DTU Fødevareinstituttet
DTU National Food Institute

The 32nd EURL-AMR Proficiency Test

Antimicrobial Susceptibility Testing of Escherichia coli, Salmonella and Campylobacter 2024

DANAK
PT Reg.nr. 516

European Union Reference Laboratory
Antimicrobial Resistance

DTU Fødevareinstituttet
DTU National Food Institute

The 32nd EURL-AMR Proficiency Test

Antimicrobial Susceptibility Testing of Escherichia coli, Salmonella and Campylobacter 2024

European Union Reference Laboratory – Antimicrobial Resistance
Final version, 1. edition, November 2025

Report by:
Athina Andrea
Susanne Karlsmose Pedersen
Rene S. Hendriksen

PT Coordinator:
Susanne Karlsmose Pedersen

Photo:
DTU National Food Institute

Published by:
DTU National Food Institute
Henrik Dams Allé
2800 Kgs. Lyngby

ISBN:
978-87-7586-067-8

food.dtu.dk

Funded by
the European Union

DANAK
PT Reg.nr. 516

European Union Reference Laboratory Antimicrobial Resistance

Table of Contents

List of Appendices

- **Appendix 1**: Pre-notification
- **Appendix 2a**: Protocol
- **Appendix 2b**: Test Forms
- **Appendix 3a**: Cover Letter
- **Appendix 3b**: Instructions for opening and reviving lyophilised cultures
- **Appendix 3c**: Subculture and maintenance of Quality Control strains
- **Appendix 4**: Expected MIC data for *test strains*

SUMMARY

The 32nd Proficiency Test (PT) for Antimicrobial Susceptibility Testing (AST) was launched in Autumn 2024 by the EU Reference Laboratory for Antimicrobial Resistance (EURL-AMR) at the National Food Institute, Technical University of Denmark (DTU Food). This PT evaluated the capacity of National Reference Laboratories (NRL-AR) of EU Member States and other European countries to accurately perform AST of *Escherichia coli*, *Salmonella*, and *Campylobacter* using broth microdilution (BMD). DTU Food is accredited by DANAK under ISO/IEC 17043:2010 for the organisation of PT schemes, ensuring high quality assurance standards.

The test comprised four components: **(1)** Phenotypic AST of *test strains* by BMD, **(2)** BMD method control using reference and additional *QC strains*, **(3)** Classification of β-lactam resistance mechanisms in *E. coli* and *Salmonella* based on phenotypic criteria, and **(4)** Species identification of *Campylobacter* strains. A total of 32 laboratories participated, using a range of recognized guidelines and standards for BMD (EUCAST, CLSI, ISO 20776-1:2019, TREK). Laboratories were instructed to report MIC values and to interpret phenotype classification based on Epidemiological cut-off values (ECOFFs) defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

AST Performance for Test Stains: Across the three organisms, participants demonstrated a high degree of concordance with expected results, achieving an overall deviation rate of 0.6% for the phenotype classification based on ECOFFs. The lowest deviation was observed in the *Campylobacter* trial (0.2%), followed by *E. coli* (0.3%) and *Salmonella* (1.0%).

AST Performance for Reference & Additional *QC strains*: Evaluation of MIC results for *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560 against CLSI-defined QC ranges showed high accuracy, with ≤0.6% deviation across organisms. For the first time, provisional QC ranges were defined for the *A. baumannii* strain 2012-70-100-69 based on cumulative data from previous PT rounds. Although not yet evaluated in 2024, these ranges are intended to be implemented in the 2025 PT to enhance standardisation of MIC assessments.

β-Lactam Resistance Mechanism Classification: Eight strains each of *E. coli* and *Salmonella* were included to assess participants' ability to correctly identify ESBL-, AmpC-, and carbapenemase-producing phenotypes. Laboratories achieved 98.7% correct identification for *E. coli* and 97.8% for *Salmonella*.

Species Identification of *Campylobacter*: All participating laboratories correctly identified the species of the *Campylobacter test strains*, confirming proficiency and consistency in species-level identification using either in-house methods or the EURL-AMR protocol.

Overall, the 2024 EURL-AMR AST PT confirmed the strong analytical capacity within the EURL-AMR network for all the PT focus areas.

1. INTRODUCTION

As the EU Reference Laboratory for Antimicrobial Resistance (EURL-AMR), the National Food Institute (DTU Food) launched the 32nd Proficiency Test (PT) in autumn 2024 for Antimicrobial Susceptibility Testing (AST) of commensal and zoonotic bacteria. DTU Food is accredited by the Danish Accreditation Fund (DANAK), under accreditation number 516, as a provider of proficiency testing schemes in accordance with the DS/EN ISO/IEC 17043 standard "Conformity assessment — General requirements for proficiency testing". The accreditation covers the organisation and delivery of PTs for zoonotic pathogens and indicator organisms, ensuring compliance with internationally recognised quality criteria.

The EURL-AMR PT for AST has been conducted annually since 2006, with the overall aim to evaluate and improve the comparability of Antimicrobial Resistance (AMR) surveillance data reported to the European Food Safety Authority (EFSA) by the National Reference Laboratories for AMR (NRL-AR) as part of the official EU AMR monitoring programme, under Commission Implementing Decision 2020/1729 (1). Specifically, the PT is designed to monitor the quality of AST results produced by participating NRL-ARs, to identify laboratories that may benefit from additional support to enhance their performance, and to highlight potential topics for further research or collaborative activities.

The 32nd EURL-AMR PT for AST is comprised of four main components:

- **COMPONENT 1 - AST of *Test strains***

  Component 1 involves AST by broth microdilution (BMD) of *E. coli*, *Salmonella* and *Campylobacter test strains*, including the determination of minimum inhibitory concentration (MIC) values and Epidemiological cut-off values (ECOFF)-based phenotypic interpretation of each strain, using a defined panel of antimicrobials, specific to each organism.

- **COMPONENT 2 - AST of Reference & QC strains**

  Component 2 involves AST by BMD of selected *reference strains* for each organism, with evaluation of the MIC values against quality control (QC) ranges published in the most recent guidelines from the Clinical and Laboratory Standards Institute (CLSI) (2,3). Additional *QC strains* are also included for further testing but are not part of the evaluation.

- **COMPONENT 3 - β-Lactam Resistance Mechanism Identification of *Test strains***

  Component 3 entails the identification of the β-lactam resistance mechanism phenotype in the relevant *E. coli* and *Salmonella test strains*, in line with EFSA guidelines (4), classified as Extended-Spectrum β-Lactamase (ESBL)-producing, AmpC β-lactamase-producing (AmpC), or Carbapenemase-producing, based on BMD results.

- **COMPONENT 4 - Species Identification of *Campylobacter Test strains***

  Component 4 covers the species identification of the *Campylobacter test strains*, specifically distinguishing between *C. jejuni* and *C. coli*.

The results of the 32nd EURL-AMR PT for AST are presented in this report, which is approved in its final version by a technical advisory group composed by representatives from all NRL-AR's, who met at the annual EURL-AMR workshop on September 23rd, 2025. The present report, at its final version, is publicly available at the EURL-AMR website (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar).

2. PARTICIPANTS

A pre-notification (**Appendix 1**), announcing the 32nd EURL-AMR Proficiency Test for AST of *E. coli*, *Salmonella*, and *Campylobacter*, was distributed via email on August 6th, 2024 to the EURL-AMR network contact list, which includes all EU Member States as well as Iceland, Moldova, North Macedonia, Norway, Serbia, Switzerland and the United Kingdom. Laboratories from non-EU countries or those not designated as their country's NRL-AR were charged a participation fee, while one NRL-AR per EU Member State participated free of charge. Participating laboratories are identified by unique codes, known only to the respective laboratories. The complete list of laboratory codes is confidential and accessible only to designated representatives of each NRL-AR and the European Commission. This report provides data from 32 countries including all 27 EU member states and 5 additional European countries. Each country is represented by a single NRL-AR for each organism, resulting in a total of 32 sets of results from each of the *E. coli* and *Salmonella* trials, and 29 sets of results for the *Campylobacter* trial.

3. STRAINS & ANTIMICROBIAL PANELS

An overview of the *test strains*, the *reference strains* as well as the additional *QC strains* included in the 32nd EURL-AMR PT for AST is presented in

Table *1*. Eight *test strains* for each organism (*E. coli*, *Salmonella* and *Campylobacter*) were selected among isolates from DTU Food's strain collection based on their AMR profiles.

To ensure long-term consistency and comparability of results across PT rounds, *internal control* strains were included in the study for each trial:

- *E. coli* (EURL EC-19.3)
- *Salmonella* (EURL S-19.5)
- *Campylobacter* (EURL C-19.2)

These strains have been used in previous PT rounds and are reintroduced each year to serve as benchmark strains. Their inclusion allows for the assessment of year-to-year reproducibility, helping to verify the stability of laboratory performance over time and to detect inconsistencies.

The *reference strains E. coli* CCM 3954 (ATCC 25922) and *C. jejuni* CCM 6214 (ATCC 33560) were provided to all laboratories upon joining the PT scheme, along with instructions for proper storage and maintenance for quality assurance and use in future proficiency tests. Additionally, the EURL-AMR distributed *Acinetobacter baumannii* (2012-70-100-69) and *C. coli* (215-QC-ETP) to support additional method QC during AST for *E. coli*/*Salmonella* and *Campylobacter*, respectively. The *A. baumannii* QC strain was included as a control for Panel 2 (EUVSEC2), due to its higher expected MIC values for most β-lactams compared to the *E. coli* ATCC 25922 reference strain. This makes it more suitable for verifying the mid-range concentrations of the antimicrobial agents tested in this panel. The *C. coli* QC strain was selected based on its expected MIC for ertapenem, which lies at the upper end of the test range (0.125–4 mg/L). In contrast, the *C. jejuni* reference strain ATCC 33560 has MIC value for ertapenem at the lower end of the range, making the *C. coli* QC strain more appropriate for QC.

The antimicrobial compounds tested in the 32nd EURL-AMR PT for AST are listed in **Table 2**, and correspond to the panel of antimicrobials defined in Commission Implementing Decision 2020/1729/EU (1). As part of the mandatory objective to identify the β-lactam resistance phenotype as ESBL-, AmpC-, or Carbapenemase-producing for *E. coli* and *Salmonella* strains, two antimicrobial panels were used for these organisms. Specifically, strains that exhibited resistance to cephalosporins (cefotaxime, ceftazidime) and/or meropenem in Panel 1 were further tested using Panel 2, in accordance with the specifications of the Implementing Decision.

Table 1. Test, reference and quality control strains included in the 32nd EURL-AMR PT for AST for *Escherichia coli*, *Salmonella* & *Campylobacter*. Internal control strains are indicated with an asterisk.

STRAIN CATEGORY	**STRAIN CODE**		
	E. coli	***Salmonella***	***Campylobacter***
TEST STRAINS	EURL EC-19.1	EURL S-19.1	EURL C-19.1
	EURL EC-19.2	EURL S-19.2	EURL C-19.2*
	EURL EC-19.3*	EURL S-19.3	EURL C-19.3
	EURL EC-19.4	EURL S-19.4	EURL C-19.4
	EURL EC-19.5	EURL S-19.5*	EURL C-19.5
	EURL EC-19.6	EURL S-19.6	EURL C-19.6
	EURL EC-19.7	EURL S-19.7	EURL C-19.7
	EURL EC-19.8	EURL S-19.8	EURL C-19.8
REFERENCE STRAINS	*E. coli* CCM3954 (ATCC 25922)		*C. jejuni* CCM6214 (ATCC33560)
QUALITY CONTROL STRAINS	*Acinetobacter baumannii 1912-70-100-69*		*C. coli* 215-QC-ETP

Table 2. Antimicrobial compounds and concentration ranges used for broth microdilution testing in the 32nd EURL-AMR PT on AST. *E. coli* or *Salmonella test strains* resistant to cefotaxime, ceftazidime and/or meropenem in Panel 1 were additionally tested in Panel 2 to confirm the β-lactam resistance phenotype.

ORGANISM	**PANEL**	**ANTIMICROBIAL CLASS**	**ANTIMICROBIAL COMPOUND**	**TEST RANGE (mg/L)**
E. coli & Salmonella	**Panel 1**	**AMINOGLYCOSIDES**	Amikacin	4-128
			Gentamicin	0.5-16
		β-LACTAMS - PENICILLINS	Ampicillin	1-32
		β-LACTAMS - CEPHALOSPORINS	Cefotaxime	0.25-4
			Ceftazidime	0.25-8
		β-LACTAMS - CARBAPENEMS	Meropenem	0.03-16
		MACROLIDES	Azithromycin	2-64
		PHENICOLS	Chloramphenicol	8-64
		POLYMYXINS	Colistin	1-16
		QUINOLONES	Ciprofloxacin	0.015-8
			Nalidixic acid	4-64
		SULFONAMIDES	Sulfamethoxazole	8-512
		TETRACYCLINES	Tetracycline	2-32
			Tigecycline	0.25-8
		TRIMETHOPRIM	Trimethoprim	0.25-16
	Panel 2	**β-LACTAMS - CEPHALOSPORINS** with or without β-lactamase inhibitor	Cefotaxime	0.25 – 64
			Cefotaxime/Clavulanic acid	0.06/4 - 64/4
			Ceftazidime	0.25 - 128
			Ceftazidime/Clavulanic acid	0.125/4 - 128/4
			Cefepime	0.06 - 32
			Cefoxitin	0.5 - 64
		β-LACTAMS - CARBAPENEMS	Ertapenem	0.015-2
			Imipenem	0.125 - 16
			Meropenem	0.03 - 16
		β-LACTAMS - PENICILLINS	Temocillin	0.5-64
Campylobacter	**EUCAMP3 (Panel 1)**	**AMINOGLYCOSIDES**	Gentamicin	0.25-16
		β-LACTAMS - CARBAPENEMS	Ertapenem	0.125-4
		MACROLIDES	Erythromycin	1-512
		PHENICOLS	Chloramphenicol	2-64
		QUINOLONES	Ciprofloxacin	0.125-32
		TETRACYCLINES	Tetracycline	0.5-64

4. TESTING PROCEDURE

In October 2024, bacterial strains were shipped to participating laboratories in transport swabs, accompanied by a cover letter (**Appendix 3a**). The shipment was classified as UN3373, biological substances–category B, and complied with IATA regulations. The PT protocol and other relevant documents, including instructions for handling test and *reference strains* (**Appendix 2**, **Appendix 3b** and **Appendix 3c**), were made available on the EURL-AMR website (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/eqas).

Participants were instructed to perform broth microdilution (BMD) and determine Minimum Inhibitory Concentrations (MICs) according to their routine procedures and the methodology outlined in Commission Implementing Decision 2020/1729/EU (1), which is described in ISO 20776-1:2019 (5). Interpretation of MIC values was to follow the ECOFFs listed in Tables 2–6 of the protocol (**Appendix 2**), as defined in the EFSA Manual for reporting 2023 antimicrobial resistance data (6), unless otherwise stated. According to these criteria, a strain is categorised as "R" - resistant if its MIC exceeds the ECOFF and "S" - susceptible if it is equal to or below it. However, as ECOFFs are not clinical breakpoints, the correct terminology is "wild-type" (WT) or "non-wild-type" (non-WT). Nevertheless, for continuity across PT reports, which historically use the terms "R" and "S", the categorisation in this PT continues to follow the R/S format.

5. EVALUATION

The results submitted by participants in the 32nd EURL-AMR Proficiency Test were evaluated based on predefined criteria specific to each component (

Table 3):

- For **COMPONENT 1** (AST of *test strains*), participants submitted MIC values and corresponding phenotype interpretations (R/S) for the relevant antimicrobial panels (**Table 2**). While MIC values themselves were not scored, the R/S interpretations were evaluated against the expected results provided by the EURL-AMR.
- For **COMPONENT 2** (AST of *reference strains* and *QC strains*), MIC values reported for the designated *reference strains* were evaluated against the CLSI quality control ranges defined in M100 (2) for *E. coli* ATCC 25922 and VET06 (3) for *C. jejuni* ATCC33560. MIC values submitted for the additional *QC strains* distributed by EURL-AMR were collected for methodological monitoring only and were not scored.
- For **COMPONENT 3** (β-lactam resistance mechanism identification), participants' phenotypic classifications (ESBL, AmpC, carbapenemase, or susceptible) were evaluated against the EURL-AMR expected results.
- For **COMPONENT 4** (*Campylobacter* species identification), participants' submitted data were also scored based on agreement with the EURL-AMR expected species.

Each submitted result under evaluation was assigned a score ("**1**", "**0**" or "**Blank**") according to

Table 3. All results were submitted electronically via the EURL-AMR webtool using a secure individual login. In March 2025, participants received access to an automatically generated individual report containing the evaluation of their submitted results.

Table 3. Overview of the results submitted, evaluation criteria, and scoring principles for each of the PT components. For all evaluated components, a score of "1" indicates concordance with expected results, "0" indicates a deviation, and "blank" represents missing or excluded data.

Component	Type of result	Evaluation basis	Scoring principle
1. AST of test strains	MIC values	Not evaluated	-
	R/S (ECOFF-based) interpretation	EURL-AMR expected data	1 = expected result 0 = unexpected blank = no result or excluded (breakpoint issue)
2. AST of reference & QC strains	MIC values of reference strains	CLSI M100 / VET06 QC ranges	1 = within QC range 0 = outside range blank = no result
	MIC values of QC strains	Not evaluated	–
3. β-lactam mechanism identification	Classification as ESBL, AmpC, carbapenemase or β-lactam-susceptible	EURL-AMR expected data	1 = expected 0 = unexpected blank = no result
4. Species identification	*Campylobacter* species (*C. jejuni* / *C. coli*)	EURL-AMR expected data	1 = expected 0 = unexpected blank = no result

5.1. Evaluation of COMPONENT 1 – AST of *Test strains*

Component 1 assessed participants' phenotype interpretation of obtained MIC values based on ECOFFs. MIC values were collected but not evaluated. Phenotypic results were compared to EURL-AMR expected data (**Appendix 4**). A score of "1" was assigned for each expected result submitted, "0" for unexpected results, and left blank if results were not submitted or the combination was excluded due to *breakpoint issues* (see below).

The expected MIC values were generated by performing broth microdilution (BMD) on all *test strains* on two separate occasions at the DTU Food. DTU Food is accredited by DANAK under the standard ISO 17025 (accreditation number 350), which specifies the general requirements for the competence of testing and calibration laboratories. MIC determination of the *E. coli* and *Salmonella test strains* was performed using the Sensititre panel EUVSEC3 (Panel 1) and EUVSEC2 (Panel 2), while for *Campylobacter* using the Sensititre panel EUCAMP3 (Trek Diagnostic Systems Ltd, UK).

The expected MIC values were verified by the Laboratory of Gastrointestinal Bacteria, Department for Bacteria, Parasites & Fungi, Statens Serum Institut, Denmark, also accredited by DANAK under the standard ISO 17025. MIC values were confirmed at DTU Food after the preparation of the transport swabs for distribution to participants. When MIC values from the different tests were not in agreement but varied plus or minus a one two-fold dilution step, the latest value obtained by DTU Food was selected as the expected value. MIC results were interpreted using the interpretative criteria listed the PT protocol (**Appendix 2**).

5.1.1. Accepted AST Method Variation

A **one two-fold dilution difference in MIC values is considered acceptable variation,** due to inherent limitations of the broth microdilution method. When a MIC result falls close to the ECOFF, such minor deviations may lead to different phenotype interpretations (i.e., S versus R or vice versa). These cases are referred to in this report as ***breakpoint issues***.

5.1.2. Network-agreed Thresholds

The evaluation of AST results for the *test strains* is based on the following **thresholds**, agreed upon within the EURL-AMR network:

- **Laboratory-Level Performance Threshold**: A maximum acceptable deviation of **5%** was defined per laboratory, for each organism, based on the full set of *test strains* for each organism (*E. coli*, *Salmonella*, and *Campylobacter*).
- **Strain–Antimicrobial Combination Threshold**: If the percentage of correct results for a given strain–antimicrobial combination fell below 75% across all laboratories, the combination was subjected to further analysis. When deviations were attributed to *breakpoint issues* (see above), all scores for that combination were blanked. For **sporadic cases**, where fewer participants are affected, the scores in the webtool for the affected strain-antimicrobial combinations were adjusted to "1".

5.2. Evaluation of COMPONENT 2 – AST of *Reference* & *QC strains*

For the *reference strains* (*E. coli* ATCC 25922 and *C. jejuni* ATCC 33560), submitted MIC values were evaluated against the CLSI QC ranges defined in **Table 4** and **Table 5**. Values within the QC range were scored as "1", and values outside the range as "0". Non-submission of results was not penalised. MIC results for the EURL-AMR additional method *QC strains* were collected in the webtool and included in individual laboratory reports but were not evaluated.

Table 4. Quality control (QC) intervals for the reference strain *E. coli* ATCC25922, according to CLSI M100, 34rd ed.(2).

***E. coli* ATCC25922**	
Antimicrobial	**MIC range mg/L**
Amikacin	0.5-4
Ampicillin	2-8
Azithromyciin	-
Cefepime	0.016-0.12
Cefotaxime	0.03-0.12
Cefotaxime+Clavulanic acid	-
Cefoxitin	2-8
Ceftazidime	0.06-0.5
Ceftazidime+Clavulanic acid	-
Chloramphenicol	2-8
Ciprofloxacin	0.004-0.016
Colistin	-
Ertapenem	0.004-0.016
Gentamicin	0.25-1
Imipenem	0.06-0.5
Meropenem	0.008-0.06
Nalidixic acid	1-4
Sulfamethoxazole(1)	8-32
Temocillin	-
Tetracycline	0.5-2
Tigecycline	0.03-0.25
Trimethoprim	0.5-2

(1)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 (Table 5A-1, CLSI M100, 34rd ed. [2]).

Table 5. Quality control (QC) intervals for the reference strain *C. jejuni* ATCC 33560, according to CLSI VET06, 1st ed., 2017 (3).

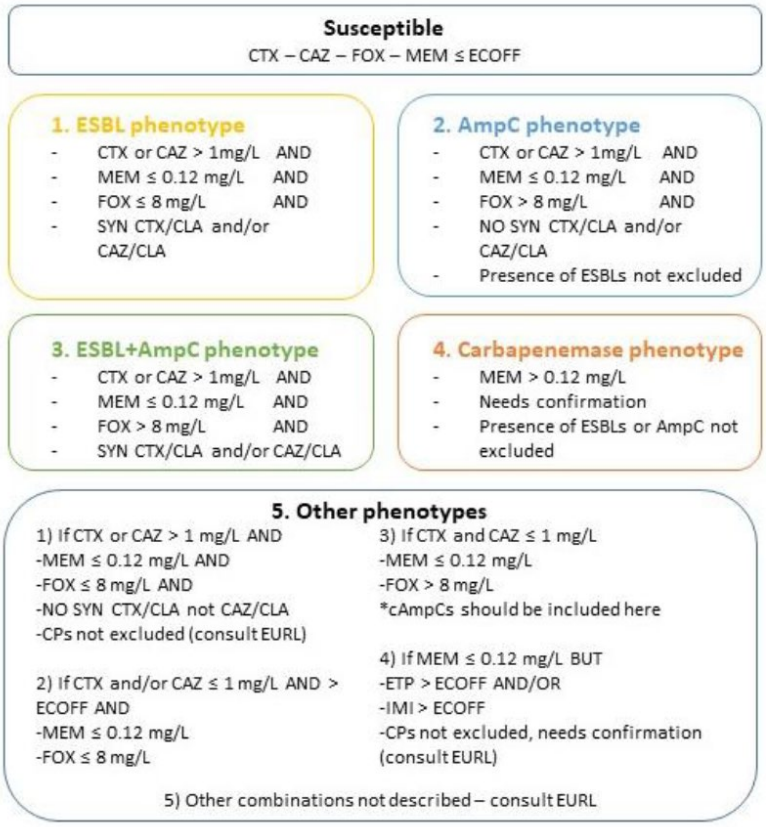
***C. jejuni* ATCC 33560**		
Antimicrobial	**MIC range mg/L**	
	36-37°C/48h	**42°C/24h**
Chloramphenicol	1-8	1-4
Ciprofloxacin	0.06-0.25	0.03-0.125
Erythromycin	0.5-2	0.25-2
Ertapenem	No data	No data
Gentamicin	0.5-2	0.25-2
Tetracycline	0.25-2	0.25-1

5.3. Evaluation of COMPONENT 3 – β-Lactam Resistance Mechanism Identification

For the *E. coli* and *Salmonella test strains*, participants were required to classify the β-lactam resistance phenotype as ESBL, AmpC, or carbapenemase producers, or as susceptible wild-type strains, using the EFSA β-lactam resistance phenotype categorisation algorithm (**Figure 1**) (4). Submitted phenotypes were evaluated against the EURL-AMR expected results. A score of “1” was assigned for submission of an expected phenotype, “0” for an unexpected phenotype, and blank if no phenotype was submitted.

The expected phenotypes for β-lactam resistance mechanisms were identified based on the MIC values for β-lactams, interpreted according to the most recent EFSA recommendations included in the PT protocol (**Appendix 2**). These phenotypes were further confirmed by Whole Genome Sequencing (WGS).

Discrepancies in phenotype interpretation might be observed near the EFSA-defined MIC thresholds for cefoxitin (>8 mg/L for AmpC) and meropenem (>0.12 mg/L for carbapenemase). Because the broth microdilution method allows for ±1 two-fold dilution variability, MIC values close to these thresholds can lead to different—but technically valid—phenotype classifications under the EFSA algorithm. All technically valid phenotypes are accepted and scored as “1”.



Susceptible
CTX – CAZ – FOX – MEM ≤ ECOFF

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

2. AmpC phenotype
- CTX or CAZ > 1mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- NO SYN CTX/CLA and/or CAZ/CLA
- Presence of ESBLs not excluded

3. ESBL+AmpC phenotype
- CTX or CAZ > 1mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- SYN CTX/CLA and/or CAZ/CLA

4. Carbapenemase phenotype
- MEM > 0.12 mg/L
- Needs confirmation
- Presence of ESBLs or AmpC not excluded

5. Other phenotypes

1) If CTX or CAZ > 1 mg/L AND
-MEM ≤ 0.12 mg/L AND
-FOX ≤ 8 mg/L AND
-NO SYN CTX/CLA not CAZ/CLA
-CPs not excluded (consult EURL)

2) If CTX and/or CAZ ≤ 1 mg/L AND > ECOFF AND
-MEM ≤ 0.12 mg/L
-FOX ≤ 8 mg/L

3) If CTX and CAZ ≤ 1 mg/L
-MEM ≤ 0.12 mg/L
-FOX > 8 mg/L
*cAmpCs should be included here

4) If MEM ≤ 0.12 mg/L BUT
-ETP > ECOFF AND/OR
-IMI > ECOFF
-CPs not excluded, needs confirmation (consult EURL)

5) Other combinations not described – consult EURL

Figure 1. EFSA β-lactam resistance phenotype categorisation algorithm (4) for the classification of *E. coli* and *Salmonella* strains based on β-lactam MIC values and combinations with the β-lactamase inhibitor clavulanic acid (CLA). The scheme defines phenotypic categories including extended-spectrum β-lactamase (ESBL), AmpC, ESBL+AmpC, and carbapenemase producers, as well as other phenotypes. Classification is based on MIC values for cefotaxime (CTX), ceftazidime (CAZ), cefoxitin (FOX), meropenem (MEM), and interpretation of synergy tests between cephalosporins and CLA, *i.e,* CTX/CLA and CAZ/CLA.

5.4. Evaluation of COMPONENT COMPONENT 4 – Species Identification of *Campylobacter*

Campylobacter species (**Table 6**) was identified by Matrix-Assisted Laser Desorption/Ionization coupled to Time-of-Flight Mass Spectrometry (MALDI-TOF MS), using MALDI Biotyper® from Bruker, server version 4.1.100 (PYTH) 174 2019-06-158_01-16-09. All *Campylobacter test strains* were species identified with score values of 2.00-3.00, *i.e.* high-confidence identification (green category), and high consistency (category A), in two different occasions at DTU Food. In addition, species identity of all strains was confirmed by whole genome sequencing (WGS). Submission of the expected species was scored with "1", submittion of an unexpected species with a "0" and non submission of species was not scored (blank).

Table 6. Expected *Campylobacter* species for the eight *test strains* of the *Campylobacter* trial.

Strain	Expected species
EURL C-19.1	*Campylobacter coli*
EURL C-19.2	*Campylobacter jejuni*
EURL C-19.3	*Campylobacter coli*
EURL C-19.4	*Campylobacter coli*
EURL C-19.5	*Campylobacter coli*
EURL C-19.6	*Campylobacter jejuni*
EURL C-19.7	*Campylobacter coli*
EURL C-19.8	*Campylobacter coli*

6. RESULTS & DISCUSSION

6.1. Broth Microdilution Standards & Guidelines

The participants were requested to report the standards and guidelines used for performing broth microdilution among the following options: EUCAST (ISO 20776-1) (5), TREK (7), and CLSI (8,9). A summary of the use of the different standards/guidelines for each organism is provided in **Figure 2**.

Among the 32 laboratories conducting BMD for *E. coli* and *Salmonella* trials, the most frequently used standards/guidelines were EUCAST (ISO 20776-1), reported by 15 laboratories for *E. coli* trial and 16 laboratories for *Salmonella*. TREK guidelines were used as a standalone method by 6 laboratories for *E. coli* and 5 for *Salmonella*, while a combination of EUCAST (ISO 20776-1) with TREK was reported by 4 laboratories for each organism. CLSI guidelines were reported alone by 5 laboratories for both trials, and a combination of CLSI and TREK was used by 2 laboratories.

Among the 29 laboratories conducting BMD for *Campylobacter*, the most used standards were TREK and CLSI, each reported by 6 laboratories. EUCAST (ISO 20776-1) was used by 7 laboratories, while the remaining laboratories applied various combinations of standards, including CLSI with TREK (n=3) or ISO 20776-1 with TREK (n=1). One laboratory reported using all four major standards. Finally, four laboratories reported using ISO 20776-1:2019 alone, which is unexpected, as this standard does not apply to fastidious organisms.

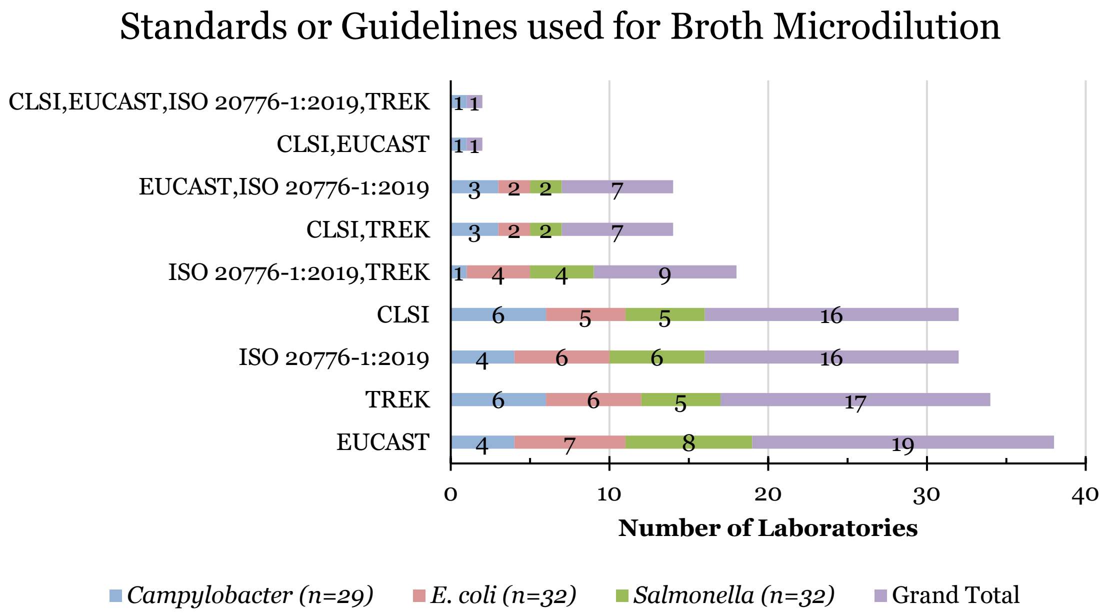


Figure 2. Standards & Guidelines used for broth microdilution (BMD) by laboratories participating in antimicrobial susceptibility testing (AST) for *Escherichia coli*, *Salmonella* and *Campylobacter* trials.

6.2. Component 1: AST Data of Test Strains

6.2.1. AST Data Omitted from the Evaluation

The percentage deviation was calculated for each strain–antimicrobial combination. Combinations with a deviation of 25% or more were further reviewed to determine whether discrepancies were due to a *breakpoint issue* (as described in **Section 0**). If deviations were primarily attributed to such issues, the combination was excluded from evaluation–meaning all associated scores (both “1” and “0”) were blanked. An overview of all strain–antimicrobial combinations omitted from scoring is provided in **Table** 7.

Table 7. Strain-antimicrobial combinations omitted from the evaluation (scores blanked).

Organism	Strain	Panel	Antimicrobial	Sum of Score	Count of Score	% Deviation (>25%)
E. coli	EURL EC-19.1	Panel 2	Cefoxitin	23	38	39
Salmonella	EURL S-19.3	Panel 1	Tigecycline	20	39	49
	EURL S-19.4	Panel 1	Tigecycline	26	39	33
	EURL S-19.8	Panel 1	Tigecycline	24	39	38
Campylobacter	EURL C-19.8	Panel 1	Ertapenem	18	34	47

6.2.2. Trend Analysis

Participants’ performance in the 2024 PT AST was consistently high. The overall deviation rates for the test strains were 0.3% for *E. coli*, 1.0% for *Salmonella* and 0.2% for *Campylobacter* (**Table 8**). The internal control strains supported these findings: the *E. coli* internal control and the *Salmonella* internal control strain EURL S-19.5 each showed a deviation of 0.6%, while the *Campylobacter* internal control strain had no deviations (**Table *8***).

Table 8. Overview of participant performance in the 2024 EURL-AMR AST Proficiency Test, showing total correct results (Sum of Score), total evaluated results (Count of Score), and the resulting percentage deviation for each trial (*E. coli*, *Salmonella*, *Campylobacter*). The table includes both the full set of results per trial ("All results"), as well as the corresponding yearly Internal Control Strains used to assess long-term consistency across PT rounds.

Trial	Category	Sum of Score	Count of Score	% Deviation
E. coli	**All results**	**6089**	**6109**	**0.3**
	Internal Control Strain	785	790	0.6
Salmonella	**All results**	**5925**	**5986**	**1.0**
	Internal Control Strain	781	786	0.6
Campylobacter	**All results**	**1257**	**1260**	**0.2**
	Internal Control Strain	172	172	0.0

In the past ten years, deviation levels for *E. coli*, *Salmonella* and *Campylobacter* have remained consistently low for both test strains and internal control strains. Year-to-year fluctuations are minor, and no systematic issues are visible. Overall, the data demonstrate stable and reliable AST performance across all three organism groups over the past decade (**Figure *3***).

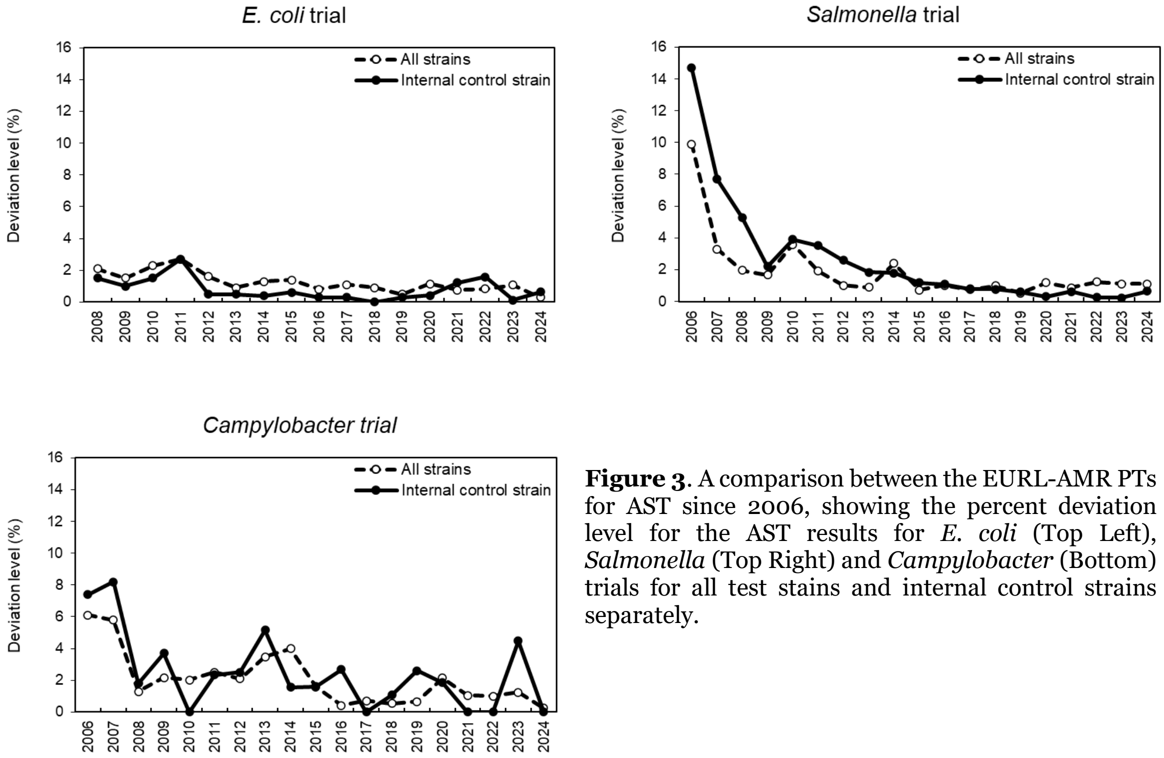


Figure 3. A comparison between the EURL-AMR PTs for AST since 2006, showing the percent deviation level for the AST results for *E. coli* (Top Left), *Salmonella* (Top Right) and *Campylobacter* (Bottom) trials for all test stains and internal control strains separately.

Comparing the log2-transformed MIC ratio (obtained/expected) across laboratories provides a clear overview of agreement between reported and expected MIC values (**Figure 4**). The distribution is sharply centred at log2 = 0, which corresponds to a MIC ratio of 1 (perfect agreement). Most results fall within log2 values of –1 to +1, reflecting deviations of only one dilution step, which is within normal methodological variation for broth microdilution. Values beyond ±1 occur only rarely and overall, the log2 distribution shows that participating laboratories report MIC values highly consistent with the expected results, with only occasional larger deviations.

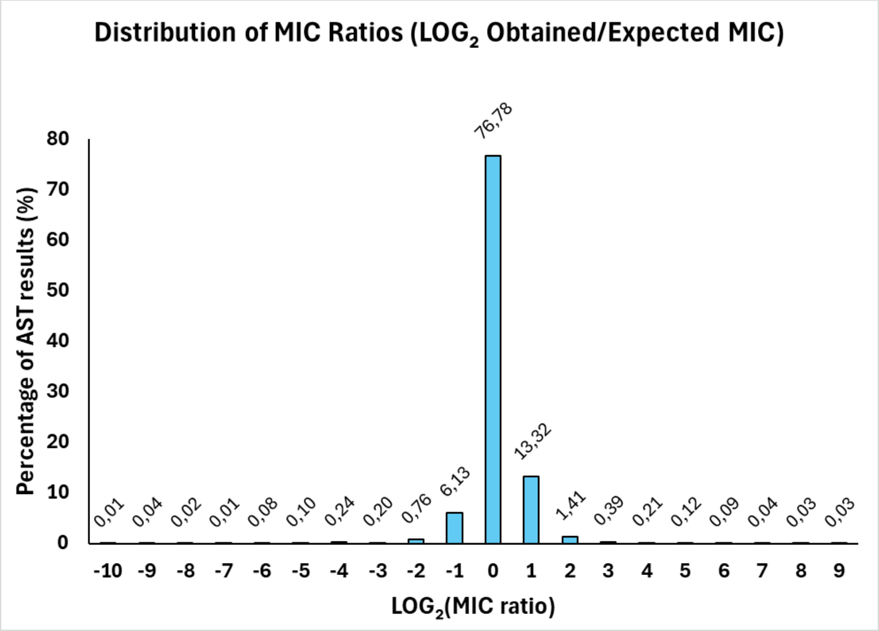


Figure 4. Distribution of MIC ratios (log2 obtained/expected MIC) expressed as percentage of all AST results. The distribution is strongly centred at 0, with smaller proportions at ±1 and very cases with more than one two-fold dilution differencer, demonstrating that the vast majority of reported MIC values are within the accepted method variation (±1 two-fold dilution).

6.2.3. Laboratory Performance

Table 9 presents the performance of participating laboratories in Component 1 (AST results) of the 2024 EURL-AMR AST PT. For each organism (E. coli, Salmonella, Campylobacter), the table reports the total number of correct results (Sum of Score), the total number of evaluated results (Count of Score), and the resulting percentage deviation. As described in **Section 0**, the maximum acceptable deviation level is 5% per laboratory per organism, based on all eight test strains. Laboratories exceeding this threshold are highlighted in red. The table also provides the mean deviation for each organism across all participating laboratories.

In the ***E. coli*** trial, 25 of the 32 laboratories reported no deviations. Seven laboratories showed minor deviations ranging from 0.5% to 2.1%. No laboratory exceeded the 5% deviation level. The average deviation across all laboratories was 0.2%. For ***Salmonella***, 18 of the 32 laboratories reported no deviations. Thirteen laboratories showed deviations ranging from 0.5% to 4.8%, and one laboratory exceeded the acceptable deviation level with 12.4%. This laboratory reported a total of 22 incorrect phenotype interpretations. Of these, 16 were associated with MIC values that were two to six two-fold dilutions **lower** than the expected MIC value, and six were due to MIC values that were two to five two-fold dilutions **higher** than expected. The average deviation across all laboratories was 0.3%. In the ***Campylobacter*** trial, 27 of the 29 laboratories reported no deviations. Two laboratories showed deviations of 2.1% and 4.3%. No laboratory exceeded the 5% deviation level. The average deviation across all laboratories was 1.1%.

Table 9. Percent deviation level for each laboratory for the AST results based on the submitted sum of score and count of score for each trial. Laboratories are listed in ascending order by NRL-AR code. Deviation levels over the limit of 5% established by the EURL-AMR network are marked in red. When laboratories obtained all results correct, the cells are makred in green. No data are indicated as "ND".

Laboratory Code	***E. coli***			***Salmonella***			***Campylobacter***		
	Sum of Score	Count of Score	**% Deviation**	Sum of Score	Count of Score	**% Deviation**	Sum of Score	Count of Score	**% Deviation**
NRL-AR-002	199	199	🟩	171	173	1,2	47	47	🟩
NRL-AR-004	189	189	🟩	178	187	4,8	47	47	🟩
NRL-AR-006	186	189	1,6	187	187	🟩	35	35	🟩
NRL-AR-009	199	199	🟩	196	196	🟩	41	41	🟩
NRL-AR-011	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-012	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-014	ND	ND	ND	ND	ND	ND	47	47	🟩
NRL-AR-016	189	189	🟩	187	187	🟩	ND	ND	ND
NRL-AR-017	199	199	🟩	194	197	1,5	46	47	2,1
NRL-AR-018	189	189	🟩	186	187	0,5	41	41	🟩
NRL-AR-019	196	199	1,5	191	197	3,0	29	29	🟩
NRL-AR-020	199	199	🟩	197	197	🟩	41	41	🟩
NRL-AR-021	120	120	🟩	113	115	1,7	35	35	🟩
NRL-AR-022	199	199	🟩	196	197	0,5	47	47	🟩
NRL-AR-023	199	199	🟩	195	197	1,0	47	47	🟩
NRL-AR-025	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-026	189	189	🟩	186	186	🟩	47	47	🟩
NRL-AR-029	189	189	🟩	187	187	🟩	41	41	🟩
NRL-AR-030	188	189	0,5	172	177	2,8	41	41	🟩
NRL-AR-033	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-034	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-037	199	199	🟩	185	187	1,1	47	47	🟩
NRL-AR-038	196	199	1,5	197	197	🟩	ND	ND	ND
NRL-AR-040	185	189	2,1	155	177	12,4	45	47	4,3
NRL-AR-041	199	199	🟩	197	197	🟩	41	41	🟩
NRL-AR-042	189	189	🟩	186	187	0,5	47	47	🟩
NRL-AR-045	189	189	🟩	185	187	1,1	47	47	🟩
NRL-AR-056	189	189	🟩	187	187	🟩	47	47	🟩
NRL-AR-059	199	199	🟩	197	197	🟩	41	41	🟩
NRL-AR-060	189	189	🟩	187	187	🟩	35	35	🟩
NRL-AR-061	197	199	1,0	197	197	🟩	40	40	🟩
NRL-AR-062	185	189	2,1	184	187	1,6	ND	ND	ND
NRL-AR-066	199	199	🟩	197	197	🟩	ND	ND	ND
		Average	**0,2**		**Average**	**0,3**		**Average**	**1,1**
		SDEVP	**0,9**		**SDEVP**	**0,7**		**SDEVP**	**2,3**

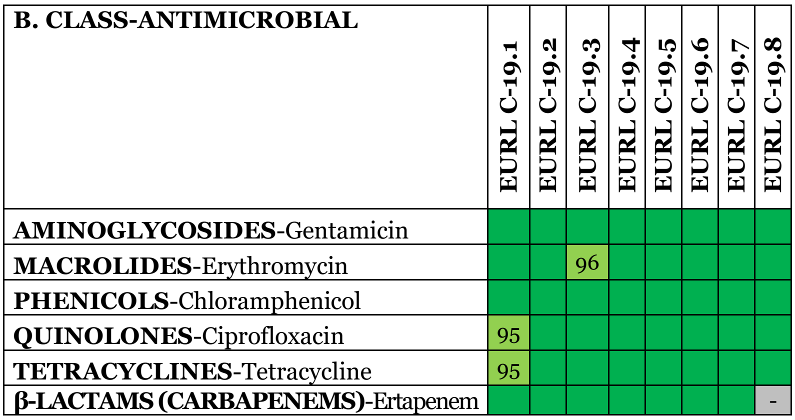
6.2.4. Performance for Strain-Antimicrobial Combinations

*The evaluation included 175 strain–antimicrobial combinations for the E. coli trial and 173 combinations for the Salmonella trial. From these, the majority achieved percent correct results above 95% (**Figure 5A**), indicating reliable testing methodologies. For E. coli, and Salmonella, four and seven combinations respectively had percent correct results ranging from 91 to 94% which were due to two or three incorrect phenotype interpretations per case. For the Campylobacter trial 47 strain-antimicrobial combinations were evaluated. From these almost all had 100% agreement (**Figure 5***

B. CLASS-ANTIMICROBIAL	**EURL C-19.1**	**EURL C-19.2**	**EURL C-19.3**	**EURL C-19.4**	**EURL C-19.5**	**EURL C-19.6**	**EURL C-19.7**	**EURL C-19.8**
AMINOGLYCOSIDES-Gentamicin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
MACROLIDES-Erythromycin	🟩	🟩	96	🟩	🟩	🟩	🟩	🟩
PHENICOLS-Chloramphenicol	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
QUINOLONES-Ciprofloxacin	95	🟩	🟩	🟩	🟩	🟩	🟩	🟩
TETRACYCLINES-Tetracycline	95	🟩	🟩	🟩	🟩	🟩	🟩	🟩
β-LACTAMS (CARBAPENEMS)-Ertapenem	🟩	🟩	🟩	🟩	🟩	🟩	🟩	-

Figure 5B). Only in three cases the percent correct results was 95-96% due to a single incorrect phenotype interpretation for each case.

	E. coli								***Salmonella***							
A. CLASS-ANTIMICROBIAL	**EURL EC-19.1**	**EURL EC-19.2**	**EURL EC-19.3**	**EURL EC-19.4**	**EURL EC-19.5**	**EURL EC-19.6**	**EURL EC-19.7**	**EURL EC-19.8**	**EURL S-19.1**	**EURL S-19.2**	**EURL S-19.3**	**EURL S-19.4**	**EURL S-19.5**	**EURL S-19.6**	**EURL S-19.7**	**EURL S-19.8**
AMINOGLYCOSIDES	🟩	🟩	🟩	🟩	🟩	🟩	98	🟩	🟩	🟩	98	🟩	🟩	🟩	🟩	🟩
Amikacin	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩
Gentamicin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
MACROLIDES - Azithromycin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	97	🟩	🟩	🟩	🟩	🟩
PHENICOLS - Chloramphenicol	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
POLYMYXINS - Colistin	🟩	🟩	94	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
QUINOLONES	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	95	🟩	97	98	🟩	97
Ciprofloxacin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	94	🟩	91	🟩	94	97	🟩	97
Nalidixic acid	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97
SULFONAMIDES - Sulfamethoxazole	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	97	97	🟩	97	97	🟩	🟩	97
TETRACYCLINES	97	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	98	97	97	98	98	98	97
Tetracycline	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	97	🟩	🟩	🟩	97
Tigecycline	94	🟩	🟩	🟩	🟩	🟩	🟩	🟩	94	97	-	-	97	97	97	-
TRIMETHOPRIM - Trimethoprim	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	97	🟩	🟩	🟩	🟩
β-LACTAMS - CARBAPENEMS	99	99	99	98	🟩	🟩	99	🟩	97	🟩	98	99	🟩	🟩	98	🟩
Ertapenem	97	🟩	🟩	94	🟩	🟩	🟩	🟩	97	🟩	97	🟩	🟩	🟩	97	🟩
Imipenem	🟩	97	97	97	🟩	🟩	92	🟩	97	🟩	🟩	97	🟩	🟩	97	🟩
Meropenem	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	97	🟩	🟩	🟩	🟩	🟩
β-LACTAMS - CEPHALOSPORINS	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	92	🟩	🟩	🟩	🟩	98
Cefotaxime	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	92	🟩	🟩	🟩	🟩	98
Cefotaxime-clavulanic acid	97	🟩	🟩	97	🟩	🟩	🟩	97	🟩	🟩	97	🟩	🟩	🟩	🟩	97
Cefepime	99	🟩	99	99	🟩	🟩	🟩	99	98	🟩	97	🟩	99	🟩	🟩	99
Cefoxitin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Ceftazidime	-	🟩	97	🟩	🟩	🟩	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Ceftazidime-clavulanic acid	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	92	🟩	98	🟩	🟩	98
β-LACTAMS - PENICILLINS	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩	97	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Ampicillin	98	🟩	🟩	🟩	🟩	🟩	🟩	🟩	98	🟩	95	🟩	🟩	🟩	🟩	🟩
Temocillin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	94	🟩	🟩	🟩	🟩	🟩



B. CLASS-ANTIMICROBIAL	**EURL C-19.1**	**EURL C-19.2**	**EURL C-19.3**	**EURL C-19.4**	**EURL C-19.5**	**EURL C-19.6**	**EURL C-19.7**	**EURL C-19.8**
AMINOGLYCOSIDES-Gentamicin	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
MACROLIDES-Erythromycin	🟩	🟩	96	🟩	🟩	🟩	🟩	🟩
PHENICOLS-Chloramphenicol	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩
QUINOLONES-Ciprofloxacin	95	🟩	🟩	🟩	🟩	🟩	🟩	🟩
TETRACYCLINES-Tetracycline	95	🟩	🟩	🟩	🟩	🟩	🟩	🟩
β-LACTAMS (CARBAPENEMS)-Ertapenem	🟩	🟩	🟩	🟩	🟩	🟩	🟩	-

Figure 5. Heat map showing participants' performance in antimicrobial susceptibility testing for the **A)** *E. coli* and *Salmonella* and **B)** *Campylobacter* test strains. Each cell displays the percentage of correct phenotype categorisations for a given antimicrobial–strain combination. Dark green indicates 100% agreement; light green, 95–99%; and yellow, 90–94%. Grey cells denote cases with no evaluable data (results excluded due to *breakpoint issues*).

6.3. Component 2: AST Data for *Reference* and QC *strains*

The AST results for *reference strains* serve as quality control, enabling assessment of methodological consistency across laboratories. Reported MIC values are evaluated against established QC ranges defined by CLSI, providing a benchmark to ensure the reliability of the obtained results. For the *reference strains* results, deviations were submitted MIC values that were outside the QC acceptance intervals according to the CLSI standards (see **Table 4** and **Table 5**).

The deviation levels for each reference strain were calculated based on the sum and count of scores of all submitted results and are presented in **Table 10**. A total of eight unexpected results were reported across the tested *reference strains*, with *E. coli* and *Salmonella* trials each accounting for three instances, while the *Campylobacter* reference strain had two instances. For *E. coli* ATCC25922 there were three MIC results outside the CLSI M100 (2) QC range and these were for ertapenem (0.15 mg/L), sulfamethoxazole (64 mg/L) and trimethoprim (0.25 mg/L). For *C. jejuni* ATCC 33560 there were three results outside the CLSI VET06 (3) QC range and these were gentamicin (0.25 mg/L) and tetracycline (0.12 mg/L).

The percent deviation levels were calculated for each laboratory, based on the sum and count of submitted scores (**Table 11**Error! Reference source not found.). The eight results outside the CLSI QC ranges were reported across six different laboratories. Two laboratories reported each two out-of-range results, while the remaining four laboratories each reported a single result outside the QC range. No particular patterns were identified, so the discrepancies were likely due to individual variability rather than systematic methodological issues in testing.

Table 10. Summary of AST results for *reference strains* across the three organism trials. The table presents the total number of correct results (Sum of Score), total results submitted (Count of Score), and the overall percentage deviation per trial. Evaluation was based on MIC values falling within the QC ranges defined by CLSI M100 (2) for *E. coli* and *Salmonella* and CLSI VET06 (3) for *Campylobacter*.

Trial	Reference strain	Sum of Score	Count of Score	% Deviation
E. coli	*E. coli* ATCC 25922	623	626	**0.5**
Salmonella	*E. coli* ATCC 25922	623	626	**0.5**
Campylobacter	*C. jejuni* ATCC 33560	138	140	**1.4**

Table 11. Percentage of correct AST results for the *reference strains* reported by participating laboratories for the *reference strains E. coli* ATCC25922 and *C. jejuni* ATCC 33560. Laboratories reporting no data are indicated as "ND". Laboratories are sorted in ascending order by NRL-AR code.

	***E. coli* ATCC25922**			***C. jejuni* (ATCC 33560)**		
Laboratory ID	Sum of Score	Count of Score	**% Correct Results**	Sum of Score	Count of Score	**% Correct Results**
NRL-AR-002	40	40	100	5	5	100
NRL-AR-004	40	40	100	5	5	100
NRL-AR-006	40	40	100	5	5	100
NRL-AR-009	40	40	100	ND	ND	ND
NRL-AR-011	40	40	100	5	5	100
NRL-AR-012	40	40	100	5	5	100
NRL-AR-014	ND	ND	ND	5	5	100
NRL-AR-016	40	40	100	ND	ND	ND
NRL-AR-017	40	40	100	5	5	100
NRL-AR-018	40	40	100	5	5	100
NRL-AR-019	40	40	100	5	5	100
NRL-AR-020	40	40	100	5	5	100
NRL-AR-021	25	26	96	5	5	100
NRL-AR-022	40	40	100	5	5	100
NRL-AR-023	40	40	100	5	5	100
NRL-AR-025	40	40	100	5	5	100
NRL-AR-026	40	40	100	5	5	100
NRL-AR-029	40	40	100	5	5	100
NRL-AR-030	40	40	100	5	5	100
NRL-AR-033	40	40	100	5	5	100
NRL-AR-034	40	40	100	4	5	80
NRL-AR-037	40	40	100	5	5	100
NRL-AR-038	40	40	100	ND	ND	ND
NRL-AR-040	38	40	95	5	5	100
NRL-AR-041	40	40	100	5	5	100
NRL-AR-042	40	40	100	5	5	100
NRL-AR-045	40	40	100	4	5	80
NRL-AR-056	40	40	100	5	5	100
NRL-AR-059	40	40	100	5	5	100
NRL-AR-060	26	26	100	5	5	100
NRL-AR-061	38	40	95	5	5	100
NRL-AR-062	39	40	98	ND	ND	ND
NRL-AR-066	40	40	100	ND	ND	ND

The additional *QC strains* included in the EURL-AMR PT for AST support internal quality assurance and help laboratories verify the consistency of their antimicrobial susceptibility testing procedures. Although results for these strains have not been formally scored, they provide important supplementary data that can reveal methodological variation and reinforce the robustness of testing.

The *A. baumannii* strain 2012-70-100-69 has been included as a QC strain for use in MIC assays with the EUVSEC2 and EUVSEC3 panels, where it serves to verify the upper concentration ranges of β-lactams. In future EURL-AMR AST proficiency tests, QC ranges for this strain are intended to be introduced, and participant results will be formally evaluated. The EURL-AMR has recently proposed QC ranges based on cumulative data collected from previous PT rounds (**Table 12**). These are included as an early reference; however the data are not evaluated. As presented in **Table 13**, the majority of laboratories reported MIC values within the proposed thresholds for *A. baumannii* (2012-70-100-69) across most tested antimicrobials. Only a small number of laboratories provided MIC values outside this range, indicating a generally high level of agreement across laboratories.

Table 12. Accepted MIC ranges (mg/L) for antimicrobial compounds used in broth microdilution testing of *Acinetobacter baumannii* (2012-70-100-69) QC strain.

Antimicrobial Compound	**Accepted MIC Range (mg/L)**	
	Lowest	**Highest**
Amikacin	≤4	8
Ampicillin	8	32
Azithromycin	≤2	4
Cefepime	1	4
Cefotaxime	4	16
Cefotaxime-clavulanic acid	4	16
Cefoxitin	32	>64
Ceftazidime	2	8
Ceftazidime-clavulanic acid	2	8
Chloramphenicol	32	>64
Ciprofloxacin	0.12	0.5
Colistin	≤1	4
Ertapenem	1	>2
Gentamicin	≤0.5	2
Imipenem	≤0.12	1
Meropenem	0.12	0.5
Nalidixic acid	≤4	8
Sulfamethoxazole	≤8	16
Temocillin	128	>128
Tetracycline	8	>32
Tigecycline	≤0.25	0.5
Trimethoprim	8	>16

Table 13. Distribution of minimum inhibitory concentration (MIC) values reported by participating laboratories for the QC strain *Acinetobacter baumannii* QC strain. The table presents the number of laboratories that reported each MIC value (mg/L) for EUVSEC3 (Panel 1) and EUVSEC2 (Panel 2) antimicrobials. The results that are within the QC ranges are highlighted in yellow.

Reported MIC (mg/L)		Amikacin	Ampicillin	Azithromycin	Cefepime	Cefotaxime	Cefotaxime-clavulanic acid	Cefoxitin	Ceftazidime	Ceftazidime-clavulanic acid	Chloramphenicol	Ciprofloxacin	Colistin	Ertapenem	Gentamicin	Imipenem	Meropenem	Nalidixic acid	Sulfamethoxazole	Temocillin	Tetracycline	Tigecycline	Trimethoprim
0.12	≤															4							
	=											9											
0.25	≤																					51	
	=											33				27	61						
0.5	≤														32							1	
	=											12			2	27	53					2	
1	≤												24										
	=				8							2	1	4	22								
2	≤			53																			
	=				44				8	9			28	47								2	
	>													7									
4	≤	50																47					
	=			1	6	4	9		82	41			3					4					

Reported MIC (mg/L)		Amikacin	Ampicillin	Azithromycin	Cefepime	Cefotaxime	Cefotaxime-clavulanic acid	Cefoxitin	Ceftazidime	Ceftazidime-clavulanic acid	Chloramphenicol	Ciprofloxacin	Colistin	Ertapenem	Gentamicin	Imipenem	Meropenem	Nalidixic acid	Sulfamethoxazole	Temocillin	Tetracycline	Tigecycline	Trimethoprim
	>					51			1														
8	≤																		52		2		
	=	4	2	2		34	42		21	8								3	1		2		7
	>								2														
16	=		29			24	7											2	1		12		43
	>																						6
32	=		22			1					4										24		
	>		3																		16		
64	=							11			20												
	>							47			32												
128	=																			15			
	>																			43			
512	>																		2				

For *C. coli* (215-QC-ETP), there are not yet sufficient data to establish QC ranges. However, the consensus MIC values based on three independent testings at DTU and one at SSI are presented in **Table 14** for reference. Given inherent accepted method variation, a deviation of plus or minus one two-fold dilution from the target values is acceptable. Most laboratories reported MIC values within this expected range (

Table *15*).

Table 14. Consensus MIC ranges for *C. coli* QC strain.

Antimicrobial Compound	**Consensus MIC** **Lowest MIC (mg/L)**	 **Highest MIC (mg/L)**
Chloramphenicol	≤2	4
Ciprofloxacin	4	16
Ertapenem	1	4
Erythromycin	≤1	2
Gentamicin	≤0.25	2
Tetracycline	32	>64

Table 15. Distribution of minimum inhibitory concentration (MIC) values reported by participating laboratories for *C. coli* QC strain. The table shows the number of laboratories that reported each MIC value (mg/L) for EUCAMP3 antimicrobials. The preliminary QC ranges are indicated in yellow.

Reported MIC (mg/L)		Chloramphenicol	Ciprofloxacin	Ertapenem	Erythromycin	Gentamicin	Tetracycline
0.25	≤					9	
0.5	=			1		23	
1	≤		1		33		
	=			9		1	
2	≤	27					
	=			22			1
4	=	5	4	2			
8	=	2	27		1		
16	=		2				1
64	=						13
	>					1	19

6.4. Component 3: Performance on β-Lactam Resistance Mechanism Identification

The 2024 EURL-AMR PT included eight *E. coli* and eight *Salmonella* strains, selected to represent distinct β-lactam resistance phenotypes. This selection enabled assessment of participants' ability to detect and classify extended-spectrum β-lactamase (ESBL), AmpC, and carbapenemase producers, as well as to differentiate these from susceptible wild-type strains in both species according to EFSA β-lactam resistance phenotype categorisation algorithm as per **Figure 1** (4).

Across all laboratories, phenotype classification was robust, with 97% expected results on average for *E. coli* strains and 91% for *Salmonella* strains. Most discrepancies occurred near classification thresholds defined by EFSA (**Figure 1**), specifically for cefoxitin (AmpC threshold >8 mg/L) and meropenem (carbapenemase threshold: >0.12 mg/L). As broth microdilution methods inherently include variability of plus or minus one two-fold dilution, borderline MIC values can result in more than one valid phenotype classification under the EFSA scheme.

The expected phenotypes for β-lactam resistance mechanisms for the *E. coli* and *Salmonella test strains* are presented in **Table 16.** Within the *E. coli* strains, three exhibited ESBL phenotypes, harbouring either *bla*$_{\text{CTX-M-1}}$ or *bla*$_{\text{SHV-12}}$. Two strains were confirmed carbapenemase producers, carrying *bla*$_{\text{OXA-181}}$ or *bla*$_{\text{NDM-5}}$. AmpC β-lactamase-mediated resistance was represented by two isolates: one carrying the acquired *bla*$_{\text{CMY-2}}$ gene and another harbouring a mutation in the *ampC* gene promoter region (−42C>T), known to lead to an upregulated expression of AmpC β-lactamase. For the *Salmonella* strains, three exhibited an ESBL-producing phenotype, associated with either *bla*$_{\text{CTX-M-1}}$ or *bla*$_{\text{CTX-M-9}}$. Two strains were identified as AmpC producers carrying *bla*$_{\text{CMY-2}}$, while carbapenemase production was represented by two strains encoding *bla*$_{\text{NDM-1}}$ or *bla*$_{\text{VIM-2}}$. One strain from each of the *E. coli* and *Salmonella* trials was fully susceptible to all β-lactams of panel 2 (EUVSEC2), serving as a wild-type control (EURL EC-19.7 and EURL S-19.2).

Table 16. Percent correct results for each strain of the *E. coli* and *Salmonella* trials for the β-lactam resistance mechanism identification based on the submitted sum of score and count of scores for each strain.

Organism	Strain	Expected phenotype	Additional Phenotype Accepted	Genotype	Sum of Score	Count of Score	% Correct Results
E. coli	EURL EC-19.1	ESBL	ESBL+AmpC	*bla*$_{\text{SHV-12}}$	32	32	100
	EURL EC-19.2	Carbapenemase	-	*bla*$_{\text{OXA-181}}$	32	32	100
	EURL EC-19.3	ESBL	-	*bla*$_{\text{CTX-M-1}}$	30	32	94
	EURL EC-19.4	AmpC	Other	*bla*$_{\text{CMY-2}}$	31	32	97
	EURL EC-19.5	Carbapenemase	-	*bla*$_{\text{NDM-5}}$	32	32	100
	EURL EC-19.6	AmpC	Other	*ampC*-42C>T	32	32	100
	EURL EC-19.7	Susceptible	-	None	32	32	100
	EURL EC-19.8	ESBL	-	*bla*$_{\text{SHV-12}}$	32	32	100
Salmonella	EURL S-19.1	ESBL	ESBL+AmpC	*bla*$_{\text{CTX-M-1}}$	31	32	97
	EURL S-19.2	Susceptible	-	None	32	32	100
	EURL S-19.3	ESBL	ESBL+AmpC	*bla*$_{\text{CTX-M-1}}$	29	31	94
	EURL S-19.4	Carbapenemase	-	*bla*$_{\text{NDM-1}}$	32	32	100
	EURL S-19.5	ESBL	-	*bla*$_{\text{CTX-M-9}}$	32	32	100
	EURL S-19.6	AmpC	-	*bla*$_{\text{CMY-2}}$	31	32	97
	EURL S-19.7	Carbapenemase	AmpC, Other	*bla*$_{\text{VIM-2}}$	32	32	100
	EURL S-19.8	AmpC	Other	*bla*$_{\text{CMY-2}}$	30	31	97

For the ***E. coli*** **trial**, performance was very high across all strains. Six strains achieved 100% correct results. Two strains showed small deviations:

- **EURL EC-19.3 (ESBL): two unexpected classifications**. Laboratory NRL-AR-038 assigned an "Other" phenotype to strain EC-19.3 after reporting an imipenem MIC of 1 mg/L, substantially higher than the expected value of 0.125 mg/L. Laboratory NRL-AR-040 reported an ESBL+AmpC phenotype for strain EC-19.3, whereas an ESBL phenotype was expected. This deviation was due to a cefoxitin MIC of 64 mg/L – thirty two times higher than the expected 2 mg/L - making it the only laboratory that classified this strain as resistant to cefoxitin.
- **EURL EC-19.4 (AmpC, Other): one unexpected classification**. Laboratory NRL-AR-040 submitted an ESBL+AmpC classification for strain EC-19.4, despite MIC results consistent with an AmpC phenotype (no observed synergy with clavulanic acid).

For the ***Salmonella*** **trial**, performance was similarly high. Five strains reached 100% correct. Three strains showed minor deviations:

- **EURL S-19.1 (ESBL, ESBL+AmpC): one unexpected classification**. Laboratory NRL-AR-004 reported a carbapenemase-producing phenotype due to a reported MIC to meropenem of 4 mg/L (expected value: 0.03 mg/L).
- **EURL S-19.3 (ESBL, ESBL+AmpC): two unexpected classifications**. Laboratory NRL-AR-019 submitted "Other phenotype" due to cefotaxime and cefotaxime/clavulanic acid MIC of 0.25 mg/L (versus 16 mg/L expected), influencing the synergy profile and leading to an "Other" classification. Laboratory NRL-AR-040 reported a carbapenemase-producing phenotype due to a reported MIC to meropenem of 0.25 mg/L (expected: 0.03 mg/L).
- **EURL S-19.6 (AmpC)**: **one unexpected classification**. Laboratory NRL-AR-038 submitted an "ESBL+AmpC" classification despite MIC values in Panel 2 fully consistent with an AmpC phenotype; likely a data entry or interpretation error.
- **EURL S-19.8** (AmpC, Other): **one unexpected classification.** Laboratory NRL-AR-038 submitted a carbapenemase phenotype despite MIC values supporting an AmpC classification.

Most laboratories achieved 100% correct β-lactam resistance mechanism categorisation for both *E. coli* and *Salmonella* (**Table *14***). Only a few laboratories showed deviations: in *E. coli* trial, one laboratory had one unexpected classification and another had two; in *Salmonella*, three laboratories had one unexpected classification each and one laboratory had two.

Table 17. Percentage of correct β-Lactam resistance mechanism phenotype categorisation reported by participating laboratories for the eight *E. coli* and eight *Salmonella test strains.* Laboratories are sorted in ascending order by NRL-AR code.

	E. coli			***Salmonella***		
Laboratory ID	Sum of Score	Count of Score	**% Correct Results**	Sum of Score	Count of Score	**% Correct Results**
NRL-AR-002	7	7	100	7	7	100
NRL-AR-004	7	7	100	6	7	86
NRL-AR-006	7	7	100	7	7	100
NRL-AR-009	7	7	100	7	7	100
NRL-AR-011	7	7	100	7	7	100
NRL-AR-012	7	7	100	7	7	100
NRL-AR-016	7	7	100	7	7	100
NRL-AR-017	7	7	100	7	7	100
NRL-AR-018	7	7	100	7	7	100
NRL-AR-019	7	7	100	6	7	86
NRL-AR-020	7	7	100	7	7	100
NRL-AR-021	7	7	100	7	7	100
NRL-AR-022	8	8	100	8	8	100
NRL-AR-023	7	7	100	7	7	100
NRL-AR-025	7	7	100	7	7	100
NRL-AR-026	7	7	100	7	7	100
NRL-AR-029	7	7	100	7	7	100
NRL-AR-030	7	7	100	6	6	100
NRL-AR-033	7	7	100	7	7	100
NRL-AR-034	7	7	100	7	7	100
NRL-AR-037	7	7	100	7	7	100
NRL-AR-038	6	7	86	5	7	71
NRL-AR-040	5	7	71	5	6	83
NRL-AR-041	8	8	100	8	8	100
NRL-AR-042	7	7	100	7	7	100
NRL-AR-045	7	7	100	7	7	100
NRL-AR-056	7	7	100	7	7	100
NRL-AR-059	7	7	100	7	7	100
NRL-AR-060	7	7	100	7	7	100
NRL-AR-061	7	7	100	7	7	100
NRL-AR-062	7	7	100	7	7	100
NRL-AR-066	7	7	100	7	7	100

6.5. *Campylobacter* Species Identification Results

Participants were instructed to determine the species of the *Campylobacter test strains* using either their routine in-house methodologies or the EURL-AMR recommended protocol. **Table 18** presents the expected species for each strain alongside a summary of submitted results and the corresponding percentage of correct identifications. All laboratories correctly identified the species for the analysed strains, reflecting a high level of proficiency and methodological reliability in *Campylobacter* species determination.

Table 18. Species identification results for *Campylobacter* strains included in the PT. The table shows the expected species, and the percentage of correct identifications for each strain.

Strain	Expected species	Sum of Score	Count of Score	%Correct Results
EURL C-19.1	*Campylobacter coli*	22	22	100.0
EURL C-19.2	*Campylobacter jejuni*	29	29	100.0
EURL C-19.3	*Campylobacter coli*	28	28	100.0
EURL C-19.4	*Campylobacter coli*	29	29	100.0
EURL C-19.5	*Campylobacter coli*	27	27	100.0
EURL C-19.6	*Campylobacter jejuni*	29	29	100.0
EURL C-19.7	*Campylobacter coli*	25	25	100.0
EURL C-19.8	*Campylobacter coli*	29	29	100.0
	Total	**218**	**218**	**100.0**

7. REFERENCES

1. 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.
2. CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, Edition 34.
3. CLSI VET06 Methods for Antimicrobial Susceptibility Testing of Bacteria Isolated From Animals, 1st ed.
4. Authority (EFSA) EFS, European Centre for Disease Prevention and Control (ECDC). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. EFSA J. 2024;22(2):e8583.
5. ISO 20776-1:2019 Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.
6. European Food Safety Authority (EFSA),Giusi Amore, Pierre-Alexandre Beloeil, Raquel Garcia Fierro, Beatriz Guerra,Valentina Rizzi and Anca-Violeta Stoicescu. Manual for reporting 2023 antimicrobial resistance data under Directive 2003/99/EC and Commission Implementing Decision (EU) 2020/1729 | EFSA [Internet]. 2024. Available from: https://www.efsa.europa.eu/en/supporting/pub/en-8585
7. Instructions for Use Thermo Scientific Sensititre Susceptibility plates for clinical Fastidious and Non-Fastidious organisms, Thermo Scientific, 012- Research Use Only –CID10120, Revision Date: May 2019.
8. CLSI standard M07, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 12th Edition, Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
9. M45 | Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 3rd Edition,Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

END OF REPORT

Appendix 1: Pre-notification

for the report of

The 32nd Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

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

G00-06-001/26.10.2020

EQAS 2024 for *E. coli*, *Salmonella* and *Campylobacter*

The EURL-AR announces the launch of another EQAS, thus 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 antimicrobial susceptibility testing of eight *Escherichia coli* isolates, eight *Salmonella* isolates and eight *Campylobacter* isolates. Additionally, quality control (QC) strains *E. coli* ATCC 25922 (CCM 3954) and *C. jejuni* ATCC 33560 (CCM 6214) will be distributed to new participants. Moreover, for the purpose of obtaining a relevant MIC value for ertapenem when performing method control for *Campylobacter*, an internal method quality control strain (*Campylobacter*) will be included in the shipment (further details on this strain will be circulated by email prior to the shipment of test 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.

This EQAS is specifically for NRL's on antimicrobial resistance (NRL-AR). Laboratories designated to be NRL-AR do not need to sign up to participate but are automatically regarded as participants. To inform of changes in relation to your level of participation in comparison to previous years, please contact the EQAS coordinator. The EURL-AR will be able to cover the expenses for one parcel, only, per EU Member State. Therefore, countries with more than one laboratory registered on the EURL-AR contact-list will be contacted directly to confirm which laboratory will be included for participation free of charge.

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 100 EUR).

TO AVOID DELAY IN SHIPPING THE ISOLATES TO YOUR LABORATORY

The content of the parcel is "UN3373, Biological Substance Category B":

- Eight *E. coli* strains,
- Eight *Salmonella* strains and
- Nine *Campylobacter* strains (eight test strains and one internal method QC strain),
- and for new participants the QC strains (ATCC strains) mentioned above.

Note, there will be nine *Campylobacter* strains included in the shipment due to the addition of an internal method quality control strain (see further details above).

Please provide the EQAS coordinator with documents or other information that can simplify customs procedures (e.g., specific text that should be written on the proforma invoice). To avoid delays, we kindly ask you to send this information already at this stage.

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

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE

Shipment of isolates and protocol: The isolates will be shipped mid-September 2024. The protocol for this proficiency test will be available for download from the website (https://www.eurl-ar.eu/eqas.aspx).

Submission of results: Results must be submitted to the National Food Institute **no later than 9 December 2024 at 16:00** via the password-protected webtool.
Upon reaching the deadline, when preliminary data validation has been performed, each participating laboratory is kindly asked to enter the password-protected webtool 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 as to the participants' obtained results. The EURL-AR and the EU Commission, only, will have access to un-coded results. The report will be publicly available.

Next EQAS: The next EQAS provided by the EURL-AR is planned for mid-October 2024 and will be the DTU Genomic PT 2024 aiming to facilitate harmonization and standardization in whole genome sequencing and data analysis of *Escherichia coli*, *Enterococcus faecium/faecalis* and *Staphylococcus aureus* isolates, with the aim to produce comparable data for monitoring and research purposes.

After this, in November 2024, the EQAS on selective isolation of presumptive ESBL-, AmpC- and carbapenemase-producing *Escherichia coli* from meat and caecal samples (Matrix EQAS) is planned to be carried out.

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

Sincerely,

Susanne Karlsmose Pedersen (suska@food.dtu.dk)
EURL-AR EQAS-coordinator

Appendix 2A: Protocol

for the report of

The 32nd Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

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*.

Table of Contents

National Food Institute
Technical University of Denmark

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:

Susanne Karlsmose Pedersen
National Food Institute,
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby
Denmark
Tel: +45 3588 6601
E-mail: suska@food.dtu.dk

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

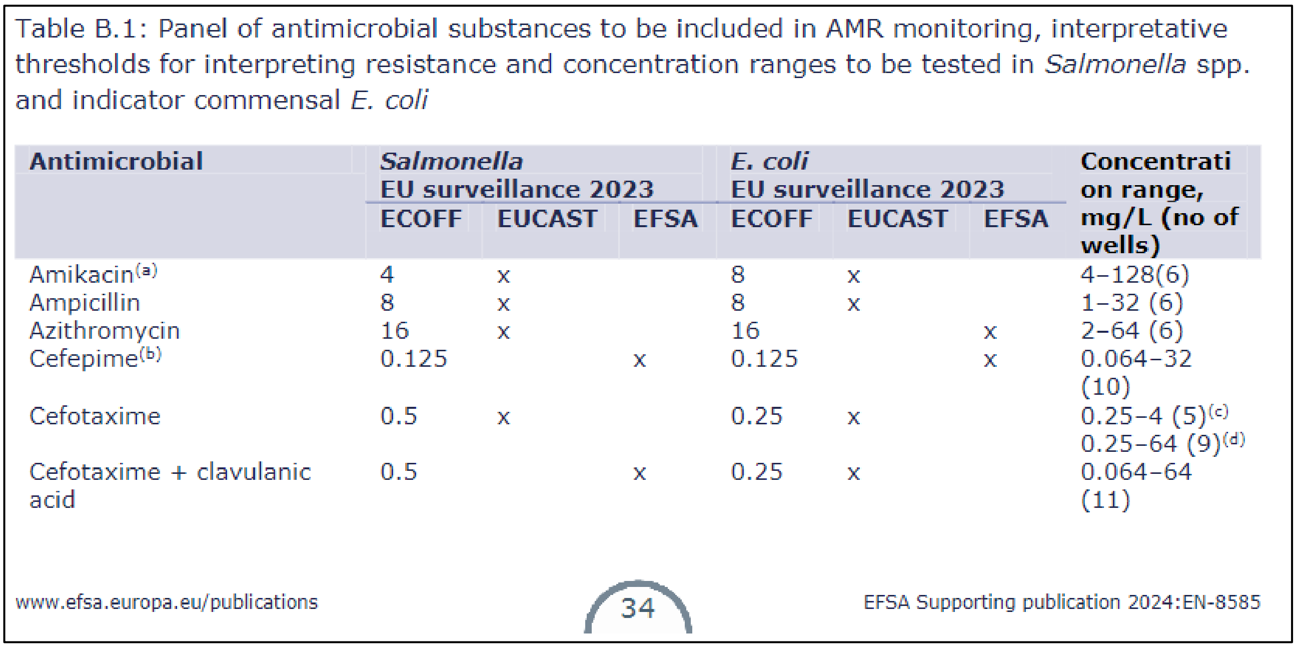
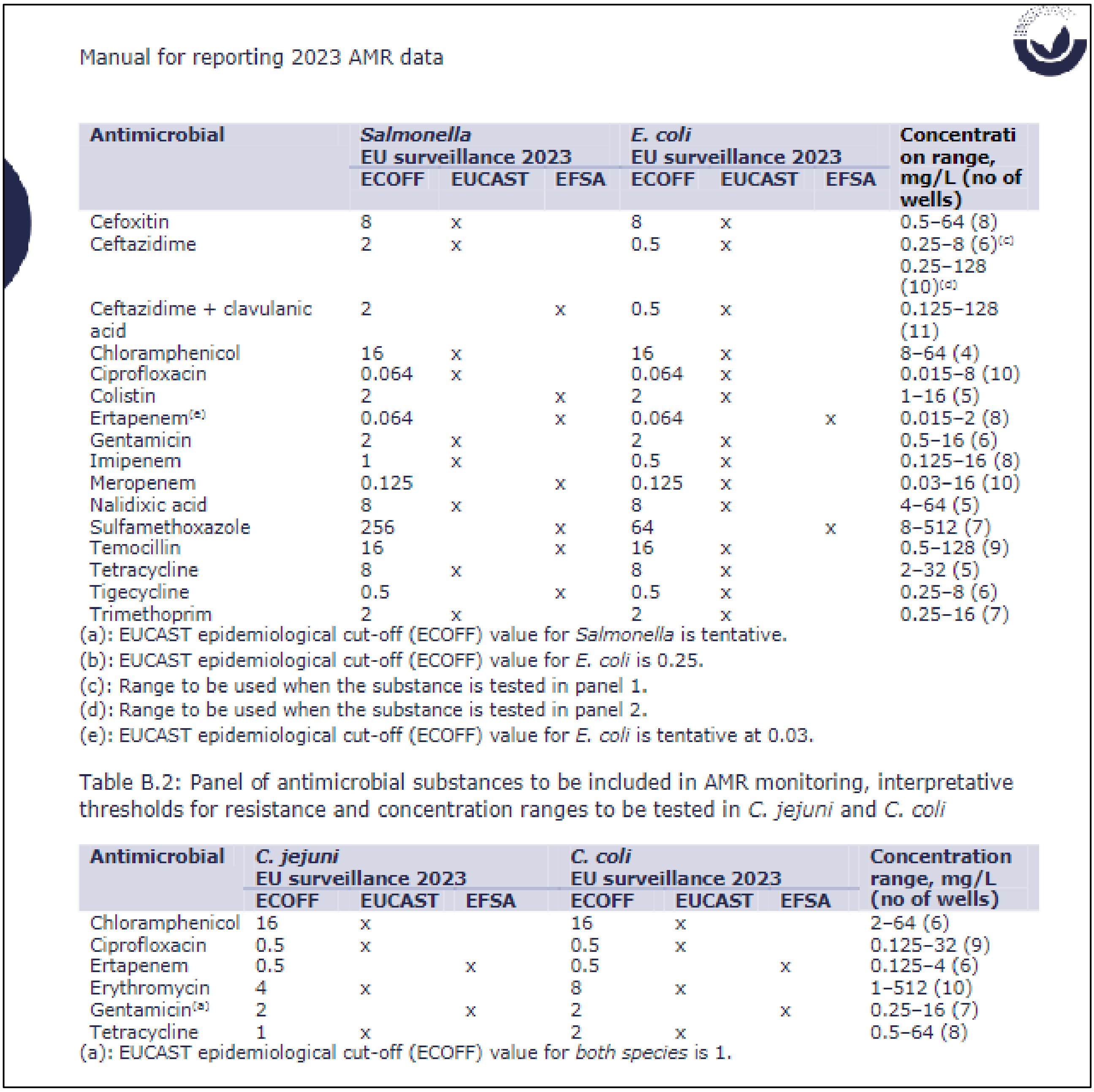


Table B.1: Panel of antimicrobial substances to be included in AMR monitoring, interpretative thresholds for interpreting resistance and concentration ranges to be tested in *Salmonella* spp. and indicator commensal *E. coli*

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)

www.efsa.europa.eu/publications 34 EFSA Supporting publication 2024:EN-8585

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*

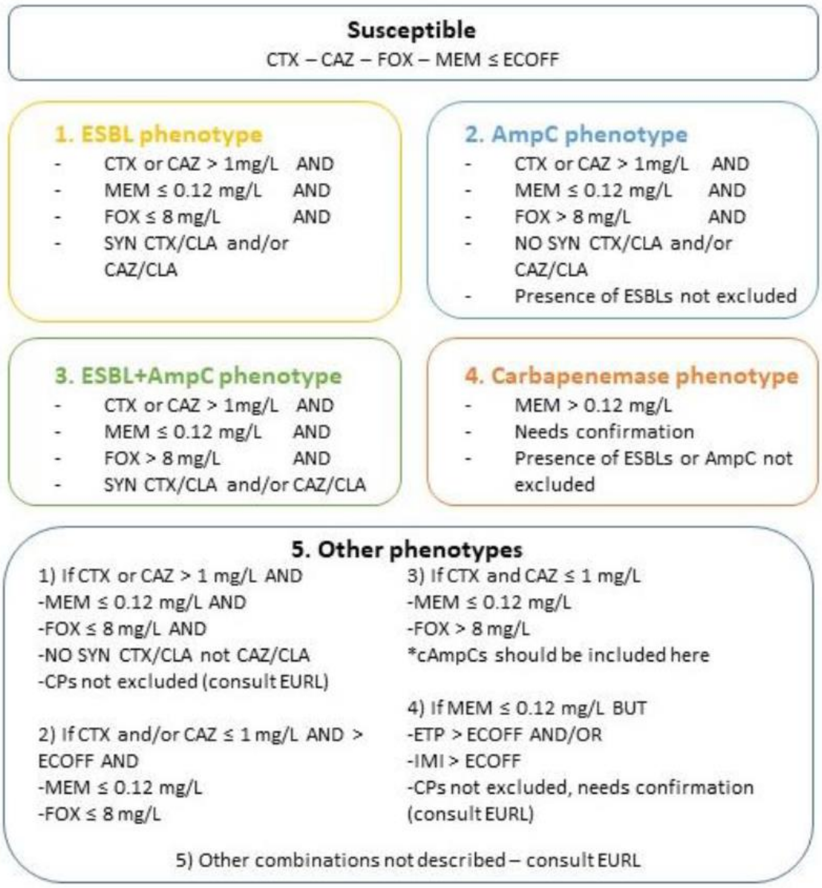
Antimicrobial	*C. jejuni* EU surveillance 2023			*C. coli* EU surveillance 2023			Concentration range, mg/L (no of wells)
	ECOFF	EUCAST	EFSA	ECOFF	EUCAST	EFSA	
Chloramphenicol	16	x		16	x		2–64 (6)
Ciprofloxacin	0.5	x		0.5	x		0.125–32 (9)
Ertapenem	0.5		x	0.5		x	0.125–4 (6)
Erythromycin	4	x		8	x		1–512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



Susceptible
CTX – CAZ – FOX – MEM ≤ ECOFF

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

2. AmpC phenotype
- CTX or CAZ > 1mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- NO SYN CTX/CLA and/or CAZ/CLA
- Presence of ESBLs not excluded

3. ESBL+AmpC phenotype
- CTX or CAZ > 1mg/L AND
- MEM ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- SYN CTX/CLA and/or CAZ/CLA

4. Carbapenemase phenotype
- MEM > 0.12 mg/L
- Needs confirmation
- Presence of ESBLs or AmpC not excluded

5. Other phenotypes

1) If CTX or CAZ > 1 mg/L AND
-MEM ≤ 0.12 mg/L AND
-FOX ≤ 8 mg/L AND
-NO SYN CTX/CLA not CAZ/CLA
-CPs not excluded (consult EURL)

2) If CTX and/or CAZ ≤ 1 mg/L AND > ECOFF AND
-MEM ≤ 0.12 mg/L
-FOX ≤ 8 mg/L

3) If CTX and CAZ ≤ 1 mg/L
-MEM ≤ 0.12 mg/L
-FOX > 8 mg/L
*cAmpCs should be included here

4) If MEM ≤ 0.12 mg/L BUT
-ETP > ECOFF AND/OR
-IMI > ECOFF
-CPs not excluded, needs confirmation (consult EURL)

5) Other combinations not described – consult EURL

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

Appendix 3A: Cover Letter

for the report of

The 32nd Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

G00-06-001/26.10.2020

EURL-AR External Quality Assurance System 2024

- *Escherichia coli*, *Salmonella*, and *Campylobacter*

Kgs. Lyngby, October 2024

Dear participant in the EURL-AR AST EQAS 2024,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2024: eight *Escherichia coli*, eight *Salmonella*, and eight *Campylobacter* spp. Additionally, you will find a new Quality Control (QC) strain, *Campylobacter coli* “215-QC-ETP” provided as a method control for broth microdilution testing of ertapenem in EUCAMP3 Sensititre panels. This strain has an expected Minimum Inhibitory Concentration (MIC) to ertapenem of 1 mg/L, which is appropriate for the ertapenem test range in the EUCAMP3 panel (0.125 - 4 mg/L).

E. coli	***Salmonella***	***Campylobacter***
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

Upon arrival to your laboratory, store the test strains in a dark place at 5-25°C until microbiological analysis.

To ensure viability, **the *Campylobacter* test strains and the additional *C. coli* QC strain “215-QC-ETP” must be subcultured immediately upon arrival**.

We ask you to test these test strains for antimicrobial susceptibility. Detailed description of the procedures to follow for antimicrobial susceptibility testing and for submitting your results via the webtool can be found in the EQAS protocol.

On the EURL-AR-website (www.eurl-ar.eu) the following documents relevant for this EURL-AR EQAS are available:

- Protocol for antimicrobial susceptibility testing of *E. coli*, *Salmonella*, and *Campylobacter*
- Test forms for collecting results prior to reporting
- Instructions for Opening and Reviving Lyophilised Cultures
- Subculture and Maintenance of Quality Control Strains
- Guideline for submission of results via the webtool

All participants registered with an account in the submission webtool will receive a separate email presenting information related to personal username and password. The email will be sent when the webtool has passed internal quality control and has been approved for user access.

We will let you know by email when to look out for it.

	Personal username	**Personal password**
Accessing the webtool (see the EQAS protocol, item 5)	*See underlined text above*	*See underlined text above*

Results should be submitted to the database no later than **9 December 2024 at 4 pm**.

Please acknowledge receipt of this parcel immediately upon arrival (to suska@food.dtu.dk).
Do not hesitate to contact me for further information.

Yours sincerely,

Susanne Karlsmose Pedersen
EURL-AR EQAS-Coordinator

Appendix 3B: Instructions for opening and reviving lyophilised cultures

for the report of

The 32nd Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

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

DTU Food
National Food Institute

INSTRUCTIONS FOR OPENING AND REVIVING LYOPHILISED CULTURES

Instructions adjusted from Czech Collection of Microorganisms (CCM) document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz.

Lyophilised cultures are supplied in vacuum-sealed ampoules. Care should be taken in opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

a. Check the number of the culture on the label inside the ampoule
b. Make a file cut on the ampoule near the middle of the plug (see Figure 1)
c. Disinfect the ampoule with alcohol-dampened gauze or alcohol-dampened cotton wool from just below the plug to the pointed end
d. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule
e. Remove the pointed end of the ampoule into disinfectant
f. Add about 0.3 ml appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosols. Transfer the contents to one or more suitable solid and /or liquid media
g. Incubate the inoculated medium at appropriate conditions for several days
h. Autoclave or disinfect effectively the used Pasteur pipette, the plug and all the remains of the original ampoule before discarding

Notes:

- Cultures should be grown on media and under conditions as recommended in the CCM catalogue (see http://www.sci.muni.cz)
- Cultures may need at least one subculturing before they can be optimally used in experiments
- Unopened ampoules should be kept in a dark and cool place!

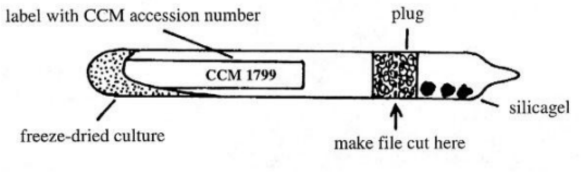


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz

Appendix 3C: Subculture and maintenance of Quality Control strains

for the report of

The 32nd Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

DTU Food
National Food Institute

European Union Reference Laboratory
Antimicrobial Resistance

SUBCULTURE AND MAINTENANCE OF QUALITY CONTROL STRAINS

1 PURPOSE AND REFERENCES

Improper storage and repeated subculturing of bacteria can produce alterations in antimicrobial susceptibility test results. The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for Quality Control (QC) stock culture maintenance to ensure consistent antimicrobial susceptibility test (AST) results.

The following can be regarded as a summary of information that should be followed for subculturing and maintaining QC-strains when performing AST by broth dilution methods. For full information related to this subject, the following standards are relevant: M100 (Performance Standards for Antimicrobial Susceptibility Testing) and M7 (Methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically; Approved Standard).

2 DEFINITION OF TERMS

Reference Culture: A reference culture is a microorganism preparation that is acquired from a culture type collection.

Reference Stock Culture: A reference stock culture is a microorganism preparation that is derived from a reference culture. Guidelines and standards outline how reference stock cultures must be processed and stored.

Working Stock Cultures: A working stock culture is growth derived from a reference stock culture. Guidelines and standards outline how working stock cultures must be processed and how often they can be subcultured.

Subcultures (Passages): A subculture is simply the transfer of established microorganism growth on media to fresh media. The subsequent growth on the fresh media constitutes a subculture or passage. Growing a reference culture or reference stock culture from its preserved status (frozen or lyophilized) is not a subculture. The preserved microorganism is not in a stage of established growth until it is thawed or hydrated and grown for the first time.

3 IMPORTANT CONSIDERATIONS

- Do not use disc diffusion strains for MIC determination.
- Obtain QC strains from a reliable source such as ATCC.
- CLSI requires that QC be performed either on the same day or weekly (after QC-validation).
- Any changes in materials or procedure must be validated with QC before implemented
- For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides.

- Periodically perform colony counts to check the inoculum preparation procedure.
- Ideally, test values should be in the middle of the acceptable range.
- Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems.

4 STORAGE OF REFERENCE STRAINS

Preparation of stock cultures

- Use a suitable stabilizer such as 50% fetal calf serum in broth, 10-15% glycerol in tryptic soy broth, defibrinated sheep blood or skim milk to prepare multiple aliquots.
- Store at -20°C, -70°C or liquid nitrogen (alternatively, freeze dry.)
- Before using rejuvenated strains for QC, subculture to check for purity and viability.

Working cultures

- Set up on agar slants with appropriate medium, store at 4-8°C and subculture weekly.
- Replace the working strain with a stock culture at least monthly.
- If a change in the organisms inherent susceptibility occurs, obtain a fresh stock culture or a new strain from a reference culture collection e.g. ATCC.

5 FREQUENCY OF TESTING

Weekly vs. daily testing

Weekly testing is possible if the laboratory can demonstrate satisfactory performance with daily testing according to the descriptions in the CLSI guidelines.

- Documentation showing reference strain results from 20 or 30 consecutive test days were within the acceptable range.
- For each antimicrobial/organism combination, no more one out of 20 or three out of 30 MIC values may be outside the acceptable range.

When the above are fulfilled, each quality control strain may be tested once a week and whenever any reagent component is changed.

Corrective Actions

If an MIC is outside the range in weekly testing, corrective action is required as follows:

- Repeat the test if there is an obvious error e.g. wrong strain or incubation conditions used
- If there is no obvious error, return to daily control testing

If five acceptable QC results are available, no additional days of QC-testing are needed.

If the problem cannot be resolved, continue daily testing until the errors are identified.

Repeat the 30 days validation before resuming weekly testing.

Appendix 4: Expected MIC data for the test strains

for the report of

The 32^{nd} Proficiency Test for the Antimicrobial Susceptibility Testing of *Escherichia coli, Salmonella* & *Campylobacter* (2024)

EU Reference Laboratory for Antimicrobial Resistance

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen, suska@food.dtu.dk

Appendix 4 - Expected MIC Values & Phenotype Interpretations as Resistant (R) or Susceptible (S)

E. coli

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL EC-19.1	Panel1	Amikacin	≤	4	S
EURL EC-19.1	Panel1	Ampicillin	>	32	**R**
EURL EC-19.1	Panel1	Azithromycin	=	8	S
EURL EC-19.1	Panel1	Cefotaxime	=	2	**R**
EURL EC-19.1	Panel1	Ceftazidime	>	8	**R**
EURL EC-19.1	Panel1	Chloramphenicol	=	64	**R**
EURL EC-19.1	Panel1	Ciprofloxacin	=	0.5	**R**
EURL EC-19.1	Panel1	Colistin	≤	1	S
EURL EC-19.1	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.1	Panel1	Meropenem	≤	0.03	S
EURL EC-19.1	Panel1	Nalidixic acid	>	64	**R**
EURL EC-19.1	Panel1	Sulfamethoxazole	>	512	**R**
EURL EC-19.1	Panel1	Tetracycline	>	32	**R**
EURL EC-19.1	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.1	Panel1	Trimethoprim	≤	0.25	S
EURL EC-19.1	Panel2	Cefepime	=	0.5	**R**
EURL EC-19.1	Panel2	Cefotaxime	=	2	**R**
EURL EC-19.1	Panel2	Cefotaxime-clavulanic acid	=	0.125	S
EURL EC-19.1	Panel2	Cefoxitin	=	8	S
EURL EC-19.1	Panel2	Ceftazidime	=	16	**R**
EURL EC-19.1	Panel2	Ceftazidime-clavulanic acid	=	0.25	S
EURL EC-19.1	Panel2	Ertapenem	≤	0.016	S
EURL EC-19.1	Panel2	Imipenem	≤	0.125	S
EURL EC-19.1	Panel2	Meropenem	≤	0.03	S
EURL EC-19.1	Panel2	Temocillin	=	8	S
EURL EC-19.2	Panel1	Amikacin	≤	4	S
EURL EC-19.2	Panel1	Ampicillin	>	32	**R**
EURL EC-19.2	Panel1	Azithromycin	=	8	S
EURL EC-19.2	Panel1	Cefotaxime	>	4	**R**
EURL EC-19.2	Panel1	Ceftazidime	>	8	**R**
EURL EC-19.2	Panel1	Chloramphenicol	>	64	**R**
EURL EC-19.2	Panel1	Ciprofloxacin	>	8	**R**
EURL EC-19.2	Panel1	Colistin	≤	1	S
EURL EC-19.2	Panel1	Gentamicin	>	16	**R**
EURL EC-19.2	Panel1	Meropenem	=	0.5	**R**
EURL EC-19.2	Panel1	Nalidixic acid	>	64	**R**
EURL EC-19.2	Panel1	Sulfamethoxazole	>	512	**R**
EURL EC-19.2	Panel1	Tetracycline	>	32	**R**
EURL EC-19.2	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.2	Panel1	Trimethoprim	>	16	**R**
EURL EC-19.2	Panel2	Cefepime	=	0.5	**R**
EURL EC-19.2	Panel2	Cefotaxime	=	32	**R**

Strain ID	**Panel**	**Antimicrobial**	**Operator**	**Value**	**Interpretation**
EURL EC-19.2	Panel2	Cefotaxime-clavulanic acid	=	16	**R**
EURL EC-19.2	Panel2	Cefoxitin	>	64	**R**
EURL EC-19.2	Panel2	Ceftazidime	=	32	**R**
EURL EC-19.2	Panel2	Ceftazidime-clavulanic acid	=	32	**R**
EURL EC-19.2	Panel2	Ertapenem	=	2	**R**
EURL EC-19.2	Panel2	Imipenem	=	1	**R**
EURL EC-19.2	Panel2	Meropenem	=	1	**R**
EURL EC-19.2	Panel2	Temocillin	>	128	**R**
EURL EC-19.3	Panel1	Amikacin	≤	4	S
EURL EC-19.3	Panel1	Ampicillin	>	32	**R**
EURL EC-19.3	Panel1	Azithromycin	=	8	S
EURL EC-19.3	Panel1	Cefotaxime	>	4	**R**
EURL EC-19.3	Panel1	Ceftazidime	=	2	**R**
EURL EC-19.3	Panel1	Chloramphenicol	≤	8	S
EURL EC-19.3	Panel1	Ciprofloxacin	≤	0.016	S
EURL EC-19.3	Panel1	Colistin	≤	1	S
EURL EC-19.3	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.3	Panel1	Meropenem	≤	0.03	S
EURL EC-19.3	Panel1	Nalidixic acid	≤	4	S
EURL EC-19.3	Panel1	Sulfamethoxazole	≤	8	S
EURL EC-19.3	Panel1	Tetracycline	≤	2	S
EURL EC-19.3	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.3	Panel1	Trimethoprim	≤	0.25	S
EURL EC-19.3	Panel2	Cefepime	=	16	**R**
EURL EC-19.3	Panel2	Cefotaxime	=	64	**R**
EURL EC-19.3	Panel2	Cefotaxime-clavulanic acid	≤	0.06	S
EURL EC-19.3	Panel2	Cefoxitin	=	2	S
EURL EC-19.3	Panel2	Ceftazidime	=	4	**R**
EURL EC-19.3	Panel2	Ceftazidime-clavulanic acid	≤	0.125	S
EURL EC-19.3	Panel2	Ertapenem	≤	0.016	S
EURL EC-19.3	Panel2	Imipenem	≤	0.125	S
EURL EC-19.3	Panel2	Meropenem	≤	0.03	S
EURL EC-19.3	Panel2	Temocillin	=	4	S
EURL EC-19.4	Panel1	Amikacin	≤	4	S
EURL EC-19.4	Panel1	Ampicillin	>	32	**R**
EURL EC-19.4	Panel1	Azithromycin	=	4	S
EURL EC-19.4	Panel1	Cefotaxime	>	4	**R**
EURL EC-19.4	Panel1	Ceftazidime	=	8	**R**
EURL EC-19.4	Panel1	Chloramphenicol	≤	8	S
EURL EC-19.4	Panel1	Ciprofloxacin	=	0.03	S
EURL EC-19.4	Panel1	Colistin	≤	1	S
EURL EC-19.4	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.4	Panel1	Meropenem	≤	0.03	S
EURL EC-19.4	Panel1	Nalidixic acid	≤	4	S
EURL EC-19.4	Panel1	Sulfamethoxazole	≤	8	S
EURL EC-19.4	Panel1	Tetracycline	≤	2	S
EURL EC-19.4	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.4	Panel1	Trimethoprim	≤	0.25	S
EURL EC-19.4	Panel2	Cefepime	=	0.25	**R**

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL EC-19.4	Panel2	Cefotaxime	=	8	**R**
EURL EC-19.4	Panel2	Cefotaxime-clavulanic acid	=	4	**R**
EURL EC-19.4	Panel2	Cefoxitin	=	64	**R**
EURL EC-19.4	Panel2	Ceftazidime	=	8	**R**
EURL EC-19.4	Panel2	Ceftazidime-clavulanic acid	=	4	**R**
EURL EC-19.4	Panel2	Ertapenem	=	0.03	S
EURL EC-19.4	Panel2	Imipenem	≤	0.125	S
EURL EC-19.4	Panel2	Meropenem	≤	0.03	S
EURL EC-19.4	Panel2	Temocillin	=	4	S
EURL EC-19.5	Panel1	Amikacin	≤	4	S
EURL EC-19.5	Panel1	Ampicillin	>	32	**R**
EURL EC-19.5	Panel1	Azithromycin	=	32	**R**
EURL EC-19.5	Panel1	Cefotaxime	>	4	**R**
EURL EC-19.5	Panel1	Ceftazidime	>	8	**R**
EURL EC-19.5	Panel1	Chloramphenicol	≤	8	S
EURL EC-19.5	Panel1	Ciprofloxacin	>	8	**R**
EURL EC-19.5	Panel1	Colistin	≤	1	S
EURL EC-19.5	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.5	Panel1	Meropenem	>	16	**R**
EURL EC-19.5	Panel1	Nalidixic acid	>	64	**R**
EURL EC-19.5	Panel1	Sulfamethoxazole	>	512	**R**
EURL EC-19.5	Panel1	Tetracycline	>	32	**R**
EURL EC-19.5	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.5	Panel1	Trimethoprim	>	16	**R**
EURL EC-19.5	Panel2	Cefepime	>	32	**R**
EURL EC-19.5	Panel2	Cefotaxime	>	64	**R**
EURL EC-19.5	Panel2	Cefotaxime-clavulanic acid	>	64	**R**
EURL EC-19.5	Panel2	Cefoxitin	>	64	**R**
EURL EC-19.5	Panel2	Ceftazidime	>	128	**R**
EURL EC-19.5	Panel2	Ceftazidime-clavulanic acid	>	128	**R**
EURL EC-19.5	Panel2	Ertapenem	>	2	**R**
EURL EC-19.5	Panel2	Imipenem	=	8	**R**
EURL EC-19.5	Panel2	Meropenem	=	16	**R**
EURL EC-19.5	Panel2	Temocillin	=	128	**R**
EURL EC-19.6	Panel1	Amikacin	≤	4	S
EURL EC-19.6	Panel1	Ampicillin	>	32	**R**
EURL EC-19.6	Panel1	Azithromycin	=	8	S
EURL EC-19.6	Panel1	Cefotaxime	=	4	**R**
EURL EC-19.6	Panel1	Ceftazidime	>	8	**R**
EURL EC-19.6	Panel1	Chloramphenicol	>	64	**R**
EURL EC-19.6	Panel1	Ciprofloxacin	=	0.25	**R**
EURL EC-19.6	Panel1	Colistin	≤	1	S
EURL EC-19.6	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.6	Panel1	Meropenem	≤	0.03	S
EURL EC-19.6	Panel1	Nalidixic acid	>	64	**R**
EURL EC-19.6	Panel1	Sulfamethoxazole	>	512	**R**
EURL EC-19.6	Panel1	Tetracycline	>	32	**R**
EURL EC-19.6	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.6	Panel1	Trimethoprim	≤	0.25	S

Strain ID	**Panel**	**Antimicrobial**	**Operator**	**Value**	**Interpretation**
EURL EC-19.6	Panel2	Cefepime	=	4	**R**
EURL EC-19.6	Panel2	Cefotaxime	=	4	**R**
EURL EC-19.6	Panel2	Cefotaxime-clavulanic acid	=	2	**R**
EURL EC-19.6	Panel2	Cefoxitin	=	16	**R**
EURL EC-19.6	Panel2	Ceftazidime	=	32	**R**
EURL EC-19.6	Panel2	Ceftazidime-clavulanic acid	=	16	**R**
EURL EC-19.6	Panel2	Ertapenem	≤	0.016	S
EURL EC-19.6	Panel2	Imipenem	=	0.5	S
EURL EC-19.6	Panel2	Meropenem	≤	0.03	S
EURL EC-19.6	Panel2	Temocillin	=	8	S
EURL EC-19.7	Panel1	Amikacin	≤	4	S
EURL EC-19.7	Panel1	Ampicillin	=	4	S
EURL EC-19.7	Panel1	Azithromycin	=	8	S
EURL EC-19.7	Panel1	Cefotaxime	≤	0.25	S
EURL EC-19.7	Panel1	Ceftazidime	≤	0.25	S
EURL EC-19.7	Panel1	Chloramphenicol	=	16	S
EURL EC-19.7	Panel1	Ciprofloxacin	≤	0.016	S
EURL EC-19.7	Panel1	Colistin	≤	1	S
EURL EC-19.7	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.7	Panel1	Meropenem	≤	0.03	S
EURL EC-19.7	Panel1	Nalidixic acid	≤	4	S
EURL EC-19.7	Panel1	Sulfamethoxazole	≤	8	S
EURL EC-19.7	Panel1	Tetracycline	≤	2	S
EURL EC-19.7	Panel1	Tigecycline	≤	0.25	S
EURL EC-19.7	Panel1	Trimethoprim	≤	0.25	S
EURL EC-19.7	Panel2	Cefepime	≤	0.06	S
EURL EC-19.7	Panel2	Cefotaxime	≤	0.25	S
EURL EC-19.7	Panel2	Cefotaxime-clavulanic acid	≤	0.06	S
EURL EC-19.7	Panel2	Cefoxitin	=	4	S
EURL EC-19.7	Panel2	Ceftazidime	≤	0.25	S
EURL EC-19.7	Panel2	Ceftazidime-clavulanic acid	=	0.25	S
EURL EC-19.7	Panel2	Ertapenem	≤	0.016	S
EURL EC-19.7	Panel2	Imipenem	=	0.25	S
EURL EC-19.7	Panel2	Meropenem	≤	0.03	S
EURL EC-19.7	Panel2	Temocillin	=	8	S
EURL EC-19.8	Panel1	Amikacin	≤	4	S
EURL EC-19.8	Panel1	Ampicillin	>	32	**R**
EURL EC-19.8	Panel1	Azithromycin	=	8	S
EURL EC-19.8	Panel1	Cefotaxime	=	2	**R**
EURL EC-19.8	Panel1	Ceftazidime	=	8	**R**
EURL EC-19.8	Panel1	Chloramphenicol	=	32	**R**
EURL EC-19.8	Panel1	Ciprofloxacin	≤	0.016	S
EURL EC-19.8	Panel1	Colistin	≤	1	S
EURL EC-19.8	Panel1	Gentamicin	≤	0.5	S
EURL EC-19.8	Panel1	Meropenem	≤	0.03	S
EURL EC-19.8	Panel1	Nalidixic acid	≤	4	S
EURL EC-19.8	Panel1	Sulfamethoxazole	>	512	**R**
EURL EC-19.8	Panel1	Tetracycline	>	32	**R**
EURL EC-19.8	Panel1	Tigecycline	≤	0.25	S

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL EC-19.8	Panel1	Trimethoprim	≤	0.25	S
EURL EC-19.8	Panel2	Cefepime	=	0.25	**R**
EURL EC-19.8	Panel2	Cefotaxime	=	2	**R**
EURL EC-19.8	Panel2	Cefotaxime-clavulanic acid	≤	0.06	S
EURL EC-19.8	Panel2	Cefoxitin	=	2	S
EURL EC-19.8	Panel2	Ceftazidime	=	16	**R**
EURL EC-19.8	Panel2	Ceftazidime-clavulanic acid	≤	0.125	S
EURL EC-19.8	Panel2	Ertapenem	≤	0.016	S
EURL EC-19.8	Panel2	Imipenem	≤	0.125	S
EURL EC-19.8	Panel2	Meropenem	≤	0.03	S
EURL EC-19.8	Panel2	Temocillin	=	4	S

Salmonella

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL S-19.1	Panel1	Amikacin	≤	4	S
EURL S-19.1	Panel1	Ampicillin	>	32	**R**
EURL S-19.1	Panel1	Azithromycin	=	8	S
EURL S-19.1	Panel1	Cefotaxime	>	4	**R**
EURL S-19.1	Panel1	Ceftazidime	=	8	**R**
EURL S-19.1	Panel1	Chloramphenicol	=	16	S
EURL S-19.1	Panel1	Ciprofloxacin	=	0.25	**R**
EURL S-19.1	Panel1	Colistin	≤	1	S
EURL S-19.1	Panel1	Gentamicin	≤	0.5	S
EURL S-19.1	Panel1	Meropenem	≤	0.03	S
EURL S-19.1	Panel1	Nalidixic acid	>	64	**R**
EURL S-19.1	Panel1	Sulfamethoxazole	=	512	**R**
EURL S-19.1	Panel1	Tetracycline	>	32	**R**
EURL S-19.1	Panel1	Tigecycline	=	1	**R**
EURL S-19.1	Panel1	Trimethoprim	>	16	**R**
EURL S-19.1	Panel2	Cefepime	=	8	**R**
EURL S-19.1	Panel2	Cefotaxime	=	32	**R**
EURL S-19.1	Panel2	Cefotaxime-clavulanic acid	=	0.25	S
EURL S-19.1	Panel2	Cefoxitin	=	8	S
EURL S-19.1	Panel2	Ceftazidime	=	8	**R**
EURL S-19.1	Panel2	Ceftazidime-clavulanic acid	=	1	S
EURL S-19.1	Panel2	Ertapenem	≤	0.016	S
EURL S-19.1	Panel2	Imipenem	=	0.25	S
EURL S-19.1	Panel2	Meropenem	≤	0.03	S
EURL S-19.1	Panel2	Temocillin	=	16	S
EURL S-19.2	Panel1	Amikacin	≤	4	S
EURL S-19.2	Panel1	Ampicillin	≤	1	S
EURL S-19.2	Panel1	Azithromycin	=	4	S
EURL S-19.2	Panel1	Cefotaxime	≤	0.25	S
EURL S-19.2	Panel1	Ceftazidime	=	0.5	S
EURL S-19.2	Panel1	Chloramphenicol	≤	8	S
EURL S-19.2	Panel1	Ciprofloxacin	≤	0.016	S

Strain ID	**Panel**	**Antimicrobial**	**Operator**	**Value**	**Interpretation**
EURL S-19.2	Panel1	Colistin	≤	1	S
EURL S-19.2	Panel1	Gentamicin	≤	0.5	S
EURL S-19.2	Panel1	Meropenem	≤	0.03	S
EURL S-19.2	Panel1	Nalidixic acid	≤	4	S
EURL S-19.2	Panel1	Sulfamethoxazole	=	32	S
EURL S-19.2	Panel1	Tetracycline	≤	2	S
EURL S-19.2	Panel1	Tigecycline	≤	0.25	S
EURL S-19.2	Panel1	Trimethoprim	≤	0.25	S
EURL S-19.2	Panel2	Cefepime	≤	0.06	S
EURL S-19.2	Panel2	Cefotaxime	≤	0.25	S
EURL S-19.2	Panel2	Cefotaxime-clavulanic acid	≤	0.06	S
EURL S-19.2	Panel2	Cefoxitin	=	4	S
EURL S-19.2	Panel2	Ceftazidime	=	0.5	S
EURL S-19.2	Panel2	Ceftazidime-clavulanic acid	=	0.25	S
EURL S-19.2	Panel2	Ertapenem	≤	0.016	S
EURL S-19.2	Panel2	Imipenem	=	0.25	S
EURL S-19.2	Panel2	Meropenem	≤	0.03	S
EURL S-19.2	Panel2	Temocillin	=	8	S
EURL S-19.3	Panel1	Amikacin	≤	4	S
EURL S-19.3	Panel1	Ampicillin	>	32	**R**
EURL S-19.3	Panel1	Azithromycin	=	8	S
EURL S-19.3	Panel1	Cefotaxime	>	4	**R**
EURL S-19.3	Panel1	Ceftazidime	=	4	**R**
EURL S-19.3	Panel1	Chloramphenicol	≤	8	S
EURL S-19.3	Panel1	Ciprofloxacin	=	0.25	**R**
EURL S-19.3	Panel1	Colistin	≤	1	S
EURL S-19.3	Panel1	Gentamicin	≤	0.5	S
EURL S-19.3	Panel1	Meropenem	≤	0.03	S
EURL S-19.3	Panel1	Nalidixic acid	>	64	**R**
EURL S-19.3	Panel1	Sulfamethoxazole	>	512	**R**
EURL S-19.3	Panel1	Tetracycline	>	32	**R**
EURL S-19.3	Panel1	Tigecycline	=	0.5	S
EURL S-19.3	Panel1	Trimethoprim	>	16	**R**
EURL S-19.3	Panel2	Cefepime	=	8	**R**
EURL S-19.3	Panel2	Cefotaxime	=	16	**R**
EURL S-19.3	Panel2	Cefotaxime-clavulanic acid	=	0.25	S
EURL S-19.3	Panel2	Cefoxitin	=	8	S
EURL S-19.3	Panel2	Ceftazidime	=	4	**R**
EURL S-19.3	Panel2	Ceftazidime-clavulanic acid	=	0.5	S
EURL S-19.3	Panel2	Ertapenem	≤	0.016	S
EURL S-19.3	Panel2	Imipenem	=	0.25	S
EURL S-19.3	Panel2	Meropenem	≤	0.03	S
EURL S-19.3	Panel2	Temocillin	=	8	S
EURL S-19.4	Panel1	Amikacin	>	128	**R**
EURL S-19.4	Panel1	Ampicillin	>	32	**R**
EURL S-19.4	Panel1	Azithromycin	>	64	**R**
EURL S-19.4	Panel1	Cefotaxime	>	4	**R**
EURL S-19.4	Panel1	Ceftazidime	>	8	**R**
EURL S-19.4	Panel1	Chloramphenicol	=	32	**R**

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL S-19.4	Panel1	Ciprofloxacin	>	8	**R**
EURL S-19.4	Panel1	Colistin	≤	1	S
EURL S-19.4	Panel1	Gentamicin	>	16	**R**
EURL S-19.4	Panel1	Meropenem	=	8	**R**
EURL S-19.4	Panel1	Nalidixic acid	>	64	**R**
EURL S-19.4	Panel1	Sulfamethoxazole	>	512	**R**
EURL S-19.4	Panel1	Tetracycline	=	4	S
EURL S-19.4	Panel1	Tigecycline	=	0.5	S
EURL S-19.4	Panel1	Trimethoprim	=	0.5	S
EURL S-19.4	Panel2	Cefepime	>	32	**R**
EURL S-19.4	Panel2	Cefotaxime	>	64	**R**
EURL S-19.4	Panel2	Cefotaxime-clavulanic acid	>	64	**R**
EURL S-19.4	Panel2	Cefoxitin	>	64	**R**
EURL S-19.4	Panel2	Ceftazidime	>	128	**R**
EURL S-19.4	Panel2	Ceftazidime-clavulanic acid	>	128	**R**
EURL S-19.4	Panel2	Ertapenem	>	2	**R**
EURL S-19.4	Panel2	Imipenem	=	8	**R**
EURL S-19.4	Panel2	Meropenem	=	8	**R**
EURL S-19.4	Panel2	Temocillin	>	128	**R**
EURL S-19.5	Panel1	Amikacin	≤	4	S
EURL S-19.5	Panel1	Ampicillin	>	32	**R**
EURL S-19.5	Panel1	Azithromycin	=	8	S
EURL S-19.5	Panel1	Cefotaxime	>	4	**R**
EURL S-19.5	Panel1	Ceftazidime	=	1	S
EURL S-19.5	Panel1	Chloramphenicol	≤	8	S
EURL S-19.5	Panel1	Ciprofloxacin	=	0.25	**R**
EURL S-19.5	Panel1	Colistin	≤	1	S
EURL S-19.5	Panel1	Gentamicin	≤	0.5	S
EURL S-19.5	Panel1	Meropenem	≤	0.03	S
EURL S-19.5	Panel1	Nalidixic acid	>	64	**R**
EURL S-19.5	Panel1	Sulfamethoxazole	=	32	S
EURL S-19.5	Panel1	Tetracycline	=	32	**R**
EURL S-19.5	Panel1	Tigecycline	≤	0.25	S
EURL S-19.5	Panel1	Trimethoprim	≤	0.25	S
EURL S-19.5	Panel2	Cefepime	=	2	**R**
EURL S-19.5	Panel2	Cefotaxime	=	8	**R**
EURL S-19.5	Panel2	Cefotaxime-clavulanic acid	≤	0.06	S
EURL S-19.5	Panel2	Cefoxitin	=	2	S
EURL S-19.5	Panel2	Ceftazidime	=	1	S
EURL S-19.5	Panel2	Ceftazidime-clavulanic acid	=	0.25	S
EURL S-19.5	Panel2	Ertapenem	≤	0.016	S
EURL S-19.5	Panel2	Imipenem	=	0.25	S
EURL S-19.5	Panel2	Meropenem	≤	0.03	S
EURL S-19.5	Panel2	Temocillin	=	4	S
EURL S-19.6	Panel1	Amikacin	≤	4	S
EURL S-19.6	Panel1	Ampicillin	>	32	**R**
EURL S-19.6	Panel1	Azithromycin	≤	2	S
EURL S-19.6	Panel1	Cefotaxime	>	4	**R**
EURL S-19.6	Panel1	Ceftazidime	>	8	**R**

Strain ID	**Panel**	**Antimicrobial**	**Operator**	**Value**	**Interpretation**
EURL S-19.6	Panel1	Chloramphenicol	≤	8	S
EURL S-19.6	Panel1	Ciprofloxacin	=	1	**R**
EURL S-19.6	Panel1	Colistin	=	2	S
EURL S-19.6	Panel1	Gentamicin	≤	0.5	S
EURL S-19.6	Panel1	Meropenem	≤	0.03	S
EURL S-19.6	Panel1	Nalidixic acid	=	32	**R**
EURL S-19.6	Panel1	Sulfamethoxazole	>	512	**R**
EURL S-19.6	Panel1	Tetracycline	>	32	**R**
EURL S-19.6	Panel1	Tigecycline	=	1	**R**
EURL S-19.6	Panel1	Trimethoprim	≤	0.25	S
EURL S-19.6	Panel2	Cefepime	=	0.5	**R**
EURL S-19.6	Panel2	Cefotaxime	=	16	**R**
EURL S-19.6	Panel2	Cefotaxime-clavulanic acid	=	16	**R**
EURL S-19.6	Panel2	Cefoxitin	=	64	**R**
EURL S-19.6	Panel2	Ceftazidime	=	32	**R**
EURL S-19.6	Panel2	Ceftazidime-clavulanic acid	=	16	**R**
EURL S-19.6	Panel2	Ertapenem	=	0.06	S
EURL S-19.6	Panel2	Imipenem	=	0.25	S
EURL S-19.6	Panel2	Meropenem	≤	0.03	S
EURL S-19.6	Panel2	Temocillin	=	8	S
EURL S-19.7	Panel1	Amikacin	=	32	**R**
EURL S-19.7	Panel1	Ampicillin	>	32	**R**
EURL S-19.7	Panel1	Azithromycin	=	4	S
EURL S-19.7	Panel1	Cefotaxime	>	4	**R**
EURL S-19.7	Panel1	Ceftazidime	>	8	**R**
EURL S-19.7	Panel1	Chloramphenicol	≤	8	S
EURL S-19.7	Panel1	Ciprofloxacin	=	8	**R**
EURL S-19.7	Panel1	Colistin	≤	1	S
EURL S-19.7	Panel1	Gentamicin	>	16	**R**
EURL S-19.7	Panel1	Meropenem	=	0.25	**R**
EURL S-19.7	Panel1	Nalidixic acid	>	64	**R**
EURL S-19.7	Panel1	Sulfamethoxazole	>	512	**R**
EURL S-19.7	Panel1	Tetracycline	>	32	**R**
EURL S-19.7	Panel1	Tigecycline	=	0.5	S
EURL S-19.7	Panel1	Trimethoprim	≤	0.25	S
EURL S-19.7	Panel2	Cefepime	=	2	**R**
EURL S-19.7	Panel2	Cefotaxime	=	64	**R**
EURL S-19.7	Panel2	Cefotaxime-clavulanic acid	=	64	**R**
EURL S-19.7	Panel2	Cefoxitin	>	64	**R**
EURL S-19.7	Panel2	Ceftazidime	=	64	**R**
EURL S-19.7	Panel2	Ceftazidime-clavulanic acid	=	32	**R**
EURL S-19.7	Panel2	Ertapenem	=	0.5	**R**
EURL S-19.7	Panel2	Imipenem	=	2	**R**
EURL S-19.7	Panel2	Meropenem	=	0.25	**R**
EURL S-19.7	Panel2	Temocillin	>	128	**R**
EURL S-19.8	Panel1	Amikacin	≤	4	S
EURL S-19.8	Panel1	Ampicillin	>	32	**R**
EURL S-19.8	Panel1	Azithromycin	=	8	S
EURL S-19.8	Panel1	Cefotaxime	>	4	**R**

Strain ID	Panel	Antimicrobial	Operator	Value	Interpretation
EURL S-19.8	Panel1	Ceftazidime	>	8	**R**
EURL S-19.8	Panel1	Chloramphenicol	≤	8	S
EURL S-19.8	Panel1	Ciprofloxacin	=	1	**R**
EURL S-19.8	Panel1	Colistin	≤	1	S
EURL S-19.8	Panel1	Gentamicin	≤	0.5	S
EURL S-19.8	Panel1	Meropenem	=	0.06	S
EURL S-19.8	Panel1	Nalidixic acid	=	32	**R**
EURL S-19.8	Panel1	Sulfamethoxazole	>	512	**R**
EURL S-19.8	Panel1	Tetracycline	>	32	**R**
EURL S-19.8	Panel1	Tigecycline	=	0.5	S
EURL S-19.8	Panel1	Trimethoprim	=	0.5	S
EURL S-19.8	Panel2	Cefepime	=	1	**R**
EURL S-19.8	Panel2	Cefotaxime	=	32	**R**
EURL S-19.8	Panel2	Cefotaxime-clavulanic acid	=	32	**R**
EURL S-19.8	Panel2	Cefoxitin	>	64	**R**
EURL S-19.8	Panel2	Ceftazidime	=	64	**R**
EURL S-19.8	Panel2	Ceftazidime-clavulanic acid	=	64	**R**
EURL S-19.8	Panel2	Ertapenem	=	0.06	S
EURL S-19.8	Panel2	Imipenem	=	0.25	S
EURL S-19.8	Panel2	Meropenem	=	0.06	S
EURL S-19.8	Panel2	Temocillin	=	8	S

Campylobacter

Strain ID	Antimicrobial	Operator	Value	Interpretation
EURL C-19.1	Chloramphenicol	=	4	S
EURL C-19.1	Ciprofloxacin	=	8	**R**
EURL C-19.1	Ertapenem	=	1	**R**
EURL C-19.1	Erythromycin	≤	1	S
EURL C-19.1	Gentamicin	≤	0.25	S
EURL C-19.1	Tetracycline	>	64	**R**
EURL C-19.2	Chloramphenicol	=	4	S
EURL C-19.2	Ciprofloxacin	=	16	**R**
EURL C-19.2	Ertapenem	=	0.5	S
EURL C-19.2	Erythromycin	≤	1	S
EURL C-19.2	Gentamicin	≤	0.25	S
EURL C-19.2	Tetracycline	=	64	**R**
EURL C-19.3	Chloramphenicol	>	64	**R**
EURL C-19.3	Ciprofloxacin	=	0.5	S
EURL C-19.3	Ertapenem	=	2	**R**
EURL C-19.3	Erythromycin	=	8	S
EURL C-19.3	Gentamicin	=	0.5	S
EURL C-19.3	Tetracycline	>	64	**R**
EURL C-19.4	Chloramphenicol	≤	2	S
EURL C-19.4	Ciprofloxacin	=	16	**R**
EURL C-19.4	Ertapenem	=	0.25	S
EURL C-19.4	Erythromycin	=	512	**R**
EURL C-19.4	Gentamicin	=	0.5	S

Strain ID	**Antimicrobial**	**Operator**	**Value**	**Interpretation**
EURL C-19.4	Tetracycline	>	64	**R**
EURL C-19.5	Chloramphenicol	≤	2	S
EURL C-19.5	Ciprofloxacin	=	8	**R**
EURL C-19.5	Ertapenem	=	0.25	S
EURL C-19.5	Erythromycin	>	512	**R**
EURL C-19.5	Gentamicin	=	16	**R**
EURL C-19.5	Tetracycline	>	64	**R**
EURL C-19.6	Chloramphenicol	=	8	S
EURL C-19.6	Ciprofloxacin	≤	0.125	S
EURL C-19.6	Ertapenem	=	0.25	S
EURL C-19.6	Erythromycin	≤	1	S
EURL C-19.6	Gentamicin	≤	0.25	S
EURL C-19.6	Tetracycline	≤	0.5	S
EURL C-19.7	Chloramphenicol	=	16	S
EURL C-19.7	Ciprofloxacin	=	16	**R**
EURL C-19.7	Ertapenem	=	0.25	S
EURL C-19.7	Erythromycin	=	2	S
EURL C-19.7	Gentamicin	>	16	**R**
EURL C-19.7	Tetracycline	>	64	**R**
EURL C-19.8	Chloramphenicol	=	4	S
EURL C-19.8	Ciprofloxacin	=	16	**R**
EURL C-19.8	Ertapenem	=	0.5	S
EURL C-19.8	Erythromycin	≤	1	S
EURL C-19.8	Gentamicin	>	16	**R**
EURL C-19.8	Tetracycline	>	64	**R**

DTU Fødevareinstituttet
DTU National Food Institute

Henrik Dams Allé
2800 Kgs. Lyngby

+45 35 88 70 00

food.dtu.dk

DANAK
PT Reg.nr. 516

European Union Reference Laboratory Antimicrobial Resistance