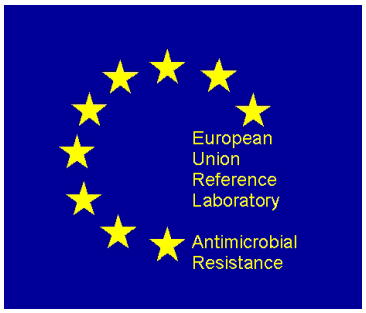
EU Reference Laboratory for Antimicrobial Resistance
(EURL-AR)

PROTOCOL

External Quality Assurance System (EQAS) 2024

For the Antimicrobial Susceptibility Testing of
Escherichia coli, *Salmonella* and *Campylobacter*.



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Introduction

The organisation and implementation of an External Quality Assurance System (EQAS) on antimicrobial susceptibility testing (AST) of *Escherichia coli*, *Salmonella* and *Campylobacter* is among the tasks of the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR). This EQAS aims to support laboratories to assess and, if necessary, to improve the quality of results obtained by AST of pathogens of food and animal origin, with special regard to *E. coli*, *Salmonella* and *Campylobacter*. Further objectives are to evaluate and improve the comparability of surveillance data on antimicrobial susceptibility of *E. coli*, *Salmonella* and *Campylobacter*, reported to EFSA by different laboratories.

The EURL-AR AST EQAS 2024 includes AST of the following types of strains, also presented in Table 1:

- **EQAS Test Strains**: eight *E. coli*, eight *Salmonella* and eight *Campylobacter* (*C. jejuni*/*C. coli*) strains
- **American Type Culture Collection (ATCC) Reference Strains**: *E. coli* ATCC 25922 (CCM 3954) for the *E. coli* and *Salmonella* trials, and *C. jejuni* ATCC 33560 (CCM 6214) for the *Campylobacter* trial.
- **EURL-AR Quality Control (QC) Strains**: *Acinetobacter baumannii* (2012-70-100-69) for the *E. coli* and *Salmonella* trials. Additionally, this year a new EURL-AR QC *C. coli* strain is included, named "215-QC-ETP". This strain is a method control for broth microdilution testing of ertapenem, as it as an expected Minimum Inhibitory Concentration (MIC) to ertapenem of 1 mg/L, which is appropriate for the ertapenem test range (0.125 - 4 mg/L).

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and the National Food Institute is responsible to the scheme participants for the subcontractor's work.

Table 1. Overview of Strains included in the current EQAS.

Category	*E. coli*	*Salmonella*	*Campylobacter*
EQAS Test strains	EURL 2024 EC-19.1	EURL 2024 S-19.1	EURL 2024 C-19.1
	EURL 2024 EC-19.2	EURL 2024 S-19.2	EURL 2024 C-19.2
	EURL 2024 EC-19.3	EURL 2024 S-19.3	EURL 2024 C-19.3
	EURL 2024 EC-19.4	EURL 2024 S-19.4	EURL 2024 C-19.4
	EURL 2024 EC-19.5	EURL 2024 S-19.5	EURL 2024 C-19.5
	EURL 2024 EC-19.6	EURL 2024 S-19.6	EURL 2024 C-19.6
	EURL 2024 EC-19.7	EURL 2024 S-19.7	EURL 2024 C-19.7
	EURL 2024 EC-19.8	EURL 2024 S-19.8	EURL 2024 C-19.8

Category	*E. coli*	*Salmonella*	*Campylobacter*
ATCC Reference strains	*E. coli* ATCC 25922 (CCM 3954)*	*E. coli* ATCC 25922 (CCM 3954)*	*C. jejuni* ATCC 33560 (CCM 6214)
EURL-AR QC strains	*A. baumannii* (2012-70-100-69)	*A. baumannii* (2012-70-100-69)	*C. coli* (215-QC-ETP)**

*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.

**New EURL-AR QC C. coli strain to be used as a method control for broth microdilution testing of ertapenem. The expected Minimum Inhibitory Concentration (MIC) to ertapenem is 1 mg/L, which is appropriate for the ertapenem test range (0.125 - 4 mg/L).

Shipping, receipt and storage of strains

In October 2024, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, eight *Salmonella* and eight *Campylobacter* strains from the National Food Institute (see Table 1). For participants who did not receive them previously, this parcel will also contain ATCC reference strains and EURL-AR internal QC strains. Additionally, this year a new EURL-AR internal *C. coli* QC strain is included, named "215-QC-ETP", as mentioned in the Introduction.

All strains belong to UN3373, Biological substance, category B. Extended spectrum beta-lactamase (ESBL)-producing strains as well as carbapenemase-producing strains are included in the selected material. It is the recipients' responsibility to comply with national legislation, rules and regulation regarding the correct use and handling of the provided strains and to possess the proper equipment and protocols to handle these strains.

The ATCC reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously and are original, CERTIFIED cultures, provided free of charge, and should be used for future internal QC for AST in your laboratory. The EURL-AR Internal QC-strains are provided for the purpose of additional QC of the broth microdilution plates. These strains will not be included in the years to come and we therefore encourage you to take proper care of these strains for example by handling and maintaining them as suggested in the manual 'Subculture and Maintenance of Quality Control Strains' available on the EURL-AR website (see https://www.eurl-ar.eu/eqas.aspx).

All test strains will be shipped as swabs of pure cultures in transport media and new laboratories to the network will receive lyophilised ATCC reference strains. Upon arrival to your laboratory, store the

strains in a dark place at 5°C to 25°C until microbiological analysis. A suggested procedure for reconstitution of the lyophilised reference strains is presented below.

Attention! The *Campylobacter* test strains must be subcultured immediately upon arrival to ensure viability.

Antimicrobial Susceptibility Testing

Participants should determine the MIC values and report results of the EQAS test strains, the ATCC Reference strains and the EURL-AR QC strains presented in **Table 1**, using the methods stated in the Commission Implementing Decision 2020/1729/EU (international reference method ISO standard 20776-1:2019). **Results should be produced according to the laboratory's routine procedures for antimicrobial susceptibility testing for MIC determination.** 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.

The participants are requested to test the requested strains by broth microdilution for specific antimicrobial compounds and for a specific concentration range for each compound, as described in the European Food Safety Authority (EFSA) "Manual for reporting 2023 antimicrobial resistance data under Directive 2003/99/EC and Commission Implementing Decision (EU) 2020/1729", Appendix B, Tables B.1 and B.2 [1]. As the current regulation and recommendations focus on broth microdilution testing only, results obtained by other methods cannot be submitted for evaluation.

To obtain the AMR phenotype of each strain for a particular antimicrobial compound as Resistant (R) or Susceptible (S), the obtained MIC values should be interpreted according to the Epidemiological Cut-Off values (ECOFFs) in Tables B.1 and B.2 from the EFSA Manual for reporting 2023 AMR data [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. For convenience, screenshots of Tables B.1 and B.2 from the EFSA Manual for reporting 2023 AMR data [1], which include the concentration ranges and ECOFFs for each antimicrobial compound, are provided in Appendix 1 of this document.

Beta-lactam Resistance Phenotype Categorisation

According to Commission Implementing Decision (EU) 2020/1729, *E. coli* and *Salmonella* isolates exhibiting a resistant phenotype to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) in Panel 1, must be further tested with a second panel of various beta-lactams including extended-spectrum cephalosporins and carbapenems (Panel 2) to identify the following beta-lactam resistance phenotypes: presumptive Extended-Spectrum Beta-Lactamase (ESBL) producers, presumptive AmpC-Beta-Lactamase (AmpC) producers and presumptive Carbapenamase (CP) producers.

The categorisation of the beta-lactam resistance phenotype should be based on the EFSA and ECDC European Union Summary Report on AMR in zoonotic and indicator bacteria from human, animals and Food 2021/2022, Annex D, Figure 1 [2] – see also Appendix 2 in this document. Note that for both *E. coli* and *Salmonella*, two cut-off values apply for cefotaxime and ceftazidime: the ECOFF values, which define R/S (see Appendix 1), and the screening cut-off values (cefotaxime >1 and ceftazidime >1) which are those applied to categorise the beta-lactam resistance phenotype, based on Panel 2 results (see Appendix 2). Likewise, this is the situation for the *E. coli* meropenem cut-off values/screening cut-off value.

Confirmatory test for ESBL-, AmpC- and CP- production requires use of both cefotaxime (FOT) and ceftazidime (TAZ) alone and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined as i) a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial compound tested in combination with clavulanic acid compared to 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.

Identification of *Campylobacter* species

Species identification of the *Campylobacter* test strains must be performed by the NRLs using in-house methods or adopting the protocol available on the EURL-AR website under: http://eurl-ar.eu/233-protocols.htm.

Reporting of Results and Evaluation

Test forms are available for recording your results before you enter them into the webtool. Please report the following results:

- MIC values and AMR phenotype interpretation for the EQAS test strains. Only AMR phenotype interpretation is evaluated, based on expected data. The MIC data are not evaluated.
- Beta-lactam resistance phenotype interpretation for the *E. coli* and *Salmonella* EQAS test strains, which are evaluated based on the expected results.
- MIC values for the ATCC Reference strains which are evaluated based on the QC range according to CLSI M100.
- MIC values for the EURL-AR QC strains. These data are not evaluated.
- *Campylobacter* species identification as *C. jejuni* or *C. coli*, which is evaluated based on the expected results.

We recommend reading carefully the webtool manual before submitting your results.

Deadline for result submission:

Results must be submitted no later than **09-12-2024** at **16:00**.

After the deadline, when all participants have uploaded results, you will be able to login to the webtool once again to view and print an automatically generated report evaluating your results. Results in agreement with the expected AMR phenotype interpretation are categorised as ‘correct’, while results deviating from the expected interpretation are categorised as ‘incorrect’.

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

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

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How to Submit Results via the Webtool

The 'guideline for submission of results via webtool' is available for download directly from the EURL-AR website (https://www.eurl-ar.eu/eqas.aspx).

Access the webtool using this address: https://amr-eqas.dtu.dk. Please follow the guideline carefully and **remember to access the webtool via an 'incognito' website**.

When you submit your results, remember to have by your side the completed test forms.

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting your input for *E. coli*, *Salmonella* and *Campylobacter*, respectively, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once for each organism!** 'Final submit' blocks data entry.

⇨ About login to the webtool:

When first given access to login to the webtool, your **personal** loginID and password were sent to you by email. This is relevant for two email addresses connected to each NRL-AR (the EURL-AR defined a primary and a secondary contact).

Note that:

a) If the EURL-AR has only one contact person for an NRL, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact suska@food.dtu.dk
b) If your laboratory has two or more contact points on the EURL-AR contact list, two have been defined as the primary and secondary contact. Should you like to make changes to the primary and secondary contact or should you like more than the two persons to be able to access the webtool, please contact suska@food.dtu.dk.

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

References

[1] EFSA (European Food Safety Authority), Amore G, Beloeil P-A, GarciaFierro R, Guerra B, Rizzi V and Stoicescu A-V, 2024. Manual for reporting 2023 antimicrobialresistance data under Directive 2003/99/EC and Commission Implementing Decision (EU)2020/1729. EFSA supporting publication 2024:EN-8585. 41 pp. https://doi.org/10.2903/sp.efsa.2024.EN-8585

[2] EFSA (European Food Safety Authority) & ECDC (European Centre for Disease Prevention and Control). (2024). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. *EFSA Journal*, 22, e8583. https://doi.org/10.2903/j.efsa.2024.8583

APPENDIX 1: INTERPRETIVE CRITERIA FOR THE AMR PHENOTYPE CATEGORISATION AS RESISTANT OR SUSCEPTIBLE

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.

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.

Screenshot 1 of 2

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)

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Screenshot 2 of 2

Manual for reporting 2023 AMR data

Antimicrobial	*Salmonella* EU surveillance 2023			*E. coli* EU surveillance 2023			Concentration range, mg/L (no of wells)
	ECOFF	EUCAST	EFSA	ECOFF	EUCAST	EFSA	
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.

Table B.2: Panel of antimicrobial substances to be included in AMR monitoring, interpretative thresholds for resistance and concentration ranges to be tested in *C. jejuni* and *C. coli*

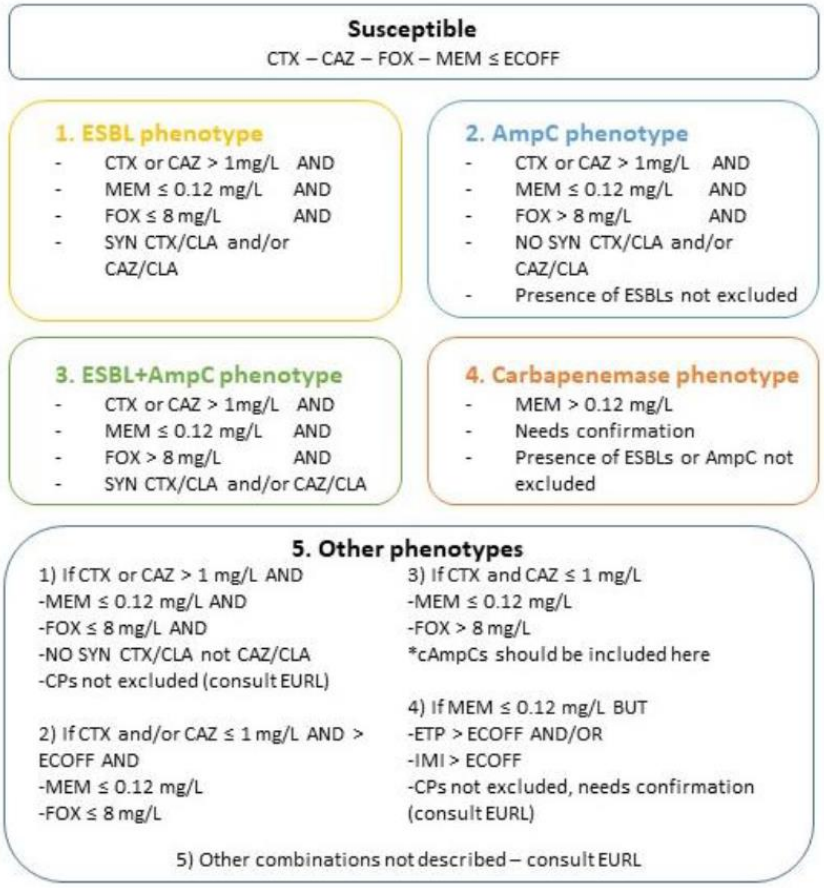
Antimicrobial	*C. jejuni* EU surveillance 2023			*C. coli* EU surveillance 2023			Concentration range, mg/L (no of wells)
	ECOFF	EUCAST	EFSA	ECOFF	EUCAST	EFSA	
Chloramphenicol	16	x		16	x		2–64 (6)
Ciprofloxacin	0.5	x		0.5	x		0.125–32 (9)
Ertapenem	0.5		x	0.5		x	0.125–4 (6)
Erythromycin	4	x		8	x		1–512 (10)
Gentamicin[a]	2		x	2		x	0.25–16 (7)
Tetracycline	1	x		2	x		0.5–64 (8)

(a): EUCAST epidemiological cut-off (ECOFF) value for *both species* is 1.

APPENDIX 2: CRITERIA FOR BETA-LACTAM RESISTANCE PHENOTYPE CATEGORISATION

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 (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 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, Annex D – Data on presumptive ESBL-, AmpC- and/or carbapenemase-producing microorganisms and their resistance occurrence (routine and specific monitoring), Figure 1.*



CTX: Cefotaxime, CAZ: Ceftazidime, MEM: Meropenem, FOX: Cefoxitin, SYS: Synergy, CLA: Clavulanic acid, ETP: Ertapenem, IMI: Imipenem, ECOFF: Epidemiological Cutoff value.