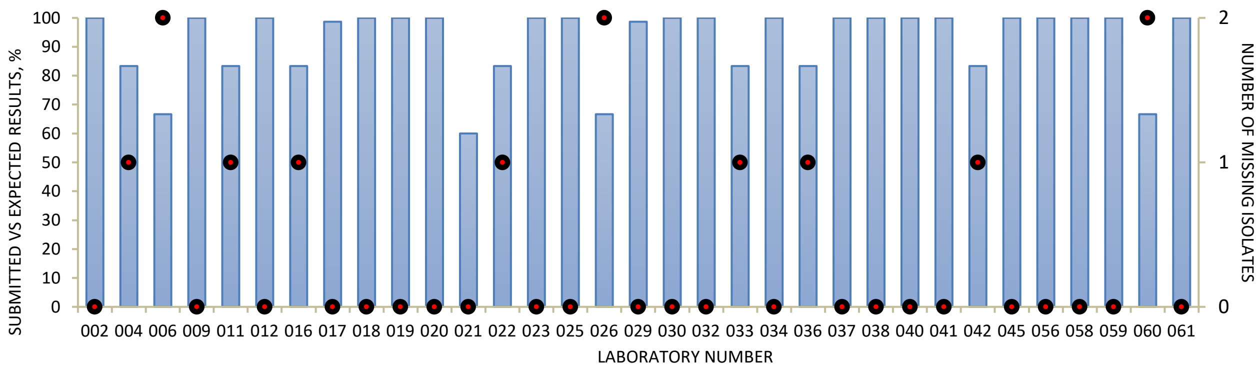

Figure 3. Percentage of submitted versus expected number of AST R/S interpretation results per participating laboratory, plotted together with the number of isolates that were not successfully retrieved, Matrix PT, 2024.

A first overview of the submitted results revealed a high number of variation and discordance for some of the strain-antibiotic combinations. For the strain combinations of M-10.3 Cefepime and M-19.7 Nalidixic acid, there was particularly high variation related to 1-fold dilution differences around the breakpoint. To account for the variation in the results, although no strains were completely omitted from the evaluation, the following strain/antibiotic combinations were not scored:

Table 2. List of antibiotic-strain combinations that were blanked (excluded from evaluation) in the results, due to high discordance (submitted results were correct for <75 % of participating laboratories).

Strain	Antibiotic	% correct results
M-10.1	Ertapenem	54 %
M-10.3	Cefepime	73 %
M-10.4	Cefotaxime-Clavulanic acid	68 %
	Cefoxitin	56 %
	Ceftazimide-Clavulanic acid	52 %
	Imipenem	52 %
M-10.6	Meropenem	47 %
	Ertapenem	58 %
	Imipenem	75 %
M-10.7	Nalidixic acid	56 %
M-10.8	Azithromycin	65 %

As a result of the exclusion of these 11 strain-antibiotic combinations and of the high number of non-submitted results for the test strains that were not isolated successfully, a considerable proportion of the deviations recorded in this PT round was not scored. From the remaining dataset, only 19 (0.5 %) deviations were recorded in total. Figure 4 shows the proportion of deviations among the evaluated results (i.e. submitted results after excluding the blanked strain-antibiotic combinations of Table 2) per laboratory. As can be seen in Figure 4, the proportion of deviations per laboratory for the evaluated results was low, with fewer than 2,5 % deviations for all laboratories and with 20 laboratories with no scored deviations.

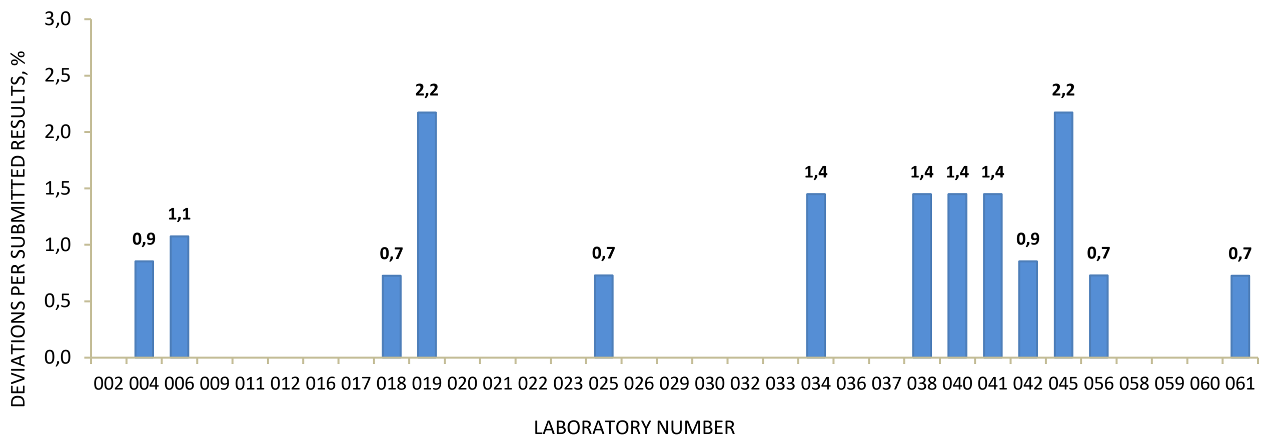

Figure 4. Proportion of deviations among evaluated (i.e. submitted and not blanked) AST R/S interpretation results per participating laboratory, Matrix PT, 2024.

The full list of deviations can be found in Appendix 6.

As the results of AST depend on the isolation and identification procedures, no threshold was set for acceptance of the performance of the participating laboratories for this PT. In general, the capacity of the laboratories to perform AST of *E. coli* is evaluated more accurately in the annual *E. coli* AST PT schemes organized by the EURL-AMR.

Figure 5 shows the percentage of scored deviations per tested antibiotic. Overall, the number of deviations was low, with no deviations for 13 of the antibiotics. The highest percentage of deviations was recorded for cefoxitin (4,9 %), followed by chloramphenicol and temocillin (1,2 %). There was particularly high discordance in the case of cefoxitin for strain M-10.1, for which 20% of the R/S interpretation results were incorrect (Fig. 6). A substantial proportion of the deviations resulted from a two-fold dilution difference around the breakpoint.

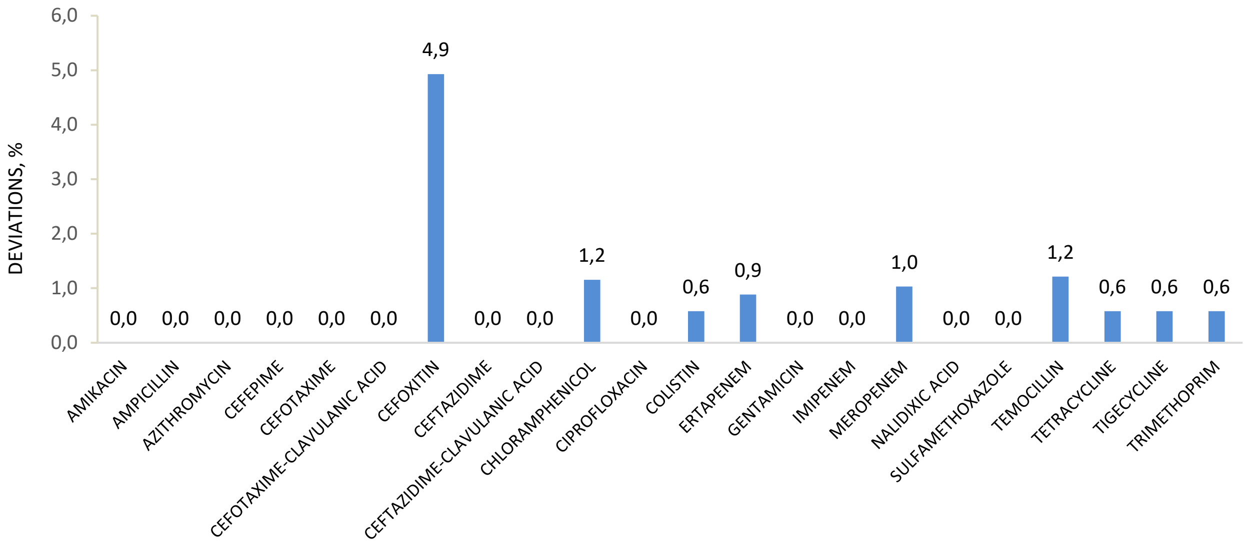

Figure 5. Proportion of deviations among submitted AST interpretation results per antibiotic, Matrix PT, 2024

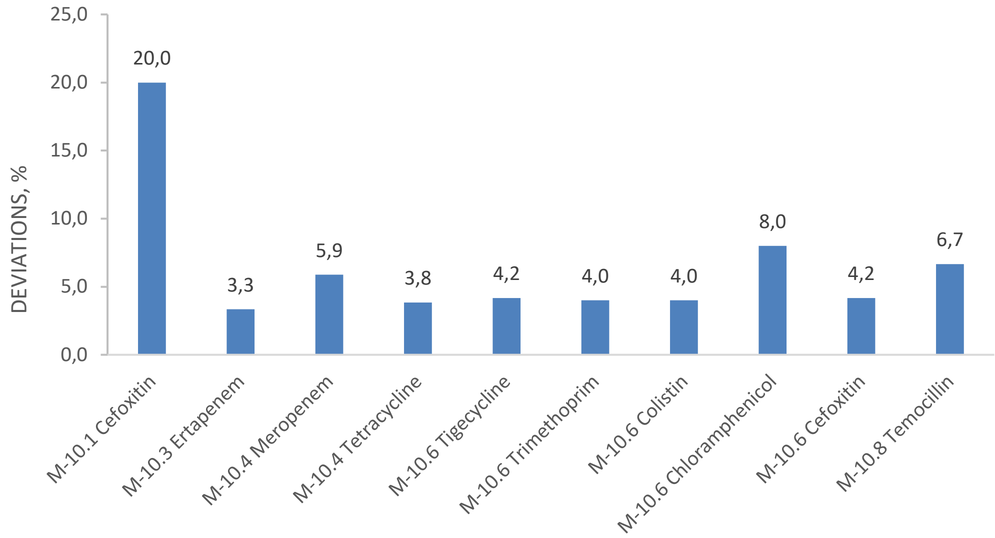

Figure 6. Proportion of deviations among submitted AST interpretation results per strain-antibiotic combination, Matrix PT, 2024

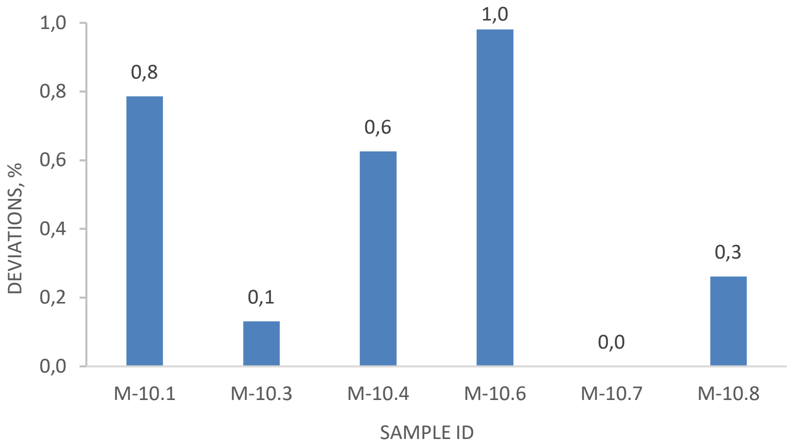

Figure 7. Proportion of deviations among submitted AST interpretation results per sample, Matrix PT, 2024

The level of deviations per sample were also low (Fig. 7). There were no deviations for sample M-10.7, whereas most deviations appeared, in decreasing order, for samples M-10.6, M-10.1 and M-10,4.

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

In total, the amount of ESBL, AmpC and CP phenotype interpretation results expected from the laboratories was 248, including 62 interpretation results for the negative control samples 10.2 and 10.5. In accordance with what was expected, laboratories did not submit any interpretation results for the negative control samples, with the exception of one laboratory that, as mentioned previously, reported a positive finding of an AmpC-producing *E. coli* strain from sample 10.2, which was inoculated with a susceptible strain only.

Excluding the negative control samples 10.2 and 10.5, the total amount of ESBL/AmpC/carbapenemase phenotype interpretation qualitative test results received for the test strains was 186. In 13 instances, laboratories submitted no results although they were expected to submit a positive result. Of the 175 evaluated positive results (174 for the control strains and one for sample 10.2), 170 (97 %) were in concordance with the expected phenotype (Figure 8 and Table 3).

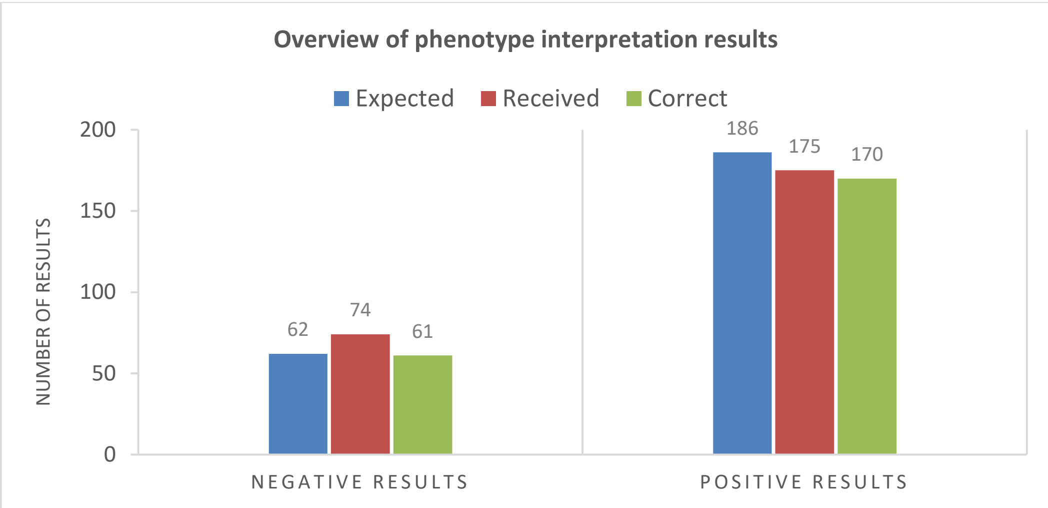

Figure 8. Overall performance on isolation and identification of ESBL/AmpC/CP-producing *E. coli*, Matrix PT, 2024

Five deviations were observed (Table 3): one for the negative control sample M-10.2 (reported as AmpC), one for sample M-10.3 (reported as ESBL+AmpC), two for M.10-4 (reported as ESBL) and one for M.10.8 (reported as AmpC).

Table 3. Overview of ESBL, AmpC and carbapenemase phenotypes, 2024

Strain ID	Expected phenotype	*bla*-gene(s)/ mutation	Additional phenotype approved	No of Deviations (%)
M-10.1	ESBL	$bla_{\text{CTX-M-55}}$	ESBL+AmpC-phenotype; Other phenotype	0
M-10.2	Susceptible		None	1 (3.2%)
M-10.3	AmpC	*ampC*-promoter (g.-42C>T)	None	1 (3.2%)
M-10.4	Carbapenemase. – Oxa-48-like	$bla_{\text{OXA-244}}$, $bla_{\text{CTX-M-27}}$	None	2 (6.5%)
M-10.6	Carbapenemase	$bla_{\text{VIM-1}}$	AmpC-phenotype; Other phenotype	0
M-10.7	ESBL	$bla_{\text{TEM-57}}$, $bla_{\text{SHV-12}}$	None	0
M-10.8	ESBL	$bla_{\text{TEM-52C}}$		1 (3.2%)

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

4. Discussion

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

In total, 13 of the spiked *E. coli* test strains were not isolated, and hereof 11 were carbapenemase producers (samples M-10.4 and M-10.6), carrying *bla*$_{OXA-244}$ and *bla*$_{VIM-1}$, which are known to confer low-level resistance to meropenem. In addition, there was high variation in the level of meropenem resistance reported for M-10.4 in this PT round (for details see below). The M-10.4 strain carried, besides the carbapenemase, an ESBL gene (*bla*$_{CTX-M-27}$), which might have caused the two categorisations as ESBL, but which should also have facilitated the isolation of this strain on MacConkey + CTX. Overall, these challenges highlight the difficulties that can be faced when isolating CP-producing strains from animal matrices, even when using selective agars that should target both ESBL- and CP- producing *E. coli*.

Besides the challenges arising from the atypical resistance phenotypes, some difficulties with isolating the strain from sample M-10.6 have likely been caused by the instability of the inoculum, which was noted also by the EURL-AMR lab during sample pre-testing.

4.2 Antimicrobial susceptibility testing

In the 2024 iteration of the matrix PT there was an unusually high number of strains not being recovered and of blanked results, leading to less data being evaluated and hence, fewer deviations. Typically, the main reason for blanking results in matrix PTs is when the AST deviations are caused by two-fold dilution differences around the ECOFF, which change the phenotype interpretation. However, this year, many of the blanked results were caused by high phenotypic variation among the strains, typically with more than one two-fold dilution difference from the ECOFF.

There has been increasing awareness of the existence of beta-lactam resistant strains that are difficult to isolate because they do not express the typical ESBL or carbapenem resistance phenotypes. To reflect that, strains with such atypical phenotypes were included in the 2024 PT panel. Some laboratories faced difficulties with successfully isolating these low-carbapenem-resistant strains, and there were some inconsistencies in the reported phenotypes.

A small number of the deviations recorded in this PT may have been caused by natural variation in the phenotype of some of the strains. For example, this may have been the case for strain M-10.4, carrying *bla*$_{OXA-244}$. This strain was expected to be reported as resistant to meropenem with an MIC of 0.25 – 0.5 mg/L, based on repeated AST determinations. However, many of the PT participants reported it with higher MIC values (1 mg/L by 12 laboratories and 2 mg/L by 9 laboratories), and three laboratories reported it as susceptible to meropenem (MIC= 0.125 mg/L). For the same strain, M-10.4, there was also a high level of variation in the reported MIC of the beta-lactams cefotaxime/clavulanic acid, ceftazidime/ clavulanic acid, cefoxitin and imipenem, leading to the respective results being blanked. This discordance could have been caused by loss of one or more of the *bla*-genes present in this strain (OXA-244 and CTX-M-27) or could have resulted from natural variation in their expression.

Besides M-10.4, there was also discrepancy in the levels of carbapenem resistance reported for the *bla*$_{VIM-1}$-carrying strain M-10.6. As a result, the MIC results for the three carbapenems were blanked.

In two cases, down from eight cases in 2023, participants reported correct MIC values but with incorrect R/S interpretations. It is unclear whether this was due to technical errors or because the participants used different guidelines for the interpretation.

4.3 ESBL /AmpC and carbapenemase phenotypic testing conclusions

Two laboratories reported the CP-producing *E. coli* isolate from sample M-10.4 as an 'ESBL', as a result of the variation in the meropenem MIC. Despite the lack of meropenem resistance in these cases, the 'ESBL' category will not be accepted, as the resistance to the other carbapenems should have led to a categorisation as 'Other phenotype' instead of 'ESBL'.

There were two cases of deviation when differentiating between the ESBL and AmpC phenotypes, highlighting known challenges in distinguishing between these two phenotypes, particularly in strains with a level of resistance around the relevant breakpoints. In the first case of deviation, the strain was only one dilution away from not expressing synergy, which made the results difficult to interpret. In the second case, the laboratory (# 021) isolated and reported on a $bla_{\mathrm{CMY\text{-}155}}$ expressing AmpC strain. However, this was a unique incidence, and this genotype was not expected from any of the test strains. Given the samples of matrix PTs include naturally contaminated matrices, there is always a risk of exchange of gene(s) from the background bacteria in the matrix.

In general, with matrix PTs it is expected to see occasional deviations in the resistance phenotypes, and possibly even in the ESBL categorisations, due to interactions with bacteria and/or mobile genetic elements from 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-AMR. In general, it is practically impossible to avoid having generic *Enterobacterales* or *E. coli*, and sometimes even other ESBL bacteria, in some parts of the meat and caecal matrix. When preparing the samples, the matrix material is screened to assess the level of background bacteria. However, this screening only serves to reveal possible ESBL/AmpC/carbapenemase contamination in the differences batches of meat/caeca and to produce a rough estimation of the level of background bacteria and cannot capture the full spectrum of the microbiota present in the matrix.

Nine laboratories indicated "Other phenotype" for the isolates from samples M-10.1 (n=4) and M-10.6 (n=5)), as the obtained phenotypes did not reflect the expected ones, i.e. ESBL and carbapenemase producers, respectively. Following the EFSA guidelines, the obtained results should be reported as "Other phenotype", and this was reflected in the webtool and approved in this report as an additional phenotype.

4.4 Performance in AST of the quality control strains

The *E. coli* ATCC 25922 QC strain as well as the *A. baumannii* 2012-70-100-69 internal control strain were included in the reporting of the PT results, but there is no evaluation for the latter. Antimicrobial susceptibility test results for the *Escherichia coli* ATCC 25922 quality control strain were evaluated based on the EUCAST quality control ranges (Appendix 5). All 33 participating labs tested *E. coli* ATCC 25922 by MIC determination. Of the reported results, 99.5% were within the acceptable range. There was a total of three deviations, for ceftazidime (n=1) and sulfamethoxazole (n=2), by laboratories #021, #060, and #061, all reporting MIC values one level higher than the QC ranges. The laboratories should carefully evaluate these deviations as part of their self-assessment of PT participation. Additionally, laboratory #021 did not provide data for Panel 2.

5. Conclusions

The 2024 Matrix PT round demonstrated that most participating laboratories have well-established methods for the isolation of ESBL/AmpC and carbapenemase-producing *E. coli* strains from meat or caecal samples, when the strains have typical resistance phenotypes. However, laboratories may face challenges in the isolation of strains with low-level carbapenem resistance, as highlighted by the high number of laboratories that failed to recover carbapenemase-producing strains from samples M-10.4 and M-10.6 in this PT round. Laboratories also faced challenges when differentiating between ESBL and AmpC-producing strains, when the strains have a level of resistance around the breakpoints.

In this PT round there was high discordance in the reported MIC results for several antibiotic-strain combinations, causing these results to be blanked. The large number of blanked results, combined with an unusually large number of strains not being recovered, lead to less data being evaluated and hence, fewer deviations. When considering just the evaluated results, the susceptibility testing results were satisfactory, although there was variation in the MIC results in several cases, such as for meropenem for sample M-10.4.

In general, the value of matrix PT schemes, as opposed to generic AST PT, is that they can simulate real-life challenges with detecting ESBL-/AmpC-/CP-producing *E. coli*, which stem from the complexity of the matrix and the background microbiota. The success in isolating the challenging strains should be part of the self-evaluation of the laboratories for this PT round.

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-AMR PT matrix 2024
Appendix 2. Test strains and reference values (MIC in mg/L)
Appendix 3. Protocol PT matrix 2024
Appendix 4. Examples of Test forms PT matrix 2024
Appendix 5. QC ranges, *E. coli* ATCC25922
Appendix 6. List of deviations

EURL-AR EQAS pre-notification

G00-06-001/26.10.2020

EQAS 2024 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 chicken meat (n=4) and/or chicken caecal content (n=4) 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 the **third week of November 2024.** 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 13 January 2025** via the password-protected webtool. Upon reaching the deadline, and the EQAS coordinator has confirmed the opening of the results, 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. Test strains and reference values (Page 1/2)

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Prediction
EURL-M-10.1	> 32	4	≤ 4	> 16	≤ 0.25	> 8	> 4	≤ 1	> 64	32	> 16	> 512	> 64	≤ 0.03	> 8	ESBL
EURL-M-10.2																Susceptible
EURL-M-10.3	>32	8	≤ 4	1	≤ 0.25	8	4	≤ 1	>64	>32	>16	>512	64	≤ 0.03	4	AmpC
EURL-M-10.4	>32	64	≤ 4	≤ 0.5	≤ 0.25	2	>4	≤ 1	≤ 4	>32	>16	>512	≤ 8	0.25	≤ 0.015	Carbapenemase
EURL-M-10.5																blank
EURL-M-10.6	>32	>64	≤ 4	2	0.5	>8	>4	≤ 1	>64	>32	>16	>512	64	0.5	>8	Carbapenemase
EURL-M-10.7	>32	8	≤ 4	>16	≤ 0.25	>8	4	≤ 1	16	>32	>16	>512	>64	≤ 0.03	0.5	ESBL
EURL-M-10.8	>32	32	≤ 4	≤ 0.5	≤ 0.25	8	>4	≤ 1	>64	>32	>16	>512	>64	≤ 0.03	1	ESBL

Strain	AMP	AZI	AMI	GEN	TGC	TAZ	FOT	COL	NAL	TET	TMP	SMX	CHL	MERO	CIP	Prediction
EURL-M-10.1	R	S	S	R	S	R	R	S	R	R	R	R	R	S	R	ESBL
EURL-M-10.2																Susceptible
EURL-M-10.3	R	S	S	S	S	R	R	S	R	R	R	R	R	S	R	AmpC
EURL-M-10.4	R	R	S	S	S	R	R	S	S	R	R	R	S	R	S	Carbapenemase
EURL-M-10.5																blank
EURL-M-10.6	R	R	S	S	S	R	R	S	R	R	R	R	R	R	R	Carbapenemase
EURL-M-10.7	R	S	S	R	S	R	R	S	R	R	R	R	R	S	R	ESBL
EURL-M-10.8	R	R	S	S	S	R	R	S	R	R	R	R	R	S	R	ESBL

Appendix 2. Test strains and reference values (Page 2/2)

Strain	FOX	FOT	TRM	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	Prediction	bla genes
EURL-M-10.1	8	>64	8	0.06	≤ 0.12	≤ 0.03	16	>32	0.12	0.25	ESBL	CTX-M-55
EURL-M-10.2											Susceptible	none
EURL-M-10.3	64	4	16	0.06	0.25	≤ 0.03	8	0.25	2	8	AmpC	CARB-2, ampC-promoter (g.-42C>T)
EURL-M-10.4	8	64	64	1	0.25	0.5	2	4	0.5	0.5	Carbapenemase	OXA-244, CTX-M-27
EURL-M-10.5											blank	none
EURL-M-10.6	32	32	64	0.25	2	0.12	>128	16	64	128	Carbapenemase	VIM-1
EURL-M-10.7	4	4	4	≤ 0.015	≤ 0.12	≤ 0.03	32	0.5	≤ 0.06	≤ 0.12	ESBL	TEM-57, SHV-12
EURL-M-10.8	8	8	16	≤ 0.015	≤ 0.12	≤ 0.03	16	1	≤ 0.06	0.25	ESBL	TEM-52C

Strain	FOX	FOT	TRM	ETP	IMI	MERO	TAZ	FEP	F/C	T/C
EURL-M-10.1	S	R	S	S	S	S	R	R	S	S
EURL-M-10.2										
EURL-M-10.3	R	R	S	S	S	S	R	R	R	R
EURL-M-10.4	S	R	R	R	S	R	R	R	R	S
EURL-M-10.5										
EURL-M-10.6	R	R	R	R	R	S	R	R	R	R
EURL-M-10.7	S	R	S	S	S	S	R	R	S	S
EURL-M-10.8	S	R	S	S	S	S	R	R	S	S

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2024

PROTOCOL

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

Table of contents

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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 2024, these eight samples will include four 25-g samples of chicken meat and four 1-g samples of chicken 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 2024

3.1 Shipping, receipt and storage of samples

In November 2024, 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 chicken meat or pooled chicken caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 10.1 to 10.8. Hereof 10.1 to 10.4 being samples of meat (each 25 g) and 10.5 to 10.8 being samples of caecal content (each 1 g).

The matrix samples will be shipped on 11 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* ATCC 25922 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* ATCC 25922 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 10.1 to 10.4.
- Follow the protocol for caecal content when testing samples 10.5 to 10.8.

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 Appendix 1 to this document). Only one *E. coli* isolate is expected to be tested for AST and these results will be evaluated in the database by comparing to expected results.

AST results to be reported should be from:

- A presumptive carbapenemase positive isolate (from carbapenemase or OXA-48 selective plates, or a strain found resistant to meropenem in the MIC test), 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 thresholds listed in Appendix 1.

Strains are categorised as "S" to a specific antimicrobial compound when the obtained MIC value for this compound is equal to, or less than, the respective ECOFF value, while strains are categorised as "R" when the obtained MIC value is greater than the ECOFF value.

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 (beta-lactams; EUVSEC2).

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 concentrations 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.

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.

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

The classification of the phenotypic results should be based on the most recent EFSA recommendations (See the Appendix 2).

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 Appendix 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 1 and 2). 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 13 January 2025.** 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:

Jette Kjeldgaard
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204

DK-2800 Lyngby

Denmark

Tel: +45 3588 6269

E-mail: jetk@food.dtu.dk

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 1 Criteria for interpretation of *Escherichia coli* MIC, panel 1 and 2 results

The following tables present the concentration range to be tested for each antimicrobial compound as well as the Epidemiological Cut-off values for the AMR phenotype categorisation as Resistant or Susceptible, as presented in Appendix B of the EFSA (European Food Safety Authority), Amore G, Beloeil P-A, Garcia Fierro R, Guerra B, Rizzi V and Stoicescu A-V, 2024. Manual for reporting 2023 antimicrobial resistance data under Directive 2003/99/EC and Commission Implementing Decision (EU) 2020/1729. *EFSA supporting publication* 2024: 21(1):EN-8585. 41 pp. doi:10.2903/sp.efsa.2024.EN-8585.

Criteria for interpretation of *E. coli* from *EFSA supporting publication* 2024: 21(1):EN-8585):

Table B.1: Panel of antimicrobial substances to be included in AMR monitoring, interpretative thresholds for interpreting resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli*

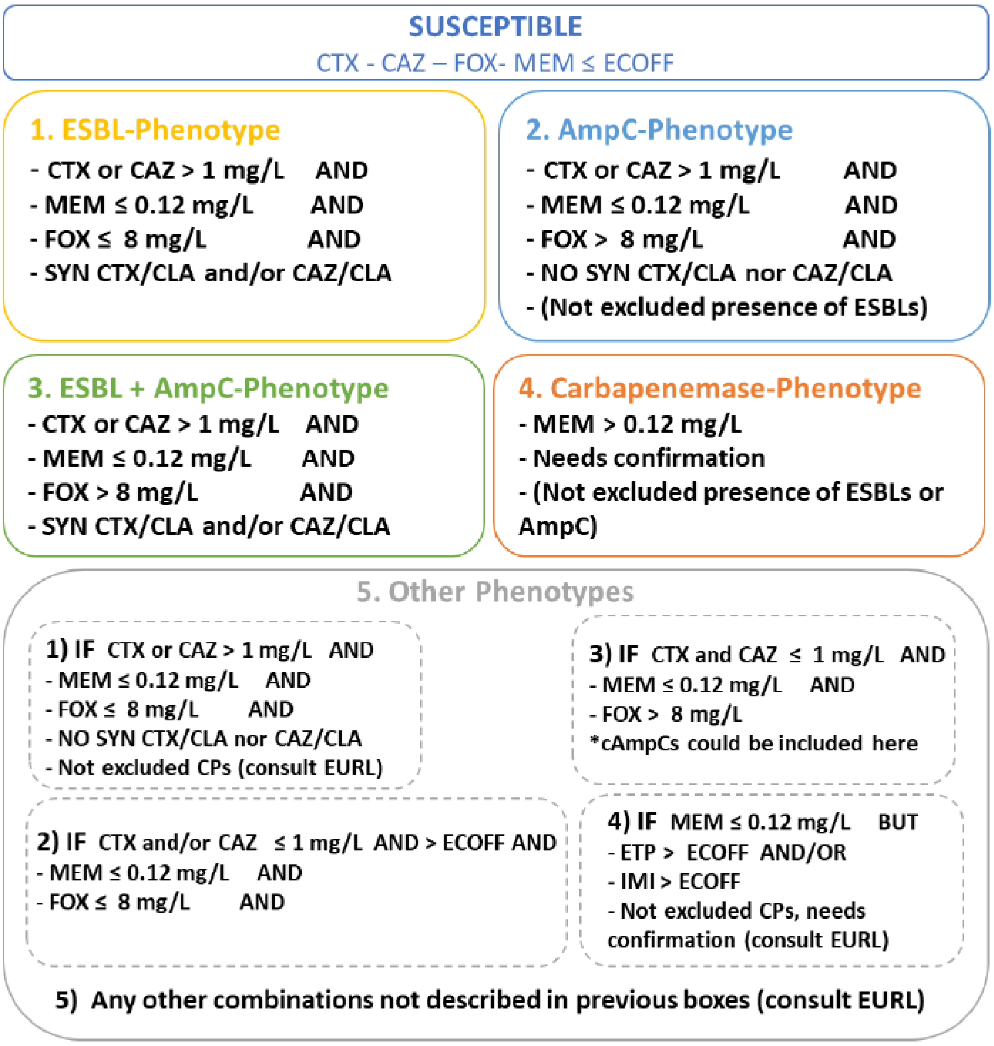
Antimicrobial	*Salmonella* EU surveillance 2023			*E. coli* EU surveillance 2023			Concentration range, mg/L (no of wells)
	ECOFF	EUCAST	EFSA	ECOFF	EUCAST	EFSA	
Amikacin[(a)]	4	x		8	x		4–128(6)
Ampicillin	8	x		8	x		1–32 (6)
Azithromycin	16	x		16		x	2–64 (6)
Cefepime[(b)]	0.125		x	0.125		x	0.064–32 (10)
Cefotaxime	0.5	x		0.25	x		0.25–4 (5)[(c)] 0.25–64 (9)[(d)]
Cefotaxime + clavulanic acid	0.5		x	0.25	x		0.064–64 (11)
Cefoxitin	8	x		8	x		0.5–64 (8)
Ceftazidime	2	x		0.5	x		0.25–8 (6)[(c)] 0.25–128 (10)[(d)]
Ceftazidime + clavulanic acid	2		x	0.5	x		0.125–128 (11)
Chloramphenicol	16	x		16	x		8–64 (4)
Ciprofloxacin	0.064	x		0.064	x		0.015–8 (10)
Colistin	2		x	2	x		1–16 (5)
Ertapenem[(e)]	0.064		x	0.064		x	0.015–2 (8)
Gentamicin	2	x		2	x		0.5–16 (6)
Imipenem	1	x		0.5	x		0.125–16 (8)
Meropenem	0.125		x	0.125	x		0.03–16 (10)
Nalidixic acid	8	x		8	x		4–64 (5)
Sulfamethoxazole	256		x	64		x	8–512 (7)
Temocillin	16		x	16	x		0.5–128 (9)
Tetracycline	8	x		8	x		2–32 (5)
Tigecycline	0.5		x	0.5	x		0.25–8 (6)
Trimethoprim	2	x		2	x		0.25–16 (7)

(a): EUCAST epidemiological cut-off (ECOFF) value for *Salmonella* is tentative.
(b): EUCAST epidemiological cut-off (ECOFF) value for *E. coli* is 0.25.
(c): Range to be used when the substance is tested in panel 1.
(d): Range to be used when the substance is tested in panel 2.
(e): EUCAST epidemiological cut-off (ECOFF) value for *E. coli* is tentative at 0.03.

APPENDIX 2 Criteria for categorisation of beta-lactam resistance phenotypes

Please use the scheme below to phenotypically identify presumptive ESBL-, AmpC-, and/or CP-producers. Five main categorizations of phenotypes are made: 1. Extended-Spectrum Beta-Lactamase-producing (ESBL phenotype), 2. AmpC Beta-Lactamase-producing (AmpC phenotype), 3. ESBL+AmpC phenotype, 4. Carbapenemase-producing (CP phenotype) and 5. Other.

The figure is from *EFSA and ECDC, 2024. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2021/2022. EFSA Journal. 2024;22:e8583. https://doi.org/10.2903/j.efsa.2024.8583, Appendix F, Figure F1.*



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 2024 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 2024 for monitoring of ESBL/AmpC detection in relation to 2020/1729/EU? (You may choose more than one option)

- ☐ caecal, poultry
- ☐ meat, poultry
- ☐ none
- ☐ other matrices, please specify:

How many samples did your laboratory process in 2024 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 2024 for monitoring of carbapenemase-production in relation to 2020/1729/EU? (you may choose more than one option)

- ☐ caecal, poultry
- ☐ meat, poultry
- ☐ 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:

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
☐ 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-10.1'

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:

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:

☐ 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 ☐

AST results

Strain	Antimicrobial	Results and interpretation		
		≤ >	MIC-value (mg/L)	S / R
E. coli EURL M-10.1	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-10.1	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 ESBL+ AmpC
☐ Presumptive AmpC
☐ Presumptive carbapenemase
☐ Other phenotype
☐ Susceptible

Comments (include optional genotype or other results):

Appendix 5 - 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

Matrix test samples with no isolate recovered – full list

Lab number	Samples with no isolate recovered
NRL-AR-004	EURL M-10.4
NRL-AR-006	EURL M-10.6, EURL M-10.7
NRL-AR-011	EURL M-10.6
NRL-AR-016	EURL M-10.6
NRL-AR-022	EURL M-10.6
NRL-AR-026	EURL M-10.4, EURL M-10.7
NRL-AR-033	EURL M-10.4
NRL-AR-036	EURL M-10.6
NRL-AR-042	EURL M-10.4
NRL-AR-060	EURL M-10.4, EURL M-10.6

Deviations, AST results - full list

Lab number	Strain	Antimicrobial	Obtained operator	Expected operator	Obtained MIC value	Expected MIC value	Obtained interpretation	Expected interpretation	Score of interpretation
NRL-AR-004	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-018	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-019	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-038	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-041	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-042	EURL M-10.1	Cefoxitin	=	=	16	8	R	S	0
NRL-AR-006	EURL M-10.3	Ertapenem	=	=	0.06	0,06	R	S	0
NRL-AR-019	EURL M-10.4	Meropenem	=	=	0.12	0,25	S	R	0
NRL-AR-019	EURL M-10.4	Meropenem	=	=	0.12	0,5	S	R	0
NRL-AR-025	EURL M-10.4	Tetracycline	<=	>	2	32	S	R	0
NRL-AR-040	EURL M-10.4	Meropenem	=	=	0.12	0,25	S	R	0
NRL-AR-034	EURL M-10.6	Tigecycline	>	=	32	0,5	R	S	0
NRL-AR-034	EURL M-10.6	Trimethoprim	<=	>	0.25	16	S	R	0
NRL-AR-040	EURL M-10.6	Colistin	=	<=	8	1	R	S	0
NRL-AR-045	EURL M-10.6	Chloramphenicol	=	=	16	32	S	R	0
NRL-AR-045	EURL M-10.6	Cefoxitin	=	=	8	32	S	R	0
NRL-AR-056	EURL M-10.6	Chloramphenicol	=	=	16	32	S	R	0
NRL-AR-045	EURL M-10.8	Temocillin	=	=	32	16	R	S	0
NRL-AR-061	EURL M-10.8	Temocillin	=	=	64	16	R	S	0

*Grey shading indicates a 2-fold dilution deviation from the expected MIC value

Deviations, ESBL categorisation - full list

Lab number	Strain	Obtained interpretation	Expected interpretation	Score of interpretation
NRL-AR-004	EURL M-10.3	ESBL+AmpC phenotype	AmpC phenotype	0
NRL-AR-006	EURL M-10.2	AmpC phenotype	Susceptible	0
NRL-AR-019	EURL M-10.4	ESBL phenotype	Carbapenemase phenotype	0
NRL-AR-021	EURL M-10.8	AmpC phenotype	ESBL phenotype	0
NRL-AR-030	EURL M-10.4	ESBL phenotype	Carbapenemase phenotype	0

DTU Fødevareinstituttet
DTU National Food Institute

Henrik Dams Allé
2800 Kgs. Lyngby

+45 35 88 70 00

food.dtu.dk

European Union Reference Laboratory Antimicrobial Resistance