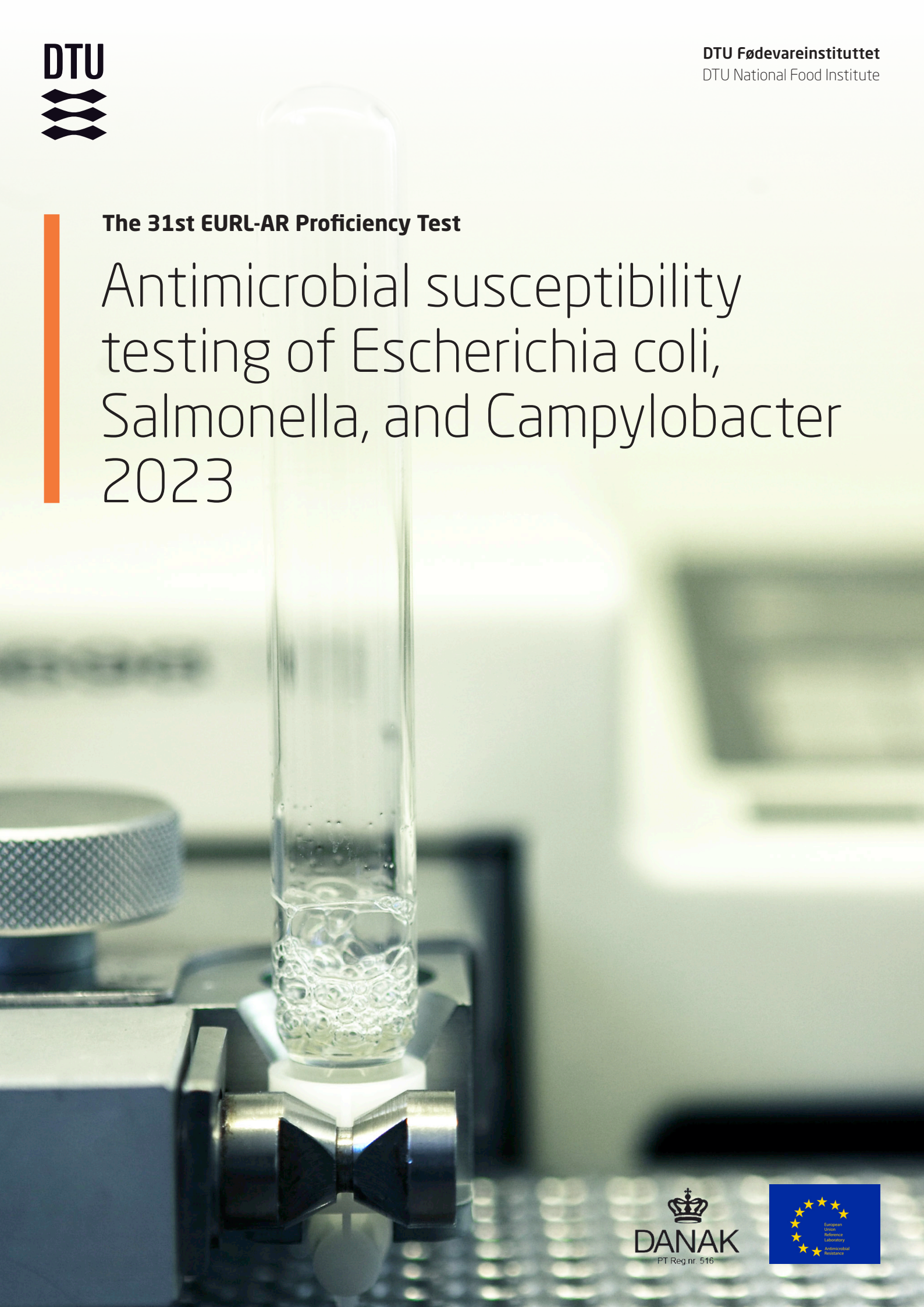
DTU Fødevareinstituttet
DTU National Food Institute

The 31st EURL-AR Proficiency Test

Antimicrobial susceptibility testing of Escherichia coli, Salmonella, and Campylobacter 2023



DANAK
PT Reg.nr. 516

European Union Reference Laboratory Antimicrobial Resistance

DTU Fødevareinstituttet
DTU National Food Institute

The 31st EURL-AR Proficiency Test

Antimicrobial susceptibility testing of Escherichia coli, Salmonella and Campylobacter 2023

European Union Reference Laboratory – Antimicrobial Resistance
Final version, 1. edition, December 2024

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

EQAS Coordinator:
Susanne Karlsmose Pedersen

Photo:
DTU National Food Institute

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

ISBN:
978-87-7586-043-2

food.dtu.dk

DANAK
PT Reg.nr. 516

Table of Contents

List of Appendices

1. Introduction

As the EU Reference Laboratory for Antimicrobial Resistance (EURL-AR), the National Food Institute (DTU Food) conducted in Autumn 2023 the 31st Proficiency Test (PT) for the antimicrobial susceptibility testing (AST) of commensal and zoonotic bacteria. The EURL-AR is accredited by the Danish Accreditation Fund (DANAK) as provider of PTs for the identification, serotyping and AST of zoonotic pathogens and indicator organisms, with accreditation number 516.

The EURL-AR PT for AST has been carried out annually since 2006 and aims to: i) monitor the quality of AST results produced by the participating National Reference Laboratories (NRL-AR), ii) identify NRL-AR's which may need assistance to improve their performance in AST and iii) determine possible topics for further research or collaboration. The overall aim of the EURL-AR PT for AST has been to evaluate and improve the comparability of the Antimicrobial Resistance (AMR) surveillance data reported to the European Food Safety Authority (EFSA) by the different NRL-ARs, as part of the official EU AMR monitoring [1].

In the 31st EURL-AR PT for AST, eight strains of each of the following three organisms were included: *Escherichia coli*, *Salmonella*, and *Campylobacter*. More specifically, the present PT includes the following four components:

1. AST by broth microdilution (BMD) of 8× *E. coli*, 8× *Salmonella* and 8× *Campylobacter* test strains, determination of the Minimum Inhibitory Concentration (MIC) value as well as phenotype interpretation for each strain as "Susceptible" (S) or "Resistant" (R) to a specific panel of antimicrobials for each organism.
2. AST by BMD of specific reference bacterial strains for each organism and evaluation of the obtained MIC values based on the QC ranges published in the most updated guidelines from the Clinical and Laboratory Standards Institute (CLSI) [2, 3].
3. Identification of the β-lactam resistance mechanism phenotype of the relevant *E. coli* and *Salmonella* strains, as Extended-Spectrum-β-Lactamase-producing (ESBL), AmpC-β-lactamase-producing (AmpC) and Carbapenemase-producing phenotypes, based on BMD data.
4. Identification of the bacterial species of the *Campylobacter* strains as *C. jejuni* or *C. coli*.

The results of the 31st EURL-AR 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 meet annually at the EURL-AR workshop. The present report, at its final version, is publicly available at the EURL-AR website (https://www.eurl-ar.eu/reports.aspx).

2. Strains and Antimicrobials

An overview of the test strains, the reference strains as well as the additional quality control (QC) strains included in the 31st EURL-AR PT for AST is presented in

Table 1. Eight test strains for each organism (*E. coli*, *Salmonella* and *Campylobacter*) were selected among isolates from the strain collection at DTU Food, based on their AMR profiles. As an internal control, one strain from each organism has been incorporated in all PT iterations conducted to date for the purpose of quality assurance.

***Table 1**. Test, reference and additional quality control (QC) strains included in the 31st EURL-AR PT for AST for E. coli, Salmonella and Campylobacter. Internal control (IC) strains have been included in all PT iterations to date and are highlighted in **bold**.*

	E. coli	***Salmonella***	***Campylobacter***
Test strains	EURL 2023 EC-18.1	EURL 2023 S-18.1	**EURL 2023 C-18.1 (IC)**
	EURL 2023 EC-18.2	EURL 2023 S-18.2	EURL 2023 C-18.2
	EURL 2023 EC-18.3	EURL 2023 S-18.3	EURL 2023 C-18.3
	EURL 2023 EC-18.4	EURL 2023 S-18.4	EURL 2023 C-18.4
	EURL 2023 EC-18.5	EURL 2023 S-18.5	EURL 2023 C-18.5
	EURL 2023 EC-18.6	EURL 2023 S-18.6	EURL 2023 C-18.6
	EURL 2023 EC-18.7	EURL 2023 S-18.7	EURL 2023 C-18.7
	EURL 2023 EC-18.8 (IC)	**EURL 2023 S-18.8 (IC)**	EURL 2023 C-18.8
Reference strains	*E. coli* CCM 3954 (ATCC 25922)	*E. coli* CCM 3954 (ATCC 25922)	*C. jejuni* CCM 6214 (ATCC 33560)
Additional QC strains	*Acinetobacter baumannii (2012-70-100-69)*	*A. baumannii (2012-70-100-69)*	*C. coli (2012-70-443-2)*

***Table 2**. Panels of antimicrobial compounds used in the 31st EURL-AR PT on AST for each organism. The abbreviations for the antimicrobial compounds and the concentration range tested are also provided. E. coli or Salmonella test strains resistant to the cephalosporins (cefotaxime and ceftazidime) and/or meropenem in EUVSEC3 were additionally tested in EUVSEC2.*

Organism(s) / Panel	**Antimicrobial**	**Abbreviaton**	**Range (mg/L)**
E. coli & *Salmonella* / EUVSEC3 (Panel 1)	Amikacin	AMI	4-128
	Ampicillin	AMP	1-32
	Azithromycin	AZI	2-64
	Cefotaxime	FOT or CTX	0.25-4
	Ceftazidime	TAZ or CAZ	0.25-8
	Chloramphenicol	CHL	8-64
	Ciprofloxacin	CIP	0.015-8
	Colistin	COL	1-16
	Gentamicin	GEN	0.5-16
	Meropenem	MERO	0.03-16
	Nalidixic acid	NAL	4-64
	Sulfonamides	SMX	8-512
	Tetracycline	TET	2-32
	Tigecycline	TGC	0.25-8
	Trimethoprim	TMP	0.25-16
E. coli & *Salmonella* / EUVSEC2 (Panel 2)	Cefepime	FEP	0.06 - 32
	Cefotaxime	FOT or CTX	0.25 – 64
	Cefotaxime/Clavulanic acid	F/C	0.06/4 - 64/4
	Cefoxitin	FOX	0.5 - 64
	Ceftazidime	TAZ or CAZ	0.25 - 128
	Ceftazidime/Clavulanic acid	T/C	0.125/4 - 128/4
	Ertapenem	ETP	0.015-2
	Imipenem	IMI	0.125 - 16
	Meropenem	MERO	0.03 - 16
	Temocillin	TRM	0.5-64
Campylobacter / EUCAMP3 (Panel 1)	Chloramphenicol	CHL	2-64
	Ciprofloxacin	CIP	0.125-32
	Ertapenem	ETP	0.125-4
	Erythromycin	ERY	1-512
	Gentamicin	GEN	0.25-16
	Tetracycline	TET	0.5-64

The reference strains *E. coli* CCM 3954 (ATCC 25922) and *C. jejuni* CCM 6214 (ATCC 33560) had been provided to all participating laboratories when they were new participants, with instructions on how to store and maintain them for quality assurance purposes and future PT trials. Moreover, the EURL-AR has distributed *Acinetobacter baumannii* (2012-70-100-69) as well as *C. coli* (2012-70-443-2) for the purpose of performing additional method QC when performing AST for *E. coli or Salmonella, and Campylobacter* respectively.

The antimicrobial compounds tested in the 31st EURL-AR PT for AST are presented in **Table 2**, as well as in the protocol (Appendix 2) and they correspond to the panel of antimicrobials listed in the Commission Implementing Decision 2020/1729/EU [1]. A mandatory part of this PT was to detect ESBL-, AmpC- and carbapenemase-producing *E. coli* and *Salmonella* strains; therefore, two antimicrobial panels were included in the testing of the *E. coli* and *Salmonella* strains, as specified in the Commission Implementing Decision mentioned above. *E. coli* or *Salmonella* test strains resistant to the cephalosporins (cefotaxime, ceftazidime) and/or meropenem in EUVSEC3 (Panel 1) were additionally tested on EUVSEC2 (Panel 2).

3. Participants

A pre-notification (Appendix 1) to announce the 31st EURL-AR PT for AST of *E. coli*, *Salmonella*, and *Campylobacter* was distributed on 18 August 2023 by e-mail to the laboratories of the EURL-AR-network contact list, including all EU countries as well as Iceland, Moldova, North Macedonia, Norway, Serbia, Switzerland, Turkey and the United Kingdom. Participating laboratories from non-EU countries or laboratories not designated as NRL-AR of their country were charged a fee for their participation, whereas the NRL-ARs from EU Member States (one per member state) participated free of charge. Participating laboratories are identified by codes and each code is known only by the corresponding laboratory. The full list of laboratory codes is confidential and known only by relevant representatives of the EURL-AR and the EU Commission.

This report provides data from thirty-four countries, with each country represented by a single laboratory. This report evaluates, in total, 33, 34, and 28 sets of results from the trials involving *E. coli*, *Salmonella*, and *Campylobacter*, respectively. This year, a number of laboratories abstained from testing one or more of the *Campylobacter* strains, despite having initially registered for this organism, because the bacterial culture(s) received in transport swabs were not viable. This was the consequence of a deviation that occurred during the EURL-AR's swab preparation process, which rendered certain swabs nonviable upon arrival. The EURL-AR promptly implemented corrective measures in response to this discovery in order to mitigate the situation. The outcomes in cases where the revival of one or more *Campylobacter* strains was unsuccessful were not assessed, i.e. laboratories did not receive a penalty for not submitting results for *Campylobacter* strains that was not viable.

4. Procedure

In October 2023, bacterial strains in transport swabs (Amies gel with charcoal), together with a cover letter (Appendix 3) were dispatched to the participating laboratories. The shipment (UN3373, biological substances category B) was sent according to International Air Transport Association (IATA) regulations. Protocols and all relevant information were uploaded to the EURL-AR website (http://www.eurl-ar.eu), allowing PT participants to access this information at any time. Moreover, information on how to handle the test and reference strains were provided to the participants (see Appendices 2 and 3).

Participants were requested to perform BMD and MIC determination according to their routine procedures as stated in the protocol (Appendix 2), by using the methods stated in the EU Commission Implementing Decision 2020/1729/EU [1], i.e., the international reference method ISO standard 20776-1:2019. Participants had to perform bacterial species identification for the *Campylobacter* test strains, according to their routine procedures. For interpretation of the results, the cut-off values listed in Tables 2, 3, 4, 5 and 6 of the protocol (Appendix 2) were used, which represent the current epidemiological cut-off values (ECOFF) developed by EUCAST (www.eucast.org), unless otherwise stated. A strain is classified as "Resistant" to a particular antimicrobial compound if the MIC value is greater than the ECOFF value and conversely, it is classified as "Susceptible" if the MIC value is equal to or less than the ECOFF value. When interpreting MIC values based on ECOFF values, bacteria are categorised as “Wild-type” or “Non-wild-type” [4] and the terms “Susceptible” and “Resistant” should be reserved for classifications made in relation to the therapeutic application of antimicrobial agents. However, to simplify the interpretation of results, throughout this report, the terms “Susceptible” and “Resistant” are used, even if referring to “Wild-type” and “Non-wild-type”, respectively.

In addition, participants were requested to submit the MIC values for the reference strains and additional control QC strains (see **Table 1**) to the relevant antimicrobial compounds for each organism. The obtained results from the EURL-AR additional method QC strains were captured in the webtool. Moreover, participants were requested to perform confirmatory tests for ESBL, AmpC β-lactamase and carbapenemase production on all *E. coli* and *Salmonella* test strains that were resistant to cefotaxime, ceftazidime and/or meropenem in EUVSEC3 (Panel 1) and proceed to testing these strains in EUVSEC2 (Panel 2) to determine the phenotype of β-lactam resistance mechanism, according to the most recent recommendations from EFSA, as described in the protocol (Appendix 2).

Overall, the participants were requested to submit:

1. MIC values and phenotype interpretation as “Susceptible” (S) or “Resistant” (R) to the relevant panel antimicrobials for each organism, as presented in **Table 2**, for each test strain.
2. MIC values of the reference strains and the additional QC strains to the relevant panel antimicrobials for each organism, as presented in **Table 2**.
3. The identified phenotype for β-lactam resistance mechanism of the relevant *E. coli* and *Salmonella* strains, as ESBL-, AmpC β-lactamase- and carbapenemase-producing phenotypes.
4. The identification of the bacterial species of the *Campylobacter* strains as *C. jejuni* or *C. coli*.

Participating laboratories were invited to submit the obtained results into an electronic record sheet at the EURL-AR webtool through a secured individual login and password. After submission of results, an evaluation of the submitted data was performed and the results were made available to participants on 19 March 2024. After this date, the participants were invited to login to the webtool and retrieve an individual, database-generated report which contained an evaluation of the submitted results.

5. Expected results

MIC determination of the *E. coli* and *Salmonella* test strains was performed using the Sensititre system EUVSEC3 and EUVSEC2, while for *Campylobacter* using the Sensititre EUCAMP3 (Trek Diagnostic Systems Ltd, UK). An overview of the antimicrobial compounds included in each panel as well as the concentration ranges is presented in **Table 2**.

The expected MIC values were generated by performing BMD for all test strains in two different occasions at the Technical University of Denmark, National Food Institute (DTU Food). The expected results were verified by the Laboratory of Gastrointestinal Bacteria, Department for Bacteria, Parasites & Fungi, Statens Serum Institut, Denmark. MIC determination was conducted at DTU Food after the preparation of the transport swabs for distribution to participants to ensured that the test material comprised the accurate strains that corresponded to the anticipated MIC values. 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) which represent ECOFFs developed by EUCAST (www.eucast.org), unless otherwise stated. The expected MIC values and phenotype interpretation for each test strain are presented in Appendix 4.

The method applied for the AST was the ISO standard, ISO 20776-1 “Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices”, and, in addition, the following guidelines/standards from the Clinical and Laboratory Standards Institute (CLSI) were applied: Document M7-A11 (2019) “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Eleventh Edition”; document M100, 33rd ed. (2023) “Performance Standards for Antimicrobial Susceptibility Testing”, document VET01 (2018) “Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals” – Fifth Edition; and document VET06 (2017) “Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated from Animals” – First Edition.

For the reference strains *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560 the expected MIC values had to be within the QC ranges according to the latest editions of CLSI documents M100 [2] and VET06 [3]. An overview of the QC ranges for each antimicrobial is presented in **Table 3** for *E. coli* ATCC25922 and in **Table 4** for *C. jejuni* ATCC 33560.

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). The expected phenotypes are presented in **Table 5** for each strain and were confirmed by Whole Genome Sequencing (WGS), i.e., genotype was identified in ResFinder-4.2 [5], software: 2023-08-22, ResFinder database: 2023-04-12, PointFinder database: 2023-05-03, using assembled short reads. The E. coli strain EURL EC-18.1 and the Salmonella strain EURL S-18.3 fall into two categories regarding the expected phenotype for β-lactam resistance mechanism. These strains were initially categorised as ESBL based on the expected MIC for cefoxitin of 8 mg/L. However, if a MIC value of 16 mg/L for cefoxitin is measured, then the strains fall into the ESBL+AmpC-producing phenotype, based on EFSA recommendations. Since a one two-fold dilution step is within the acceptable BMD method limitations, both phenotypes are accepted for these strains.

Finally, *Campylobacter* species 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. Five of the *Campylobacter* test strains were identified as *C. jejuni* (EURL C-18.1, EURL C-18.2, EURL C-18.4, EURL C-18.5 and EURL C-18.7) while EURL C-18.3, EURL C-18.6 and EURL C-18.8 were identified as *C. coli*.

***Table 3**. Quality control (QC) intervals for the reference strain E. coli ATCC25922, according to CLSI M100, 33rd ed. [2].*

***E. coli* ATCC25922**	
Antimicrobial	**MIC range mg/L**
Amikacin	0.5-4
Ampicillin	2-8
Azithromyciin	No data
Cefepime	0.016-0.125
Cefotaxime	0.03-0.125
Cefotaxime+Clavulanic acid	No data
Cefoxitin	2-8
Ceftazidime	0.06-0.5
Ceftazidime+Clavulanic acid	No data
Chloramphenicol	2-8
Ciprofloxacin	0.004-0.016
Colistin	0.25-2
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	No data
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, 33rd ed. [2]).

***Table 4**. 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

***Table 5**. Expected phenotype for β-lactam resistance mechanism for each strain,as determined by the obtained MIC values. The phenotypes were confirmed by whole genome sequencing using ResFinder-4.2. The GenBank accession number of the hit with 100% identity and coverage appears in parenthesis next to the gene name.*

	Strain	Expected phenotype for β-lactam resistance mechanisms	Detected β-lactam resistance gene (GenBank accession number)
E. coli	**EURL EC-18.1**	ESBL **or** ESBL+AmpC producing	*bla*$_{CTX-M-1}$ (DQ915955) *bla*$_{TEM-1B}$ (AY458016)
	EURL EC-18.2	AmpC producing	*ampC* promoter: n.-42C>T
	EURL EC-18.3	ESBL+AmpC producing	*bla*$_{TEM-1B}$ (AY458016) *bla*$_{CTX-M-32}$ (AJ557142) *bla*$_{CMY-2}$ (X91840)
	EURL EC-18.4	ESBL+AmpC producing	*bla*$_{TEM-1B}$ (AY458016), *bla*$_{CTX-M-1}$ (DQ915955), *ampC* promoter: n.-42C>T
	EURL EC-18.5	Carbapenemase producing	*bla*$_{NDM-5}$ (JN104597) *bla*$_{TEM-1A}$ (HM749966)
	EURL EC-18.6	Carbapenemase producing	*bla*$_{NDM-5}$ (JN104597) *bla*$_{CTX-M-15}$ (AY044436) *bla*$_{OXA-1}$ (HQ170510)
	EURL EC-18.7	Susceptible to β-lactams	None
	EURL EC-18.8	ESBL producing	*bla*$_{CTX-M-1}$ (DQ915955)
Salmonella	**EURL S-18.1**	AmpC producing	*bla*$_{CMY-2}$ (X91840)
	EURL S-18.2	AmpC producing	*bla*$_{CMY-2}$ (X91840) *bla*$_{TEM-1B}$ (AY458016)
	EURL S-18.3	ESBL **or** ESBL+AmpC producing	*bla*$_{CTX-M-1}$ (DQ915955)
	EURL S-18.4	Carbapenemase producing	*bla*$_{OXA-48}$ (AY236073)
	EURL S-18.5	Carbapenemase producing	*bla*$_{NDM-1}$ (FN396876)
	EURL S-18.6	Carbapenemase producing	*bla*$_{NDM-1}$ (FN396876) *bla*$_{OXA-9}$ (KQ089875) *bla*$_{OXA-10}$ (J03427)
	EURL S-18.7	Susceptible to β-lactams	None
	EURL S-18.8	ESBL producing	*bla*$_{CTX-M-9}$ (AF174129) *bla*$_{TEM-1B}$ (AY458016)

6. Scoring system and evaluation of submitted results

The evaluation of the submitted AST results was based on the phenotype interpretation for each strain as susceptible (S) or resistant (R), based on the MIC values determined by the participating NRL-ARs. Only the phenotype interpretations as “R” or “S” were evaluated, whereas the MIC values were used as supplementary information. According to the criteria presented in the table below, each submitted result was assigned a score. For the phenotype interpretation (R or S), the identification of the β-lactam resistance mechanism phenotype and the *Campylobacter* species identification, the submission of expected results was scored with a “1”, the submission of not expected results with a “0”, while non-submission of data had no penalty (blank score).

Result type	Score
Submission of expected result	1
Submission of non-expected result	0
Non-submission of result	Blank

For the reference strains *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560 submitted MIC values were evaluated towards the QC ranges according to **Table 3** and **Table 4**. If the submitted MIC value for each antimicrobial was within the QC range, results were scored with a “1”, while when the submitted MIC

value was outside the accepted range, results were scored with a “0”. For non-submission of results for the reference strains, there was no penalty (blank score). The obtained results from the EURL-AR additional method QC strains were captured in the webtool and are presented in the laboratories’ individual evaluation report. However, no further overall analysis of the EURL-AR additional method QC strain results is performed in the present PT report.

The assessment of an incorrect AST phenotype interpretation (R or S) should be meticulously examined through a self-evaluation process carried out by the participants. This should also encompass deliberations concerning any corrective measures implemented within the laboratory. It is important to note that one two-fold dilution difference in the MIC value of a particular antimicrobial is not regarded as an error when testing the same strains, due to the inherent limitations of the methods used for MIC determination. In case the expected MIC is close to the ECOFF value, one two-fold dilution difference, which is within the acceptable method limitations, may result in two different interpretations, *i.e.*, the same strain can be categorised as susceptible or resistant. The present report is based on evaluation of phenotype interpretations as susceptible or resistant; therefore, some participants may find their results classified as incorrect even though the actual MIC value they reported is only one two-fold dilution away from the expected MIC value. In the present report, these cases are referred to as “breakpoint issues”.

The submitted AST data, *i.e.*, the phenotype interpretation for each strain as “susceptible” or “resistant”, were further evaluated based on the following criteria:

1) The EURL-AR network reached a consensus on establishing the maximum acceptable deviation level for laboratory performance on AST for each microorganism tested (*E. coli*, *Salmonella, Campylobacter* and *S. aureus*) at 5%.

2) In 2008, the EURL-AR network reached a consensus that a thorough investigation into the underlying causes of incorrect results for a particular strain/antimicrobial combination is mandatory when the rate of correct results falls below 75% (25% deviation level). For each strain-antimicrobial combination with less than 75% correct results, that the deviations were due to a “breakpoint issue”, all scores for that strain-antimicrobial combination were blanked.

7. Results and Discussion

7.1. AST data omitted from the evaluation

Several laboratories did not test one or more of the *Campylobacter* strains this year, even though they had initially registered for this organism, due to the fact that the bacteria they received in transport swabs were not viable. This was the result of an error that occurred during the preparation of swabs at DTU Food, resulting in some swabs being nonviable upon arrival. Following this discovery, the EURL-AR initiated immediate a corrective action, and results in situations where the recovery of one or more strains of *Campylobacter* failed were not evaluated, therefore there is no impact on the evaluation of the laboratory performance. All scores for strain EURL C-18.2 were blanked for all participants as the majority of the submitted data for this strain were of poor quality.

Moreover, isolate EURL EC-18.7 was identified after the test material distribution as a Shiga toxin-producing *E. coli* (STEC) and therefore participants were notified to discard it unless having the required biosafety level facilities. The submitted results are still analysed in the present report.

The percent deviation level was calculated for each strain-antimicrobial combination, per organism, *i.e.*, for the *E. coli*, *Salmonella* and *Campylobacter* trial. For strain-antimicrobial combinations with more than or equal to 25% deviation level, the discrepancies were further analysed to evaluate if they were caused by a breakpoint issue (see above). In cases for which the deviations were mainly attributed to a breakpoint issue, the results were omitted from the evaluation, *i.e.*, all scores (both 1 and 0) for that strain-antimicrobial combination were blanked. The overview of all the strain-antimicrobial combinations that were omitted from the evaluation are presented in **Table 6**.

***Table 6**. Strain-antimicrobial combinations omitted from the evaluation.*

Trial	Strain/antimicrobial combination omitted from the evaluation (Scores blanked)	%Expected results
E. coli	EURL EC-18.1/Cefoxitin	70
	EURL EC-18.3/Ertapenem	58
Salmonella	EURL S-18.3/Cefoxitin	50
	EURL S-18.3/Tigecycline	44
	EURL S-18.6/Tigecycline	50
Campylobacter	EURL C-18.4/Ertapenem	60
	EURL C-18.5/Ertapenem	50

7.2. Overall Performance on AST Results

The deviation level for all AST results for each trial as well as for the AST results of the internal control strains (EURL EC-18.8, EURL S-18.8 and EURL C-18.1) are presented in **Table 7**. The 31st EURL-AR PT for AST is the sixteenth iteration for *E. coli* and the eighteenth for *Salmonella* and *Campylobacter*; the deviation levels of all previous iterations are presented in **Figure 1**. The AST data from 2023 reflect a high level of performance, with the overall deviation level for each trial ranging from 1.1% (*E. coli* and *Salmonella* trial) to 1.3% (*Campylobacter* trial), similar to the results of the previous years. For the *E. coli* and *Salmonella* internal control strains, the percent deviation level was very low (0.1 and 0.2 % respectively) while for the *Campylobacter* internal control strain an increase of 4.5% was observed (149 correct results from 156 submitted) compared to the levels of 2021 and 2022 where the deviation level was zero. Six out of seven deviations were due to incorrect MIC values reported for ertapenem for this strain (one or two two-fold dilutions greater than the expected value).This increase should be also viewed as an artifact of the lower number of results submitted in the *Campylobacter* internal control strain (156 results submitted) compared to the *E. coli* and *Salmonella* internal control strains (806 and 841 results submitted respectively), due to the lower level of participation in the *Campylobacter* trial in general.

***Table 7**. Overall* deviation *levels for the AST results of the E. coli, Salmonella and Campylobacter trials as well as for the internal control strains for each trial of the 31st EURL-AR PT.*

Trial or Internal control strain	Sum of Score	Count of Score	%Deviation level
E. coli	6140	6206	1.1
Salmonella	6277	6348	1.1
Campylobacter	943	955	1.3
EURL EC-18.8	805	806	0.1
EURL S-18.8	839	841	0.2

EURL C-18.1	149	156	4.5

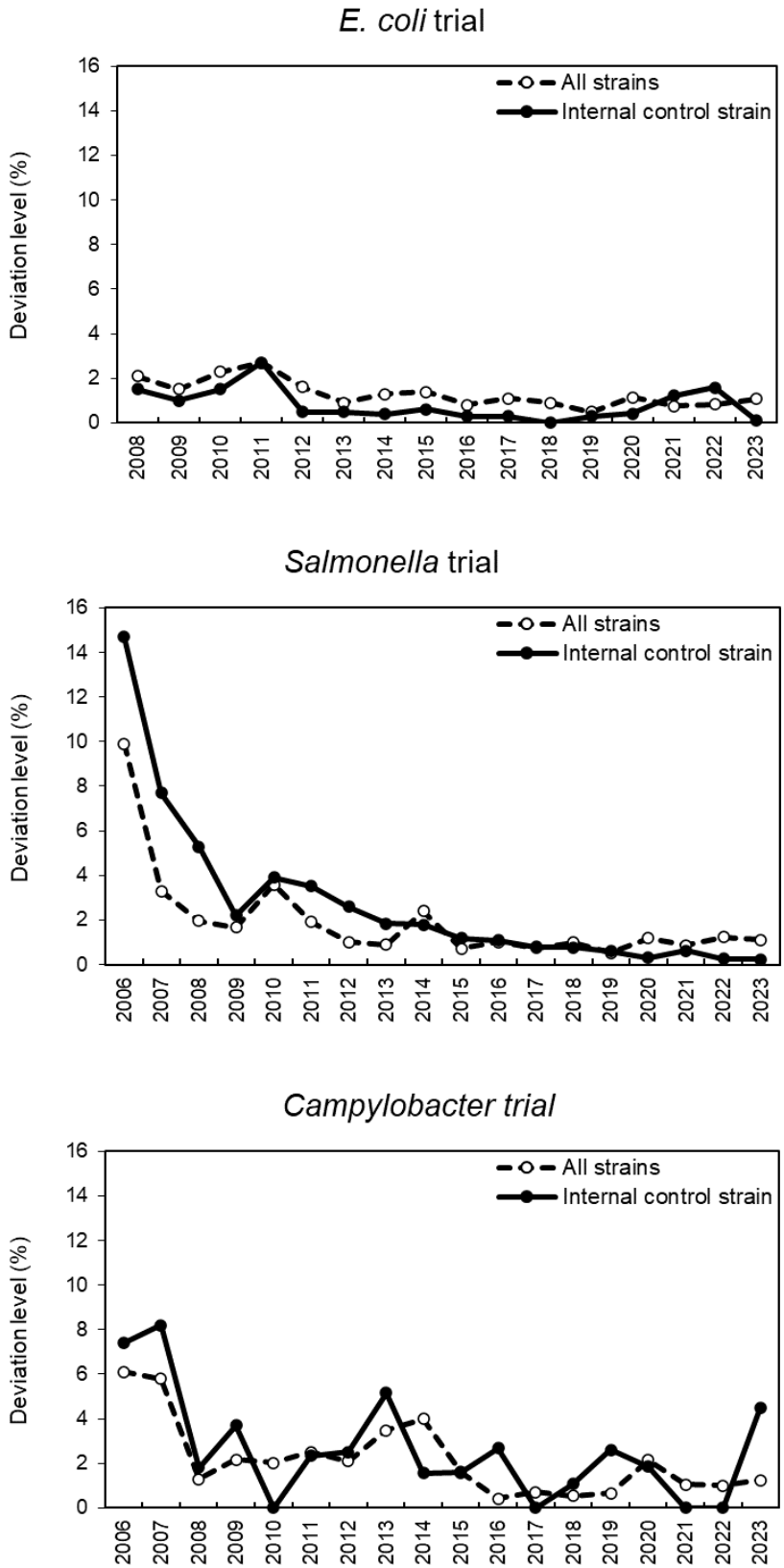


***Figure 1**. A comparison between the EURL-AR PTs for AST since 2006, showing the percent* deviation *level for the AST results for E. coli (Top), Salmonella (Middle) and Campylobacter (Bottom) trials.*

7.3. Individual Laboratory Performance on AST Results

The percent deviation level for each laboratory for the AST results was calculated based on the submitted sum of scores and count of scores for each trial (**Table 8**). The laboratories with non-zero percent deviation level are presented in the bar charts in **Figure 2**, for each organism. The outcomes were assessed in accordance with the EURL-AR network's consensus regarding the establishment of a 5% maximum acceptable deviation level for laboratory performance on AST for each organism. For the *E. coli* trial one of thirty-three participating laboratories exceeded this limit, obtaining a deviation level of 9.0%. This seems to be attributed to a mix of results between strain EURL EC-18.1 and EURL EC-18.2 when submitting the data. For *Salmonella* and *Campylobacter* two laboratories out of thirty-four and twenty-eight respectively, obtained a deviation level between 5.4 and 10.0%.

***Table 8**. 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 with decreasing percent* deviation *level.* Deviation levels over the limit of 5% established by the EURL-AR network are marked in red.

***E. coli* trial**				***Salmonella* trial**				***Campylobacter* trial**			
Lab. code	**Score (Sum)**	**Score (Count)**	**% Dev.**	**Lab. code**	**Score (Sum)**	**Score (Count)**	**% Dev.**	**Lab. code**	**Score (Sum)**	**Score (Count)**	**% Dev.**
NRL-AR-037	171	188	9.0	NRL-AR-045	174	187	7.0	NRL-AR-061	9	10	10.0
NRL-AR-045	167	173	3.5	NRL-AR-064	140	148	5.4	NRL-AR-019	37	40	7.5
NRL-AR-017	169	173	2.3	NRL-AR-018	180	187	3.7	NRL-AR-011	38	40	5.0
NRL-AR-034	169	173	2.3	NRL-AR-040	172	177	2.8	NRL-AR-036	33	34	2.9
NRL-AR-042	169	173	2.3	NRL-AR-062	182	187	2.7	NRL-AR-002	39	40	2.5
NRL-AR-032	178	182	2.2	NRL-AR-006	193	197	2.0	NRL-AR-018	39	40	2.5
NRL-AR-056	184	188	2.1	NRL-AR-012	184	187	1.6	NRL-AR-025	39	40	2.5
NRL-AR-002	194	198	2.0	NRL-AR-017	184	187	1.6	NRL-AR-045	39	40	2.5
NRL-AR-061	194	198	2.0	NRL-AR-021	184	187	1.6	NRL-AR-059	39	40	2.5
NRL-AR-021	184	187	1.6	NRL-AR-034	175	177	1.1	NRL-AR-004	40	40	0.0
NRL-AR-040	185	188	1.6	NRL-AR-037	184	186	1.1	NRL-AR-012	40	40	0.0
NRL-AR-030	186	188	1.1	NRL-AR-033	185	187	1.1	NRL-AR-017	40	40	0.0
NRL-AR-062	186	188	1.1	NRL-AR-060	185	187	1.1	NRL-AR-020	29	29	0.0
NRL-AR-018	196	198	1.0	NRL-AR-026	176	177	0.6	NRL-AR-021	40	40	0.0
NRL-AR-020	187	188	0.5	NRL-AR-032	180	181	0.6	NRL-AR-022	23	23	0.0
NRL-AR-004	197	198	0.5	NRL-AR-022	184	185	0.5	NRL-AR-023	35	35	0.0
NRL-AR-066	197	198	0.5	NRL-AR-002	186	187	0.5	NRL-AR-026	34	34	0.0
NRL-AR-006	198	198	0.0	NRL-AR-011	186	187	0.5	NRL-AR-029	23	23	0.0
NRL-AR-009	188	188	0.0	NRL-AR-016	186	187	0.5	NRL-AR-030	40	40	0.0
NRL-AR-011	188	188	0.0	NRL-AR-030	186	187	0.5	NRL-AR-032	40	40	0.0
NRL-AR-012	188	188	0.0	NRL-AR-042	186	187	0.5	NRL-AR-033	40	40	0.0
NRL-AR-016	173	173	0.0	NRL-AR-004	196	197	0.5	NRL-AR-034	34	34	0.0
NRL-AR-019	188	188	0.0	NRL-AR-023	196	197	0.5	NRL-AR-037	34	34	0.0
NRL-AR-022	182	182	0.0	NRL-AR-058	196	197	0.5	NRL-AR-040	12	12	0.0
NRL-AR-023	198	198	0.0	NRL-AR-066	196	197	0.5	NRL-AR-042	35	35	0.0
NRL-AR-025	188	188	0.0	NRL-AR-009	186	186	0.0	NRL-AR-056	23	23	0.0
NRL-AR-026	188	188	0.0	NRL-AR-019	187	187	0.0	NRL-AR-058	35	35	0.0
NRL-AR-029	188	188	0.0	NRL-AR-020	187	187	0.0	NRL-AR-060	34	34	0.0
NRL-AR-033	188	188	0.0	NRL-AR-025	187	187	0.0				
NRL-AR-036	188	188	0.0	NRL-AR-029	186	186	0.0				
NRL-AR-058	198	198	0.0	NRL-AR-036	187	187	0.0				
NRL-AR-059	198	198	0.0	NRL-AR-056	187	187	0.0				
NRL-AR-060	188	188	0.0	NRL-AR-059	197	197	0.0				
				NRL-AR-061	197	197	0.0				

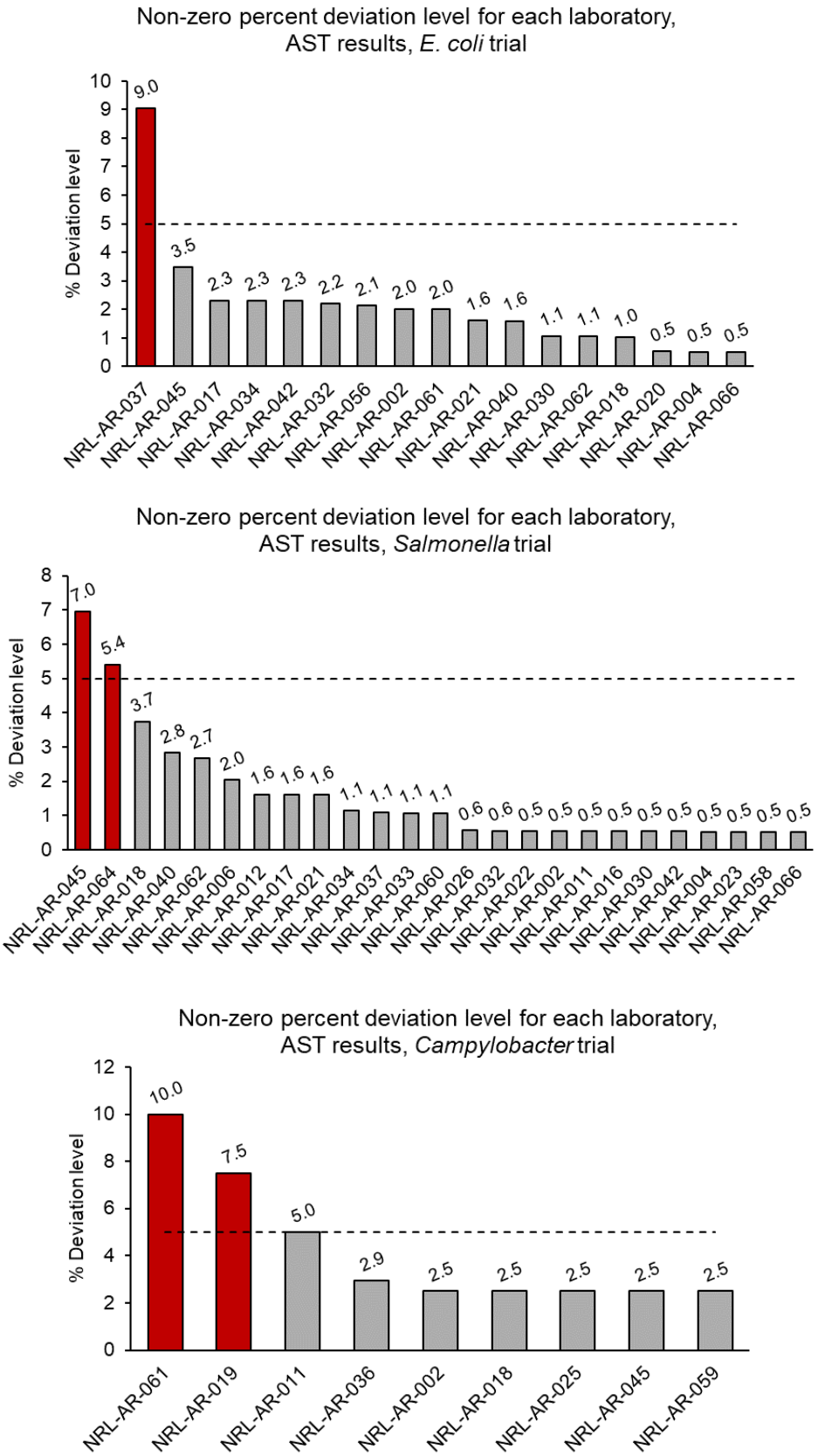


***Figure 2**. Non-zero percent* deviation *level for the AST results of each trial, for each laboratory. The maximum acceptable* deviation *level is 5%. Laboratories that are above the 5% are marked in red. Laboratories with 0.0* deviation *level are omitted from the bar charts but are presented in **Table 8**.*

7.4. Performance on AST Results for Strain-Antimicrobial Combinations

The percent deviation level for each strain-antimicrobial combination was calculated based on the sum and count of all submitted scores. For several strain-antimicrobial combinations, non-zero percent deviation levels were obtained, and these cases are presented in **Table 11**, **Table 12** and **Table 13** for the *E. coli*, *Salmonella* and the *Campylobacter* trials respectively. Strain-antimicrobial combinations for which all submitted results were correct data are not displayed.

In the *E. coli* trial, 102 out of 120 (85.0%) and 66 out of 80 (82.5%) strain-antimicrobial combinations in Panel 1 and Panel 2 respectively had 100% correct results. For the remaining combinations, deviation levels ranged from 3.0% to 9.1%, except for four combinations (ciprofloxacin, chloramphenicol, tetracycline and trimethoprim) from strain EC-18.1, which exhibited deviation levels higher than 20%. In these cases, the obtained MIC values were two to five two-fold dilutions lower than the expected values – see **Table 9**.

Table 9*. Summary of strain/antimicrobial cominations with higher deviation levels for for E. coli strain EC-18.1.*

Antimicrobial	Deviation level (%)	Number of laboratories out of 33 (%)	Submitted MIC mg/L (phenotype)	Expected MIC (phenotype)	Difference of MIC values
Ciprofloxacin	27.3	8 (24)	0.015 (S)	0.125 (R)	**× 2^3** ↓
Chloramphenicol	21.2	6 (18)	16 (S)	64 (R)	**× 2^2**↓
		1 (3)	8 (S)	64 (R)	**× 2^3** ↓
Tetracycline	21.2	7 (21)	2 (S)	32 (R)	**× 2^4** ↓
Trimethoprim	21.2	7 (21)	0.5 (S)	16 (R)	**× 2^5** ↓

To further investigate these cases, assembled short reads of EC-18.1 were run in MOB-Recon in Galaxy (Version 3.0.3+galaxy0) [6] for mobile genetic element identification. Additionally, ResFinder [5] (Database versions ResFinder-2.3.1, PointFinder-4.1.0) was used to identify the AMR genotype. The results, summarized in **Table 10**, showed that the resistance phenotype to ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim is associated with plasmid-encoded genes, which can be sporadically lost. The EURL-AR obtained whole genome sequencing (WGS) data from two EC-18.1 isolates reported to exhibit a susceptible phenotype, from two different NRLs. Both isolates were found to belong to cgST 204049, the same as the original EC-18.1 isolate, as determined by cgMLSTFinder-1.2 (Software version: 1.0.1, 2021-08-29) [7, 8]. Furthermore, single nucleotide polymorphism (SNP) analysis (CSI Phylogeny 1.4 [9]) revealed one to twelve SNP differences between the susceptible EC-18.1 isolates and the original EC-18.1 isolate, suggesting that it is the same isolate that lost plasmid DNA and, consequently, the associated AMR genes.

***Table 10**. Hits for AMR genes and plasmid prediction as outputted in ResFinder and MOB-Recon respectively, from the analysis of assembled short reads for strain EC-18.1.*

Putative AMR phenotype	AMR gene	Contig	%Identity	%Coverage	Accession no.	Molecule	Primary cluster id
Ciprofloxacin	*qnrS1*	Node 63	100.0	100.0	AB187515	Plasmid 1	AA998
Chloramphenicol	*cmlA1*	Node 47	99.9	100.0	M64556	Plasmid 1	AA998
	floR	Node 68	98.1	99.9	AF118107	Plasmid 2	AA474
Tetracycline	*tet(A)*	Node 64	99.9	100.0	AJ517790	Plasmid 2	AA474
Tetracycline	*tet(M)*[1]	Node 65	96.2	100.0	X04388	Chromosome	-
Trimethoprim	*dfrA12*	Node 47	100.0	100.0	AM040708	Plasmid 1	AA998

***Table 11**. Non-zero percent* deviation *levels for strain-antimicrobial combinations for the AST results in E. coli trial, based on the submitted sum of score and count of score.*

Strain	Panel	Antimicrobial	Sum of Score	Count of Score	%Deviation level
EURL EC-18.1	Panel 1	Ciprofloxacin	24	33	27.3
		Chloramphenicol	26	33	21.2
		Tetracycline	26	33	21.2
		Trimethoprim	26	33	21.2
		Ceftazidime	31	33	6.1
		Azithromycin	32	33	3.0
		Colistin	32	33	3.0
		Sulfamethoxazole	32	33	3.0
	Panel 2	Ceftazidime	30	33	9.1
		Cefotaxime-clavulanic acid	30	31	3.2
		Ceftazidime-clavulanic acid	30	31	3.2
		Cefepime	32	33	3.0
EURL EC-18.2	Panel 1	Azithromycin	31	33	6.1
		Chloramphenicol	32	33	3.0
		Ciprofloxacin	32	33	3.0
		Colistin	32	33	3.0
		Sulfamethoxazole	32	33	3.0
	Panel 2	Ceftazidime-clavulanic acid	28	30	6.7
		Cefotaxime-clavulanic acid	29	31	6.5
		Cefepime	31	33	6.1
		Cefoxitin	32	33	3.0
EURL EC-18.3	Panel 2	Temocillin	32	33	3.0
EURL EC-18.4	Panel 1	Tigecycline	32	33	3.0
	Panel 2	Ertapenem	32	33	3.0
EURL EC-18.5	Panel 1	Tigecycline	30	33	9.1
		Azithromycin	31	33	6.1
		Nalidixic acid	32	33	3.0
		Sulfamethoxazole	32	33	3.0
	Panel 2	Temocillin	32	33	3.0
EURL EC-18.8	Panel 2	Ceftazidime	32	33	3.0

In the *Salmonella* trial, 95 out of 120 (79.2%) and 63 out of 80 (78.8%) strain-antimicrobial combinations in Panel 1 and Panel 2 respectively had 100% correct results. For the rest of the strain-antimicrobial combinations, the percent deviation level ranged from 2.9 to 18.2 percent. All deviations are presented in Appendix 5. In the *Campylobacter* trial, 33 out of 48 (68.8%) strain-antimicrobial combinations had 100% correct results. For the rest of the strain-antimicrobial combination, the percent deviation level ranged from 3.6 to 5.6 percent apart from EURL C-18.1/Ertapenem for which the deviation level was 23.1% (20 out of 26 submitted results correct). Participants submitted one or two two-fold dilution steps

[1] The scientific literature does not conclusively demonstrate that the tetracycline resistance ribosomal protection protein Tet(M) alone confers high levels of resistance to tetracycline, therefore when alone it might not be sufficient in conferring a resistant phenotype.

higher than the expected value for this strain/antimicrobial combination: 0.25 (R) or 0.5 (R) instead of 0.125 mg/L (S) that was expected for this strain.

***Table 12**. Non-zero percent* deviation *levels for strain-antimicrobial combinations for the AST results in the Salmonella trial, based on the submitted sum of score and count of score.*

Strain	Panel	Antimicrobial	Sum of Score	Count of Score	%Deviation level
EURL S-18.1	Panel 1	Sulfamethoxazole	30	34	11.8
		Tetracycline	33	34	2.9
	Panel 2	Cefepime	27	33	18.2
EURL S-18.2	Panel 1	Meropenem	33	34	2.9
	Panel 2	Cefepime	32	33	3.0
		Ertapenem	32	33	3.0
		Temocillin	32	33	3.0
EURL S-18.3	Panel 1	Ceftazidime	32	34	5.9
		Azithromycin	33	34	2.9
		Sulfamethoxazole	33	34	2.9
	Panel 2	Ceftazidime-clavulanic acid	28	31	9.7
		Ceftazidime	33	34	2.9
EURL S-18.4	Panel 1	Meropenem	29	34	14.7
		Cefotaxime	30	34	11.8
		Azithromycin	33	34	2.9
		Sulfamethoxazole	33	34	2.9
		Tetracycline	33	34	2.9
		Trimethoprim	33	34	2.9
	Panel 2	Imipenem	25	30	16.7
		Cefotaxime	26	30	13.3
		Cefoxitin	28	30	6.7
		Cefotaxime-clavulanic acid	27	28	3.6
		Cefepime	29	30	3.3
		Ceftazidime	29	30	3.3
		Ertapenem	29	30	3.3
		Meropenem	29	30	3.3
		Temocillin	29	30	3.3
EURL S-18.5	Panel 1	Amikacin	33	34	2.9
		Chloramphenicol	33	34	2.9
		Meropenem	33	34	2.9
		Tigecycline	33	34	2.9
	Panel 2	Imipenem	31	33	6.1
EURL S-18.6	Panel 1	Sulfamethoxazole	32	34	5.9
		Tetracycline	32	34	5.9
		Trimethoprim	32	34	5.9
EURL S-18.7	Panel 1	Sulfamethoxazole	31	34	8.8
		Amikacin	33	34	2.9
EURL S-18.8	Panel 1	Ceftazidime	33	34	2.9
		Sulfamethoxazole	33	34	2.9

***Table 13**. Non-zero percent* deviation *levels for strain-antimicrobial combinations for the AST results in the Campylobacter trial, based on the submitted sum of score and count of score.*

Strain	Antimicrobial	Sum of Score	Count of Score	%Deviation level
EURL C-18.1	Ertapenem	20	26	23.1
	Tetracycline	25	26	3.8
EURL C-18.3	Gentamicin	17	18	5.6
EURL C-18.4	Ciprofloxacin	25	26	3.8
EURL C-18.7	Chloramphenicol	25	26	3.8
EURL C-18.8	Ertapenem	26	27	3.7
	Ciprofloxacin	27	28	3.6

7.5. Performance on β-Lactam Resistance Mechanism Identification

The expected phenotypes for the β-Lactam resistance mechanism of the *E. coli* and *Salmonella* trial are presented in **Table 14** along with the deviation levels per strain, calculated based on the submitted sum of scores and count of scores. The deviation level was at 4.7% for the *E. coli* trial (225 correct results out of 236) and 1.3% for the *Salmonella* trial (235 correct results out of 238).

For the *E. coli* trial, all submitted results were as expected for four of the strains (EURL EC-18.5, EURL EC-18.6, EURL EC-18.7 and EURL EC-18.8). For two strains (EURL EC-18.2 and EURL EC-18.4) there were two deviations for each, from the 33 submitted results. For EURL EC-18.2, for which an AmpC phenotype was expected, one laboratory identified it as ESBL due to unexpected MIC values reported to panel 2 antimicrobials and another as ESBL+AmpC due to incorrect interpretation of the obtained MIC values, or a typing mistake. For EURL EC-18.1 one participant idenitified it as AmpC-phenotype due to unexpected MIC values. Finally, for EC-18.3, the obtained deviation level was 18.2%, which was the highest observed in the PT (27 correct results out of 33). For this strain, three participants identified it as AmpC-phenotype, and three as Other phenotype, attributed to either unexpected MIC values or typing mistakes.

***Table 14**. Percent* deviation *levels for each strain of the E. coli and Salmonella trial for the β-Lactam resistance mechanism identification based on the submitted sum of score and count of scores for each strain.*

	Strain	Expected β-lactam resistance mechanism phenotype	Sum of Score	Count of Score	% Deviation level
E. coli	EURL EC-18.1	ESBL- **or** ESBL+AmpC-producing	32	33	3.0
	EURL EC-18.2	AmpC-producing	31	33	6.1
	EURL EC-18.3	ESBL+AmpC-producing	27	33	18.2
	EURL EC-18.4	ESBL+AmpC-producing	31	33	6.1
	EURL EC-18.5	Carbapenemase-producing	33	33	0.0
	EURL EC-18.6	Carbapenemase-producing	33	33	0.0
	EURL EC-18.7	Susceptible to panel 2 antimicrobials	5	5	0.0
	EURL EC-18.8	ESBL-producing	33	33	0.0
		***E. coli* Total**	**225**	**236**	**4.7**
Salmonella	EURL S-18.1	AmpC-producing	33	34	2.9
	EURL S-18.2	AmpC-producing	34	34	0.0
	EURL S-18.3	ESBL- **or** ESBL+AmpC-producing	33	34	2.9
	EURL S-18.4	Carbapenemase-producing	29	30	3.3
	EURL S-18.5	Carbapenemase-producing	34	34	0.0
	EURL S-18.6	Carbapenemase-producing	34	34	0.0
	EURL S-18.7	Susceptible to panel 2 antimicrobials	4	4	0.0
	EURL S-18.8	ESBL-producing	34	34	0.0
		***Salmonella* Total**	**235**	**238**	**1.3**

For the *Salmonella* trial, five of the eight test strains obtained 100% correct results (EURL S-18.2, EURL S-18.5, EURL S-18.6, EURL S-18.7 and EURL S-18.8). One deviating result was submitted for each of the following strains:

- EURL S-18.1, for which one participant identified it as ESBL+AmpC-phenotype due to incorrect

interpretation of the obtained MIC values or due to a typing mistake.

- EURL S-18.3, for which one participant identified it as Other phenotype even though they obtained the expected MIC values
- EURL S-18.4, for which one participant identified it as ESBL-phenotype due to unexpected MIC valies

The percent deviation level was calculated per laboratory for the β-lactam resistance mechanism phenotype identification, and the results are summarized in **Table 15**. Among the laboratories for which deviations were observed there were one or two unexpected phenotype identifications for each case.

***Table 15**. Non-zero percent* deviation *levels per laboratory for the β-lactam resistance mechanism identification for the E. coli and Salmonella trial, baseded on the submitted sum of scores and count of scores.*

Organism	Lab ID	Sum of Score	Count of Score	% Dev. level
E. coli	NRL-AR-037	5	7	28.6
	NRL-AR-040	5	7	28.6
	NRL-AR-022	6	8	25.0
	NRL-AR-016	6	7	14.3
	NRL-AR-018	6	7	14.3
	NRL-AR-021	6	7	14.3
	NRL-AR-025	6	7	14.3
	NRL-AR-042	6	7	14.3
Salmonella	NRL-AR-020	6	7	14.3
	NRL-AR-045	6	7	14.3
	NRL-AR-061	7	8	12.5

7.6. Reference Strain Results

For the reference strains results, deviations were defined as submitted MIC values that were outside the QC acceptance intervals according to the CLSI standards (see **Table 3** and **Table 4**). For the *C. jejuni* ATCC 33560, 85.7% of the participants (24/28) reported all the results correctly. For the reference strain results 85.7% (24 out of 28), 84.9% (28 out of 33) and 82.4% (28 out of 34) of the participants submitted MIC values within the QC range.

The percent deviation levels were calculated for each laboratory per trial, based on the sum and count of submitted scores (**Table 16**). For the reference strain results in the *Campylobacter* trial, four laboratories submitted one incorrect result out of four results submitted, leading to a higher deviation level percentwise (25.0%). For the *E. coli* and *Salmonella* trial, the deviation levels for the reference stain results were below 5% in general apart from one case where the same laboratory obtained 33.3% deviation for the reference strain in the *Salmonella* trial and 9.5% of the same reference strain in the *E. coli* trial.

The deviation levels for each reference strain-antimicrobial combination were also calculated based on the sum and count of scores of all submitted results and are presented in **Table 17**. The deviation level for the reference strain results was 3.6% for *C. jejuni* ATCC33560 (108 correct out of 112), 0.6% for the *E. coli* ATCC25922 (658 correct out of 664, *E. coli* trial) and 1.7% for *E. coli* ATCC25922 (680 correct out of 692, *Salmonella* trial). From the non-zero percent deviation levels for each reference strain/antimicrobial combination, there were one to three incorrect results submitted, leading to a maximum deviation level of 9.1%.

***Table 16**. Non-zero percent* deviation *levels per laboratory for the reference strain results for each organism based on the submitted sum of scores and count of scores.*

Organism	Lab ID	Sum of Score	Count of Score	%Deviation level
Campylobacter	NRL-AR-004	3	4	25.0
	NRL-AR-011	3	4	25.0
	NRL-AR-030	3	4	25.0
	NRL-AR-045	3	4	25.0
E. coli	NRL-AR-061	19	21	9.5
	NRL-AR-029	19	20	5.0
	NRL-AR-009	20	21	4.8
	NRL-AR-018	20	21	4.8
	NRL-AR-040	20	21	4.8
Salmonella	NRL-AR-061	14	21	33.3
	NRL-AR-029	19	20	5.0
	NRL-AR-009	20	21	4.8
	NRL-AR-018	20	21	4.8
	NRL-AR-040	20	21	4.8
	NRL-AR-062	20	21	4.8

***Table 17**. Non-zero percent* deviation *levels for each reference strain-antimicrobial combination, based on the submitted sum of score and count of scores for each strain.*

Reference strain	Antimicrobial	Score sum	Score count	% Deviation level
C. jejuni ATCC 33560	Ciprofloxacin	26	28	7.1
	Gentamicin	26	28	7.1
	Total	**108**	**112**	**3.6**
E. coli ATCC25922 (*E. coli* trial)	Tigecycline	30	33	9.1
	Trimethoprim	31	33	6.1
	Imipenem	28	29	3.4
	Total	**658**	**664**	**0.9**
E. coli ATCC25922 (*Salmonella* trial)	Tigecycline	32	34	5.9
	Trimethoprim	32	34	5.9
	Cefepime	30	31	3.2
	Cefoxitin	30	31	3.2
	Ertapenem	30	31	3.2
	Sulfamethoxazole	33	34	2.9
	Tetracycline	33	34	2.9
	Cefotaxime	64	65	1.5
	Ceftazidime	64	65	1.5
	Meropenem	64	65	1.5
	Total	**680**	**692**	**1.7**

7.7. *Campylobacter* Species Identification Results

The participants were instructed to identify the species of the *Campylobacter* test strains utilizing either in-house techniques or the protocol provided on the EURL-AR website. **Table 18** displays the anticipated species for each strain, in addition to the overview of the submitted result and the percent deviation level that was computed. The bacterial species of five test strains, namely EURL C-18.1, EURL C-18.2 (despite its exclusion from the evaluation), EURL C-18.4, EURL C-18.5, and EURL C-18.6, were accurately identified by all participants. For each of the remaining three test strains (EURL C-18.3, EURL C-18.7, and EURL C-18.8) one laboratory erroneously identified the species. The erroneous species identification was the outcome of two laboratories; one laboratory provided two

incorrect results out of three submitted, while the other laboratory produced one incorrect result out of eight results submitted.

***Table 18**. Expected species identification for each strain of the Campylobacter trial.*

Strain	Expected species	Sum of Score	Count of Score	%Correct results
EURL C-18.1	*C. jejuni*	26	26	100.0
EURL C-18.2	*C. jejuni*	25	25	100.0
EURL C-18.3	*C. coli*	17	18	94.4
EURL C-18.4	*C. jejuni*	26	26	100.0
EURL C-18.5	*C. jejuni*	20	20	100.0
EURL C-18.6	*C. coli*	23	23	100.0
EURL C-18.7	*C. jejuni*	26	27	96.3
EURL C-18.8	*C. coli*	27	28	96.4

8. Concluding remarks

The EU Reference Laboratory for Antimicrobial Resistance conducted the 31st PT for AST of zoonotic and commensal bacteria at DTU Food in Autumn 2023, aiming to improve the comparability of AMR surveillance data by assessing the quality of AST results. The EURL-AR network established a 5% maximum acceptable deviation level for laboratory performance in the AST results per organism, which was achieved this year with a few exceptions. One of thirty-three participants in the *E. coli* trial exceeded this limit, while two laboratories for *Salmonella* and two for *Campylobacter* had deviation levels between 5.4 and 10.0%. The test covering the identification of the phenotype of *E. coli* and *Salmonella* strains producing β-lactamases of the ESBL-, AmpC, and carbapenemase type obtained 97% correct phenotype identifications. This is a priority area within the EURL-AR activities, and the focus on identifying ESBL-, AmpC-, and carbapenemase-producing organisms is encouraged. In conclusion, the EURL-AR welcomes recommendations from all members of the network regarding ways to enhance forthcoming PT trials and solicits suggestions for training courses and specific areas of emphasis that would broaden the network's understanding of antimicrobial resistance.

9. References

[1] European Commission Implementing Decision 2020/1729/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

[2] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2023.

[3] CLSI. Methods for Antimicrobial Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria Isolated From Animals. 1st ed. CLSI supplement VET06. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

[4] Schwarz S, Silley P, Simjee S, Woodford N, van, DE, Johnson AP & Gaastra W. (2010) Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 65: 601-604.

[5] Bortolaia V, Kaas RF, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AR, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I,

Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup F. M. ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy (2020) 75:12,3491-3500.

[6] Daniel Blankenberg, Gregory Von Kuster, Emil Bouvier, Dannon Baker, Enis Afgan, Nicholas Stoler, the Galaxy Team, James Taylor and Anton Nekrutenko. Dissemination of scientific software with Galaxy ToolShed. Genome Biology (2014) 15:403 doi:10.1186/gb4161.

[7] Clausen PTLC, Aarestrup FM, Lund O. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics (2018) 19(1):307.

[8] Zhou Z., Alikhan NF, Mohamed K, the Agama Study Group, Achtman M. The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny and Escherichia core genomic diversity. Genome Res. (2020) 30(1) 138-152.

[9] Rolf S. Kaas, Pimlapas Leekitcharoenphon, Frank M. Aarestrup, Ole Lund. Solving the Problem of Comparing Whole Bacterial Genomes across Different Sequencing Platforms. PLoS ONE (2014); 9(8): e104984.

Appendix 1: Pre-notification

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

G00-06-001/26.10.2020

EQAS 2023 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.

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 eight *Campylobacter* strains, and for new participants the QC strains mentioned 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.

TIMELINE FOR RESULTS TO BE RETURNED TO THE NATIONAL FOOD INSTITUTE
Shipment of isolates and protocol: The isolates will be shipped late September 2023. 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 12 December 2023 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'

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

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 to be launched mid-October 2023 and will be the DTU Genomic PT 2023 aiming to facilitate harmonization and standardization in whole genome sequencing and data analysis of *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* isolates, with the aim to produce comparable data for monitoring and research purposes.

After this, at the beginning of November 2023, 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

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

PROTOCOL

For antimicrobial susceptibility testing of *Escherichia coli, Salmonella* and *Campylobacter*.

---

1 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). The current EQAS 2023 will include AST of eight *E. coli, Salmonella* and *Campylobacter* strains and AST of reference strains *E. coli* ATCC 25922 (CCM 3954) and *Campylobacter jejuni* ATCC 33560 (CCM 6214) together with AST of the internal EURL reference strain, *Acinetobacter baumannii* (2012-70-100-69).

The reference strains are included in the parcel only for new participants of the EQAS who did not receive them previously. The ATCC reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The EURL-AR QC-strains are provided for the purpose of additional QC of the broth microdilution plates. The reference 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).

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.

2 OBJECTIVES

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.

3 OUTLINE OF THE EC/SALM/CAMP EQAS 2023

3.1 Shipping, receipt and storage of strains

In September 2023, the National Reference Laboratories for Antimicrobial Resistance (NRL-AR) will receive a parcel containing eight *E. coli*, eight *Salmonella* and eight *C.jejuni/C.coli* (referred to as *Campylobacter* in this document) strains, respectively from the National Food Institute (see Table 1). For participants who did not receive them previously, this parcel will also contain reference strains.

Table 1: Codes for the test strains included in the current EQAS

E. coli	***Salmonella***	***Campylobacter***
EURL 2023 EC-18.1	EURL 2023 S-18.1	EURL 2023 C-18.1
EURL 2023 EC-18.2	EURL 2023 S-18.2	EURL 2023 C-18.2
EURL 2023 EC-18.3	EURL 2023 S-18.3	EURL 2023 C-18.3
EURL 2023 EC-18.4	EURL 2023 S-18.4	EURL 2023 C-18.4
EURL 2023 EC-18.5	EURL 2023 S-18.5	EURL 2023 C-18.5
EURL 2023 EC-18.6	EURL 2023 S-18.6	EURL 2023 C-18.6
EURL 2023 EC-18.7	EURL 2023 S-18.7	EURL 2023 C-18.7
EURL 2023 EC-18.8	EURL 2023 S-18.8	EURL 2023 C-18.8

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.

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.

<u>Attention!</u> The *Campylobacter* test strains must be subcultured immediately upon arrival.

3.2 QC reference strains

Include the ATCC reference strains as well as the internal EURL reference strain for the MIC testing and report results of these together with the isolates obtained from the EQAS samples. I.e., for the *E. coli* and *Salmonella* testing, include *E. coli* ATCC 25922 (CCM 3954) together with *Acinetobacter baumannii* (2012-70-100-69).

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.

For a suggested procedure for reconstitution of the lyophilised cultures, please refer to the document 'Instructions for opening and reviving lyophilised cultures' on the EURL-AR-website (see https://www.eurl-ar.eu/eqas.aspx).

3.3 Antimicrobial susceptibility testing

Participants should perform minimum inhibitory concentration (MIC) determination 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 by MIC determination.** For interpretation of the results, please use the cut-off values listed in Tables 2, 3, 4, 5 and 6 in this document. Except where specifically indicated, these values represent the current epidemiological cut-off values (ECOFF) developed by EUCAST (www.eucast.org) and allow categorisation of bacterial isolates into two categories: resistant and susceptible. A categorisation as intermediate is not accepted**.**

Attention! Please note that for the purpose of this EQAS and to be in alignment with the ESBL-phenotype categorization scheme (see Appendix), the MIC-value used for interpretation of cefoxitin for *E. coli* is set at 8 mg/L.

As the current regulation and recommendations focus on broth microdilution testing only, results obtained by other methods cannot be submitted for evaluation.

Beta-lactam and carbapenem resistance

Confirmatory tests for ESBL/AmpC/carbapenemase production are mandatory on all *E.coli* and *Salmonella* test strains resistant to cefotaxime (FOT), ceftazidime (TAZ) and/or meropenem (MERO) and should be performed by testing the second panel of antimicrobials (Table 3 and Table 5 of this document corresponding to Table 5 in Commission Implementing Decision 2020/1729/EU).

Confirmatory test for AmpC-, ESBL- and carbapenemase 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 agent tested in combination with clavulanic acid vs. the MIC of the agent when tested alone (MIC FOT:FOT/Cl or TAZ:TAZ/Cl ratio ≥ 8) (CLSI M100 Table 3A, Tests for ESBLs). The presence of synergy indicates ESBL production.

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

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

The classification of the phenotypic beta-lactam resistance results should be based on the most recent EFSA recommendations (see appendix to this protocol). Importantly: Note that for *both E. coli* and *Salmonella*, two cut-off values apply for cefotaxime and ceftazidime: the EUCAST cut-off values, those that define R/S (see Tables 2, 3, 4 and 5), and the screening cut-off values (cefotaxime >1 and ceftazidime >1) which are those applied to categorise bacterial phenotypes as ESBL, AmpC, carbapenemase, etc., based on panel 2 results (see Appendix). Likewise this is the situation for the *E.coli* meropenem cut-off values/screening cut-off value.

For phenotypes that are non-susceptible to CTX, CAZ, FOX or MEM and do not fall into any of the categories above (ESBL, AmpC, ESBL+AmpC and Carbapenemase), strains should for the current EQA be categorized as "Other phenotype".

E. coli

The interpretative criteria that should be applied for categorizing the *E. coli* test strain as resistant or susceptible are those listed in Tables 2 and 3.

Table 2: Panel 1 antimicrobials recommended for AST of *E. coli* spp. and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (accessed on 20.09.2023) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	8
Ampicillin (AMP)	8
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.25
Ceftazidime (TAZ or CAZ)	1
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.06
Colistin (COL)	2
Gentamicin (GEN)	2
Meropenem (MERO or MEM)	0.06
Nalidixic acid (NAL)	8
Sulfonamides (SMX)	64*
Tetracycline (TET)	8
Tigecycline (TGC)	0.5
Trimethoprim (TMP)	2

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

Table 3: Panel 2 antimicrobials recommended for AST of *E. coli* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (20.09.2023)

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.125
Cefotaxime (FOT or CTX)	0.25
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.25
Cefoxitin (FOX)	8*
Ceftazidime (TAZ or CAZ)	1
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	1
Ertapenem (ETP)	0.03
Imipenem (IMI)	0.5
Meropenem (MERO or MEM)	0.06
Temocillin (TRM)	16

*For the purpose of this EQAS and to be in alignment with the ESBL-phenotype categorization scheme (see Appendix), the MIC-value used for interpretation of cefoxitin for *E. coli* is set at 8 mg/L, and is therefore not according to the latest EUCAST update

Salmonella

The interpretative criteria that should be applied for categorizing the *Salmonella* test strain as resistant or susceptible are those listed in Tables 4 and 5.

Table 4: Panel 1 antimicrobials recommended for AST of *Salmonella* spp. and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (20.09.2023) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Amikacin (AMI)	4
Ampicillin (AMP)	4
Azithromycin (AZI)	16
Cefotaxime (FOT or CTX)	0.5
Ceftazidime (TAZ or CAZ)	2
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.125
Colistin (COL)	2*
Gentamicin (GEN)	2
Meropenem (MERO or MEM)	0.125*
Nalidixic acid (NAL)	8
Sulfonamides (SMX)	256*
Tetracycline (TET)	8
Tigecycline (TGC)	0.5*
Trimethoprim (TMP)	2

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

Table 5: Panel 2 antimicrobials recommended for AST of *Salmonella* spp. resistant to cefotaxime, ceftazidime or meropenem in panel 1 antimicrobials and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (20.09.2023) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.1

Antimicrobial	MIC (µg/mL) (R>)
Cefepime (FEP)	0.25
Cefotaxime (FOT or CTX)	0.5
Cefotaxime + clavulanic acid (F/C or CTX/CLA)	0.5*
Cefoxitin (FOX)	8
Ceftazidime (TAZ or CAZ)	2
Ceftazidime + clavulanic acid (T/C or CAZ/CLA)	2*
Ertapenem (ETP)	0.06*
Imipenem (IMI)	1
Meropenem (MERO or MEM)	0.125*
Temocillin (TRM)	16

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

Campylobacter

The interpretative criteria to be applied for categorizing the *Campylobacter* test strain as resistant or susceptible are those listed in Table 6.

The obtained values of the *C. jejuni* QC reference strain will be evaluated according to the values listed in the CLSI document VET06, 1st ed., i.e., based on incubation at 36-37ºC for 48 hours or 42ºC for 24 hours.

Table 6: Antimicrobials recommended for AST of *Campylobacter jejuni* and *C. coli* and interpretative criteria ((T)ECOFFs) according to latest updates from EUCAST (20.09.2023) supplemented with ECOFFs from the EFSA Technical Report 2021, Table B.2

Antimicrobial	*C. jejuni*	*C. coli*
	MIC (µg/mL) (R>)	MIC (µg/mL) (R>)
Chloramphenicol (CHL)	16	16
Ciprofloxacin (CIP)	0.5	0.5
Ertapenem (ETP)	0.125	0.5*
Erythromycin (ERY)	4	8
Gentamicin (GEN)	2	2
Tetracycline (TET)	1	2

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

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.

4 REPORTING OF RESULTS AND EVALUATION

Test forms are available for recording your results before you enter them into the web tool.

We recommend reading carefully the web tool manual before submitting your results.

Results must be submitted no later than December 12th 2023 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 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

5 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.

--- --- ---

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

APPENDIX

Criteria for interpretation of *E. coli* and *Salmonella*, panel 2 results

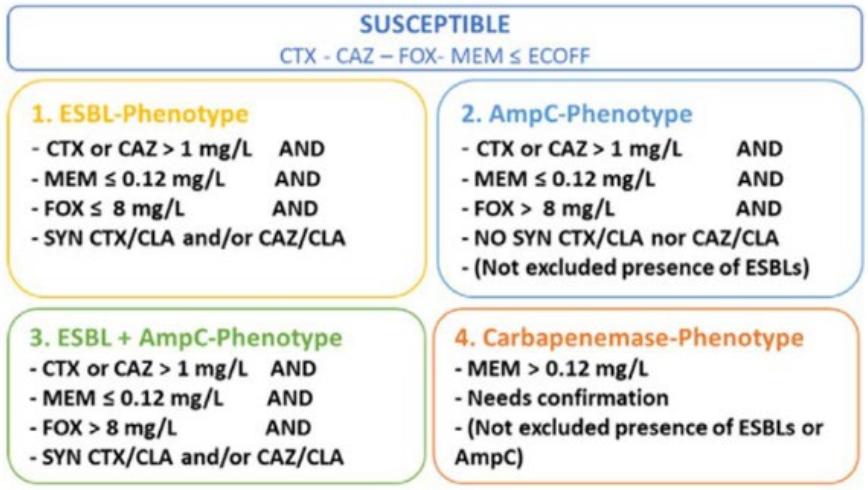


Figure F.1: Phenotypes inferred based on the resistance to the b-lactams included in Panel 2

Please refer to: EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), **2023**, The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2020/2021. *EFSA Journal* **21**(*3*):7867, page 226, Figure F.1, https://doi.org/10.2903/j.efsa.2023.7867.

For phenotypes that are non-susceptible to CTX, CAZ, FOX or MEM and do not fall into any of the categories above (ESBL, AmpC, ESBL+AmpC and Carbapenemase), strains should for the current EQA be categorized as "Other phenotype".

Appendix 2b: Test Forms

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

E. coli, Salmonella and *Campylobacter*

TEST FORMS

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM – *E. coli*

Which method did you use for antimicrobial susceptibility testing of *E. coli* in this EQAS?

- ☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?

- ☐ CLSI
- ☐ EUCAST
- ☐ ISO 20776-1:2019
- ☐ TREK

Which incubation conditions did you use? °C/ h

Which solvent was used for the preparation of the 0.5 McFarland solution

- ☐ Water
- ☐ Saline
- ☐ Mueller Hinton broth

The inoculum was prepared by adding µl of 0.5 McFarland solution in mL CAMHB broth

What was the expected inoculum size? * ^ CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM - *Salmonella*

Which method did you use for antimicrobial susceptibility testing of *Salmonella* in this EQAS?

- ☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?

- ☐ CLSI
- ☐ EUCAST
- ☐ ISO 20776-1:2019
- ☐ TREK

Which incubation conditions did you use? °C/ h

Which solvent was used for the preparation of the 0.5 McFarland solution

- ☐ Water
- ☐ Saline
- ☐ Mueller Hinton broth

The inoculum was prepared by adding µl of 0.5 McFarland solution in mL cation-adjusted Mueller Hinton broth (CAMHB).

What was the expected inoculum size? * ^ CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM - *Campylobacter*

Which method did you use for antimicrobial susceptibility testing of *Campylobacter* in this EQAS?

- ☐ MIC - Broth microdilution

Which standard(s)/guideline(s) did you use when performing AST?

- ☐ CLSI
- ☐ EUCAST
- ☐ ISO 20776-1:2019
- ☐ TREK

Which incubation conditions did you use?

- ☐ 36-37ºC, 48 hours
- ☐ 42ºC, 24 hours

Which solvent was used for the preparation of the 0.5 McFarland solution

- ☐ Water
- ☐ Saline
- ☐ Mueller Hinton broth

The inoculum was prepared by adding µl of 0.5 McFarland solution in mL cation-adjusted Mueller Hinton broth supplemented with lysed horse blood (CAMHB-LHB).

What was the expected inoculum size? * ^ CFU/mL (indicate for example 5 times 10 to the power of 5 using this format '5 * 10 ^ 5')

Comments or additional information:

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
E. coli EURL EC-18.X	Amikacin, AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

All strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem (MERO) must be included for testing in the second panel as part of confirmatory tests for ESBL-, AmpC or carbapenemase production. See further description in the protocol, section '3.3'.

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
E. coli EURL EC-18.X	Cefepime, FEP			
	Cefotaxime, FOT			
	Cefotaxime + clavulanic acid, F/C			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid, T/C			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			

Interpretation of PANEL 2 results:

☐ ESBL-phenotype
☐ ESBL+AmpC-phenotype
☐ AmpC-phenotype
☐ Carbapenemase-phenotype
☐ Other phenotype
☐ Susceptible (to panel 2 antimicrobials)

Comments:

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM

AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value (µg/ml)
1st panel	Amikacin, AMI	
	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* for the testing of the *E. coli* ATCC25922 reference strain, 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 (CLSI M100, Table 5).

AST of reference strain *Acinetobacter baumannii* (2012-70-100-69)

	Antimicrobial	MIC-value (µg/ml)
1st panel	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* 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 (CLSI M100, Table 5).

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
Salmonella EURL S-18.X	Amikacin, AMI			
	Ampicillin, AMP			
	Azithromycin, AZI			
	Cefotaxime, FOT			
	Ceftazidime, TAZ			
	Chloramphenicol, CHL			
	Ciprofloxacin CIP			
	Colistin, COL			
	Gentamicin, GEN			
	Meropenem, MERO			
	Nalidixic acid, NAL			
	Sulfamethoxazole, SMX			
	Tetracycline, TET			
	Tigecycline, TGC			
	Trimethoprim, TMP			

All strains resistant to cefotaxime (FOT), ceftazidime (TAZ) or meropenem (MERO) must be included for testing in the second panel as part of confirmatory tests for ESBL-, AmpC or carbapenemase production. See further description in the protocol, section '3.3'.

Strain	Antimicrobial	Results and interpretation		
		≤ / >	MIC-value (µg/ml)	S / R
Salmonella EURL S-18.X	Cefepime, FEP			
	Cefotaxime, FOT			
	Cefotaxime + clavulanic acid, F/C			
	Cefoxitin, FOX			
	Ceftazidime, TAZ			
	Ceftazidime+ clavulanic acid, T/C			
	Ertapenem, ETP			
	Imipenem, IMI			
	Meropenem, MERO			
	Temocillin, TRM			

Interpretation of PANEL 2 results:

☐ ESBL-phenotype
☐ ESBL+AmpC-phenotype
☐ AmpC-phenotype
☐ Carbapenemase-phenotype
☐ Other phenotype
☐ Susceptible (to panel 2 antimicrobials)

Comments:

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM

AST of reference strain *E. coli* ATCC 25922

	Antimicrobial	MIC-value (µg/ml)
1st panel	Amikacin, AMI	
	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* for the testing of the *E. coli* ATCC25922 reference strain, 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 (CLSI M100, Table 3).

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

AST of reference strain *Acinetobacter baumannii* (2012-70-100-69)

	Antimicrobial	MIC-value (µg/ml)
1st panel	Ampicillin, AMP	
	Azithromycin, AZI	
	Cefotaxime, FOT	
	Ceftazidime, TAZ	
	Chloramphenicol, CHL	
	Ciprofloxacin, CIP	
	Colistin, COL	
	Gentamicin, GEN	
	Meropenem, MERO	
	Nalidixic acid, NAL	
	Sulfamethoxazole, SMX*	
	Tetracycline, TET	
	Tigecycline, TGC	
	Trimethoprim, TMP	
2nd panel	Cefepime, FEP	
	Cefotaxime, FOT	
	Cefotaxime + clavulanic acid, F/C	
	Cefoxitin, FOX	
	Ceftazidime, TAZ	
	Ceftazidime+ clavulanic acid, T/C	
	Ertapenem, ETP	
	Imipenem, IMI	
	Meropenem, MERO	
	Temocillin, TRM	

* 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 (CLSI M100, Table 3).

EU Reference Laboratory for Antimicrobial Resistance
External Quality Assurance System (EQAS) 2023

TEST FORM

Strain	Antimicrobial	Interpretation	
		MIC-value (µg/ml)	S / R
Campylobacter EURL C-18.X ☐ *C. jejuni* ☐ *C. coli*	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		

TEST FORM

Susceptibility testing of *Campylobacter jejuni* reference strain ATCC 33560

Strain	Antimicrobial	MIC-value (µg/ml)	
		36 °C/48 hours	42 °C/24 hours
C. jejuni ATCC 33560	Chloramphenicol		
	Ciprofloxacin		
	Ertapenem		
	Erythromycin		
	Gentamicin		
	Tetracycline		

Appendix 3a: Cover Letter

G00-06-001/26.10.2020

EURL-AR External Quality Assurance System 2023

- *E. coli, Salmonella*, and *Campylobacter*

Lyngby, September 2023

Dear participant in the EURL-AR AST EQAS 2023,

Please find enclosed the bacterial strains for the EURL-AR EQAS 2023: eight *E. coli*, eight *Salmonella,* and eight *Campylobacter* spp.

E. coli	***Salmonella***	***Campylobacter***
EURL 2023 EC-18.1	EURL 2023 S-18.1	EURL 2023 C-18.1
EURL 2023 EC-18.2	EURL 2023 S-18.2	EURL 2023 C-18.2
EURL 2023 EC-18.3	EURL 2023 S-18.3	EURL 2023 C-18.3
EURL 2023 EC-18.4	EURL 2023 S-18.4	EURL 2023 C-18.4
EURL 2023 EC-18.5	EURL 2023 S-18.5	EURL 2023 C-18.5
EURL 2023 EC-18.6	EURL 2023 S-18.6	EURL 2023 C-18.6
EURL 2023 EC-18.7	EURL 2023 S-18.7	EURL 2023 C-18.7
EURL 2023 EC-18.8	EURL 2023 S-18.8	EURL 2023 C-18.8

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 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.

I will let you know 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 **12 December 2023**.

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

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!

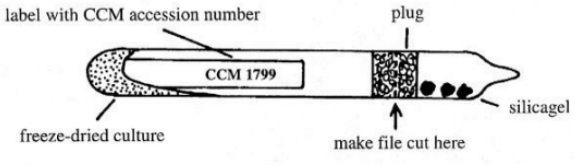


Figure 1: from CCM document 'Instructions for Opening and Reviving of Freeze-Dried Bacteria and Fungi' available on http://www.sci.muni.cz

Appendix 3c: Subculture and maintenance of Quality Control strains

DTU Food
National Food Institute

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 values and phenotype interpretation as resistant (R) or susceptible (S)

Epidemiological cut-off (ECOFF) values are according to the Protocol for the 31st EURL-AR Proficiency Test for the antimicrobial susceptibility testing of *E. coli*, *Salmonella* and *Campylobacter* 2023.

I. E. coli

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
EURL EC-18.1	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	**R**
		Azithromycin	>	64	16	**R**
		Cefotaxime	>	4	0.25	**R**
		Ceftazidime	=	2	1	**R**
		Chloramphenicol	>	64	16	**R**
		Ciprofloxacin	=	0.125	0.06	**R**
		Colistin	=	8	2	**R**
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	>	512	64	**R**
		Tetracycline	>	32	8	**R**
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	>	16	2	**R**
	EUVSEC2	Cefepime	=	8	0.125	**R**
		Cefotaxime	=	32	0.25	**R**
		Cefotaxime-clavulanic acid	=	0.125	0.25	S
		Cefoxitin	=	8	8	S
		Ceftazidime	=	2	1	**R**
		Ceftazidime-clavulanic acid	=	0.25	1	S
		Ertapenem	≤	0.015	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S
		Temocillin	=	8	16	S
EURL EC-18.2	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	**R**
		Azithromycin	=	8	16	S
		Cefotaxime	=	2	0.25	**R**
		Ceftazidime	=	4	1	**R**
		Chloramphenicol	=	32	16	**R**
		Ciprofloxacin	≤	0.015	0.06	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	≤	8	64	S
		Tetracycline	>	32	8	**R**
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	>	16	2	**R**
	EUVSEC2	Cefepime	≤	0.06	0.125	S
		Cefotaxime	=	2	0.25	**R**

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Cefotaxime-clavulanic acid	=	1	0.25	R
		Cefoxitin	=	32	8	R
		Ceftazidime	=	4	1	R
		Ceftazidime-clavulanic acid	=	2	1	R
		Ertapenem	≤	0.015	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S
		Temocillin	=	4	16	S
EURL EC-18.3	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	R
		Azithromycin	=	4	16	S
		Cefotaxime	>	4	0.25	R
		Ceftazidime	>	8	1	R
		Chloramphenicol	=	32	16	R
		Ciprofloxacin	≤	0.015	0.06	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	>	512	64	R
		Tetracycline	>	32	8	R
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	>	16	2	R
	EUVSEC2	Cefepime	=	8	0.125	R
		Cefotaxime	>	64	0.25	R
		Cefotaxime-clavulanic acid	=	4	0.25	R
		Cefoxitin	=	64	8	R
		Ceftazidime	=	16	1	R
		Ceftazidime-clavulanic acid	=	4	1	R
		Ertapenem	=	0.03	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S
		Temocillin	=	4	16	S
EURL EC-18.4	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	R
		Azithromycin	=	64	16	R
		Cefotaxime	>	4	0.25	R
		Ceftazidime	=	8	1	R
		Chloramphenicol	=	32	16	R
		Ciprofloxacin	>	8	0.06	R
		Colistin	=	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	>	64	8	R
		Sulfamethoxazole	>	512	64	R
		Tetracycline	>	32	8	R
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	>	16	2	R
	EUVSEC2	Cefepime	=	4	0.125	R
		Cefotaxime	=	16	0.25	R
		Cefotaxime-clavulanic acid	=	1	0.25	R
		Cefoxitin	=	32	8	R
		Ceftazidime	=	8	1	R
		Ceftazidime-clavulanic acid	=	4	1	R
		Ertapenem	=	0.03	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Temocillin	=	4	16	S
EURL EC-18.5	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	**R**
		Azithromycin	=	8	16	S
		Cefotaxime	>	4	0.25	**R**
		Ceftazidime	>	8	1	**R**
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	≤	0.015	0.06	S
		Colistin	=	2	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	=	8	0.06	**R**
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	≤	8	64	S
		Tetracycline	>	32	8	**R**
		Tigecycline	=	0.5	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	16	0.125	**R**
		Cefotaxime	>	64	0.25	**R**
		Cefotaxime-clavulanic acid	>	64	0.25	**R**
		Cefoxitin	>	64	8	**R**
		Ceftazidime	>	128	1	**R**
		Ceftazidime-clavulanic acid	>	128	1	**R**
		Ertapenem	>	2	0.03	**R**
		Imipenem	=	8	0.5	**R**
		Meropenem	=	8	0.06	**R**
		Temocillin	=	32	16	**R**
EURL EC-18.6	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	**R**
		Azithromycin	=	64	16	**R**
		Cefotaxime	>	4	0.25	**R**
		Ceftazidime	>	8	1	**R**
		Chloramphenicol	>	64	16	**R**
		Ciprofloxacin	=	8	0.06	**R**
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	=	4	0.06	**R**
		Nalidixic acid	>	64	8	**R**
		Sulfamethoxazole	>	512	64	**R**
		Tetracycline	>	32	8	**R**
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	>	16	2	**R**
	EUVSEC2	Cefepime	>	32	0.125	**R**
		Cefotaxime	>	64	0.25	**R**
		Cefotaxime-clavulanic acid	>	64	0.25	**R**
		Cefoxitin	>	64	8	**R**
		Ceftazidime	>	128	1	**R**
		Ceftazidime-clavulanic acid	>	128	1	**R**
		Ertapenem	>	2	0.03	**R**
		Imipenem	=	4	0.5	**R**
		Meropenem	=	8	0.06	**R**
		Temocillin	=	128	16	**R**
EURL EC-18.7	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	=	2	8	S
		Azithromycin	=	4	16	S
		Cefotaxime	≤	0.25	0.25	S
		Ceftazidime	≤	0.25	1	S
		Chloramphenicol	≤	8	16	S

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Ciprofloxacin	≤	0.015	0.06	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	≤	8	64	S
		Tetracycline	≤	2	8	S
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	≤	0.06	0.125	S
		Cefotaxime	≤	0.25	0.25	S
		Cefotaxime-clavulanic acid	≤	0.06	0.25	S
		Cefoxitin	=	2	8	S
		Ceftazidime	≤	0.25	1	S
		Ceftazidime-clavulanic acid	≤	0.125	1	S
		Ertapenem	≤	0.015	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S
		Temocillin	=	8	16	S
EURL EC-18.8	**EUVSEC3**	Amikacin	≤	4	8	S
		Ampicillin	>	32	8	**R**
		Azithromycin	=	8	16	S
		Cefotaxime	>	4	0.25	**R**
		Ceftazidime	=	2	1	**R**
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	≤	0.015	0.06	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.06	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	≤	8	64	S
		Tetracycline	≤	2	8	S
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	16	0.125	**R**
		Cefotaxime	=	64	0.25	**R**
		Cefotaxime-clavulanic acid	≤	0.06	0.25	S
		Cefoxitin	=	4	8	S
		Ceftazidime	=	2	1	**R**
		Ceftazidime-clavulanic acid	≤	0.125	1	S
		Ertapenem	≤	0.015	0.03	S
		Imipenem	≤	0.125	0.5	S
		Meropenem	≤	0.03	0.06	S
		Temocillin	=	4	16	S

II. Salmonella

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
EURL S-18.1	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	=	4	16	S
		Cefotaxime	>	4	0.5	**R**
		Ceftazidime	>	8	2	**R**
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	=	0.03	0.125	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.125	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	=	16	256	S
		Tetracycline	≤	2	8	S
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	0.25	0.25	S
		Cefotaxime	=	16	0.5	**R**
		Cefotaxime-clavulanic acid	=	16	0.5	**R**
		Cefoxitin	=	32	8	**R**
		Ceftazidime	=	16	2	**R**
		Ceftazidime-clavulanic acid	=	16	2	**R**
		Ertapenem	=	0.03	0.06	S
		Imipenem	=	0.25	1	S
		Meropenem	≤	0.03	0.125	S
		Temocillin	=	8	16	S
EURL S-18.2	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	=	8	16	S
		Cefotaxime	>	4	0.5	**R**
		Ceftazidime	>	8	2	**R**
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	=	0.03	0.125	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	=	0.06	0.125	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	≤	8	256	S
		Tetracycline	>	32	8	**R**
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	0.5	0.25	**R**
		Cefotaxime	=	32	0.5	**R**
		Cefotaxime-clavulanic acid	=	32	0.5	**R**
		Cefoxitin	=	64	8	**R**
		Ceftazidime	=	32	2	**R**
		Ceftazidime-clavulanic acid	=	32	2	**R**
		Ertapenem	=	0.03	0.06	S
		Imipenem	=	0.25	1	S
		Meropenem	=	0.06	0.125	S
		Temocillin	=	8	16	S
EURL S-18.3	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	=	8	16	S
		Cefotaxime	>	4	0.5	**R**

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Ceftazidime	=	4	2	**R**
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	=	0.25	0.125	**R**
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.125	S
		Nalidixic acid	>	64	8	**R**
		Sulfamethoxazole	>	512	256	**R**
		Tetracycline	>	32	8	**R**
		Tigecycline	=	1	0.5	**R**
		Trimethoprim	>	16	2	**R**
	EUVSEC2	Cefepime	=	8	0.25	**R**
		Cefotaxime	=	32	0.5	**R**
		Cefotaxime-clavulanic acid	=	0.25	0.5	S
		Cefoxitin	=	8	8	S
		Ceftazidime	=	4	2	**R**
		Ceftazidime-clavulanic acid	=	0.5	2	S
		Ertapenem	≤	0.015	0.06	S
		Imipenem	≤	0.125	1	S
		Meropenem	≤	0.03	0.125	S
		Temocillin	=	8	16	S
EURL S-18.4	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	=	32	16	**R**
		Cefotaxime	=	0.5	0.5	S
		Ceftazidime	=	0.5	2	S
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	=	8	0.125	**R**
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	=	0.25	0.125	**R**
		Nalidixic acid	>	64	8	**R**
		Sulfamethoxazole	≤	8	256	S
		Tetracycline	≤	2	8	S
		Tigecycline	=	0.5	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	0.125	0.25	S
		Cefotaxime	=	0.5	0.5	S
		Cefotaxime-clavulanic acid	=	0.25	0.5	S
		Cefoxitin	=	8	8	S
		Ceftazidime	=	0.5	2	S
		Ceftazidime-clavulanic acid	=	0.5	2	S
		Ertapenem	=	0.5	0.06	**R**
		Imipenem	=	0.5	1	S
		Meropenem	=	0.25	0.125	**R**
		Temocillin	>	128	16	**R**
EURL S-18.5	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	>	64	16	**R**
		Cefotaxime	>	4	0.5	**R**
		Ceftazidime	>	8	2	**R**
		Chloramphenicol	>	64	16	**R**
		Ciprofloxacin	>	8	0.125	**R**
		Colistin	≤	1	2	S
		Gentamicin	=	1	2	S
		Meropenem	=	1	0.125	**R**
		Nalidixic acid	>	64	8	**R**

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Sulfamethoxazole	>	512	256	R
		Tetracycline	>	32	8	R
		Tigecycline	=	0.5	0.5	S
		Trimethoprim	>	16	2	R
	EUVSEC2	Cefepime	=	32	0.25	R
		Cefotaxime	>	64	0.5	R
		Cefotaxime-clavulanic acid	>	64	0.5	R
		Cefoxitin	>	64	8	R
		Ceftazidime	>	128	2	R
		Ceftazidime-clavulanic acid	>	128	2	R
		Ertapenem	=	1	0.06	R
		Imipenem	=	2	1	R
		Meropenem	=	1	0.125	R
		Temocillin	=	64	16	R
EURL S-18.6	**EUVSEC3**	Amikacin	>	128	4	R
		Ampicillin	>	32	4	R
		Azithromycin	>	64	16	R
		Cefotaxime	>	4	0.5	R
		Ceftazidime	>	8	2	R
		Chloramphenicol	=	64	16	R
		Ciprofloxacin	>	8	0.125	R
		Colistin	≤	1	2	S
		Gentamicin	>	16	2	R
		Meropenem	=	8	0.125	R
		Nalidixic acid	>	64	8	R
		Sulfamethoxazole	>	512	256	R
		Tetracycline	=	4	8	S
		Tigecycline	=	0.5	0.5	S
		Trimethoprim	=	0.5	2	S
	EUVSEC2	Cefepime	>	32	0.25	R
		Cefotaxime	>	64	0.5	R
		Cefotaxime-clavulanic acid	>	64	0.5	R
		Cefoxitin	>	64	8	R
		Ceftazidime	>	128	2	R
		Ceftazidime-clavulanic acid	>	128	2	R
		Ertapenem	>	2	0.06	R
		Imipenem	=	4	1	R
		Meropenem	=	4	0.125	R
		Temocillin	>	128	16	R
EURL S-18.7	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	≤	1	4	S
		Azithromycin	=	4	16	S
		Cefotaxime	≤	0.25	0.5	S
		Ceftazidime	=	0.5	2	S
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	≤	0.015	0.125	S
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.125	S
		Nalidixic acid	≤	4	8	S
		Sulfamethoxazole	=	16	256	S
		Tetracycline	≤	2	8	S
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	≤	0.06	0.25	S
		Cefotaxime	≤	0.25	0.5	S
		Cefotaxime-clavulanic acid	=	0.125	0.5	S

Strain	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Cefoxitin	=	4	8	S
		Ceftazidime	=	0.5	2	S
		Ceftazidime-clavulanic acid	=	0.25	2	S
		Ertapenem	≤	0.015	0.06	S
		Imipenem	=	0.25	1	S
		Meropenem	≤	0.03	0.125	S
		Temocillin	=	4	16	S
EURL S-18.8	**EUVSEC3**	Amikacin	≤	4	4	S
		Ampicillin	>	32	4	**R**
		Azithromycin	=	4	16	S
		Cefotaxime	>	4	0.5	**R**
		Ceftazidime	=	1	2	S
		Chloramphenicol	≤	8	16	S
		Ciprofloxacin	=	0.25	0.125	**R**
		Colistin	≤	1	2	S
		Gentamicin	≤	0.5	2	S
		Meropenem	≤	0.03	0.125	S
		Nalidixic acid	>	64	8	**R**
		Sulfamethoxazole	=	16	256	S
		Tetracycline	=	32	8	**R**
		Tigecycline	≤	0.25	0.5	S
		Trimethoprim	≤	0.25	2	S
	EUVSEC2	Cefepime	=	2	0.25	**R**
		Cefotaxime	=	8	0.5	**R**
		Cefotaxime-clavulanic acid	=	0.125	0.5	S
		Cefoxitin	=	2	8	S
		Ceftazidime	=	1	2	S
		Ceftazidime-clavulanic acid	=	0.25	2	S
		Ertapenem	≤	0.015	0.06	S
		Imipenem	≤	0.125	1	S
		Meropenem	≤	0.03	0.125	S
		Temocillin	=	4	16	S

III. Campylobacter

Strain (species)	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
EURL C-18.1 (*C. jejuni*)	**EUCAMP3**	Chloramphenicol	=	4	16	S
		Ciprofloxacin	=	8	0.5	**R**
		Ertapenem	≤	0.125	0.125	S
		Erythromycin	≤	1	4	S
		Gentamicin	≤	0.25	2	S
		Tetracycline	=	64	1	**R**
EURL C-18.2 (*C. jejuni*)	**EUCAMP3**	Chloramphenicol	=	4	16	S
		Ciprofloxacin	≤	0.125	0.5	S
		Ertapenem	≤	0.125	0.125	S
		Erythromycin	≤	1	4	S
		Gentamicin	≤	0.25	2	S
		Tetracycline	≤	0.5	1	S
EURL C-18.3 (*C. coli*)	**EUCAMP3**	Chloramphenicol	=	4	16	S
		Ciprofloxacin	≤	0.125	0.5	S
		Ertapenem	>	4	0.5	**R**
		Erythromycin	=	512	8	**R**

Strain (species)	Panel type	Antimicrobial	Operator	MIC value mg/L	ECOFF mg/L	R/S
		Gentamicin	=	0.5	2	S
		Tetracycline	>	64	2	**R**
EURL C-18.4 (*C. jejuni*)	**EUCAMP3**	Chloramphenicol	≤	2	16	S
		Ciprofloxacin	≤	0.125	0.5	S
		Ertapenem	=	0.25	0.125	**R**
		Erythromycin	>	512	4	**R**
		Gentamicin	>	16	2	**R**
		Tetracycline	>	64	1	**R**
EURL C-18.5 (*C. jejuni*)	**EUCAMP3**	Chloramphenicol	=	4	16	S
		Ciprofloxacin	=	16	0.5	**R**
		Ertapenem	≤	0.125	0.125	S
		Erythromycin	>	512	4	**R**
		Gentamicin	>	16	2	**R**
		Tetracycline	=	64	1	**R**
EURL C-18.6 (*C. coli*)	**EUCAMP3**	Chloramphenicol	=	4	16	S
		Ciprofloxacin	=	32	0.5	**R**
		Ertapenem	=	4	0.5	**R**
		Erythromycin	>	512	8	**R**
		Gentamicin	≤	0.25	2	S
		Tetracycline	>	64	2	**R**
EURL C-18.7 (*C. jejuni*)	**EUCAMP3**	Chloramphenicol	=	64	16	**R**
		Ciprofloxacin	=	32	0.5	**R**
		Ertapenem	=	4	0.125	**R**
		Erythromycin	>	512	4	**R**
		Gentamicin	≤	0.25	2	S
		Tetracycline	=	32	1	**R**
EURL C-18.8 (*C. coli*)	**EUCAMP3**	Chloramphenicol	=	8	16	S
		Ciprofloxacin	=	32	0.5	**R**
		Ertapenem	=	2	0.5	**R**
		Erythromycin	≤	2	8	S
		Gentamicin	=	0.5	2	S
		Tetracycline	=	1	2	S

Appendix 5 – Deviations

I. List of deviations per laboratory, AST Results

Lab number	Organism	Strain	Panel type	Antimicrobial	MIC		Phenotype (R/S)		Score
					Obtained	Expected	Obtained	Expected	
NRL-AR-002	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	=0.5	≤0.125	**R**	S	0
	E. coli	EURL EC-18.1	EUVSEC3	Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
	Salmonella	EURL S-18.2	EUVSEC2	Cefepime	=0.25	=0.5	S	**R**	0
NRL-AR-004	***E. coli***	EURL EC-18.5	EUVSEC3	Tigecycline	=1	=0.5	**R**	S	0
	Salmonella	EURL S-18.1	EUVSEC2	Cefepime	=0.5	=0.25	**R**	S	0
NRL-AR-006	***Salmonella***	EURL S-18.1	EUVSEC2	Cefepime	=0.5	=0.25	**R**	S	0
		EURL S-18.4	EUVSEC3	Cefotaxime	=1	=0.5	**R**	S	0
			EUVSEC2	Cefotaxime	=1	=0.5	**R**	S	0
				Imipenem	=2	=0.5	**R**	S	0
NRL-AR-011	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	>64	≤0.125	**R**	S	0
		EURL C-18.4	EUCAMP3	Ciprofloxacin	=4	≤0.125	**R**	S	0
	Salmonella	EURL S-18.3	EUVSEC3	Sulfamethoxazole	=256	>512	S	**R**	0
NRL-AR-012	***Salmonella***	EURL S-18.4	EUVSEC3	Cefotaxime	=1	=0.5	**R**	S	0
			EUVSEC2	Cefotaxime	=1	=0.5	**R**	S	0
				Cefotaxime-clav.acid	=1	=0.25	**R**	S	0
NRL-AR-016	***Salmonella***	EURL S-18.3	EUVSEC2	Ceftazidime-clav.acid	=0.5	=0.5	**R**	S	0
NRL-AR-017	***E. coli***	EURL EC-18.1	EUVSEC3	Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
	Salmonella	EURL S-18.2	EUVSEC2	Ertapenem	=0.06	=0,03	**R**	S	0
		EURL S-18.3	EUVSEC2	Ceftazidime-clav.acid	=1	=0.5	**R**	S	0
		EURL S-18.4	EUVSEC2	Cefoxitin	=16	=8	**R**	S	0
NRL-AR-018	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	=0.25	≤0.125	**R**	S	0
	E. coli	EURL EC-18.2	EUVSEC2	Cefepime	=1	≤0.06	**R**	S	0
		EURL EC-18.5	EUVSEC3	Azithromycin	=16	=8	**R**	S	0
	Salmonella	EURL S-18.1	EUVSEC3	Sulfamethoxazole	>512	=16	**R**	S	0
		EURL S-18.2	EUVSEC2	Temocillin	=32	=8	**R**	S	0
		EURL S-18.4	EUVSEC3	Cefotaxime	=1	=0.5	**R**	S	0
			EUVSEC2	Cefotaxime	=2	=0.5	**R**	S	0
				Imipenem	=2	=0.5	**R**	S	0
		EURL S-18.6	EUVSEC3	Tetracycline	>32	=4	**R**	S	0
				Trimethoprim	>16	=0.5	**R**	S	0
NRL-AR-019	***Campylobacter***	EURL C-18.1	EUCAMP3	Tetracycline	≤0.5	=64	S	**R**	0
		EURL C-18.7	EUCAMP3	Chloramphenicol	=16	=64	S	**R**	0
		EURL C-18.8	EUCAMP3	Ertapenem	=1	=2	S	**R**	0
NRL-AR-020	***E. coli***	EURL EC-18.4	EUVSEC3	Tigecycline	≤0.25	≤0.25	**R**	S	0
NRL-AR-021		EURL EC-18.1	EUVSEC3	Ceftazidime	>1	=2	S	**R**	0
				Ciprofloxacin	=0.12	=0.125	S	**R**	0
			EUVSEC2	Ceftazidime	=1	=2	S	**R**	0
	Salmonella	EURL S-18.1	EUVSEC3	Tetracycline	=16	≤2	**R**	S	0
		EURL S-18.6	EUVSEC3	Tetracycline	=32	=4	**R**	S	0
				Trimethoprim	>16	=0.5	**R**	S	0
NRL-AR-022	***Salmonella***	EURL S-18.4	EUVSEC2	Imipenem	=1	=0.5	**R**	S	0
NRL-AR-023	***Salmonella***	EURL S-18.1	EUVSEC2	Cefepime	=0.5	=0.25	**R**	S	0
NRL-AR-025	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	=0.5	≤0.125	**R**	S	0

Lab number	Organism	Strain	Panel type	Antimicrobial	MIC		Phenotype (R/S)		Score
					Obtained	Expected	Obtained	Expected	
NRL-AR-026	***Salmonella***	EURL S-18.4	EUVSEC3	Meropenem	=0.12	=0.25	S	**R**	0
NRL-AR-030	***E. coli***	EURL EC-18.5	EUVSEC3	Azithromycin	>16	=8	**R**	S	0
				Tigecycline	=1	=0.5	**R**	S	0
	Salmonella	EURL S-18.3	EUVSEC2	Ceftazidime-clav.acid	=0.5	=0.5	**R**	S	0
NRL-AR-032	***E. coli***	EURL EC-18.1	EUVSEC3	Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
	Salmonella	EURL S-18.6	EUVSEC3	Sulfamethoxazole	=32	>512	S	**R**	0
NRL-AR-033	***Salmonella***	EURL S-18.5	EUVSEC3	Chloramphenicol	≤8	>64	S	**R**	0
		EURL S-18.6	EUVSEC3	Sulfamethoxazole	=256	>512	S	**R**	0
NRL-AR-034	***E. coli***	EURL EC-18.1	EUVSEC3	Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
	Salmonella	EURL S-18.4	EUVSEC3	Meropenem	=0.12	=0.25	S	**R**	0
		EURL S-18.8	EUVSEC3	Ceftazidime	=1	=1	**R**	S	0
NRL-AR-036	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	=0.25	≤0.125	**R**	S	0
NRL-AR-037	***E. coli***	EURL EC-18.1	EUVSEC3	Azithromycin	=8	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Colistin	≤1	=8	S	**R**	0
				Sulfamethoxazole	≤8	>512	S	**R**	0
			EUVSEC2	Cefepime	=0.12	=8	S	**R**	0
				Cefotaxime-clav.acid	=1	=0.125	**R**	S	0
				Ceftazidime-clav.acid	=4	=0.25	**R**	S	0
		EURL EC-18.2	EUVSEC3	Azithromycin	>64	=8	**R**	S	0
				Ciprofloxacin	=0.12	≤0,015	**R**	S	0
				Colistin	=8	≤1	**R**	S	0
				Sulfamethoxazole	>512	≤8	**R**	S	0
			EUVSEC2	Cefepime	>32	≤0.06	**R**	S	0
				Cefotaxime-clav.acid	=0.12	=1	S	**R**	0
				Cefoxitin	=8	=32	S	**R**	0
				Ceftazidime-clav.acid	≤0.12	=2	S	**R**	0
		EURL EC-18.4	EUVSEC2	Ertapenem	=0.06	=0,03	**R**	S	0
		EURL EC-18.8	EUVSEC2	Ceftazidime	=4	=2	S	**R**	0
	Salmonella	EURL S-18.1	EUVSEC3	Sulfamethoxazole	=32	=16	**R**	S	0
			EUVSEC2	Cefepime	=0.5	=0.25	**R**	S	0
NRL-AR-040	***E. coli***	EURL EC-18.1	EUVSEC2	Ceftazidime	=1	=2	S	**R**	0
		EURL EC-18.5	EUVSEC3	Sulfamethoxazole	=128	≤8	**R**	S	0
		EURL EC-18.5	EUVSEC2	Temocillin	=16	=32	S	**R**	0
	Salmonella	EURL S-18.1	EUVSEC3	Sulfamethoxazole	>512	=16	**R**	S	0
		EURL S-18.3	EUVSEC3	Ceftazidime	=2	=4	S	**R**	0
			EUVSEC2	Ceftazidime	=2	=4	S	**R**	0
		EURL S-18.4	EUVSEC3	Meropenem	=0.12	=0.25	S	**R**	0
		EURL S-18.5	EUVSEC2	Imipenem	=1	=2	S	**R**	0
NRL-AR-042	***E. coli***	EURL EC-18.1	EUVSEC3	Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
	Salmonella	EURL S-18.1	EUVSEC2	Cefepime	=0.5	=0.25	**R**	S	0
NRL-AR-045	***Campylobacter***	EURL C-18.1	EUCAMP3	Ertapenem	=1	≤0.125	**R**	S	0
	E. coli	EURL EC-18.1	EUVSEC3	Ceftazidime	=1	=2	S	**R**	0
				Chloramphenicol	=16	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
			EUVSEC2	Ceftazidime	=1	=2	S	**R**	0

Lab number	Organism	Strain	Panel type	Antimicrobial	MIC		Phenotype (R/S)		Score
					Obtained	Expected	Obtained	Expected	
	Salmonella	EURL S-18.4	EUVSEC3	Azithromycin	=16	=32	S	**R**	0
				Cefotaxime	>4	=0.5	**R**	S	0
				Meropenem	≤0.03	=0.25	S	**R**	0
				Sulfamethoxazole	>512	≤8	**R**	S	0
				Tetracycline	>32	≤ 2	**R**	S	0
				Trimethoprim	>16	≤0.25	**R**	S	0
			EUVSEC2	Cefepime	=8	=0.125	**R**	S	0
				Cefotaxime	=32	=0.5	**R**	S	0
				Ceftazidime	=4	=0.5	**R**	S	0
				Ertapenem	≤0.015	=0.5	S	**R**	0
				Meropenem	≤0.03	=0.25	S	**R**	0
				Temocillin	=16	>128	S	**R**	0
		EURL S-18.5	EUVSEC3	Meropenem	=1	=1	S	**R**	0
NRL-AR-056	***E. coli***	EURL EC-18.1	EUVSEC3	Chloramphenicol	≤8	>64	S	**R**	0
				Ciprofloxacin	≤0.015	=0.125	S	**R**	0
				Tetracycline	≤2	>32	S	**R**	0
				Trimethoprim	=0.5	>16	S	**R**	0
NRL-AR-058	***Salmonella***	EURL S-18.4	EUVSEC2	Cefoxitin	=16	=8	**R**	S	0
NRL-AR-059	***Campylobacter***	EURL C-18.3	EUCAMP3	Gentamicin	>16	=0.5	**R**	S	0
NRL-AR-060	***Salmonella***	EURL S-18.4	EUVSEC2	Imipenem	=2	=0.5	**R**	S	0
		EURL S-18.5	EUVSEC3	Tigecycline	=1	=0.5	**R**	S	0
NRL-AR-061	***Campylobacter***	EURL C-18.8	EUCAMP3	Ciprofloxacin	≤0.12	=32	S	**R**	0
	E. coli	EURL EC-18.2	EUVSEC3	Chloramphenicol	=64	=32	S	**R**	0
			EUVSEC2	Cefotaxime-clav.acid	=1	=1	S	**R**	0
				Ceftazidime-clav.acid	=4	=2	S	**R**	0
		EURL EC-18.5	EUVSEC3	Nalidixic acid	≤4	≤4	**R**	S	0
NRL-AR-062	***E. coli***	EURL EC-18.2	EUVSEC3	Azithromycin	=4	=8	**R**	S	0
		EURL EC-18.3	EUVSEC2	Temocillin	=64	=4	**R**	S	0
	Salmonella	EURL S-18.1	EUVSEC2	Cefepime	=0.25	=0.25	**R**	S	0
		EURL S-18.4	EUVSEC2	Imipenem	=2	=0.5	**R**	S	0
		EURL S-18.7	EUVSEC3	Amikacin	=8	≤4	**R**	S	0
				Sulfamethoxazole	>512	=16	**R**	S	0
		EURL S-18.8	EUVSEC3	Sulfamethoxazole	>512	=16	**R**	S	0
NRL-AR-064	***Salmonella***	EURL S-18.1	EUVSEC3	Sulfamethoxazole	=512	=16	**R**	S	0
		EURL S-18.2	EUVSEC3	Meropenem	≤0.03	=0.06	**R**	S	0
		EURL S-18.3	EUVSEC3	Azithromycin	=32	=8	**R**	S	0
				Ceftazidime	=2	=4	S	**R**	0
		EURL S-18.4	EUVSEC3	Meropenem	=0.06	=0.25	S	**R**	0
		EURL S-18.5	EUVSEC3	Amikacin	=8	≤4	**R**	S	0
			EUVSEC2	Imipenem	=1	=2	S	**R**	0
		EURL S-18.7	EUVSEC3	Sulfamethoxazole	=128	=16	**R**	S	0
NRL-AR-066	***E. coli***	EURL EC-18.5	EUVSEC3	Tigecycline	=1	=0.5	**R**	S	0
	Salmonella	EURL S-18.7	EUVSEC3	Sulfamethoxazole	>512	=16	**R**	S	0

II. List of deviations per laboratory, β-lactam resistance mechanism phenotype identification results

Lab number	Strain	Obtained esbl-phenotype value	Expected esbl-phenotype value	Score
NRL-AR-016	EURL EC-18.3	Other phenotypes	ESBL+AmpC-phenotype	0
NRL-AR-018	EURL EC-18.3	AmpC-phenotype	ESBL+AmpC-phenotype	0
NRL-AR-020	EURL S-18.3	Other phenotypes	ESBL-phenotype	0
NRL-AR-021	EURL EC-18.4	ESBL-phenotype	ESBL+AmpC-phenotype	0

Lab number	Strain	Obtained esbl-phenotype value	Expected esbl-phenotype value	Score
NRL-AR-022	EURL EC-18.3	AmpC-phenotype	ESBL+AmpC-phenotype	0
NRL-AR-022	EURL EC-18.4	AmpC-phenotype	ESBL+AmpC-phenotype	0
NRL-AR-025	EURL EC-18.3	Other phenotypes	ESBL+AmpC-phenotype	0
NRL-AR-037	EURL EC-18.1	AmpC-phenotype	ESBL-phenotype	0
NRL-AR-037	EURL EC-18.2	ESBL-phenotype	AmpC-phenotype	0
NRL-AR-040	EURL EC-18.2	ESBL+AmpC-phenotype	AmpC-phenotype	0
NRL-AR-040	EURL EC-18.3	AmpC-phenotype	ESBL+AmpC-phenotype	0
NRL-AR-042	EURL EC-18.3	Other phenotypes	ESBL+AmpC-phenotype	0
NRL-AR-045	EURL S-18.4	ESBL-phenotype	Carbapenemase-phenotype	0
NRL-AR-061	EURL S-18.1	ESBL+AmpC-phenotype	AmpC-phenotype	0

III. List of deviations per laboratory, reference strain results

Lab number number	Reference strain	Panel type	Antimicrobial	Obtained mic value	Min value	Max value	Score
NRL-AR-004	*C. jejuni* (ATCC 33560)	EUCAMP3	Gentamicin	≤0.25	0.5	2	0
NRL-AR-009	*E. coli* ATCC25922	EUVSEC3	Tigecycline	=0.5	0.03	0.25	0
NRL-AR-009	*E. coli* ATCC25922	EUVSEC3	Tigecycline	=0.5	0.03	0.25	0
NRL-AR-011	*C. jejuni* (ATCC 33560)	EUCAMP3	Ciprofloxacin	=0.5	0.06	0.25	0
NRL-AR-018	*E. coli* ATCC25922	EUVSEC3	Trimethoprim	≤0.25	0.5	2	0
NRL-AR-018	*E. coli* ATCC25922	EUVSEC3	Trimethoprim	≤0.25	0.5	2	0
NRL-AR-029	*E. coli* ATCC25922	EUVSEC3	Tigecycline	≤0.5	0.03	0.25	0
NRL-AR-029	*E. coli* ATCC25922	EUVSEC3	Tigecycline	≤0.5	0.03	0.25	0
NRL-AR-030	*C. jejuni* (ATCC 33560)	EUCAMP3	Ciprofloxacin	=0.5	0.06	0.25	0
NRL-AR-040	*E. coli* ATCC25922	EUVSEC3	Trimethoprim	≤0.25	0.5	2	0
NRL-AR-040	*E. coli* ATCC25922	EUVSEC3	Trimethoprim	≤0.25	0.5	2	0
NRL-AR-045	*C. jejuni* (ATCC 33560)	EUCAMP3	Gentamicin	≤0.25	0.5	2	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC3	Tigecycline	=0.5	0.03	0.25	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Imipenem	≤0.012	0.06	0.5	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC3	Tetracycline	=4	0.5	2	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Cefepime	=2	0.016	0.12	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Cefotaxime	=16	0.03	0.12	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Cefoxitin	>64	2	8	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Ceftazidime	=4	0.06	0.5	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Ertapenem	=2	0.004	0.016	0
NRL-AR-061	*E. coli* ATCC25922	EUVSEC2	Meropenem	=0.25	0.008	0.06	0
NRL-AR-062	*E. coli* ATCC25922	EUVSEC3	Sulfamethoxazole	=64	8	32	0

IV. List of deviations per laboratory, *Campylobacter* species identification

Lab number	Strain	Obtained test strain id	Expected test strain id	Score
NRL-AR-040	EURL C-18.7	*C. coli*	*C. jejuni*	0
NRL-AR-059	EURL C-18.3	*C. jejuni*	*C. coli*	0

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