



# PROTOCOL

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

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## **1 INTRODUCTION**

The organisation and implementation of a Proficiency Testing for the 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-AMR). The PT assesses the selective isolation procedures and antimicrobial susceptibility testing (AST) of isolates obtained from eight samples of either meat or caecal content. In 2025, these eight samples will comprise four 25-g samples of beef meat and four 1-g samples of cattle caecal content, which may or may not contain *E. coli* producing either ESBL-, AmpC- or carbapenemase-enzymes.

Participating laboratories are expected to apply the same analytical procedures as for the EU monitoring activities, described in the regulation 2020/1729/EU, and to perform the selective isolation following the EU-recommended methods, published on the EURL-AMR website: <https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols>.

## **2 OBJECTIVES**

This PT aims to assess and, if necessary, improve the quality of results obtained from the selective isolation of presumptive ESBL-, AmpC- or carbapenemase-producing *E. coli* 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 PT 2025**

### **3.1 Shipping, receipt and storage of samples**

In November 2025, the National Reference Laboratories for Antimicrobial Resistance (NRL-AMR) will receive a parcel from the National Food Institute containing eight samples that may or may not be spiked with *E. coli* test and control strains. All strains used for spiking fall under UN3373, Biological substance, category B. Participants should expect that some of the strains will be producing ESBL, AmpC and/or carbapenemases.

The samples will be spiked matrices of either beef meat or cattle caecal content and will be distributed already weighed and ready to be tested, in tubes labelled from 11.1 to 11.8. Of those, 11.1 to 11.4 will be samples of meat (each 25 g) and 11.5 to 11.8 will be samples of caecal content (each 1 g).

It is expected that the matrix samples will be shipped on 03 November in frozen/chilled state in separate tubes contained in a cooling box together with a temperature logging device and freezing elements.

Upon arrival, it is very important to open the parcel as soon as possible and proceed with 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 of opening**
- **Proceed with sample analysis immediately after opening the parcel**
- **Register the start date of analysis for each sample.**
- **Collect the temperature logging device from the parcel (small disc-shaped device located inside a bag inserted in a labelled tube);** open the tube and take out the bag containing the device. Place this bag with the device into the labelled bubble envelope provided and return it as normal letter to the EURL-AMR as soon as possible. Please note that you will have to arrange for stamps/postage (postal systems differ from country to country, so EURL-AMR cannot prepay or arrange shipping for this 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 the results from these strains together with the results obtained from the PT isolates. 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 (CP)-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 appropriate volume of media (225 ml of Buffered Peptone water for the meat samples and 9 ml for the caecal samples) as described in the EURL-AMR protocols. **Results should be generated according to the laboratory's routine procedures for antimicrobial susceptibility testing by MIC determination.** All subsequent procedures should be in accordance with the methods used for the monitoring for ESBL-, AmpC and CP-producing *E. coli* as outlined in the 2020/1729/EU Decision. If any modifications are made to the official protocols, these must be described in detail in the online database that can be found under the methods page of the webtool that will be used for result submission. The participants are responsible for ensuring the validity of the selective plates; therefore, the protocols entitled "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" and "Validation of selective and indicative agar plates for monitoring of carbapenemase-producing *E. coli*" should be run ahead of the analysis, as stated in the protocols found on the EURL-AMR webpage (see <https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols>).

The officially recommended protocols can be found on the EURL-AMR webpage (<https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols>):



- Follow the protocol for meat when testing samples 11.1 to 11.4.
- Follow the protocol for caecal content when testing samples 11.5 to 11.8.

As described in these protocols, any isolates obtained from the selective isolation procedure should be confirmed as being *E. coli* using the species identification methods routinely applied by your laboratory for the specific monitoring of ESBL-, AmpC-, and CP-producing *E. coli*.

Please store the isolates obtained during the isolation procedure and document the entire process, including all findings at each step.

As part of the online results submission, you will be requested to describe the enrichment and selective isolation processes you followed along with all findings, including the level of observed growth in the media, the isolation of suspected colonies, the results from the species identification and any findings of presumptive ESBL-, AmpC-, or CP-producing *E. coli* isolates (the latter will be evaluated as qualitative results by comparison with the expected results). These data can all be collected in the supporting document Test Forms.

### **3.4 Antimicrobial susceptibility testing (AST)**

If the sample is deemed positive for ESBL-, AmpC- or CP -producing *E. coli*, one *E. coli* isolate per sample should be tested further for susceptibility to antimicrobials, as stated in the EU regulation (the targeted antimicrobials are listed in the Appendix 1 of this document). These results will be evaluated in the database against the expected results.

Reported AST results (from one isolate per sample) should be from:

- Any presumptive carbapenemase-positive *E. coli* isolates detected (isolated from carbapenemase or OXA-48 selective plates or found resistant to meropenem in the MIC test).
- Any presumptive ESBL- or AmpC-producing *E. coli* isolate detected, if no carbapenemase-positive isolate was found.

The AST should be performed exactly as performed by your laboratory as for the EFSA monitoring according to Decision 2020/1729/EU. Specifically, the two-step approach should be followed, i.e. including both testing panels, using 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 for all strains resistant to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) and should be performed by AST on the second panel of antimicrobials (beta-lactams; EUVSEC2).

Confirmatory testing for ESBL production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone as well as in combination with a  $\beta$ -lactamase inhibitor (clavulanic acid). Synergy is defined as a  $\geq 3$  two-fold-concentration decrease in the MIC for either antimicrobial agent (FOT or



TAZ) tested in combination with clavulanic acid vs. the MIC of the agent when tested alone (MIC FOT: FOT/CL or TAZ: TAZ/CL ratio  $\geq 8$ ). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases can be performed by testing bacterial susceptibility to cefoxitin (FOX). Resistance to FOX may indicate the presence of an AmpC-type beta-lactamase.

Confirmatory testing for carbapenemase production requires susceptibility testing to meropenem (MERO).

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

Important note: For *E. coli*, there are two sets of relevant cut-off values for cefotaxime, ceftazidime and meropenem: 1) the EUCAST epidemiological cut-off values, which are used to define R/S phenotypes (see Appendix 1 and 2), and 2) the screening cut-off values (cefotaxime  $>1$  and ceftazidime  $>1$ ), which are used to categorise the resistance phenotypes, i.e. as ESBL, AmpC, carbapenemase, etc., based on the results from panel 2 (see Appendix 1 and 2).



## 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 in paragraph 5 before entering your results in the web database. **Results must be submitted no later than 16 December 2025.** After the deadline, when all participants have uploaded results, you will be able to login to the database once again to view and print an automatically generated report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

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

All results will be summarized in a report which will be made publicly available. The data in the report will be presented under laboratory codes instead of laboratory names, to ensure anonymity. Each laboratory code will be known only to the respective laboratory, while the complete list of laboratories and their corresponding codes will remain confidential and known only to the EURL-AMR and the EU Commission. All conclusions will be public.

If you have any questions, please do not hesitate to contact the PT 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-AMR website (<https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/protocols>).

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 submission' blocks further data entry.

⇒ About login to the webtool:

When you were first given access to login to the webtool, your **personal** loginID and password were sent to you by email on two email addresses (for primary and secondary contact) connected to each NRL-AMR.

Note that:

- a) If the EURL-AMR 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](mailto:jetk@food.dtu.dk).
- b) If your laboratory has two or more contact points on the EURL-AMR contact list, two of those have been defined as primary and secondary contact. Should you like to make changes to the primary and secondary contact or should you like to have more than two persons able to access the webtool, please contact [jetk@food.dtu.dk](mailto:jetk@food.dtu.dk).

All participants registered with an account in the submission webtool will receive a separate email with their personal username and password. The email will be sent after 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 manual for reporting AMR data: EFSA (European Food Safety Authority), Amore G, Beloeil P-A, Garcia Fierro R, Rizzi V and Stoicescu A-V, 2025. Manual for reporting 2024 antimicrobial resistance data under Directive 2003/99/EC and Commission Implementing Decision (EU) 2020/1729. *EFSA supporting publication 2025:EN-9238*. 39 pp.. doi:10.2903/sp.efsa.2025.EN-9238

**Criteria for interpretation of *E. coli* from EFSA supporting publication 2025:EN-9238**. 39 pp.2024 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	<i>Salmonella</i> EU surveillance 2024			<i>E. coli</i> EU surveillance 2024			Concentration range, mg/L (no of wells)
	ECOFF	EUCAST	EFSA	ECOFF	EUCAST	EFSA	
Amikacin <sup>(a)</sup>	4	x		8	x		4–128(6)
Ampicillin	8	x		8	x		1–32 (6)
Azithromycin	16	x		16		x	2–64 (6)
Cefepime <sup>(b)</sup>	0.125		x	0.125		x	0.064–32 (10)
Cefotaxime	0.5	x		0.25	x		0.25–4 (5) <sup>(c)</sup> 0.25–64 (9) <sup>(d)</sup>
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) <sup>(c)</sup> 0.25–128 (10) <sup>(d)</sup>
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 <sup>(e)</sup>	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, 2025. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2022/2023. EFSA Journal. 2025;23:e9237. <https://doi.org/10.2903/j.efsa.2025.9237>, Appendix A, Figure A.3.*

