

External Quality Assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2024

National summary report for Estonia

Provider of the EARS-Net EQA:

Research group for Global Capacity Building

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1. edition, February 2025



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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
DTU Food	Technical University of Denmark, National Food Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EQA	External quality assessment
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	Susceptible, increased exposure
MIC	Minimum inhibitory concentration
ME	Major error
R	Resistant
S	Susceptible, standard dosing regimen
s.d.	Standard deviation
VME	Very major error

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1. Introduction

This report describes and summarises national results from the external quality assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2024. Participating laboratories are identified by codes known by the corresponding laboratory, the national EQA coordinator and the EQA provider.

The 2024 EARS-Net EQA exercise aimed to: 1) assess the quality of species identification by participating laboratories; 2) assess the accuracy of the qualitative antimicrobial susceptibility testing (AST) results reported by participating laboratories; and 3) evaluate the overall comparability of routinely collected AST results between laboratories and European Union/European Economic Area (EU/EEA) countries.

The report provides a summary of results, including a short conclusion on the capacity of participating laboratories, and if needed, recommendations for improvement.

The 2024 EQA focused on species identification and interpretation of the AST of the six strains shared with the participating laboratories (*Acinetobacter baumannii*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*).

1.1 Participation

Overall, in the 2024 EARS-Net EQA, 978 laboratories from all 30 EU/EEA countries signed up for participation, and 912 laboratories submitted data. This is a little more than in 2023 EARS-Net EQA, when 957 laboratories signed up for participation and 871 submitted data, from all 30 EU/EEA countries.

In Estonia, 11 laboratories signed up for participation in the 2024 EARS-Net EQA and received the six strains for analysis, and 10 laboratories submitted data for evaluation. No results were submitted by 1 laboratory (EE001). Following the EUCAST guideline is mandatory when participating in the EARS-Net EQA. All laboratories reported using the EUCAST guideline.

2. Materials and Methods

2.1 Strains and antimicrobial susceptibility testing

The *Acinetobacter baumannii*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* strains were selected for this EQA from the strain collection at the Technical University of Denmark, National Food Institute (DTU Food) based on their antimicrobial resistance profiles and the recommendations from European Centre for Disease Prevention and Control (ECDC).

The expected results were determined by examining the consensus AST results obtained by DTU Food through broth microdilution and/or disk diffusion, and results from confirmatory testing provided by two reference laboratories. These were the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory, Växjö, Sweden and the Microbiological Diagnostic Unit Public Health Laboratory, The Doherty Institute, Australia. Subsequently, the consensus phenotypic AST profile was compared with whole-genome sequencing (WGS) data on acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) obtained at DTU Food using the bioinformatics tools ResFinder v4.5, AMRFinderPlus and CARD RGI (Tables 1–6). Finally, after the preparation of the agar swab cultures/charcoal swabs for shipment to participants, MIC determinations were performed at DTU Food, to confirm that the vials contained the correct strains with the expected AST results.

The antimicrobial agents selected for this EQA correspond to the panel of pathogen and antimicrobial agent combinations under surveillance by EARS-Net presented in the antimicrobial resistance (AMR) reporting protocol 2024¹. The exceptions were testing of cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam and meropenem-vaborbactam for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp, which were included in the original table, but is not part of the 2024 EARS-Net EQA exercise.

Participating laboratories should perform AST according to the laboratory's applied routine procedures, i.e., automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient-diffusion, or others following EUCAST recommendations (https://www.eucast.org/ast_of_bacteria/).

The EUCAST clinical breakpoints tables v14.0 were used for the interpretation of AST results (https://www.eucast.org/clinical_breakpoints/) (Tables 1-6). This permitted categorisation of the AST results into three categories: "resistant" (R), "susceptible, increased exposure" (I), and "susceptible, standard dosing regimen" (S).

Table 1. EUCAST clinical breakpoints for *Acinetobacter baumannii* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2024 EARS-Net 1' (*A. baumannii*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Imipenem	2	4	24	21	Easy	>16	R	<i>bla</i> _{OXA-23}
Meropenem	2	8	21	15	Easy	>64	R	<i>bla</i> _{OXA-23}
Ciprofloxacin	0.001	1	50	21	Easy	>8	R	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Levofloxacin	0.5	1	23	20	Easy	16	R	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Amikacin	8	8	19	19	Easy	128	R	<i>aac</i> (6')-Ib3, <i>aph</i> (3')-Via
Gentamicin	4	4	17	17	Easy	2	S	<i>aph</i> (3')-Via
Tobramycin	4	4	17	17	Difficult	8	R	<i>aac</i> (6')-Ib3
Colistin	2	2	Note ****	Note ****	Easy	0.5	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**The expected value corresponds to the MIC expressed in 'mg/L'.

***ND: Not detected. Additional ARGs or chromosomal PMs: *sul1*, *dfrA7*, *bla*_{GES-11}, *bla*_{OXA-65} (intrinsic), *bla*_{ADC-25} (likely intrinsic). MALDI-TOF by DTU: *Acinetobacter baumannii* (score 2,37). MLST: ST-499 (scheme *A. baumannii* #1) / ST-158 (scheme *A. baumannii* #2).

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

Table 2. EUCAST clinical breakpoints for *Enterococcus faecium* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2024 EARS-Net 2' (*E. faecium*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Ampicillin	4	8	10	8	Easy	>64	R	PBP5-R
Amoxicillin	4	8	Note ****	Note ****	Easy	64	R	PBP5-R
Vancomycin	4	4	12	12	Easy	>16	R	<i>VanHBX</i>
Teicoplanin	2	2	16	16	Easy	1	S	ND
Linezolid	4	4	20	20	Easy	2	S	ND
Gentamicin (HLAR)	128	128	8	8	Easy	≤8	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**The expected value corresponds to the MIC expressed in 'mg/L'.

***ND: Not detected. PBP5-R: *pbp5* M485A, *pbp5* D204G, *pbp5* S27G, *pbp5* R34Q, *pbp5* E525D, *pbp5* N496K, *pbp5* V24A, *pbp5* T324A, *pbp5* A499T, *pbp5* E100Q, *pbp5* L177I, *pbp5* E629V, *pbp5* A216S, *pbp5* A68T, *pbp5* P667S, *pbp5* E85D, *pbp5* G66E, *pbp5* K144Q, *pbp5* T172A, *pbp5* V586L. Additional ARGs or chromosomal PMs: *msr(C)*, *tet(M)*, *gyrA* S83Y, *parC* S80I, *aac(6)-II* (intrinsic). MALDI-TOF by DTU: *Enterococcus faecium* (score 2,42). MLST: ST-17.

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

Table 3. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2024 EARS-Net 3' (*E. coli*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)			EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result*	Expected interpretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Ampicillin	8	8		14	14		Easy	>32	R	<i>bla</i> _{OXA-1} , <i>bla</i> _{CTX-M-15}
Amoxicillin	8	8		Note ****	Note ****		Easy	>64	R	<i>bla</i> _{OXA-1} , <i>bla</i> _{CTX-M-15}
Amoxicillin-clavulanic acid*****	8	8		19	19	19-20	Easy	>64/2	R	<i>bla</i> _{OXA-1}
Piperacillin-tazobactam** ***	8	8	16	20	20	19	Difficult	16/4	R	<i>bla</i> _{OXA-1}
Cefepime	1	4		27	24		Difficult	2	I	<i>bla</i> _{OXA-1} , <i>bla</i> _{CTX-M-15}
Cefotaxime	1	2		20	17		Easy	>4	R	<i>bla</i> _{CTX-M-15}
Ceftazidime	1	4		22	19		Difficult	2	I	<i>bla</i> _{CTX-M-15}
Ceftriaxone	1	2		25	22		Easy	>16	R	<i>bla</i> _{CTX-M-15}
Ertapenem	0.5	0.5		23	23		Easy	≤0.03	S	ND
Imipenem	2	4		22	19		Easy	≤0.25	S	ND
Meropenem	2	8		22	16		Easy	≤0.03	S	ND
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	>4	R	<i>aac</i> (6')-Ib-cr, <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Levofloxacin	0.5	1		23	19		Easy	>8	R	<i>aac</i> (6')-Ib-cr, <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Moxifloxacin	0.25	0.25		22	22		Easy	>8	R	<i>aac</i> (6')-Ib-cr, <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	<i>aac</i> (6')-Ib-cr, <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Amikacin	8	8		18	18		Difficult	8	S	<i>aac</i> (6')-Ib-cr
Gentamicin	2	2		17	17		Easy	1	S	ND
Tobramycin	2	2		16	16		Easy	>16	R	<i>aac</i> (6')-Ib-cr
Tigecycline	0.5	0.5		18	18		Easy	≤0.25	S	ND
Colistin	2	2		Note ****	Note ****		Easy	≤0.25	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different

interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**The expected value corresponds to the MIC expressed in 'mg/L'.

***ND: Not detected. Additional ARGs or chromosomal PMs: *mph(A)*, *catB3*, *aadA5*, *sul1*, *dfrA17*. MALDI-TOF by DTU: *Escherichia coli* (score 2,26). MLST: ST-131 (scheme *E. coli* #1) / ST-43 (scheme *E. coli* #2).

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

*****Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.

Table 4. EUCAST clinical breakpoints, expected AST results for *Pseudomonas aeruginosa* and the level of difficulty in interpretation and expected interpretations for strain '2024 EARS-Net 4' (*P. aeruginosa*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≤	R >	ATU				
Piperacillin	0.001	16	50	18	18-19	Difficult	128	R	ND
Piperacillin-tazobactam *****	0.001	16	50	18	18-19	Difficult	≤16/4	I	ND
Cefepime	0.001	8	50	21		Difficult	8	I	ND
Ceftazidime	0.001	8	50	17		Difficult	>8	R	ND
Imipenem	0.001	4	50	20		Easy	>8	R	<i>oprD</i> W339STOP
Meropenem	2	8	20	14		Difficult	8	I	<i>oprD</i> W339STOP
Ciprofloxacin	0.001	0.5	50	26		Easy	>4	R	<i>crpP</i> , <i>gyrA</i> T83I
Levofloxacin	0.001	2	50	18		Easy	8	R	<i>gyrA</i> T83I
Amikacin	16	16	15	15		Easy	4	S	ND
Tobramycin	2	2	18	18		Easy	0.5	S	ND
Colistin	4	4	Note ****	Note ****		Easy	1	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**The expected value corresponds to the MIC expressed in 'mg/L'.

***ND: Not detected. Additional ARGs or chromosomal PMs: *aph(3)-IIB*, *fosA* (intrinsic), *catB7* (intrinsic), *bla_{PAO}* (intrinsic), *bla_{OXA-48B}* (likely intrinsic). MALDI-TOF by DTU: *Pseudomonas aeruginosa* (score 2,45). MLST: ST-395.

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

*****Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.

Table 5. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2024 EARS-Net 5' (*K. pneumoniae*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)			EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result*	Expected interpretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Amoxicillin-clavulanic acid*****	8	8		19	19	19-20	Easy	>64/2	R	<i>bla</i> _{VEB-1} , <i>bla</i> _{SHV-11}
Piperacillin-tazobactam** ***	8	8	16	20	20	19	Easy	>128/4	R	<i>bla</i> _{VEB-1} , <i>bla</i> _{SHV-11} , <i>bla</i> _{OXA-10}
Cefepime	1	4		27	24		Difficult	2	I	<i>bla</i> _{VEB-1} , <i>bla</i> _{SHV-11}
Cefotaxime	1	2		20	17		Difficult	4	R	<i>bla</i> _{VEB-1} , <i>bla</i> _{SHV-11}
Ceftazidime	1	4		22	19		Easy	>16	R	<i>bla</i> _{VEB-1} , <i>bla</i> _{SHV-11}
Ceftriaxone	1	2		25	22		Easy	8	R	<i>bla</i> _{SHV-11}
Ertapenem	0.5	0.5		23	23		Easy	2	R	ND
Imipenem	2	4		22	19		Difficult	4	I	ND
Meropenem	2	8		22	16		Difficult	2	S	ND
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	0.03	S	ND
Levofloxacin	0.5	1		23	19		Easy	0.06	S	ND
Moxifloxacin	0.25	0.25		22	22		Easy	0.06	S	ND
Ofloxacin	0.25	0.5		24	22		Difficult	≤0.25	S	ND
Amikacin	8	8		18	18		Easy	4	S	<i>aac</i> (6')-Ia
Gentamicin	2	2		17	17		Difficult	4	R	<i>ant</i> (2'')-Ia
Tobramycin	2	2		16	16		Easy	8	R	<i>aac</i> (6')-Ia, <i>ant</i> (2'')-Ia
Colistin	2	2		Note ****	Note ****		Easy	0.5	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**The expected value corresponds to the MIC expressed in 'mg/L'.

***ND: Not detected. *bla*_{SHV-11} was an imperfect match (other identified variants: *bla*_{SHV-40}, *bla*_{SHV-56}, *bla*_{SHV-79}, *bla*_{SHV-85}, *bla*_{SHV-89}). Additional ARGs or chromosomal PMs: *bla*_{OXA-436}, *ARR-2*, *aadA1*, *cml*, *cmlA1*, *sul1*, *OqxA* (intrinsic), *OqxB* (intrinsic), *fosA6* (intrinsic), *fosA7* (intrinsic), *ompK36* N49S, *ompK36* L59V, *ompK36* G189T, *ompK36* F198Y, *ompK36* F207Y, *ompK36* A217S, *ompK36* T222L, *ompK36* D223G, *ompK36* E232R, *ompK36* N304E, *ompK37* I70M, *ompK37* I128M, *acrR* P161R, *acrR* G164A, *acrR* F172S, *acrR* R173G, *acrR* L195V, *acrR* F197I, *acrR* K201M (*ompK36* A217S, *ompK37* I70M and *ompK37* I128M potentially associated with carbapenem resistance). MALDI-TOF by DTU: *Klebsiella pneumoniae* (score 2,32), and MLST: ST-37.

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

*****Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.

Table 6. EUCAST clinical breakpoints for *Staphylococcus aureus* and the expected MIC value, level of difficulty in interpretation and interpretation for strain '2024 EARS-Net 6' (*S. aureus*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Oxacillin	Note ****	2	Note ****	Note ****	Easy	8	R	ND
Cefoxitin	Note ****	4	22	22	Difficult	27 mm	S	ND
Ciprofloxacin	0.001	2	50	17	Difficult	1	I	ND
Levofloxacin	0.001	1	50	22	Easy	≤0.5	I	ND
Norfloxacin	NA	NA	17	17	Easy	24 mm	S	ND
Vancomycin	2	2	Note ****	Note ****	Easy	1	S	ND
Linezolid	4	4	21	21	Easy	2	S	ND
Daptomycin	1	1	Note ****	Note ****	Easy	≤0.5	S	ND
Rifampicin	0.06	0.06	26	26	Easy	0.015	S	ND

*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

**For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and cefoxitin the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

***ND: Not detected. Additional ARGs or chromosomal PMs: *blaZ*, *fusA* L461K. MALDI-TOF by DTU: *Staphylococcus aureus* (score 2.26). MLST: ST-188.

****Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

2.2 Procedure

The protocol, test forms and webtool user guide are available on the 2024 EARS-Net EQA website (antimicrobialresistance.dk/ears_net_EQA.aspx).

All participating laboratories were invited to enter results into the EARS-Net EQA web-based database using a secure personal login and password. The deadline for submission of data was 11 August 2024. The results were evaluated using a scoring algorithm considering the difficulty of classification and the severity of error.

All participants were encouraged to complete an electronic evaluation form using a link forwarded to contact persons for the participating laboratories with the aim of improving future EQA exercises. The evaluation questions were provided by ECDC.

2.3 Scoring antimicrobial susceptibility results

In the 2024 EARS-Net EQA, the implemented scoring system for the evaluation of interpreted results took "level of difficulty" and "severity of error" into account for each organism-antimicrobial combination.

The level of difficulty indicated the magnitude of the risk of getting the categorisation wrong and consisted of two levels: easy and difficult. 'Difficult' were situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' were situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table. The scoring of a result reflected the level of difficulty (Table 7).

The severity of error was divided into three levels: very major error (VME), major error (ME) and no error. Both VME and ME were penalised. VME was reporting false susceptibility – expecting an R but obtaining an S or I. If the only categories were I and R, then reporting I instead of R was also a VME. ME was reporting false resistance – expecting an S or I but obtaining an R. The scoring of a result reflected the severity of an error (Table 7).

This scoring system is the same as applied in the 2023 EARS-Net EQA.

Table 7. Exercise scoring system for reported AST results in the 2024 EARS-Net EQA

		Difficulty of result and expected interpretation					
		Easy			Difficult		
		R	I	S	R	I	S
Obtained interpretation	R	1	-3 (ME)	-3 (ME)	4	0 (ME)	0 (ME)
	I	-4 (VME)	1	-1	-1 (VME)	4	2
	S	-4 (VME)	-1	1	-1 (VME)	2	4
	Not reported	-	-	-	-	-	-

Note: R: resistant, I: susceptible, increased exposure, S: susceptible, standard dosing regimen; VME: very major error, ME: major error; - : no data.

3. Results

3.1 Participation

In the 2024 EARS-Net EQA, it was decided to include species relevant for the EARS-Net surveillance for species identification. In total, six strains were included in the 2024 EARS-Net EQA and all six were covered by EARS-Net surveillance. Therefore, results on the species identification and interpretation of AST results should be reported for all six strains. Overall, 10 out of 11 (90.9%) laboratories from Estonia submitted results for one or more of the six strains.

A total of 10 laboratories (100.0%) submitted AST results for all six strains, and all laboratories provided interpretation for all submitted strains. Providing interpretation of the six strains was a minimum requirement for receiving a certificate of participation.

3.2 Species identification results

For each strain, the species should be identified. In total, 60 out of 60 (100.0%) strains submitted with interpretation had the correct species identification. An overview of the species identification for the six strains and the number of laboratories reporting the correct identification is given in Table 8.

Only the interpretation of AST results submitted for strains with correct species identification could be evaluated (Table 8).

Table 8. Number and percentage of laboratories reporting the correct species in the 2024 EARS-Net EQA

Estonia Strain ID	Expected species	No. of labs submitting data with interpretation	No. of labs reporting correct species identification	% of labs reporting correct species identification
2024 EARS-Net 1	<i>Acinetobacter baumannii</i>	10	10	100.0
2024 EARS-Net 2	<i>Enterococcus faecium</i>	10	10	100.0
2024 EARS-Net 3	<i>Escherichia coli</i>	10	10	100.0
2024 EARS-Net 4	<i>Pseudomonas aeruginosa</i>	10	10	100.0
2024 EARS-Net 5	<i>Klebsiella pneumoniae</i>	10	10	100.0
2024 EARS-Net 6	<i>Staphylococcus aureus</i>	10	10	100.0

3.3 Antimicrobial susceptibility testing (AST) results

AST results were evaluated for strains with correct species identification. In the 2024 EARS-Net EQA, the species for the six strains were included in the EARS-Net surveillance.

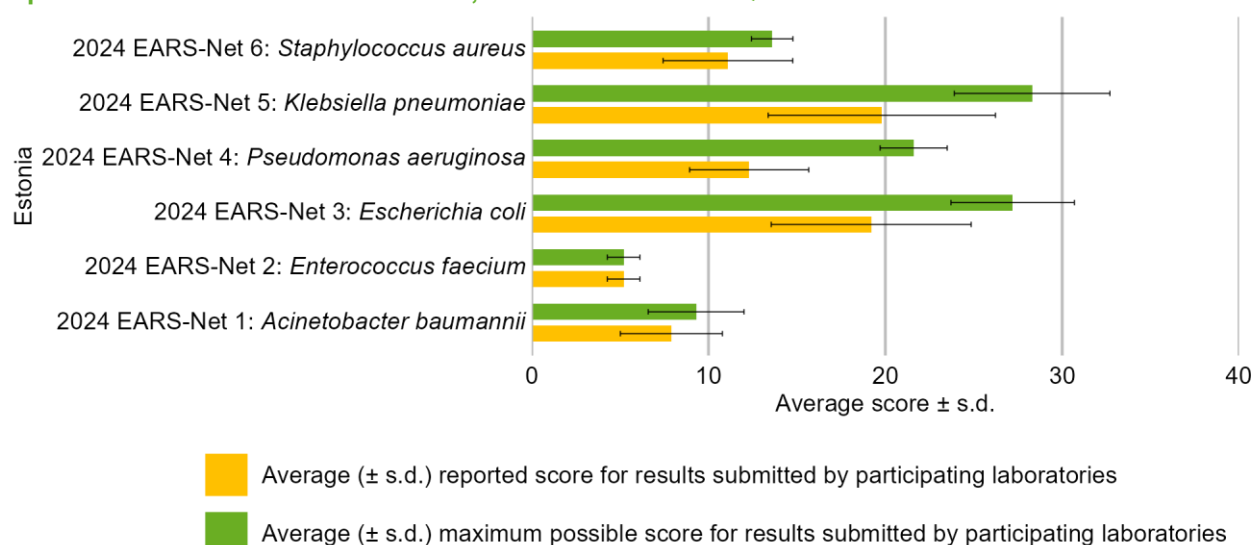
Participants were asked to report AST results, i.e., MIC or zone diameter values and their categorisation as “resistant” (R), “susceptible, increased exposure” (I), and “susceptible, standard dosing regimen” (S) for the species covered by EARS-Net surveillance. Only the categorisation was evaluated, whereas the quantitative values were used as supplementary information.

For the 2024 EARS-Net EQA, each laboratory could report interpretation for 71 different strain-antimicrobial combinations with a total maximum score of 125.

For the 10 laboratories submitting results with correct species identification, interpretation of AST results were reported for 581 out of the 710 possible strain-antimicrobial combinations, and 529 (91.0%) were reported with the correct interpretation with an average score for submitted results of 75.5 ± 18.2 . The maximum possible score for the reported results was 105.2 ± 12.8 .

Figure 1 presents the average maximum possible score for reported results $\pm$ std, and the average score for reported results $\pm$ std for the laboratories reporting results for each of the six strains.

Figure 1. Average maximum possible score of reported results $\pm$ s.d., and average score of the reported results $\pm$ s.d. for each strain, in 2024 EARS-Net EQA



Key: s.d. – standard deviation.

An overview of the methods used for the determination of the antimicrobial resistance for the six strains and the percentage of correct interpretations is given in [Tables 9-11](#). The most commonly used method was disk/tablet diffusion (53.9%) ([Table 12](#)). The lowest level of concordance with expected interpretations was reported when using the gradient test (83.3%) ([Table 12](#)).

Table 9. Overview of methods used for determination of the AST results for strains '2024 EARS-Net 1' and '2024 EARS-Net 2'

Estonia	2024 EARS-Net 1 <i>Acinetobacter baumannii</i>			2024 EARS-Net 2 <i>Enterococcus faecium</i>		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Automated system	5	7.2	80.0	15	28.8	100.0
Broth microdilution	17	24.6	94.1	2	3.8	100.0
Disk/Tablet diffusion	41	59.4	97.6	25	48.1	100.0
Gradient test	6	8.7	100.0	10	19.2	100.0
Total	69	100.0	95.7	52	100.0	100.0

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 10. Overview of methods used for determination of the AST results for strains '2024 EARS-Net 3' and '2024 EARS-Net 4'

Estonia	2024 EARS-Net 3 <i>Escherichia coli</i>			2024 EARS-Net 4 <i>Pseudomonas aeruginosa</i>		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Automated system	30	19.4	93.3	18	19.4	77.8
Broth microdilution	25	16.1	92.0	14	15.1	92.9
Disk/Tablet diffusion	89	57.4	87.6	43	46.2	83.7
Gradient test	11	7.1	100.0	18	19.4	61.1
Total	155	100.0	90.3	93	100.0	79.6

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 11. Overview of methods used for determination of the AST results for strains '2024 EARS-Net 5' and '2024 EARS-Net 6'

Estonia	2024 EARS-Net 5 <i>Klebsiella pneumoniae</i>			2024 EARS-Net 6 <i>Staphylococcus aureus</i>		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Automated system	32	23.5	96.9	21	27.6	100.0
Broth microdilution	17	12.5	100.0	0	-	-
Disk/Tablet diffusion	73	53.7	87.7	42	55.3	100.0
Gradient test	14	10.3	85.7	13	17.1	76.9
Total	136	100.0	91.2	76	100.0	96.1

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 12. Overview of methods used for determination of the AST results for all six strains

Estonia	Total		
Method	No. of AST performed	% of total AST performed	% correct interpretation
Automated system	121	20.8	93.4
Broth microdilution	75	12.9	94.7
Disk/Tablet diffusion	313	53.9	91.1
Gradient test	72	12.4	83.3
Total	581	100.0	91.0

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Reported intention of participating laboratories to send a strain to a reference laboratory

When submitting AST results, the participating laboratories could indicate whether they would send the strain to a reference laboratory for further microbiological analysis.

For strain '2024 EARS-Net 1' (*A. baumannii*), 7 (70.0%) of the 10 laboratories submitting results would send the strain to a reference or other laboratory for further testing. Only 1 (50%) of the 2 laboratories reporting VME would send the strain for further analysis.

For strain '2024 EARS-Net 2' (*E. faecium*), 7 (70.0%) of the 10 laboratories submitting results would send the strain for further testing. None of the laboratories had any VME.

For strain '2024 EARS-Net 3' (*E. coli*), 5 (50.0%) of the 10 laboratories submitting results would send the strain for further testing. Only 1 (25%) of the 4 laboratories reporting VME would send the strain for further analysis.

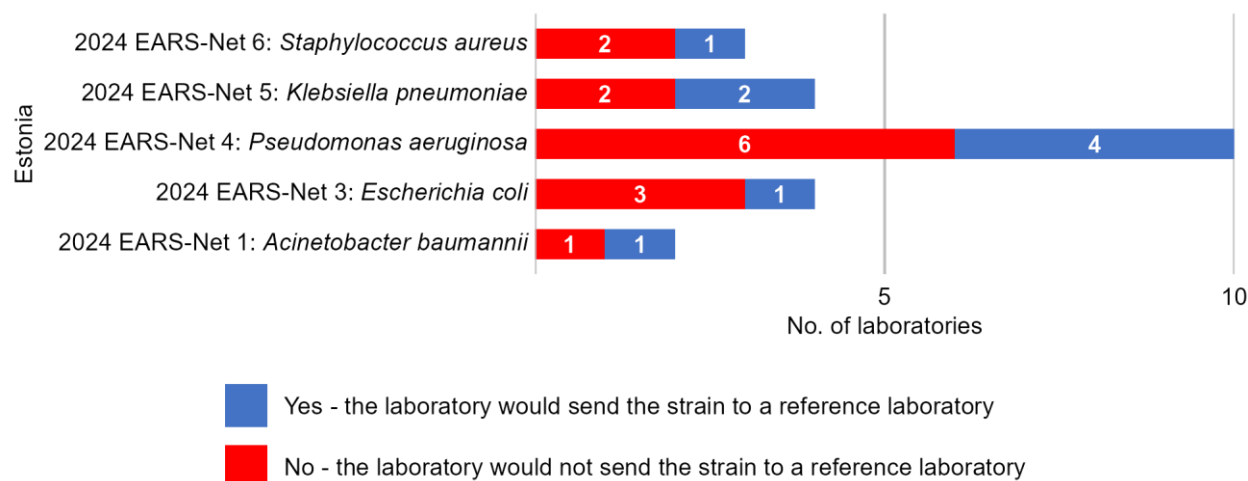
For strain '2024 EARS-Net 4' (*P. aeruginosa*), 4 (40.0%) of the 10 laboratories submitting results would send the strain for further testing. Only 4 (40%) of the 10 laboratories reporting VME would send the strain for further analysis.

For strain '2024 EARS-Net 5' (*K. pneumoniae*), 6 (60.0%) of the 10 laboratories submitting results would send the strain for further testing. Only 2 (50%) of the 4 laboratories reporting VME would send the strain for further analysis.

For strain '2024 EARS-Net 6' (*S. aureus*), 4 (40.0%) of the 10 laboratories submitting results would send the strain for further testing. Only 1 (33.3%) of the 3 laboratories reporting VME would send the strain for further analysis.

Figure 2 provides an overview of the laboratories with very major errors (VME) that would send the strains for further testing.

Figure 2. Laboratories with very major errors (VME) intention to send a strain to a reference laboratory for further testing in 2024 EARS-Net EQA, by strain



Number in the columns: Number of laboratories

Antimicrobial agents tested for each EQA strain

The EQA protocol [1] states that participating laboratories should perform AST on the species-antimicrobial agent combination that can be reported to EARS-Net if they perform that test within their standard practice. The overwhelming majority of clinical laboratories in the EU/EEA are unlikely to perform, as standard practice, AST on all these combinations. For example, many laboratories will utilise the services of reference laboratories.

For strain '2024 EARS-Net 1' (*A. baumannii*), 5 out of 10 laboratories tested all 8 antimicrobials (Figure 4).

For strain '2024 EARS-Net 2' (*E. faecium*), 5 out of 10 laboratories tested all 6 antimicrobials (Figure 6).

For strain '2024 EARS-Net 3' (*E. coli*), none out of 10 laboratories tested all 20 antimicrobials (Figure 9).

For strain '2024 EARS-Net 4' (*P. aeruginosa*), 1 out of 10 laboratories tested all 11 antimicrobials (Figure 11).

For strain '2024 EARS-Net 5' (*K. pneumoniae*), none out of 10 laboratories tested all 17 antimicrobials (Figure 14).

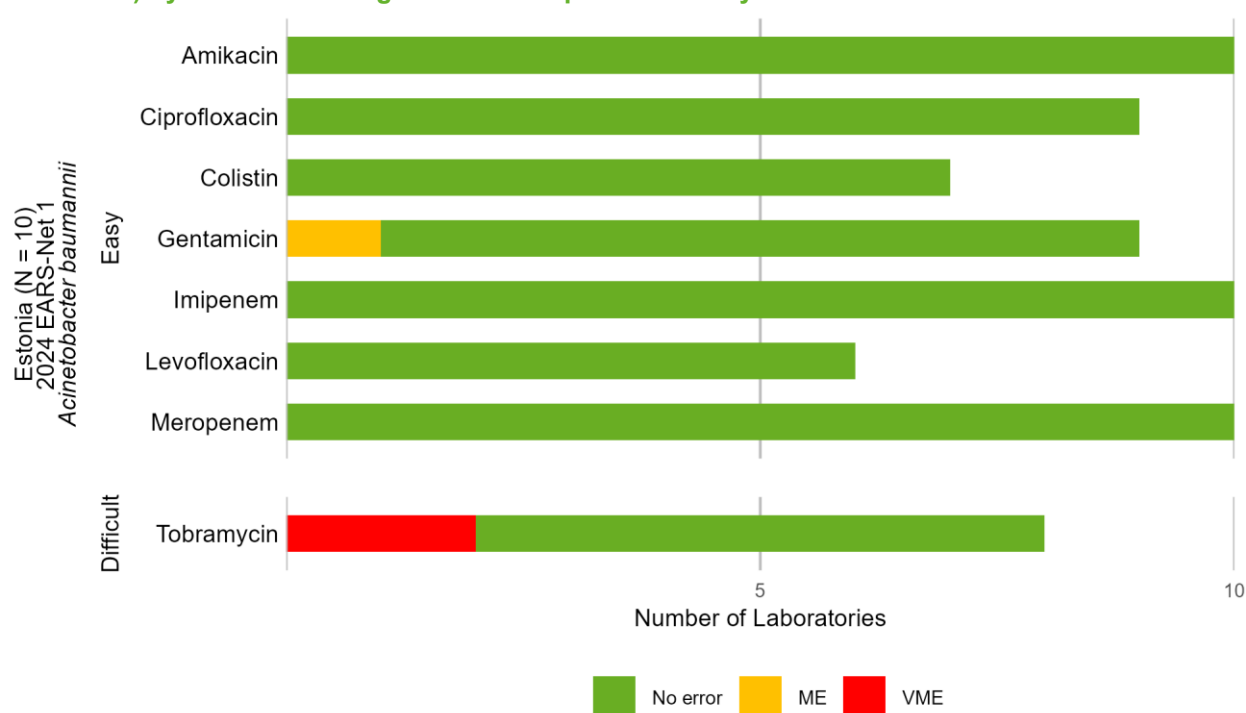
For strain '2024 EARS-Net 6' (*S. aureus*), 3 out of 10 laboratories tested all 9 antimicrobials (Figure 16).

Strain '2024 EARS-Net 1' (*A. baumannii*)

The 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 1'. Each laboratory could submit results from 8 antimicrobials (maximum 80 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

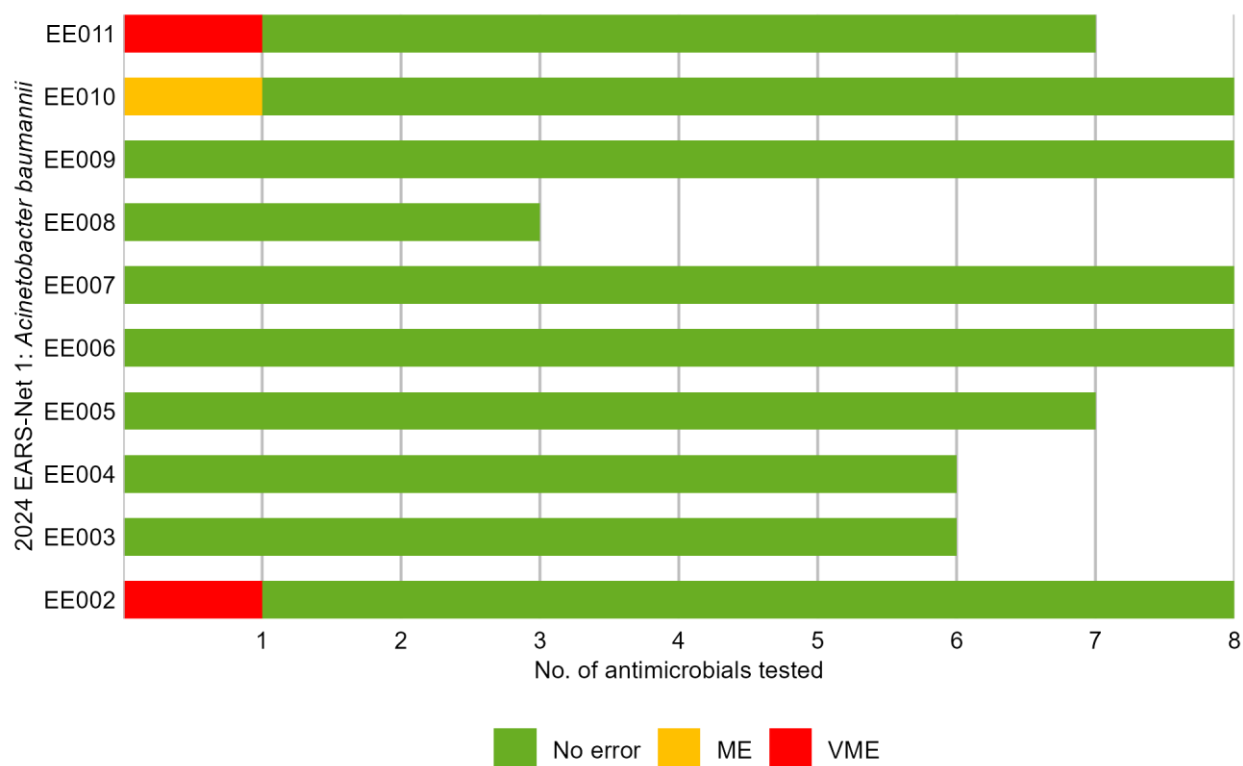
Overall, 69 AST results were submitted and the interpretations were correct for 66 (95.7%) of the results; 1 (1.4%) of the interpretations were ME and 2 (2.9%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2024 EARS-Net 1' were reported for tobramycin (Figure 3). An overview of the reported results for all laboratories is presented in Figure 4.

Figure 3. Reported interpretation of AST results for strain '2024 EARS-Net 1' (*Acinetobacter baumannii*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 4. Reported interpretation of AST results for strain '2024 EARS-Net 1' (*Acinetobacter baumannii*) by laboratory



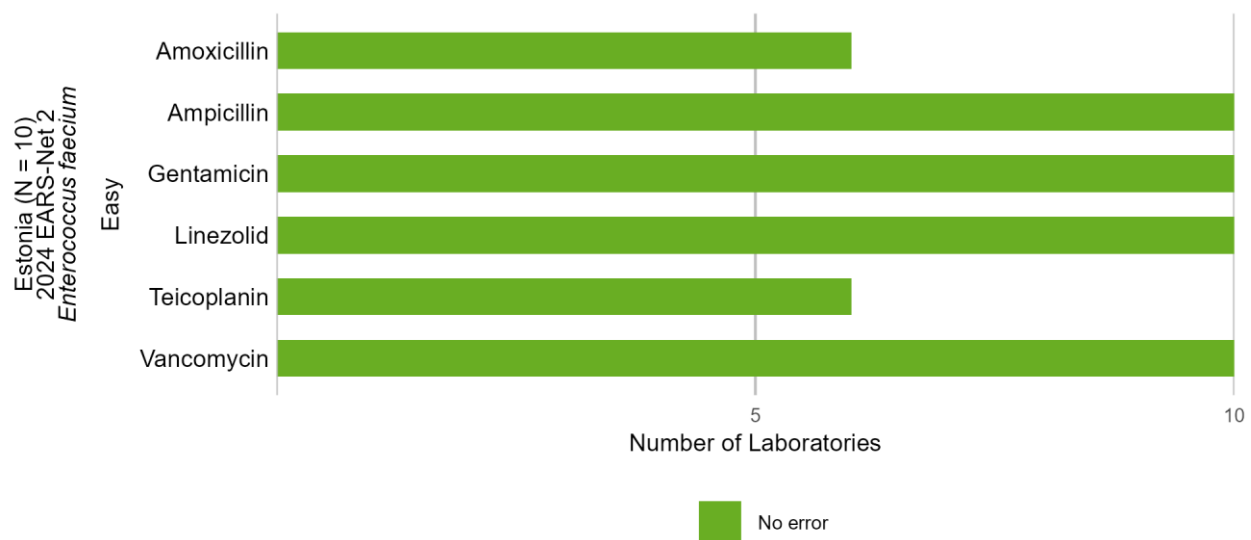
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain '2024 EARS-Net 2' (*Enterococcus faecium*)

The 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 2'. Each laboratory could submit results from 6 antimicrobials (maximum 60 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

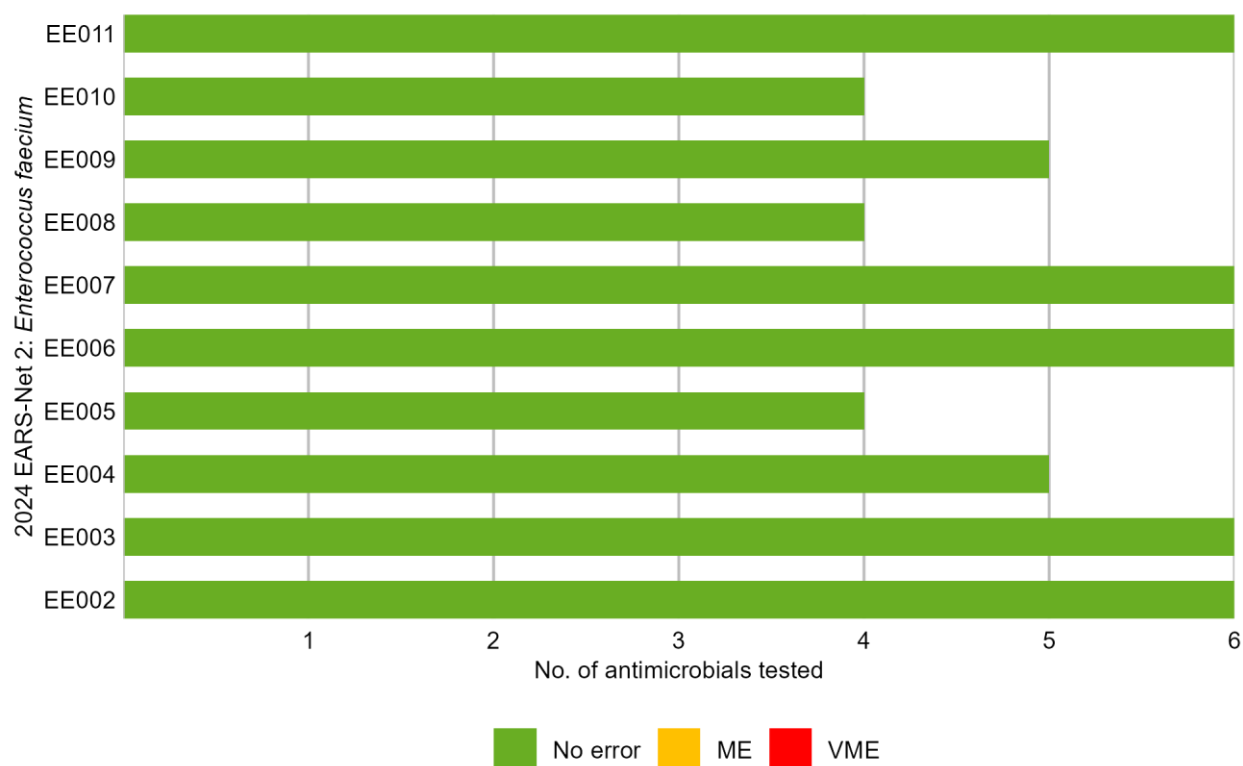
Overall, 52 AST results were submitted and the interpretations were correct for 52 (100.0%) of the results. None of the laboratories reported any ME or VME (Figure 5). An overview of the reported results for all laboratories is presented in Figure 6.

Figure 5. Reported interpretation of AST results for strain '2024 EARS-Net 2' (*Enterococcus faecium*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 6. Reported interpretation of AST results for strain '2024 EARS-Net 2' (*Enterococcus faecium*) by laboratory



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

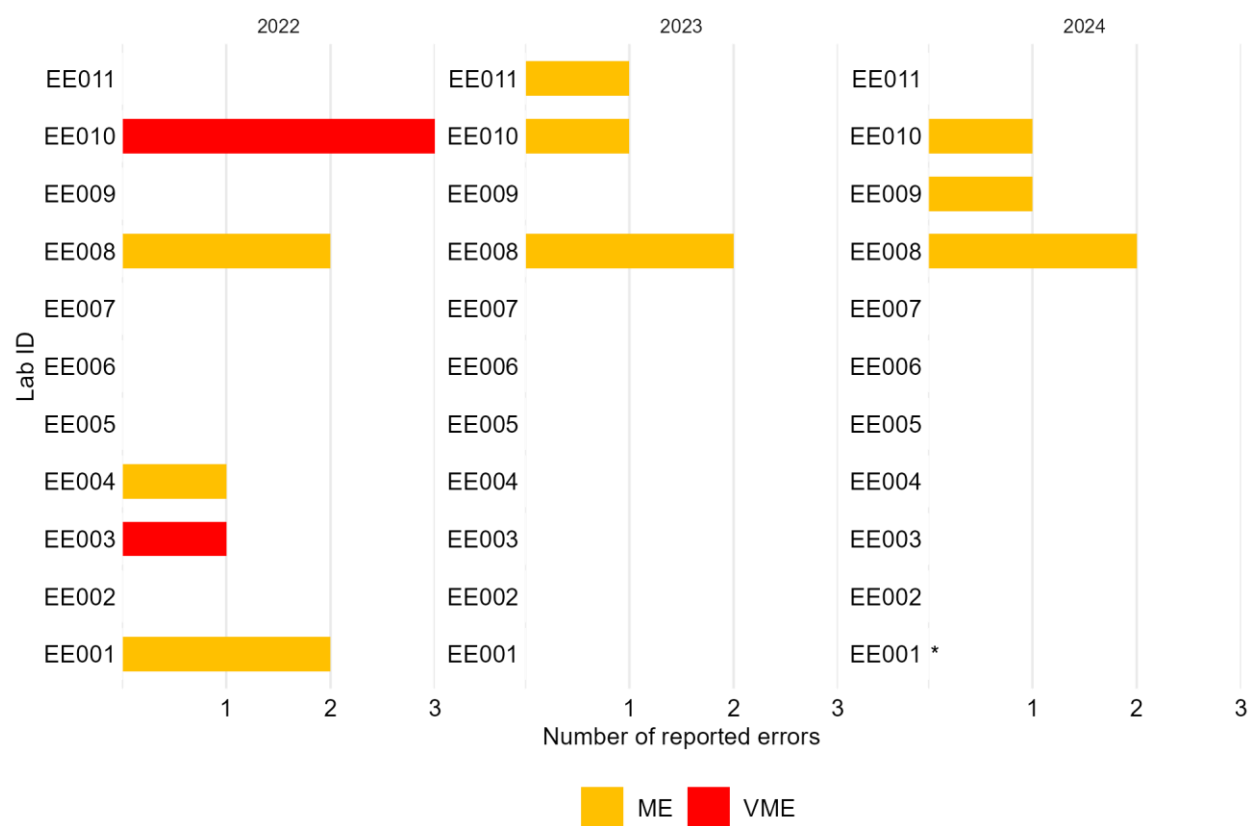
Strain '2024 EARS-Net 3' (*Escherichia coli*)

The strain '2022 EARS-Net 2' (*E. coli*) and the strain '2023 EARS-Net 1' (*E. coli*) from the 2022 and 2023 EARS-Net EQAs, respectively, were the same strain and most challenging for participating laboratories. Therefore, it was decided to include the exact same *E. coli* strain in the panel for the 2024 EARS-Net EQA (strain '2024 EARS-Net 3'). To ensure harmonisation between expected results included in the 2024 EQA, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA. The obtained expected results were essentially in agreement with the results obtained and described in the 2022 and 2023 EARS-Net EQA. However, three differences existed between expected results in different years. Firstly, the obtained consensus for piperacillin-tazobactam (const. 4) for both the 2024 and the 2023 EARS-Net EQAs was MIC=16/4 mg/L, and therefore received an interpretation as Resistant, whereas for the 2022 EARS-Net EQA the expected result was MIC=8/4 mg/L with an interpretation of Susceptible, standard dosing regimen. Furthermore, the obtained consensus for amikacin for both the 2024 and the 2023 EARS-Net EQAs was MIC=8 mg/L, and therefore received an interpretation as Susceptible, standard dosing regimen, whereas for the 2022 EARS-Net EQA the expected result was MIC>8 mg/L with an interpretation of Resistant. Finally, the expected result for cefepime in the 2024 EARS-Net EQA was MIC=2 mg/L with the interpretation of Susceptible, increased exposure, whereas for the 2023 and 2022 EARS-Net EQAs the expected result was MIC=1 mg/L with the interpretation Susceptible, standard dosing regimen. These results further illustrate the variability of the strain and the difficulty of obtaining concordant AST results.

At the EU/EEA level, when comparing results between the 2022, 2023 and the 2024 EARS-Net EQAs, there was little variability of results for this strain (excluding the results obtained for amikacin and for piperacillin-tazobactam). The highest variation was the decrease in ME for cefepime, from 20% of the participating laboratories in 2022 to 17% of laboratories in 2023 and 2024.

In Estonia, 11 laboratories submitted interpretation of AST results minimum two years, and 7 laboratories reported results with VME/ME at least one year (the results on piperacillin-tazobactam (const. 4) and amikacin were excluded). An overview of the laboratories reporting results with VME/ME (leaving out piperacillin-tazobactam (const. 4) and amikacin results) is presented in [Figure 7](#).

Figure 7. Reported errors of interpretation of AST results (not including piperacillin-tazobactam (const. 4) and amikacin) for strain '2022 EARS-Net 2', '2023 EARS-Net 1' and '2024 EARS-Net 3' by laboratories providing results for at least two of the three years

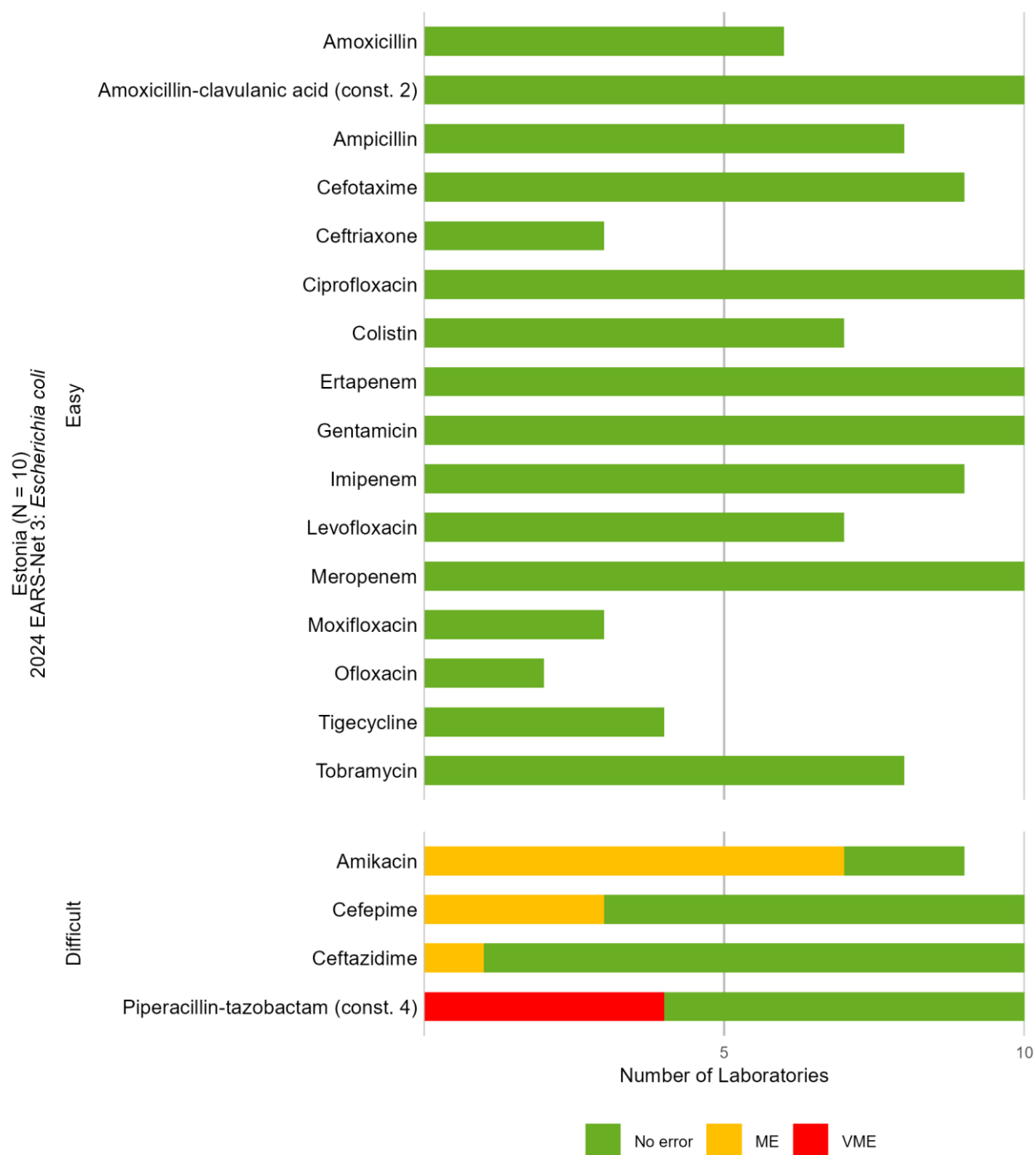


Key: VME – very major error; ME – major error, * - no data was submitted this year

For the 2024 EARS-Net EQA, the 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 3'. Each laboratory could submit results from 20 antimicrobials (maximum 200 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

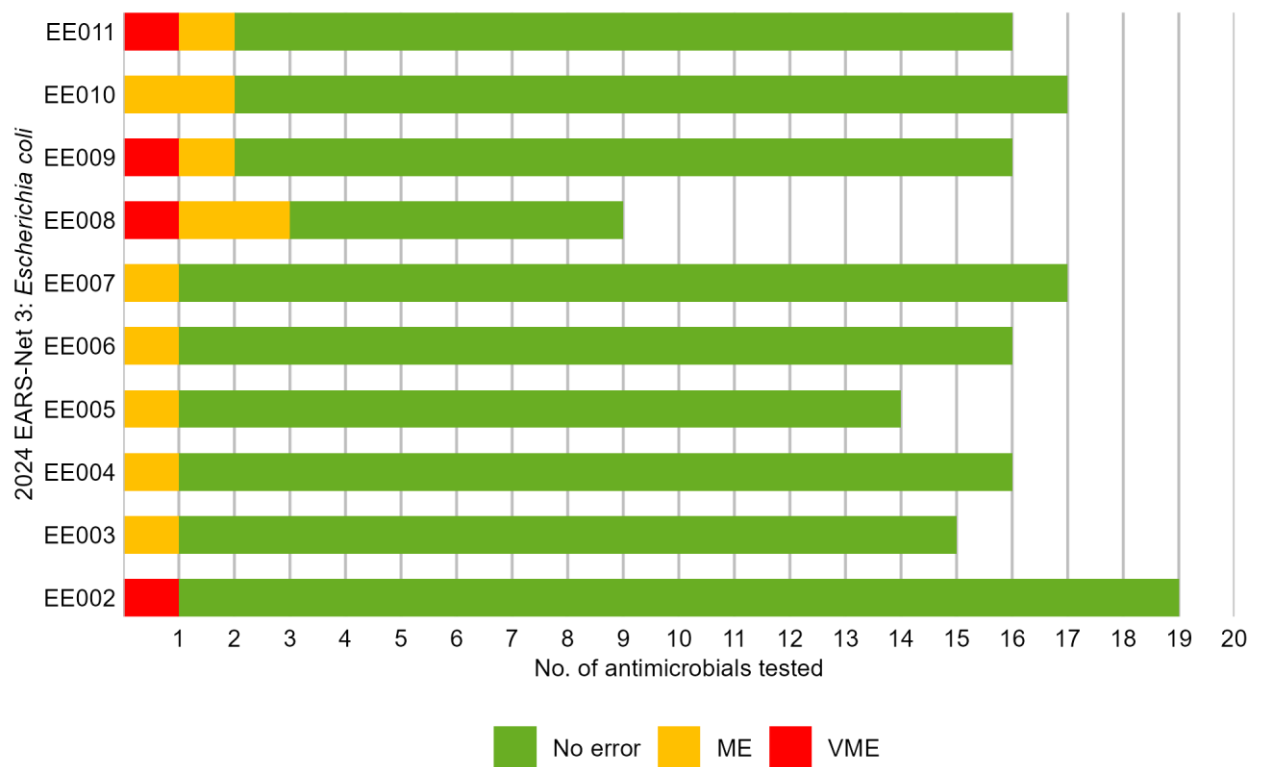
Overall, 155 AST results were submitted and the interpretations were correct for 140 (90.3%) of the results; 11 (7.1%) of the interpretations were ME and 4 (2.6%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2024 EARS-Net 3' were reported for piperacillin-tazobactam (const. 4) (Figure 8). An overview of the reported results for all laboratories is presented in Figure 9.

Figure 8. Reported interpretation of AST results for strain '2024 EARS-Net 3' (*Escherichia coli*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 9. Reported interpretation of AST results for strain '2024 EARS-Net 3' (*Escherichia coli*) by laboratory



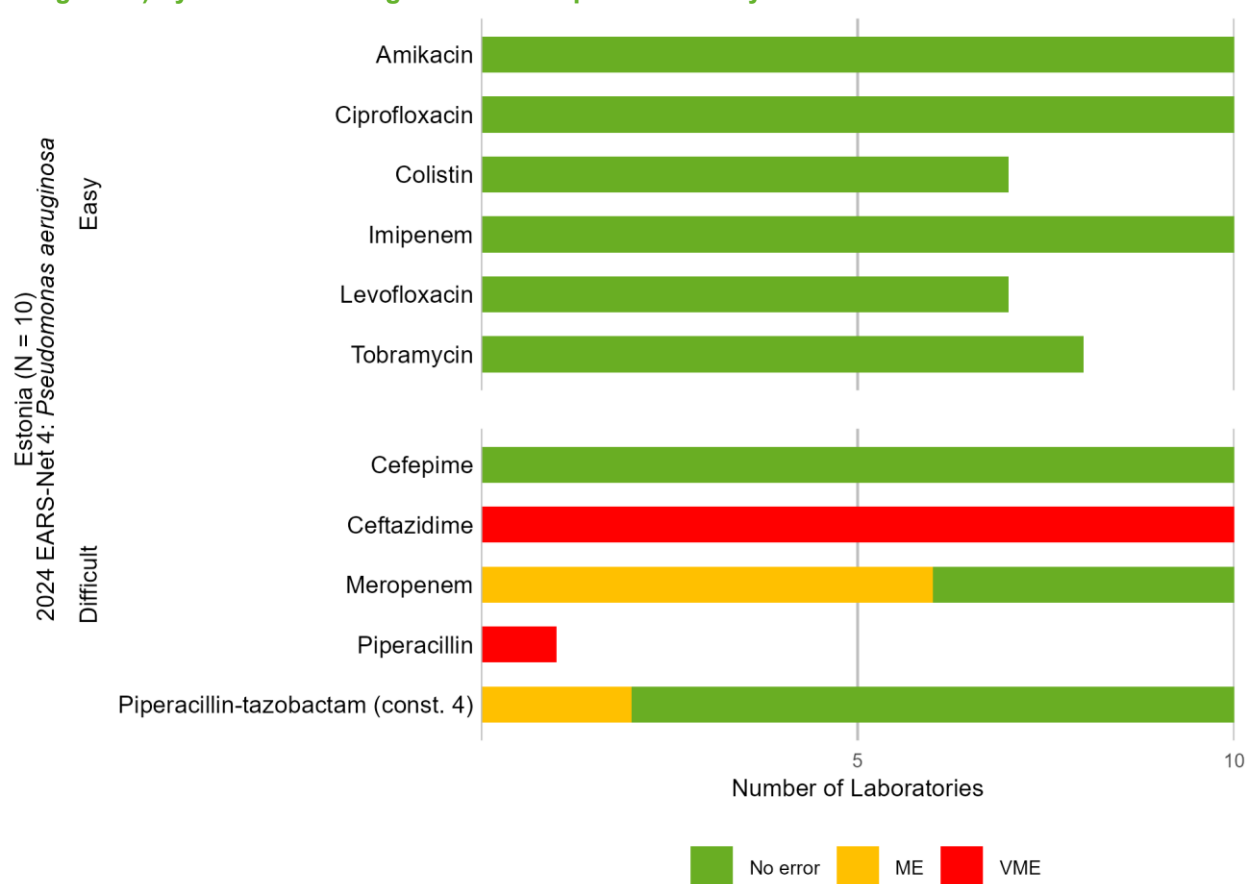
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain '2024 EARS-Net 4' (*Pseudomonas aeruginosa*)

The 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 4'. Each laboratory could submit results from 11 antimicrobials (maximum 110 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

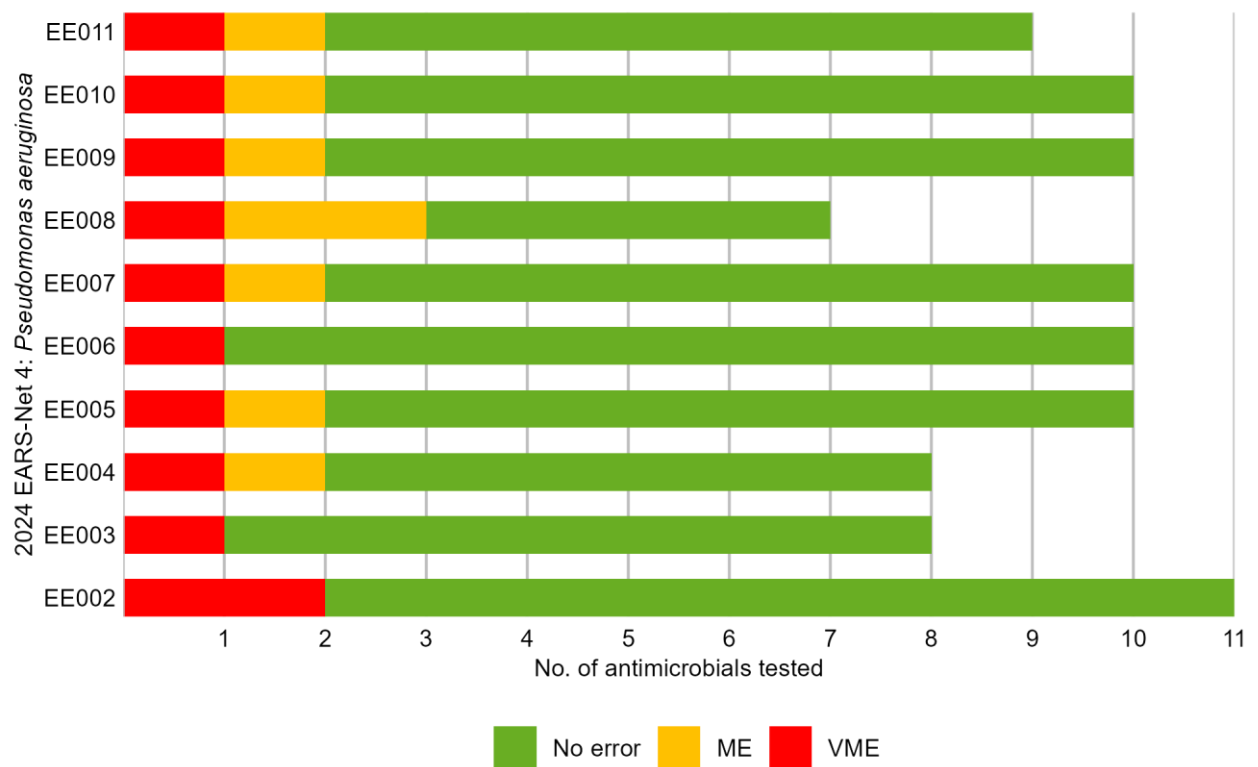
Overall, 93 AST results were submitted and the interpretations were correct for 74 (79.6%) of the results; 8 (8.6%) of the interpretations were ME and 11 (11.8%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2024 EARS-Net 4' were reported for ceftazidime, piperacillin (Figure 10). An overview of the reported results for all laboratories is presented in Figure 11.

Figure 10. Reported interpretation of AST results for strain '2024 EARS-Net 4' (*Pseudomonas aeruginosa*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 11. Reported interpretation of AST results for strain '2024 EARS-Net 4' (*Pseudomonas aeruginosa*) by laboratory



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

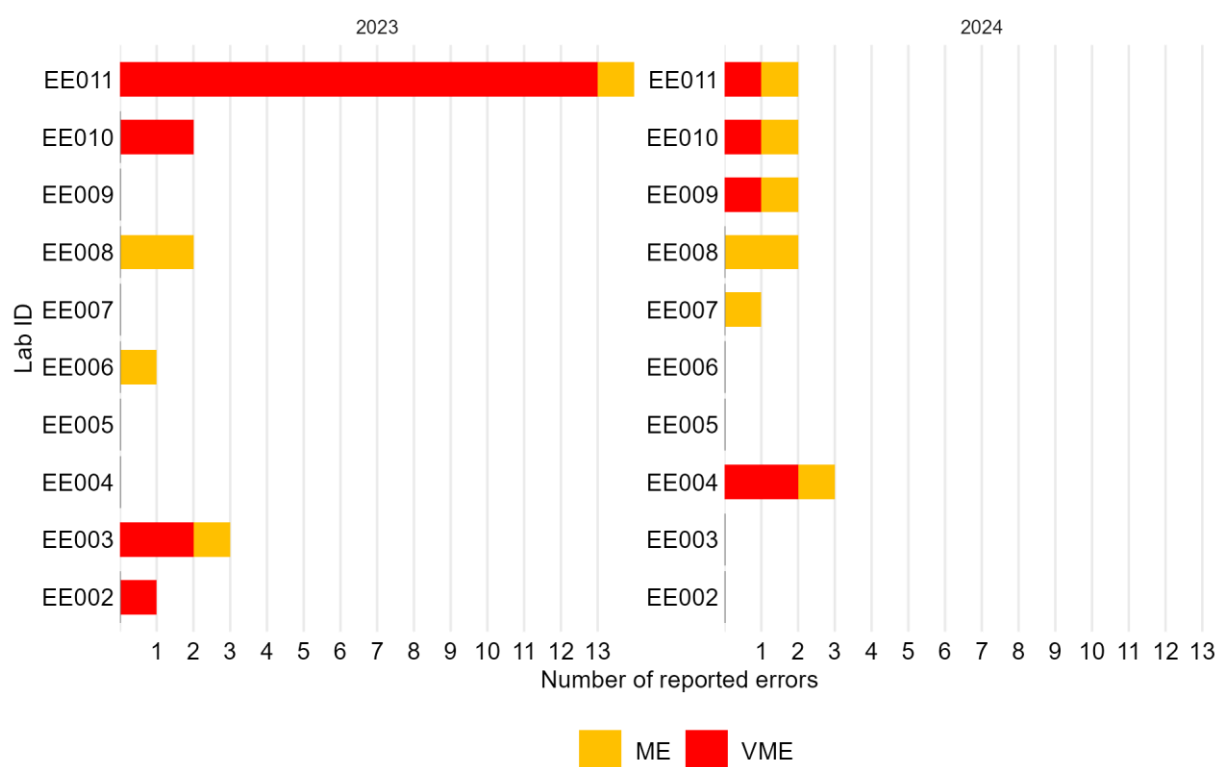
Strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*)

In the 2023 EARS-Net EQA, the strain '2023 EARS-Net 2' (*K. pneumoniae*) was challenging for participating laboratories. Therefore, it was decided to include the exact same *K. pneumoniae* strain in the panel for the 2024 EARS-Net EQA (strain '2024 EARS-Net 5'). To ensure harmonisation between expected results included in the 2024 EQA, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA. The obtained expected results were essentially in agreement with the results obtained and described in the 2023 EARS-Net EQA. However, the obtained consensus for imipenem for the 2024 EARS-Net EQA was MIC=4 mg/L, and therefore received an interpretation as Susceptible, increased exposure, whereas for the 2023 EARS-Net EQA the expected result was MIC=2 mg/L with an interpretation of Susceptible, standard dosing regimen.

At the EU/EEA level, when comparing results between the 2023 and the 2024 EARS-Net EQAs, there was little variability of results for this strain. The highest variation was the decrease in ME for amikacin, from 33% of the participating laboratories in 2023 to 29% in 2024.

In Estonia, 10 laboratories submitted interpretation of AST results both years, and 9 laboratories reported results with VME/ME at least one year. An overview of the laboratories reporting results with VME/ME is presented in Figure 12.

Figure 12. Reported errors of interpretation of AST results for strain '2023 EARS-Net 2' and '2024 EARS-Net 5' by laboratories providing at results for both years

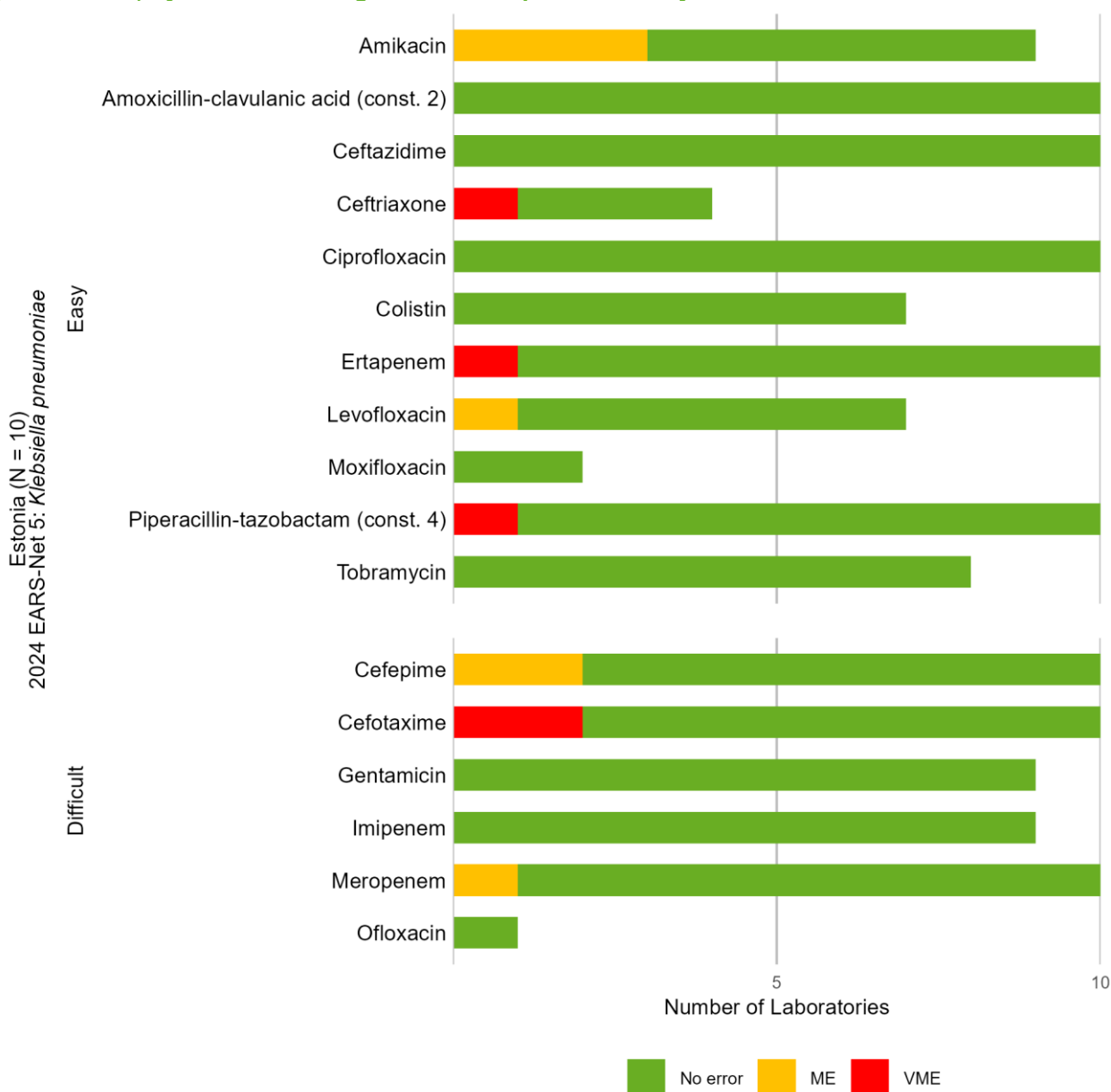


Key: VME – very major error; ME – major error

In the 2024 EARS-Net EQA, the 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 5'. Each laboratory could submit results from 17 antimicrobials (maximum 170 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

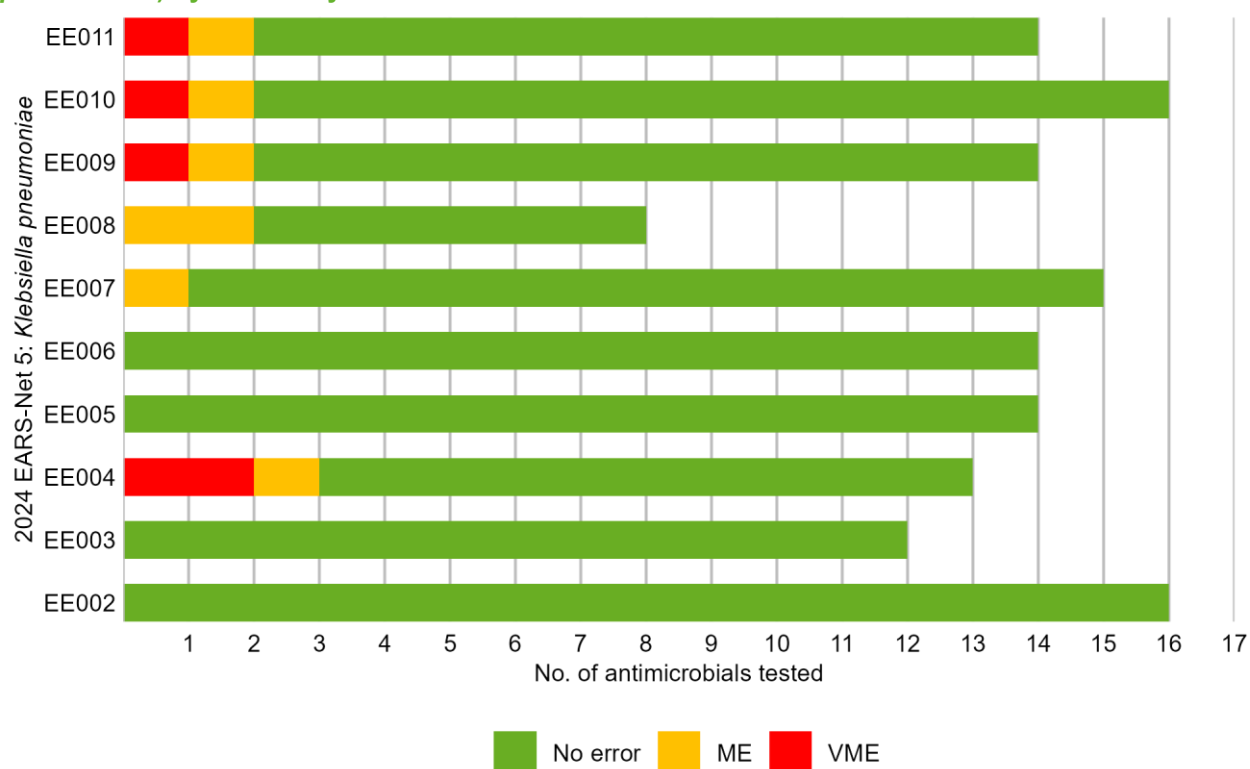
Overall, 136 AST results were submitted and the interpretations were correct for 124 (91.2%) of the results; 7 (5.1%) of the interpretations were ME and 5 (3.7%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2024 EARS-Net 5' were reported for cefotaxime, ceftriaxone, ertapenem, piperacillin-tazobactam (const. 4) (Figure 13). An overview of the reported results for all laboratories is presented in Figure 14.

Figure 13. Reported interpretation of AST results for strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 14. Reported interpretation of AST results for strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*) by laboratory



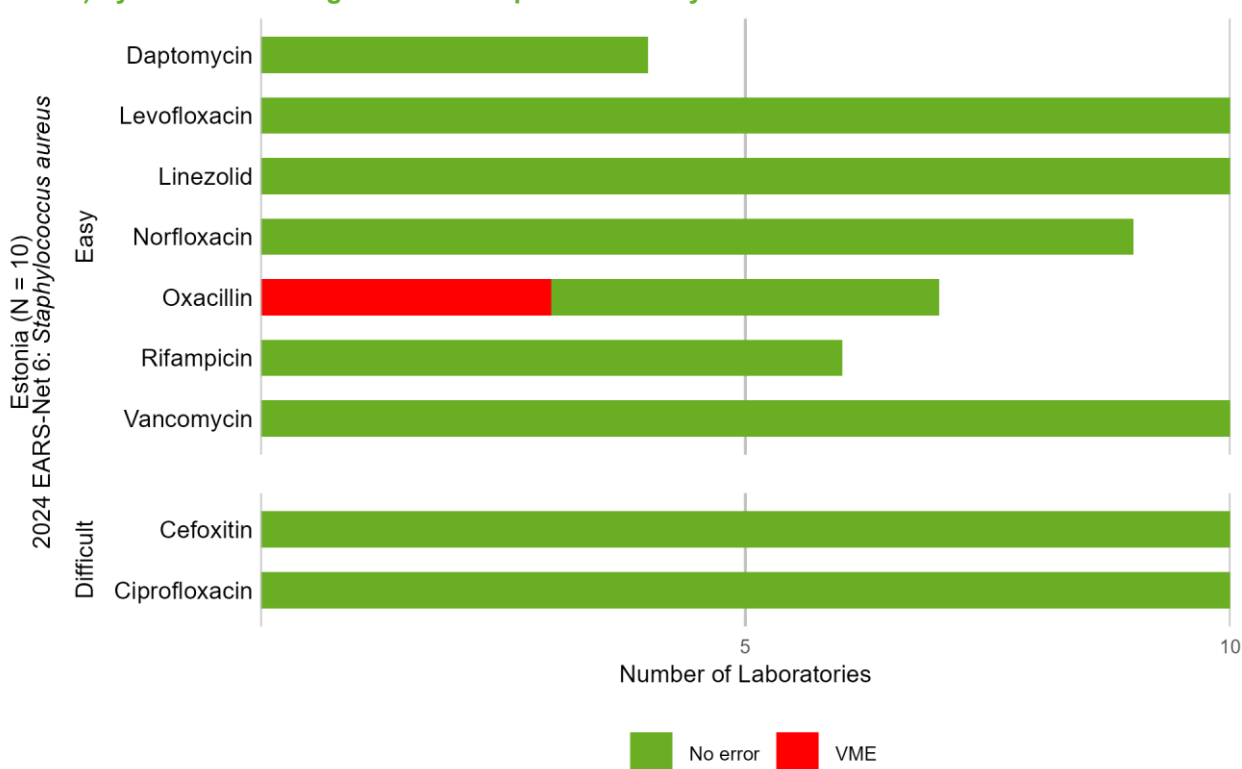
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain 2024 EARS-Net 6 (*Staphylococcus aureus*)

The 10 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain '2024 EARS-Net 6'. Each laboratory could submit results from 9 antimicrobials (maximum 90 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2024.

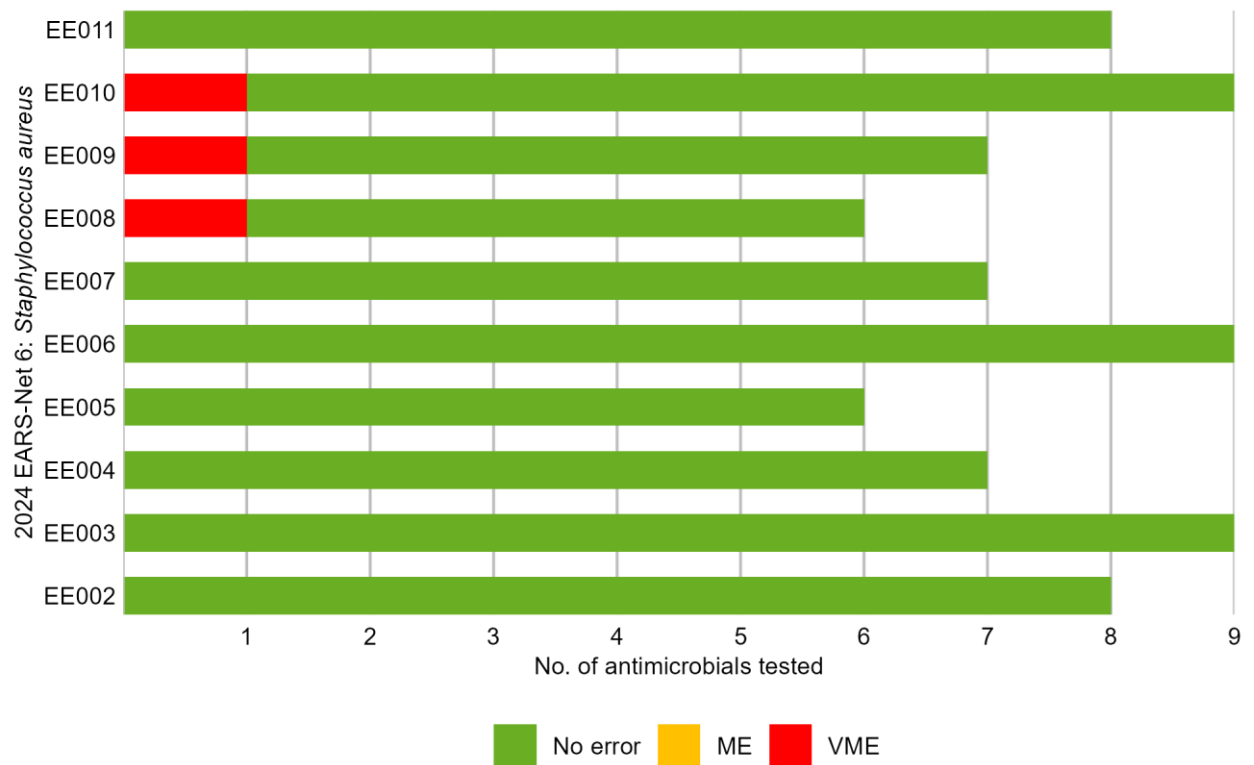
Overall, 76 AST results was submitted and the interpretation were correct for 73 (96.1%) of the results. None of the laboratories reported any ME however 3 (3.9%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2024 EARS-Net 6' were reported for oxacillin (Figure 15). An overview of the reported results for all laboratories is presented in Figure 16.

Figure 15. Reported interpretation of AST results for strain '2024 EARS-Net 6' (*Staphylococcus aureus*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 16. Reported interpretation of AST results for strain '2024 EARS-Net 6' (*Staphylococcus aureus*) by laboratory



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

4. Conclusions and recommendation for improvement

For the 2024 EARS-Net EQA, correct species identification was submitted for 60 strains (100.0%) out of 60 strains, submitted by the 10 laboratories in Estonia.

Interpretation of AST results was reported for 581 out of the 710 possible strain-antimicrobial combinations. Overall, there was 'very good' concordance with the expected interpretations as 529 (91.0%) were correct out of the 581 tests performed with 2 (20%) laboratories meeting the 'excellent' level of 95% concordance for the reported interpretations.

The following methodologies were applied by the laboratories when performing the tests: automated system (20.8%), broth microdilution (12.9%), disk/tablet diffusion (53.9%), and gradient test (12.4%)

Strain '2024 EARS-Net 1' (*Acinetobacter baumannii*)

For the strain '2024 EARS-Net 1', 7 laboratories were in full concordance with the expected interpretations, and 3 laboratories had a 'good' concordance (< 90% and ≥ 85%).

In Estonia, for the strain '2024 EARS-Net 1', VMEs were observed for tobramycin. These corresponded to 25.0% of all submitted interpretations for that antimicrobial and were reported when using the automated system and broth microdilution. The expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures. A high proportion of MEs were observed for gentamicin (11.1% of submitted results) and were reported when using disk/tablet diffusion. The expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures.

Strain '2024 EARS-Net 2' (*Enterococcus faecium*)

For the strain '2024 EARS-Net 2', all laboratories were in full concordance with the expected interpretations.

In Estonia, for the strain '2024 EARS-Net 2', no VMEs and no MEs were observed.

Strain '2024 EARS-Net 3' (*Escherichia coli*)

For the strain '2024 EARS-Net 3', 6 laboratories had a 'very good' concordance (< 95% and ≥ 90%), 3 laboratories had a 'good' concordance (< 90% and ≥ 85%), and 1 laboratory had < 80% concordance.

In Estonia, for the strain '2024 EARS-Net 3', VMEs were observed for piperacillin-tazobactam. These deviations corresponded to 40.0% of all submitted interpretations for that antimicrobial and were reported when using the automated system and disk/tablet diffusion. The expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 3). A

high proportion of MEs were observed for amikacin (77.8% of submitted results), cefepime (30.0% of submitted results) and ceftazidime (10.0% of submitted results) and were reported mainly when using disk/tablet diffusion. For all three antimicrobials the expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 3).

Strain '2024 EARS-Net 4' (*Escherichia coli*)

For the '2024 EARS-Net 4', 1 laboratory had a 'very good' concordance (< 95% and ≥ 90%), 1 laboratory had a 'good' concordance (< 90% and ≥ 85%), 5 laboratories had a 'satisfactory' concordance (< 85% and ≥ 80%), and 3 laboratories had < 80% concordance.

In Estonia, for the strain '2024 EARS-Net 4', VMEs were observed for ceftazidime (100.0% of all submitted interpretations for that antimicrobial) and piperacillin (100.0% of submitted results) and were reported throughout all methods. For both antimicrobials the expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the complex antimicrobial resistance mechanism harboured by the strain (Table 4). A high proportion of MEs were observed for meropenem (60.0% of submitted results) and piperacillin-tazobactam (20.0% of submitted results) and were reported when using the automated system, disk/tablet diffusion and gradient test. For both antimicrobials the expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the complex antimicrobial resistance mechanism harboured by the strain (Table 4).

Strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*)

For the '2024 EARS-Net 5', 4 laboratories were in full concordance with the expected interpretations, 1 laboratory had a 'very good' concordance (< 95% and ≥ 90%), 3 laboratories had a 'good' concordance (< 90% and ≥ 85%), and 2 laboratories had < 80% concordance.

In Estonia, for the strain '2024 EARS-Net 5', VMEs were observed for cefotaxime, ceftriaxone, ertapenem and piperacillin-tazobactam. Deviations in cefotaxime corresponded to 20.0% of all submitted interpretations for that antimicrobial and were reported when using disk/tablet diffusion and gradient test. Deviations in ceftriaxone corresponded to 25.0% of all submitted interpretations for that antimicrobial and were reported when using the automated system. Deviations in ertapenem corresponded to 10.0% of all submitted interpretations for that antimicrobial and were reported when using disk/tablet diffusion. Deviations in piperacillin-tazobactam corresponded to 10.0% of all submitted interpretations for that antimicrobial and were reported when using disk/tablet diffusion. For cefotaxime the expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 5). For the remaining antimicrobials, the expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures, and they can also be

derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 5). A high proportion of MEs were observed for amikacin (33.3% of submitted results), cefepime (20.0% of submitted results), levofloxacin (14.3% of submitted results) and meropenem (10.0% of submitted results) and were reported mainly when using disk/tablet diffusion. For amikacin and levofloxacin the expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures. For cefepime and meropenem the expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures, and they can also be derived from the complex antimicrobial resistance mechanism harboured by the strain (Table 5).

Strain '2024 EARS-Net 6' (*Staphylococcus aureus*)

For the '2024 EARS-Net 6', 7 laboratories were in full concordance with the expected interpretations, 2 laboratories had a 'good' concordance ($< 90\%$ and $\geq 85\%$), and 1 laboratory had a 'satisfactory' concordance ($< 85\%$ and $\geq 80\%$).

In Estonia, for the strain '2024 EARS-Net 6', VMEs were observed for oxacillin. These deviations corresponded to 42.9% of all submitted interpretations for that antimicrobial and were reported when using gradient test. The expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures. No MEs were observed for this strain.

4.1 Recommendations

We recommend the following actions to identify root causes to address the observed deviations:

- Confirm the protocols in use are in accordance with the latest EUCAST recommendations and guidelines;
- Ensure the adequate control strains are being applied and monitored to guarantee reliability of results;
- Ensure that relevant quality management systems and control measures are in place;
- Be aware of method variability when applying the different AST methods, especially the automated system, gradient test and disk/tablet diffusion methods;
- Be aware and potentially seek consultancy regarding the testing and reading of results for aminoglycosides, due to random and systematic deviations derived from variations in media composition or other factors;
- Be aware and potentially seek consultancy regarding the testing and reading of results for cephalosporins and carbapenems, due to differential expression of beta-lactamase genes or other factors;
- Consider additional training of technical staff to enhance capabilities and performance.

5. References

- 1) Antimicrobial resistance (AMR) reporting protocol 2024. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2024

6. Annex 1. Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2024

Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2024: Microorganism and antimicrobial agent combinations under surveillance by EARS-Net (isolates from blood and/or cerebrospinal fluid). Available at: <https://www.ecdc.europa.eu/sites/default/files/documents/EARS-Net-reporting-protocol-2024.pdf>. As indicated in the text preceding the table, “When, according to the EUCAST guidelines, a specific type of test is to be used, the method is indicated next to the antimicrobial.” Testing of testing of cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam and meropenem-vaborbactam for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp. are included in the original table but are not part of the 2024 EARS-Net EQA exercise.

Microorganism	Antimicrobial agent
<i>Acinetobacter</i> species (ACISPP)	Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
<i>Enterococcus faecalis</i> (ENCFAE)	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
<i>Enterococcus faecium</i> (ENCFAI)	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
<i>Escherichia coli</i> (ESCCOL)	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Tigecycline (TGC) Colistin (COL) - Broth microdilution
<i>Klebsiella pneumoniae</i>	Amoxicillin-clavulanic acid (AMC)

Microorganism	Antimicrobial agent
(KLEPNE)	Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Colistin (COL) - Broth microdilution
<i>Pseudomonas aeruginosa</i> (PSEAER)	Piperacillin/Tazobactam (TZP) Piperacillin (PIP) Ceftazidime (CAZ) Cefepime (FEP) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
<i>Staphylococcus aureus</i> (STAAUR)	Cefoxitin (FOX) – Disk diffusion Oxacillin (OXA)* – MIC test Levofloxacin (LVX) Ciprofloxacin (CIP) Norfloxacin (NOR) – Disk diffusion Vancomycin (VAN) – MIC test Rifampin (RIF) Linezolid (LNZ) Daptomycin (DAP) – MIC test
<i>Streptococcus pneumoniae</i> (STRPNE)	Oxacillin (OXA) – Disk diffusion Penicillin (PEN) – MIC test Clarithromycin (CLR) – MIC test Erythromycin (ERY) Azithromycin (AZM) – MIC test Levofloxacin (LVX) Moxifloxacin (MFX) Norfloxacin (NOR) – Disk diffusion Cefotaxime (CTX) – MIC test Ceftriaxone (CRO) – MIC test