SURVEILLANCE & MONITORING

External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2024

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Abbreviations

AMC	amoxicillin-clavulanic acid
AMK	amikacin
AMP	ampicillin
AMR	Antimicrobial resistance
AMX	amoxicillin
ARG	Antimicrobial resistance genes
AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
CAZ	ceftazidime,
CIP	ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
COL	colistin
CRO	ceftriaxone
CTX	cefotaxime
DAP	daptomycin
DTU Food	Technical University of Denmark, National Food Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological cut-off value
EQA	External quality assessment
ETP	ertapenem
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FEP	cefepime
FOX	cefoxitin
GEN	gentamicin
HLAR	high-level aminoglycoside resistance
I	'Susceptible, increased exposure'
IPM	imipenem
LNZ	linezolid
LVX	levofloxacin
ME	Major error
MEM	meropenem
MFX	moxifloxacin
MIC	Minimum inhibitory concentration
NOR	norfloxacin
OXA	oxacillin
OFX	ofloxacin
PIP	piperacillin
PM	Point mutation (chromosomal)
R	'Resistant'
RIF	rifampicin
S	'Susceptible, standard dosing regimen'
s.d.	Standard deviation
TEC	teicoplanin
TGC	tigecycline
TOB	tobramycin
TZP	piperacillin-tazobactam
VAN	vancomycin
VME	Very major error

Executive summary

This report describes the results of the 2024 external quality assessment (EQA) exercise for antimicrobial susceptibility testing (AST) by clinical laboratories that participate in the European Antimicrobial Resistance Surveillance Network (EARS-Net). It includes a short conclusion on the capacities of the participating laboratories, and recommendations for improvement. All 30 European Union/European Economic Area (EU/EEA) countries participated in this EARS-Net EQA exercise.

The aims of the EARS-Net EQA exercises are: 1) to assess the accuracy of species identification reported by individual participating laboratories; 2) to assess the accuracy of qualitative AST results reported by individual participating laboratories, and 3) to evaluate the overall comparability of routinely collected test results, between laboratories and EU/EEA countries. In EARS-Net EQA exercises, eligible laboratories are identified by national EARS-Net EQA coordinators, designated by the Coordinating Competent Body in each EU/EEA country. Participating laboratories identify the species of six bacterial strains and submit AST results for the antimicrobial agents included in EARS-Net surveillance, using the methods routinely applied.

In 2024, the panel of six EQA strains consisted of *Acinetobacter baumannii, Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Staphylococcus aureus* (Table 1). The *E. coli* and the *K. pneumoniae* strains had been included in previous EARS-Net EQA exercises. The *E. coli* strain ('2024 EARS-Net 3') was the most challenging strain in 2022 (strain '2022 EARS-Net 2') and 2023 (strain '2023 EARS-Net 1') – i.e. the strain with the most incorrect results. The *K. pneumoniae* strain (strain '2024 EARS-Net 5') was a challenging strain in 2023 (strain '2023 EARS-Net 2') [2,3].

On 10 June 2024, the six strains were distributed via the national EARS-Net EQA coordinators to 980 laboratories in all 30 EU/EEA countries. An EQA webpage was opened to receive submission of results between 11 June and 11 August 2024.

As in previous EARS-Net EQA exercises [2-4], concordance of species and AST interpretations with the expected results was defined as 'excellent' (≥95% of interpretations in concordance with expected results), 'very good' (>90% to <95%), or 'good' (>85 to ≤90%). There was also the category 'satisfactory' (>80 to ≤85%) for results that could be improved.

Results were submitted by 912 laboratories. Species identification was evaluated for the laboratories, and 5 408 (99.2%) of the 5 451 reported species were correct. There was 'excellent' concordance for each of the six strains (98.5 to 99.5% concordance). Two laboratories reported the wrong species for every submitted strain.

The interpretation of AST results was only evaluated if the species had been correctly identified. The evaluation was performed according to the clinical breakpoints in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints Tables v14.0 [6], with the EUCAST categories 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R).

In the 2024 EARS-Net EQA exercise, the scoring system for the evaluation of interpreted results included an assessment of the 'level of difficulty' and the 'severity of error' of the submitted AST result for each strain-antimicrobial agent combination. The scoring system was the same as in the 2023 EARS-Net EQA. There were two 'levels of difficulty' ('easy' and 'difficult'), reflecting the magnitude of the risk of getting the AST result wrong. 'Easy' results were those with expected AST results far from the breakpoint, where the categorisation was obvious. Conversely, 'difficult' results were those close to the breakpoint or inside the area of technical uncertainty (ATU), or those for which breakpoints had been recently changed or added. Consequently, the scoring system allocated a higher score to 'difficult' results than 'easy' results, and penalised errors for 'easy' results more severely than errors for 'difficult' results. The severity of error was divided into three levels: very major error (VME), which indicated reporting false susceptibility (i.e. reporting S or I, instead of R); major error (ME), which indicated reporting false resistance (i.e. reporting R, instead of S or I) and no error. The scoring system penalised VMEs more severely for 'easy' results than for 'difficult' results and did not penalise MEs if the test was considered 'difficult'.

The reported interpretations of AST results were evaluated for 910 laboratories (excluding the two laboratories that reported the wrong species for all submitted strains).

Among the 54 044 AST results evaluated, the most frequently reported methods for AST had very good concordance with the expected result (Table 13). These were automated systems (55.8% of all tests, 91.5% of which were correct), followed by disk or tablet diffusion (26.0% of all tests, 91.9% of which were correct) and minimum inhibitory concentration (MIC) methods, including broth microdilution and gradient test (17.6% of all tests, 92.1% of which were correct).

Overall, the AST interpretations submitted achieved a 'very good' level of concordance with the expected results, with 91.7% (49 579 out of 54 044) correct. Otherwise, MEs and VMEs were observed for 4.1% and 4.2% of interpretations, respectively. At country level, all countries achieved a 'very good ' level of concordance with the expected interpretation of AST results except for two countries (Cyprus and Latvia) which achieved a 'good' level concordance. At laboratory level, 15.7% (n=143) of the laboratories achieved an 'excellent' level of concordance; 57.9% (n=527) achieved a 'very good' level of concordance; 23.4% (n=213) achieved a 'good' level of concordance; 2.5% (n=23) achieved a 'satisfactory' level (>80 to ≤85%), and 0.4% (n=4) were below the 'satisfactory' level (<80%).

There were 71 strain-antimicrobial agent combinations tested for antimicrobial susceptibility in the 2024 EARS-Net EQA exercise, and the vast majority had results in 'excellent' concordance with the expected results (n=53 or 74.6% of the combinations). A 'very good' level of concordance was achieved for four combinations (5.6%).

Overall, the results of the 2024 EARS-Net EQA exercise did not show a systematic overestimation or underestimation of AMR in the EU/EEA, with deviations distributed across both types of errors (MEs and VMEs). However, these results show that there are still inconsistencies between laboratories and that there has been no improvement in the prediction of AST profiles for beta-lactam antimicrobials for the *E. coli* and *K. pneumoniae* EQA strains since the previous EARS-Net EQAs. The results also support a continuing trend, across different species, of difficulties in predicting AST results for aminoglycosides. The results imply that AST of *S. aureus* and *P. aeruginosa* bacterial isolates with difficult or unexpected resistance profiles can be problematic.

There were three situations where a specific method seemed to influence the percentage of correct results. The use of disk/tablet diffusion for AST of cephalosporins resistance in *E. coli* did not perform as well as other methods; the use of MIC methods (broth microdilution and gradient test) and of automated systems was not adequate for the prediction of cefoxitin susceptibility in *S. aureus*; and gradient tests had particularly poor performance for the prediction of AST results of beta-lactam agents for *P. aeruginosa.*

As standard practice, laboratories should confirm that their laboratory protocols are in accordance with the latest EUCAST recommendations and guidelines, applying the most recent EUCAST breakpoints. In addition, AMR surveillance and control activities should note and consider the specific deviations in AST results observed for each species and antimicrobial agent/group during this EQA exercise.

Table 1. Overview of species identification results and antimicrobial susceptibility testing results reported by clinical laboratories participating in the 2024 EARS-Net EQA exercise

Strain ID	Species and expected AST results for tested antimicrobial agents*	Species identification		AST results			
		Labs reporting species (N)	Labs reporting correct species (N(%))	Reported AST results (N)	Correct AST interpretations (N(%))	Major errors (N (%))	Very major errors (N (%))
2024 EARS-Net 1	*Acinetobacter baumannii* S: COL, GEN R: AMK, CIP, IPM, LVX, MEM, TOB	907	900 (99.2%)	6 571	6 318 (96.1%)	93 (1.4%)	160 (2.4%)
2024 EARS-Net 2	*Enterococcus faecium* S: GEN (no HLAR), LNZ, TEC R: AMP, AMX, VAN	908	894 (98.5%)	4 651	4 564 (98.1%)	71 (1.5%)	16 (0.3%)
2024 EARS-Net 3	*Escherichia coli* ** S: AMK, COL, ETP, GEN, IPM, MEM, TGC I: CAZ, FEP R: AMC, AMP, AMX, CIP, CRO, CTX, LVX, MFX, OFX, TOB, TZP	912	907 (99.5%)	14 777	13 373 (90.5%)	830 (5.6%)	574 (3.9%)
2024 EARS-Net 4	*Pseudomonas aeruginosa* S: AMK, COL, TOB I: FEP, MEM, TZP R: CAZ, CIP, IPM, LVX, PIP	908	902 (99.3%)	8 700	7 038 (80.9%)	516 (5.9%)	1 146 (13.2%)
2024 EARS-Net 5	*Klebsiella pneumoniae**** S: AMK, CIP, COL, LVX, MEM, MFX, OFX I: FEP, IPM R: AMC, CAZ, CRO, CTX, ETP, GEN, TOB, TZP	912	906 (99.3%)	12 812	12 071 (94.2%)	571 (4.5%)	170 (1.3%)
2024 EARS-Net 6	*Staphylococcus aureus* S: DAP, FOX, LNZ, NOR, RIF, VAN I: CIP, LVX R: OXA	904	899 (99.4%)	6 533	6 215 (95.1%)	127 (1.9%)	191 (2.9%)
Total		**912**	**5 408 (99.2%)**	**54 044**	**49 579 (91.7%)**	**2 208 (4.1%)**	**2 257 (4.2%)**

** All samples were considered to be obtained from patients with bloodstream infections. The expected AST results were generated using EUCAST Breakpoint Table 14.0. To describe the expected results of the strains included in the 2024 EARS-Net EQA the following adaptations were made to the EUCAST reporting recommendations, as described in the 2024 EARS-Net EQA protocol: breakpoints based on epidemiological cut-off (ECOFF) values (i.e. breakpoints in brackets) were used for interpretation of results when no other relevant EUCAST clinical breakpoints existed and it was assumed that the antimicrobials would be administered in combination with other agents; for Enterobacterales and enterococci it was assumed that penicillins would be administered intravenously; for enterococci, absence of high-level aminoglycoside resistance (HLAR) was registered as 'S' and presence of HLAR was registered as 'R'; breakpoints were applied for screening agents regardless of their status as 'screen only'; results from screening agents were not used for interpretation of other antimicrobials belonging to the same class and instead all AST was performed individually.*

*** The '2024 EARS-Net 3' strain was identical to the '2023 EARS-Net 1' strain and '2022 EARS-Net 2' strain. See explanation on page 22.*

**** The '2024 EARS-Net 5' strain was identical to the '2023 EARS-Net 2' strain. See explanation on page 29.*

AST: antimicrobial susceptibility testing; NA: not applicable; S: susceptible, standard dosing regimen; I: susceptible, increased exposure; R: resistant; HLAR: high-level aminoglycoside resistance; AMC: amoxicillin-clavulanic acid, AMK: amikacin, AMP: ampicillin, AMX: amoxicillin, CAZ: ceftazidime, CIP: ciprofloxacin, COL: colistin, CRO: ceftriaxone, CTX: cefotaxime, DAP: daptomycin, ETP: ertapenem, FEP: cefepime, FOX: cefoxitin, NOR: norfloxacin, GEN: gentamicin, IPM: imipenem, LNZ: linezolid, LVX: levofloxacin, MEM: meropenem, MFX: moxifloxacin, OXA: oxacillin, OFX: ofloxacin, PIP: piperacillin, RIF: rifampicin, TEC: teicoplanin, TGC: tigecycline, TOB: tobramycin, TZP: piperacillin-tazobactam, VAN: vancomycin.

Summary of results for each EQA strain

Strain '**2024 EARS-Net 1' (*Acinetobacter baumannii*)** was resistant to imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin and tobramycin, and susceptible to gentamicin and colistin (Table 1, Table 2).

In total, 99.2% (900/907) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (96.1%). MEs and VMEs were observed for 1.4% and 2.4% of the reported interpretations, respectively.

There was a 'very good' level of concordance with the expected results (>90% of concordance) for every reported AST method.

Prediction of resistance to tobramycin was problematic: 80.4% of the results submitted were in concordance with the expected result. These deviations can be attributed to the inherent method variability, since results within the acceptable variation range (+/-1 dilution) would lead to an incorrect AST interpretation.

Strain '**2024 EARS-Net 2' *(Enterococcus faecium)*** was resistant to ampicillin, amoxicillin and vancomycin. The strain was susceptible to teicoplanin and linezolid and did not present high-level aminoglycoside resistance to gentamicin (Table 1, Table 3).

In total, 98.5% (894/908) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for this strain were in 'excellent' concordance with expected results (98.1%). MEs and VMEs were observed for 1.5% and 0.3% of the reported interpretations, respectively.

Every reported AST method had a level of concordance of at least 85% with the expected results (i.e. 'good', 'very good', or 'excellent').

There were no systematic methodological issues identified from the submitted AST results for any of the antimicrobial agents tested for this strain.

Strain '**2024 EARS-Net 3' (*Escherichia coli*)** was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin and tobramycin. It was susceptible to ertapenem, imipenem, meropenem, amikacin, gentamicin, tigecycline and colistin, and the expected MIC values for ceftazidime and cefepime were in the I range (Table 1, Table 4).

In total, 99.5% (907/912) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for this strain were in 'very good' concordance with expected results (90.5%). MEs and VMEs were observed for 5.6% and 3.9% of the reported interpretations, respectively.

Every reported AST method had a level of concordance of at least >80% with the expected results (i.e. 'satisfactory').

Prediction of susceptibility to amikacin (34.0% concordance), cefepime (83.3%) and ceftazidime (87.4%) and prediction of resistance to piperacillin-tazobactam (39.9%) were the problematic issues identified with this strain. These deviations can be attributed to the inherent method variability, since results within the acceptable variation range (+/-1 dilution) would lead to incorrect AST interpretations. Furthermore, variations in results for the beta-lactam agents can also be derived from the differential expression of the $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$ genes that were harboured by the strain.

In general, there was no method that systematically performed worse than others for AST of the *E. coli* strain, however the use of disk/tablet diffusion for AST of cephalosporins resulted in poorer performance than other methods.

The strain had been included in previous EARS-Net EQAs exercises (2022 and 2023). In comparison with results from 2023, there was little variability in the submitted results and some small improvements. Comparison with results from 2022 is more complex due to different expected results and interpretation for certain antimicrobials, but overall there were improvements from 2022 to 2023 and 2024, except for the antimicrobials with different expected interpretations (amikacin and piperacillin-tazobactam).

Strain '**2024 EARS-Net 4' (*Pseudomonas aeruginosa*)** was resistant to piperacillin, ceftazidime, imipenem, ciprofloxacin and levofloxacin. It was susceptible to amikacin, tobramycin and colistin, and the expected MIC values for piperacillin-tazobactam, cefepime and meropenem were in the I range (Table 1, Table 5).

The strain harbours a chromosomal point mutation that affects the expression of porins, contributing to a complex and potentially variable AMR profile towards carbapenems. Databases of genetic determinants of AMR for *Pseudomonas* spp. remain incomplete. Consequently, it is unknown whether the strain harbours additional mechanisms that contribute to its overall difficult susceptibility profile, for other beta-lactam agents.

In total, 99.3% (902/908) of laboratories correctly identified the species of this test strain and, overall, the AST interpretations reported for the strain were in 'satisfactory' concordance with the expected results (80.9%). MEs and VMEs were observed for 5.9% and 13.2% of the reported interpretations, respectively.

There was a least a 'satisfactory' level of concordance with expected results (>80%) for agar dilution, automated systems, broth microdilution and macro broth dilution. The concordance was below 'satisfactory' for gradient test (61.5%), disk/tablet diffusion (76.6%), and 'other' (68.2%).

The worst performances of all strain-antimicrobial combination included in the 2024 EARS-Net EQA were for this strain. These were the prediction of resistance to ceftazidime (6.2% concordance) and piperacillin (22.9%). Prediction of susceptibility to meropenem and piperacillin-tazobactam were also suboptimal (61.8% and 86.2% had concordance, respectively). All four of these AST determinations were classified as 'difficult', and so is possible to attribute many of the incorrect results to the inherent variability in AST methods, as results within the acceptable variation range (+/-1 dilution) would have an incorrect AST interpretation.

In general, there was no method that systematically performed much worse than others for AST of the *P. aeruginosa* strain, although the use of gradient tests for AST of beta-lactam agents was associated with more incorrect results than the other methods reported.

Strain '**2024 EARS-Net 5' (*Klebsiella pneumoniae*)** was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin and tobramycin. It was susceptible to meropenem, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, amikacin and colistin, and the expected MIC values for cefepime and imipenem were in the I range (Table 1, Table 5).

In total, 99.3% (906/912) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for the strain were in 'very good' concordance with expected results (94.2%). MEs and VMEs were observed for 4.5% and 1.3% of the reported interpretations, respectively.

Every reported AST method had a level of concordance of at least >90% with the expected results (i.e. 'very good').

Characterisation of susceptibility to amikacin was challenging (71.5% concordance). The expected result was not close to the clinical breakpoint, and so incorrect results are unlikely to be due to inherent method variability. One explanation, applicable to other problematic determinations of results for aminoglycoside across species, is the variations in methods and/or material used for testing. Suboptimal proportions of concordance were also observed for cefepime (83.0%) and imipenem (86.4%). These can be attributed to the inherent method variability, since results within the acceptable variation range (+/-1 dilution) would lead to incorrect AST interpretations. Furthermore, variations in results for the beta-lactam agents can also be derived from the differential expression of the $bla_{\text{VEB-1}}$ and $bla_{\text{OXA-10}}$ genes that were harboured by this strain.

The strain had been included in the previous EARS-Net EQA exercise in 2023. The proportion of correct results for each strain-antimicrobial combination was very similar between 2023 and 2024, with some small improvements in 2024 compared to 2023.

Strain '**2024 EARS-Net 6' (*Staphylococcus aureus*)** was resistant to oxacillin and susceptible to cefoxitin, norfloxacin, vancomycin, linezolid, daptomycin and rifampicin. Its expected MIC values for ciprofloxacin and levofloxacin were in the I range (Table 1, Table 7).

In total, 99.4% (899/904) of laboratories correctly identified the species of this test strain and, overall, the reported interpretations were in 'excellent' concordance with expected results (95.1%). MEs and VMEs were observed for 1.9% and 2.9% of the reported interpretations, respectively.

There was a 'good' level of concordance with the expected results (>85% of concordance) for every reported AST method except for 'other' (60.0%).

The strain was a 'borderline oxacillin resistant *S. aureus*' (BORSA), resistant to oxacillin but susceptible to cefoxitin. Prediction of this profile was problematic for the participating laboratories. Concordance of results for oxacillin was low (72.8%) and results for cefoxitin achieved 86.6% of correct interpretations.

The results for oxacillin cannot be due to inherent method variability because the expected MIC value was not close to the clinical breakpoints – i.e. it was classified as 'easy'. Some laboratories may potentially have missed the specific requirements for broth microdilution of oxacillin in staphylococci. Alternatively, some laboratories might have inferred oxacillin susceptibility from cefoxitin susceptibility.

Incorrect results for cefoxitin could partially be due to inherent method variability, but deviations for these AST results were observed for automated systems and broth microdilution. These are MIC methods, and not compliant with the EUCAST recommendation to use disk diffusion for AST of cefoxitin. This recommendation appears to be supported by the AST results for this EQA. Results that were reported to have been generated using disk/tablet diffusion had high concordance (95.2%).

1. Introduction

From 2000 to 2009, an annual EQA exercise for AST was delivered to clinical laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS). In 2010, this activity was renamed as the European Antimicrobial Resistance System Network (EARS-Net) and transferred to ECDC. This report describes and summarises the results of the EQA performance by laboratories participating in EARS-Net in 2024.

In 2024, the EARS-Net EQA exercise was carried out in collaboration with the Technical University of Denmark, National Food Institute (DTU Food). Since 2000, DTU Food has provided capacity-building for diagnostics and AST as well as EQA services globally in its capacity as a World Health Organization Collaborating Centre for antimicrobial resistance (AMR) and Genomics, the European Union Reference Laboratory for AMR, and the Food and Agriculture Organization of the United Nations Reference Laboratory for AMR.

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 AST results reported by participating laboratories; and 3) evaluate the overall comparability of routinely collected AST results between laboratories and EU/EEA countries.

2. Study design and methods

Antimicrobial susceptibility testing, and selected antimicrobial agents

The 2024 EARS-Net EQA protocol [5] specified that laboratories should perform AST according to their routine procedures, using methods such as broth microdilution, agar dilution, automated systems, disk or tablet diffusion, gradient tests, or other methods.

The antimicrobial agents selected for this EQA exercise correspond to the panel of species–antimicrobial agent combinations under surveillance by EARS-Net [1]. 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 are not part of the 2024 EARS-Net EQA exercise.

When performing their standard practices, clinical laboratories in the EU/EEA are highly unlikely to perform AST on every species-antimicrobial agent combination that can be reported to EARS-Net. For example, many will use the services of reference laboratories. This is discussed in more detail in the section 'Evaluation of EQA results'.

Selection and characteristics of the EQA strains

In the 2024 EQA exercise, participating laboratories were asked to consider all six samples as if they had been obtained from patients with bloodstream infections.

The EUCAST Clinical Breakpoints Tables v14.0 [6] were used for the interpretation of AST results. This permitted categorisation of the expected AST results into three categories: susceptible, standard dosing regimen (S), susceptible, increased exposure (I), and resistant (R). EUCAST breakpoints are generally based on clinical breakpoints to delineate S/I/R, or, if no relevant EUCAST clinical breakpoints are available, epidemiological cutoff (ECOFF) values are used. To describe the expected results of the strains included in the 2024 EARS-Net EQA, the following adaptations were made to the EUCAST reporting recommendations, as described in the 2024 EARS-Net EQA protocol: breakpoints based on ECOFF values (i.e. breakpoints in brackets) were used for interpretation of results when no other relevant EUCAST clinical breakpoints existed and it was assumed that the antimicrobials would be administered in combination with other agents; for Enterobacterales and enterococci it was assumed that penicillins would be administered intravenously; for enterococci, absence of high-level aminoglycoside resistance (HLAR) was registered as 'S' and presence of HLAR was registered as 'R'; breakpoints were applied for screening agents regardless of their status as 'screen only'; results from screening agents were not used for interpretation of other antimicrobials belonging to the same class and instead all AST were performed individually [5,6].

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 other 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. The consensus phenotypic AST profile was then 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 (Table 2 – Table 7). 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.

Table 2. EUCAST clinical breakpoints for *Acinetobacter baumannii* and the expected AST results, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 <				
Amikacin	8	8	19	19	Easy	128	R	*aac(6')-Ib3, aph(3')-Via*
Ciprofloxacin	0.001	1	50	21	Easy	>8	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin	2	2	Note****	Note****	Easy	0.5	S	ND
Gentamicin	4	4	17	17	Easy	2	S	*aph(3')-Via*
Imipenem	2	4	24	21	Easy	>16	R	bla_{OXA-23}
Levofloxacin	0.5	1	23	20	Easy	16	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	>64	R	bla_{OXA-23}
Tobramycin	4	4	17	17	Difficult	8	R	*aac(6')-Ib3*

MALDI-TOF by DTU: Acinetobacter baumannii (score 2,37). MLST: ST-499 (scheme A. baumannii #1) / ST-158 (scheme A. baumannii #2).

**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, or 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} (probably intrinsic).*
*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.*

Table 3. EUCAST clinical breakpoints for *Enterococcus faecium* and the expected AST results, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 inter pretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Amoxicillin	4	8	Note****	Note****	Easy	64	R	PBP5-R
Ampicillin	4	8	10	8	Easy	>64	R	PBP5-R
Gentamicin (HLAR)	128	128	8	8	Easy	<=8	S	ND
Linezolid	4	4	20	20	Easy	2	S	ND
Teicoplanin	2	2	16	16	Easy	1	S	ND
Vancomycin	4	4	12	12	Easy	>16	R	*VanHBX*

MALDI-TOF by DTU: Enterococcus faecium (score 2,42). MLST: ST-17.

**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, or 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).*
*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. Most relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening. For enterococci, absence of high-level aminoglycoside resistance (HLAR) was registered as 'S' and presence of HLAR was registered as 'R'.*

Table 4. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 inter-pretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Amikacin	8	8		18	18		Difficult	8	S	*aac(6')-Ib-cr*
Amoxicillin	8	8		Note****	Note****		Easy	>64	R	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Amoxicillin-clavulanic acid*****	8	8		19	19	19-20	Easy	>64/2	R	*bla*$_{OXA-1}$
Ampicillin	8	8		14	14		Easy	>32	R	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefepime	1	4		27	24		Difficult	2	I	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefotaxime	1	2		20	17		Easy	>4	R	*bla*$_{CTX-M-15}$
Ceftazidime	1	4		22	19		Difficult	2	I	*bla*$_{CTX-M-15}$
Ceftriaxone	1	2		25	22		Easy	>16	R	*bla*$_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	>4	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Colistin	2	2		Note****	Note****		Easy	<=0.25	S	ND
Ertapenem	0.5	0.5		23	23		Easy	<=0.03	S	ND
Gentamicin	2	2		17	17		Easy	1	S	ND
Imipenem	2	4		22	19		Easy	<=0.25	S	ND
Levofloxacin	0.5	1		23	19		Easy	>8	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Meropenem	2	8		22	16		Easy	<=0.03	S	ND
Moxifloxacin	0.25	0.25		22	22		Easy	>8	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Piperacillin-tazobactam*****	8	8	16	20	20	19	Difficult	16-Apr	R	*bla*$_{OXA-1}$
Tigecycline	0.5	0.5		18	18		Easy	<=0.25	S	ND
Tobramycin	2	2		16	16		Easy	>16	R	*aac(6')-Ib-cr*

MALDI-TOF by DTU: Escherichia coli (score 2,26). MLST: ST-131 (scheme E. coli #1) / ST-43 (scheme E. coli #2).
**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, or 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.*
*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.*
******Reference results for amoxicillin-clavulanic acid MICs relate to a test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to a test with a fixed concentration of 4 mg/L tazobactam.*

Table 5. EUCAST clinical breakpoints for *Pseudomonas aeruginosa*, and the expected AST results, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 interpre-tation	ARGs and PMs***
	S ≤	R >	S ≤	R >	ATU				
Amikacin	16	16	15	15		Easy	4	S	ND
Cefepime	0.001	8	50	21		Difficult	8	I	ND
Ceftazidime	0.001	8	50	17		Difficult	>8	R	ND
Ciprofloxacin	0.001	0.5	50	26		Easy	>4	R	*crpP*, *gyrA* T83I
Colistin	4	4	Note****	Note****		Easy	1	S	ND
Imipenem	0.001	4	50	20		Easy	>8	R	*oprD* W339STOP
Levofloxacin	0.001	2	50	18		Easy	8	R	*gyrA* T83I
Meropenem	2	8	20	14		Difficult	8	I	*oprD* W339STOP
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
Tobramycin	2	2	18	18		Easy	0.5	S	ND

MALDI-TOF by DTU: Pseudomonas aeruginosa (score 2,45). MLST: ST-395.

**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, or 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), $bla_{OXA-488}$ (probably intrinsic).*
*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.*
******Reference results for amoxicillin-clavulanic acid MICs relate to a test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to a test with a fixed concentration of 4 mg/L tazobactam.*

Table 6. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 inter-pretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Amikacin	8	8		18	18		Easy	4	S	*aac(6')-Ia*
Amoxicillin-clavulanic acid*****	8	8		19	19	19-20	Easy	>64/2	R	bla_{VEB-1}, bla_{SHV-11}
Cefepime	1	4		27	24		Difficult	2	I	bla_{VEB-1}, bla_{SHV-11}
Cefotaxime	1	2		20	17		Difficult	4	R	bla_{VEB-1}, bla_{SHV-11}
Ceftazidime	1	4		22	19		Easy	>16	R	bla_{VEB-1}, bla_{SHV-11}
Ceftriaxone	1	2		25	22		Easy	8	R	bla_{SHV-11}
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	0.03	S	ND
Colistin	2	2		Note****	Note****		Easy	0.5	S	ND
Ertapenem	0.5	0.5		23	23		Easy	2	R	ND
Gentamicin	2	2		17	17		Difficult	4	R	*ant(2")-Ia*
Imipenem	2	4		22	19		Difficult	4	I	ND
Levofloxacin	0.5	1		23	19		Easy	0.06	S	ND
Meropenem	2	8		22	16		Difficult	2	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
Piperacillin-tazobactam*****	8	8	16	20	20	19	Easy	>128/4	R	bla_{VEB-1}, bla_{SHV-11}, bla_{OXA-10}
Tobramycin	2	2		16	16		Easy	8	R	*aac(6')-Ia*, *ant(2")-Ia*

MALDI-TOF by DTU: Klebsiella pneumoniae (score 2,32), and MLST: ST-37.

**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, or 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 (instrinsic), 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).*
*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.*
******Reference results for amoxicillin-clavulanic acid MICs relate to a test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to a test with a fixed concentration of 4 mg/L tazobactam.*

Table 7. EUCAST clinical breakpoints for *Staphylococcus aureus* and the expected MIC value, level of difficulty of AST determinations, expected AST interpretations, species identification and subtyping results 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 <				
Cefoxitin	Note****	4	22	22	Difficult	27 mm	S	ND
Ciprofloxacin	0.001	2	50	17	Difficult	1	I	ND
Daptomycin	1	1	Note****	Note****	Easy	<=0.5	S	ND
Levofloxacin	0.001	1	50	22	Easy	<=0.5	I	ND
Linezolid	4	4	21	21	Easy	2	S	ND
Norfloxacin	NA	NA	17	17	Easy	24 mm	S	ND
Oxacillin	Note****	2	Note****	Note****	Easy	8	R	ND
Rifampicin	0.06	0.06	26	26	Easy	0.015	S	ND
Vancomycin	2	2	Note****	Note****	Easy	1	S	ND

MALDI-TOF by DTU: Staphylococcus aureus (score 2.26). MLST: ST-188.

**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, or 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.*

*****Please refer to notes in the EUCAST clinical breakpoints tables v14.0. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.*

Procedure for participating laboratories

The 2024 EARS-Net EQA protocol [5] specified that participating laboratories should identify the species of six bacterial strains, and then perform AST, following EUCAST recommendations [6] on species that are included in EARS-Net surveillance. If the species identification was incorrect, the reported AST results were not evaluated.

Identification of eligible laboratories

Each participating country designated a 'national EARS-Net EQA coordinator' for the 2024 EARS-Net EQA exercise. The national EARS-Net EQA coordinators were asked to provide a list of laboratories that were eligible to participate, and those laboratories received an information letter. Since 2019, only laboratories using EUCAST guidelines to perform AST can participate in the EARS-Net EQA exercise.

Distribution of EQA strains to laboratories

On 10 June 2024, DTU Food sent a shipment to each national EARS-Net EQA coordinator in accordance with International Air Transport Association regulations (UN3373, biological substances category B), containing individual packages for further national distribution. Each individual package (double pack containers (class UN 6.2)) was labelled with the address of a laboratory that had enrolled to participate. Every individual package contained six swabs (Copan Transystem™), with each swab containing a pure culture of one of the six EQA strains. Each package also contained a cover letter with safety instructions and information on how to process the swabs on arrival at a laboratory.

Reporting EQA results

The 2024 EARS-Net EQA protocol, test forms and a guide on how to access the password-protected webpage for submission of results were available on the EARS-Net EQA website (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net). The dedicated password-protected EARS-Net EQA webpage for participating laboratories to submit EQA results for evaluation, using a personal login and password, was developed and hosted by DTU Food.

The EARS-Net EQA protocol specified that participants should report AST results, specifically minimum inhibitory concentration (MIC) or zone diameter values, and their respective categorisation as S, I, or R, based on the most recent clinical breakpoints in EUCAST guidelines (v14.0). Participants were instructed to apply the following

adaptations to EUCAST reporting recommendations, as described in the 2024 EARS-Net EQA protocol: to use breakpoints based on ECOFF values (i.e. breakpoints in brackets) for interpretation of results where no other relevant EUCAST clinical breakpoints exist, and to assume that the antimicrobials would be administered in combination with other agents; for Enterobacterales and enterococci to assume that penicillins would be administered intravenously; for enterococci, to report absence of high-level aminoglycoside resistance (HLAR) as 'S' and presence of HLAR as 'R'; to apply breakpoints for screening agents regardless of their status as 'screen only', and to not use results from screening agents for interpretation of other antimicrobials belonging to the same class, but instead to perform all AST individually. [5,6].

Participants were also asked to provide information on the standard guideline they used, the method for undertaking AST (agar dilution, automated systems, broth microdilution, disk or tablet diffusion, gradient test, macro broth dilution, or other), and whether they would send the strain to a reference laboratory for further testing.

The deadline for submission of results was 5 August 2024, however, the submission period was extended until 11 August 2024. After submission of results, an email was automatically forwarded to all contacts from the respective laboratory with a report containing their submitted results.

Laboratories acquired a certificate of participation if they had reported interpretation of AST results for the six strains included in the 2024 EARS-Net EQA. Laboratories only had access to the certificate for their own laboratory, via the password protected webpage. National EARS-Net EQA coordinators received copies of all certificates issued for their country only.

Participants were also encouraged to complete an electronic feedback survey using a link sent via email, with the aim of improving future EQA exercises. The evaluation questions were provided by ECDC (Annex 2).

Evaluation of reported EQA results

Scoring antimicrobial susceptibility results

The participants were asked to report AST results (i.e. MIC or zone diameter values and their categorisation) as S, I or R. Only these interpretations of AST results were evaluated using the scoring system; quantitative values were used as supplementary information. If a laboratory reported the incorrect species for an EQA strain, the interpretations of AST results were not evaluated for that strain.

The 2024 EARS-Net EQA protocol specified the scoring system for the evaluation of submitted results (Table 8). It assigned scores for each strain-antimicrobial agent combination based on the 'level of difficulty' and the 'severity of error' for the submitted AST interpretation.

The level of difficulty indicated the magnitude of risk of getting the categorisation wrong and consisted of two levels: easy and difficult. 'Easy' were results far from the breakpoint, where the categorisation was obvious and therefore the error was considered severe. 'Difficult' were results close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint had been recently changed or added. The categorisation was difficult and therefore the error was considered mild. The scoring of a result reflected the level of difficulty.

The severity of error was divided into three levels: very major error (VME), major error (ME) and no error. VME was reporting false susceptibility – expecting an R, but obtaining an S or I. ME was reporting false resistance – expecting an S or I, but obtaining an R. The scoring system penalised VMEs more severely for 'easy' results than for 'difficult' results and did not penalise MEs if the test was considered 'difficult'. The classification of 'no error' included situations where one susceptibility category (S or I) was expected, but the other susceptibility category was reported. However, this resulted in a lower positive score than if the expected susceptibility category had been reported (Table 8).

This report presents the scores of results for all participating laboratories, by EQA strain. However, the total score for each laboratory was not calculated because these total scores cannot always be compared between laboratories. For example, a laboratory that performed excellently, reporting correct AST interpretations for a small subset of strain-antimicrobial agent combinations, could achieve the same score as a laboratory that tested more combinations, but reported some incorrect AST interpretations. Therefore, the EQA protocol recommended that laboratories analyse scores for each strain-antimicrobial agent combination individually. The national EARS-Net EQA coordinators received the raw data with the scores for all laboratories in their countries, to enable national analyses that incorporate appropriate knowledge of the (sub-)national setting.

For EARS-Net EQA exercises, the definition of an appropriate minimum set of species–antimicrobial agent combinations that is relevant for all (sub-)national settings in all 30 EU/EEA countries has always been a methodological challenge. The EARS-Net EQA methodology is designed to provide information to support assessment of EARS-Net surveillance data quality. Therefore, every species-antimicrobial agent combination that can be reported to EARS-Net is included in the EQA exercise, but laboratories were not penalised for missing results in the 2024 EARS-Net EQA exercise.

Table 8. 2024 EARS-Net EQA exercise scoring system for reported AST results

Reported interpretation	Difficulty of result, and expected interpretation					
	Easy			Difficult		
	R	I	S	R	I	S
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	-	-	-	-	-	-

R: resistant; I: susceptible, increased exposure; S: susceptible, standard dosing regimen.
VME: very major error; ME: major error.

Scoring concordance

As in the previous EARS-Net EQA exercises, the concordance of submitted species identification and AST interpretations with the expected results was categorised as 'excellent' (≥95% of interpretations in concordance with expected results), 'very good' (>90% to <95%) or 'good' (>85 to ≤90%). There was also the category 'satisfactory' (>80 to ≤85%) for results that could be improved [2–4].

Reporting EQA results

Laboratories that reported using EUCAST guidelines for this EQA received a laboratory evaluation report and were included in the analysis for this report and the national summary reports.

The contacts from each participating laboratory were notified via email when their evaluation report could be downloaded from the webpage using their personal login and password, and that an overview of the expected results was available for download on the EARS-Net EQA website. Contacts only had access to the evaluation reports for their own laboratory.

The individual laboratory evaluation reports from each country were also shared with the national EARS-Net EQA coordinators together with a detailed, country-specific national summary of the performance of the laboratories in the respective country. The national summary reports included an overview of reported results, discussion and recommendations for improvements where relevant. Participating laboratories were identified by codes known only to the corresponding laboratory, the national EARS-Net EQA coordinator and the EQA provider. A national database with all the reported results was also shared with the national EARS-Net EQA coordinators. ECDC received the anonymised national summary reports, as well as an anonymised database containing all submitted results.

3. Results

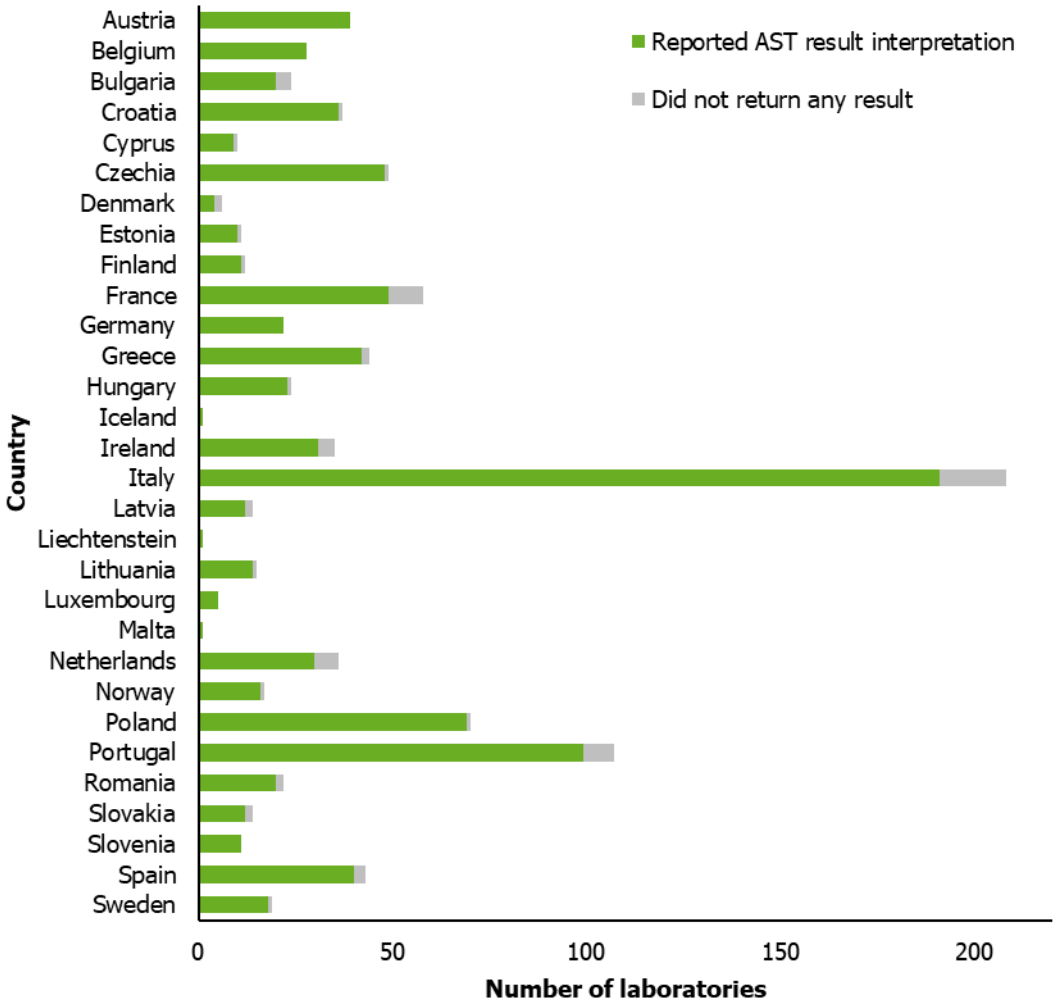
Participation

In 2024, all 30 EU/EEA countries participated in the EARS-Net EQA exercise. DTU Food sent information letters to the 983 laboratories identified by the national EARS-Net EQA coordinators, and 980 (99.7%) laboratories enrolled. National EARS-Net EQA coordinators then received packages from DTU Food for each of the 980 laboratories, containing the six EQA strains for distribution to the laboratories in their country.

One week before the submission deadline, a reminder email was sent to the laboratories that had enrolled but not yet submitted results, with a one-week extension of the submission deadline. After the expiry of the extended deadline (11 August 2024), 912 (93.1%) laboratories from 30 countries had submitted results (Figure 1, Annex 1). One laboratory reported using the CLSI guideline for one sample, therefore data submitted for this specific strain were not included in any of the evaluations. Overall, results were evaluated for 912 laboratories, corresponding to 93.1% of all laboratories that received the EQA strains. Almost all laboratories submitted AST result interpretations for all six isolates (n=903; 99.0%), which was the minimum criterion for receiving a certificate of participation.

Nine (1.3%) laboratories in seven countries (Bulgaria, Czechia, Estonia, France (n=2), Italy (n=2), Portugal and Slovakia) entered results on the EQA webpage but did not finalise submission, so their data could not be validated and were not included.

Figure 1. Number of participating laboratories returning interpretation of AST results, based on EUCAST guidelines, by country, 2024 EARS-Net EQA exercise



AST= antimicrobial susceptibility testing.

Species identification results

The overall concordance between the submitted and expected results for species identification was 'excellent' (≥95%) because 912 laboratories submitted speciation results for 5 451 strains and 99.2% (5 408 strains) were correct.

An overview of the species identification for the six strains and the number of laboratories reporting the correct identification is provided in Table 9. There was 'excellent' concordance between the submitted species identification and the expected results for all six EQA strains, with the lowest concordance reported for strain '2024 EARS-Net 2' *Enterococcus faecium* (98.5%).

Table 9. Number and percentage of laboratories reporting the correct species in the 2024 EARS-Net EQA exercise

Strain ID	Expected species	No. of reporting laboratories	No. of laboratories reporting correct species identification	Percentage of laboratories reporting correct species identification
2024 EARS-Net 1	*Acinetobacter baumannii*	907	900	99.2
2024 EARS-Net 2	*Enterococcus faecium*	908	894	98.5
2024 EARS-Net 3	*Escherichia coli*	912	907	99.5
2024 EARS-Net 4	*Pseudomonas aeruginosa*	908	902	99.3
2024 EARS-Net 5	*Klebsiella pneumoniae*	912	906	99.3
2024 EARS-Net 6	*Staphylococcus aureus*	904	899	99.4

Antimicrobial susceptibility testing results

EQA results were submitted to the EARS-Net EQA webpage by 912 laboratories. As AST results were evaluated for strains with correct species identification, data were not analysed for two of the 912 laboratories as they had reported an incorrect species for every submitted EQA strain. Therefore AST results from 910 laboratories were included in the analyses.

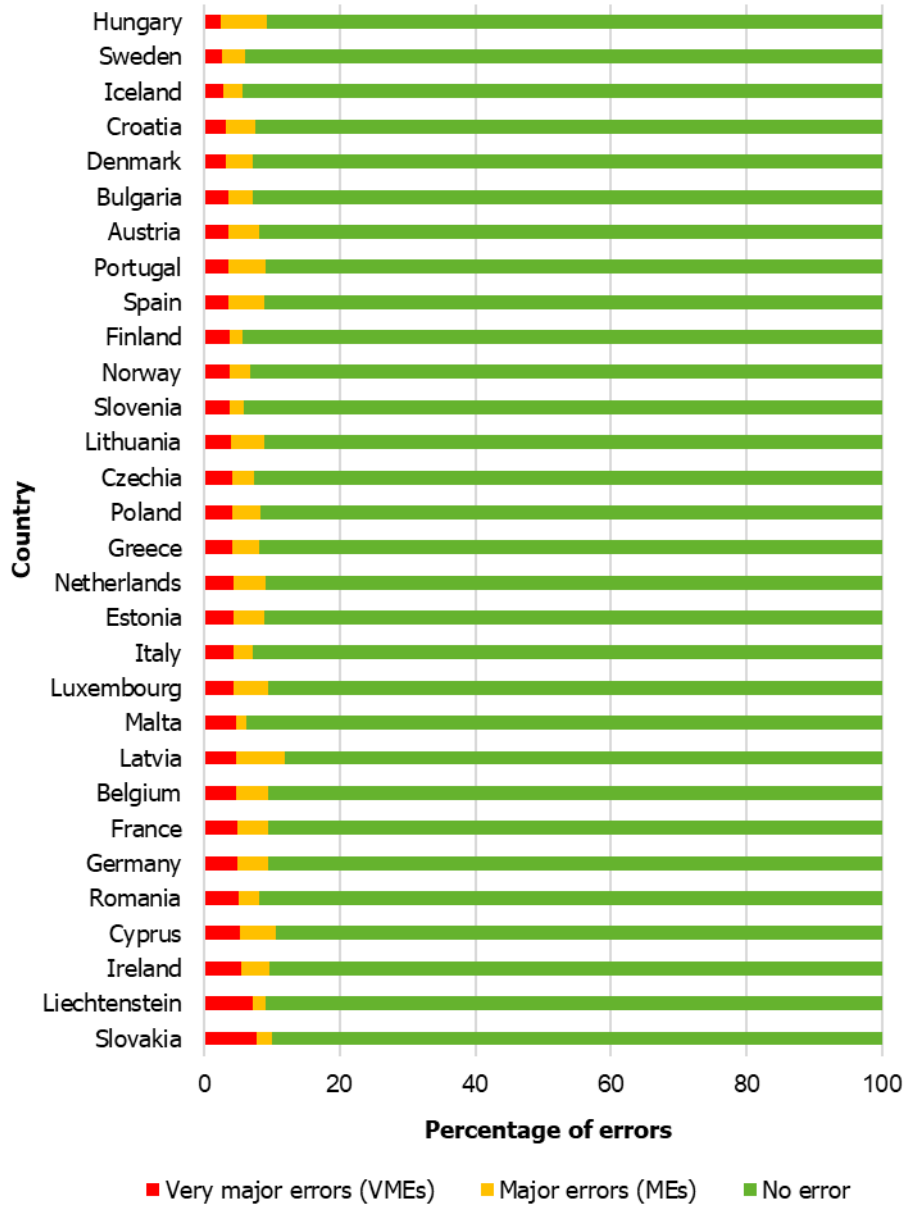
If every participating laboratory had reported data for every strain-antimicrobial agent combination included in this EQA, for every strain tested, with a correct species identification, they would have submitted a grand total of 64 119 AST results. Ultimately, the participating laboratories reported 54 044 AST result interpretations, which is equivalent to 84.3% of the theoretical maximum.

Overall, the interpretations were in 'very good' concordance, as 91.7% (n=49 579) of the 54 044 reported interpretations were correct. MEs were observed for 4.1% (n=2 208) of the reported interpretations and VMEs were observed for 4.2% (n=2 257) of the interpretations.

By country, concordance with the expected interpretation of AST results varied from 88.1% ('good') to 94.4% ('very good'). All countries achieved a 'very good' level of concordance except for two (Cyprus and Latvia) which achieved a 'good' level concordance. The range of MEs in the countries was 1.6% to 7.2%, and the range of VMEs was 2.4% to 7.8% (Figure 2).

At laboratory level, 15.7% (n=143) of the laboratories achieved an 'excellent' level of concordance, 57.9% (n=527) achieved a 'very good' level of concordance, 23.4% (n=213) achieved a 'good' level of concordance, 2.5% (n=23) achieved a 'satisfactory' level, and 0.4% (n=4) were below the 'satisfactory' level.

Figure 2. Percentage of errors among the reported interpretation of AST results, by country, 2024 EARS-Net EQA exercise, sorted by country according to the proportion of AST results representing very major errors

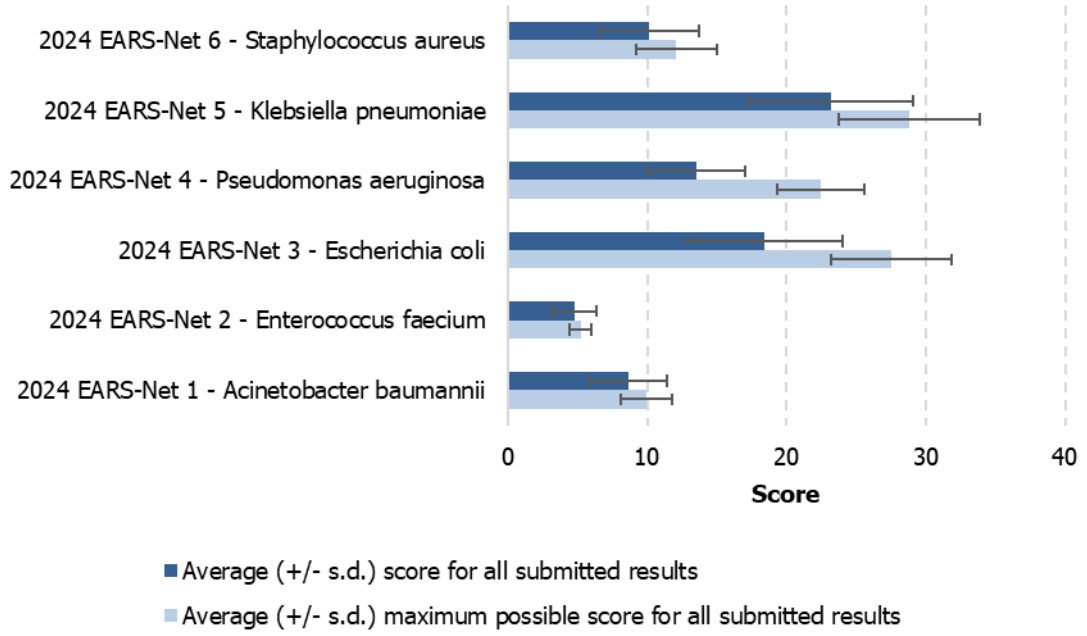


AST = antimicrobial susceptibility testing

In the 2024 EARS-Net EQA, each laboratory could report an interpretation for 71 different strain-antimicrobial combinations. According to the EQA scoring system, the maximum possible score that a laboratory could obtain, if they had submitted correct AST results for every strain-antimicrobial agent combination for all six strains, was 125. However, as expected, in this EQA, the participating laboratories did not report results for every strain-antimicrobial combination or strain. Therefore, the maximum possible score was lower than 125. To be more specific, if the participating laboratories had reported a correct AST result for every result they submitted, the average of their maximum possible scores, at laboratory level, would be 105.2 (± 15.8).

In the 2024 EARS-Net EQA exercise, ultimately, the average score of the 910 laboratories that participated was 78.2 (± 15.4). Figure 3 presents the averages of maximum possible score and the reported scores for the participating laboratories, for each strain.

Figure 3. Average maximum possible score, and average total scores, for the AST results reported by participating laboratories, by EQA strain, 2024 EARS-Net EQA exercise



AST= antimicrobial susceptibility testing; s.d. = standard deviation.

Tables 10 to 12 present the distribution of the methods used per strain and the percentage of correct AST interpretations for each method, for each strain. The most commonly used method was automated systems (55.8%), followed by disk or tablet diffusion (26.0%), and MIC methods including broth microdilution and gradient test (17.6%) (Table 13). Excellent concordance was observed for macro broth dilution (96.3%), and 'very good' concordance was observed for broth microdilution (93.1%), disk/tablet diffusion (91.9%), agar dilution (91.5%), automated systems (91.5%) and gradient test (90.7%) (Table 13).

Table 10. Overview of methods used for determination of AST results for strains '2024 EARS-Net 1' and '2024 EARS-Net 2'

Method	2024 EARS-Net 1 *Acinetobacter baumannii*			2024 EARS-Net 2 *Enterococcus faecium*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	13	0.2	100.0	8	0.2	87.5
Automated systems	3 599	54.8	94.8	2 575	55.4	98.0
Broth microdilution	978	14.9	96.4	304	6.5	98.0
Disk/tablet diffusion	1 664	25.3	98.7	1 095	23.5	98.5
Gradient test	297	4.5	96.6	634	13.6	98.1
Macro broth dilution (tubes)	5	0.1	100.0	6	0.1	100.0
Other	15	0.2	93.3	29	0.6	100.0
Total	**6 571**	**100.0**	**96.1**	**4 651**	**100.0**	**98.1**

Percentages may not total 100% due to rounding.

Table 11. Overview of methods used for determination of AST results for strains '2024 EARS-Net 3' and '2024 EARS-Net 4'

Method	2024 EARS-Net 3 *Escherichia coli*			2024 EARS-Net 4 *Pseudomonas aeruginosa*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	35	0.2	94.3	21	0.2	81.0
Automated systems	8 317	56.3	88.8	4 974	57.2	83.3
Broth microdilution	1 532	10.4	93.4	1 136	13.1	85.7
Disk/tablet diffusion	3 880	26.3	91.9	2 145	24.7	76.6
Gradient test	955	6.5	94.6	397	4.6	61.5
Macro broth dilution (tubes)	5	0.03	80.0	5	0.1	100.0
Other	53	0.4	94.3	22	0.3	68.2
Total	**14 777**	**100.0**	**90.5**	**8 700**	**100.0**	**80.9**

Percentages may not total 100% due to rounding.

Table 12. Overview of methods used for determination of AST results for strains '2024 EARS-Net 5' and '2024 EARS-Net 6'

Method	2024 EARS-Net 5 *Klebsiella pneumoniae*			2024 EARS-Net 6 *Staphylococcus aureus*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	31	0.2	96.8	21	0.3	85.7
Automated systems	7 126	55.6	94.7	3 560	54.5	95.1
Broth microdilution	1 426	11.1	95.1	432	6.6	93.3
Disk/tablet diffusion	3 374	26.3	93.4	1 880	28.8	97.0
Gradient test	814	6.4	91.2	615	9.4	92.4
Macro broth dilution (tubes)	6	0.05	100.0	-	-	-
Other	35	0.3	97.1	25	0.4	60.0
Total	**12 812**	**100.0**	**94.2**	**6 533**	**100.0**	**95.1**

Percentages may not total 100% due to rounding.

Table 13. Total overview of methods used for determination of AST results for all six EQA strains

Method	Total		
	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	129	0.2	91.5
Automated systems	30 151	55.8	91.5
Broth microdilution	5 808	10.7	93.1
Disk/tablet diffusion	14 038	26.0	91.9
Gradient test	3 712	6.9	90.7
Macro broth dilution (tubes)	27	0.05	96.3
Other	179	0.3	87.7
Total	**54 044**	**100.0**	**91.7**

Percentages may not total 100% due to rounding.

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

The strain **'2024 EARS-Net 1' (*Acinetobacter baumannii*)** was described as being obtained from a patient with a bloodstream infection. This strain was resistant to imipenem, meropenem, ciprofloxacin, levofloxacin, amikacin and tobramycin. The strain was susceptible to gentamicin and colistin (Table 2). The level of difficulty was considered 'difficult' for tobramycin since the expected MIC value was less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'. The strain harboured the *bla*$_{OXA-23}$ gene which confers resistance to carbapenems, as well as aminoglycoside resistance genes *aac(6')-Ib3* and *aph(3')-Via*. The strain also harboured various chromosomal point mutations, contributing to fluoroquinolone resistance.

Interpretation of AST results for the *A. baumannii* strain were analysed for the 900 laboratories with correct species identification (Table 9). In total, 44.7% of the laboratories (n=402) would have sent the strain to a reference or other laboratory for further testing. A total of 6 571 tests were performed, and 6 318 reported interpretations were correct. Therefore the reported interpretations were in 'excellent' concordance with expected results (96.1%) (Table 14). MEs were observed for 1.4% (n=93) and VMEs for 2.4% (n=160) of the reported interpretations (Figure 4).

The following methods were applied: automated systems (54.8%), disk/tablet diffusion (25.3%), broth microdilution (14.9%), gradient test (4.5%), agar dilution (0.2%), other (0.2%), macro broth dilution (tubes) (0.1%) (Table 10). Overall, most methods achieved, as a minimum, a 'excellent' level of concordance with the expected results (>95% of concordance). The exception was automated systems, which achieved a 'very good' concordance (94.8%), and other methods (93.3%) (Table 14).

VMEs were observed for five of the six antimicrobials with an expected interpretation of R: amikacin, ciprofloxacin, imipenem, meropenem and tobramycin (Figure 4). VMEs in tobramycin (19.6% of all submitted interpretations for that antimicrobial) were reported for all methods, with a lower proportion of errors for disk/tablet diffusion. For the other antimicrobials, VMEs represented <1% of all submitted interpretations. The only antimicrobial with an expected interpretation of R for which no VMEs were reported was levofloxacin (Table 14).

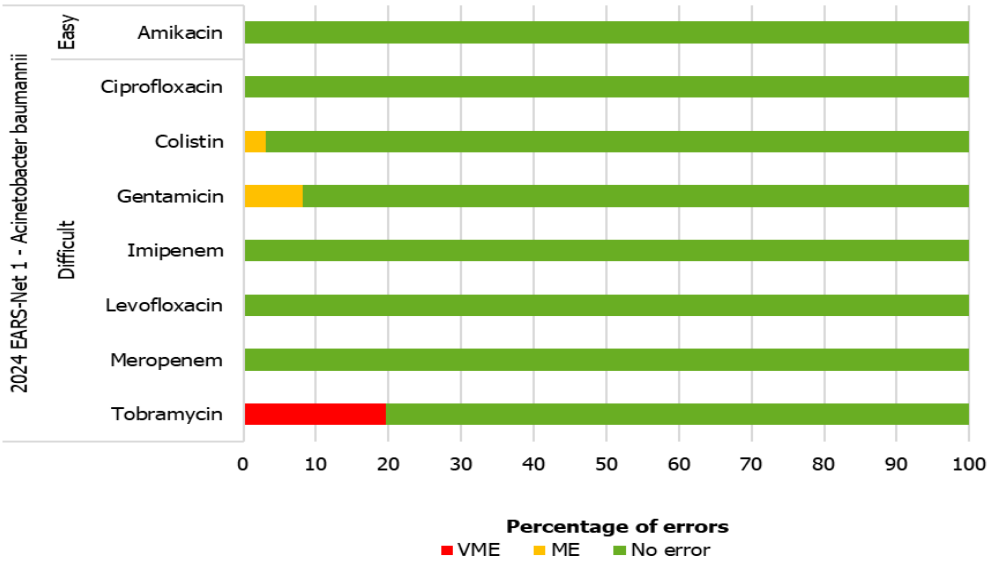
A moderate proportion of MEs was observed for gentamicin (8.2% of submitted results) and reported for most methods, especially automated systems. A low proportion of MEs was observed for colistin (3.1%). For the remaining antimicrobial agents there were no MEs (Figure 4).

Discussion

Strain **'2024 EARS-Net 1' (*Acinetobacter baumannii*)** was resistant to tobramycin, but concordance of results for this antimicrobial agent was worse than for the other agents (80.4%) and only just reached a 'satisfactory' level (>80%). These deviations corresponded to VMEs (R → S) and were observed for most methods, with a significantly lower proportion of errors for disk/tablet diffusion. The expected MIC result (MIC = 8 mg/L) was very close to the clinical breakpoints (S ≤ 4 mg/L and R > 4 mg/L). Therefore the prediction of tobramycin resistance was considered difficult and the observed deviations could be attributed to the inherent method variability, since the expected MIC value corresponds to a borderline concentration, increasing the likelihood of misclassification. The deviations could also represent, or have been exacerbated by, variations in the methods and/or material used for testing [7-9].

For strain '2024 EARS-Net 1', concordance of results for most of the remaining antimicrobial agents was excellent (≥95%). The exception were results for gentamicin that achieved a 'very good' concordance (91.8%).

Figure 4. Reported interpretation of AST results for strain '2024 EARS-Net 1' (*Acinetobacter baumannii*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).

Table 14. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2024 EARS-Net 1' (*Acinetobacter baumannii*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty*	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	R	2	100.0	463	99.8	90	100.0	244	100.0	25	100.0	-	-	2	100.0	**826**	**99.9**
Ciprofloxacin	Easy	R	2	100.0	552	99.8	82	100.0	244	100.0	15	100.0	-	-	2	100.0	**897**	**99.9**
Colistin	Easy	S	1	100.0	253	93.7	453	98.5	6	100.0	15	100.0	5	100.0	2	100.0	**735**	**96.9**
Gentamicin	Easy	S	1	100.0	508	90.0	74	93.2	226	94.7	40	95.0	-	-	2	100.0	**851**	**91.8**
Imipenem	Easy	R	2	100.0	463	100.0	67	98.5	227	100.0	61	100.0	-	-	2	100.0	**822**	**99.9**
Levofloxacin	Easy	R	3	100.0	398	100.0	57	100.0	243	100.0	52	100.0	-	-	1	100.0	**754**	**100.0**
Meropenem	Easy	R	2	100.0	521	99.8	86	98.8	223	100.0	60	100.0	-	-	2	100.0	**894**	**99.8**
Tobramycin	Difficult	R	-	-	441	73.7	69	69.6	251	96.4	29	72.4	-	-	2	50.0	**792**	**80.4**
Total			**13**	**100.0**	**3 599**	**94.8**	**978**	**96.4**	**1 664**	**98.7**	**297**	**96.6**	**5**	**100.0**	**15**	**93.3**	**6 571**	**96.1**

n: number of reporting laboratories; '-': no data; shaded cells: Below the threshold of satisfactory concordance (80%).

**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, or added to the latest EUCAST clinical breakpoint table.*

Percentages may not total 100% due to rounding. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

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

The strain **'2024 EARS-Net 2' (*Enterococcus faecium*)** was described as being obtained from a patient with a bloodstream infection. This strain was resistant to ampicillin, amoxicillin and vancomycin. The strain was susceptible to teicoplanin and linezolid, and did not present high-level aminoglycoside resistance to gentamicin (Table 3). The level of difficulty was considered 'easy' for all antimicrobials. The strain harboured the *vanHBX* operon which confers resistance to vancomycin, as well as the chromosomal point mutations profile associated with resistance toward penicillins.

Interpretation of AST results for the *E. faecium* strain were analysed for the 894 laboratories with correct species identification (Table 9). In total, 36.8% of the laboratories (n=329) would have sent the strain to a reference or other laboratory for further testing. In total, 4 651 tests were performed, and 4 564 reported interpretations were correct. Therefore the reported interpretations were in 'excellent' concordance with expected results (98.1%) (Table 15). MEs were observed for 1.5% (n=71) and VMEs for 0.3% (n=16) of the reported interpretations (Figure 5).

The following methods were applied: automated systems (55.4%), disk/tablet diffusion (23.5%), gradient test (13.6%), broth microdilution (6.5%), other (0.6%), agar dilution (0.2%), macro broth dilution (tubes) (0.1%) (Table 10). Overall, most methods achieved an 'excellent' level of concordance with the expected results (>95% of concordance) as a minimum. The exception was agar dilution, which achieved a 'good' concordance (87.5%) (Table 15).

VMEs were observed for two of the three antimicrobials with an expected interpretation of R: ampicillin and vancomycin (Figure 5). VMEs in vancomycin (1.7% of all submitted interpretations for that antimicrobial) were reported when using agar dilution, automated systems, disk/tablet diffusion and gradient tests. For ampicillin, VMEs represented <1% of all submitted interpretations. The only antimicrobial with an expected interpretation of R for which no VMEs were reported was amoxicillin (Table 15).

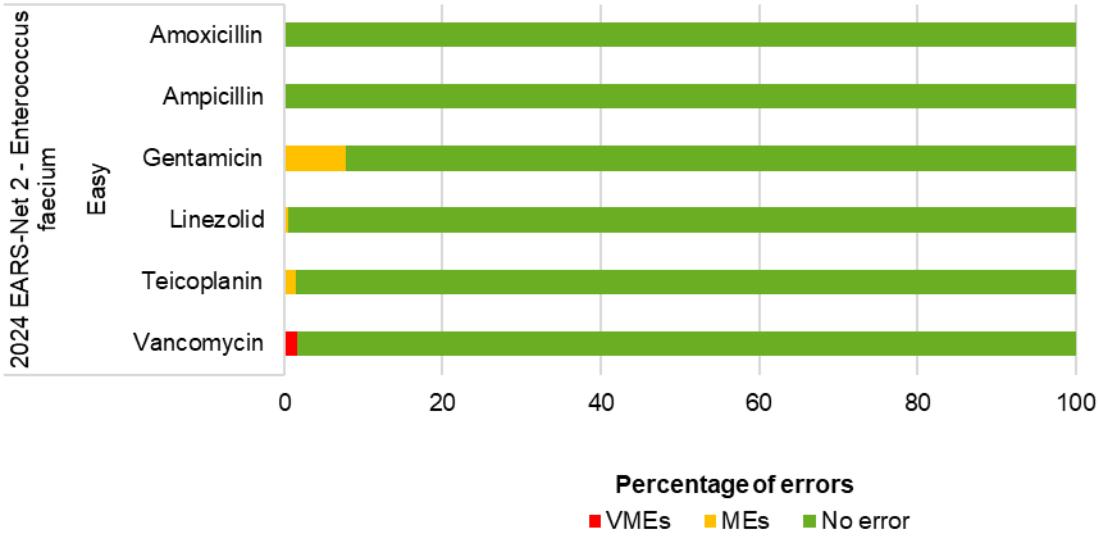
A moderate proportion of MEs was observed for gentamicin (7.9% of submitted results) and these were reported for the automated systems, broth microdilution, disk/tablet diffusion and gradient tests. Very low proportions of MEs were observed for teicoplanin (1.4%) and linezolid (0.5%) (Figure 5, Table 15).

Discussion

Strain **'2024 EARS-Net 2' (*Enterococcus faecium*)** was naturally resistant to gentamicin but did not present high-level aminoglycoside resistance (HLAR) to the antimicrobial. Prediction of this profile was the most problematic for the strain, although it still achieved a 'very good' level of concordance (92.1%). These deviations corresponded to MEs (S → R) and did not seem to be associated with a specific AST method. One of the main reasons for the lower concordance was misinterpretation of the EQA protocol, which instructed participants to report isolates not presenting HLAR as susceptible (S) to gentamicin, but the information might have been missed by some laboratories, which then reported the natural aminoglycoside resistance, including gentamicin resistance, of the test strain. The expected gentamicin MIC ≤ 8 mg/L should be easily identifiable as not being categorised as HLAR, therefore methodological variability should not be a justification for these deviations. These results do not seem to indicate anomalous reporting of aminoglycoside resistance in *E. faecium* in the EU/EEA, nor do they illustrate problems with the methods applied by the laboratories.

For strain '2024 EARS-Net 2', concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

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



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).

Table 15. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2024 EARS-Net 2' (*Enterococcus faecium*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty**	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amoxicillin	Easy	R	-	-	140	100.0	17	100.0	66	100.0	218	100.0	-	-	17	100.0	**458**	**100.0**
Ampicillin	Easy	R	2	100.0	559	100.0	45	100.0	247	99.6	28	100.0	2	100.0	1	100.0	**884**	**99.9**
Gentamicin	Easy	S	1	100.0	317	87.7	40	90.0	268	97.8	67	91.0	1	100.0	5	100.0	**699**	**92.1**
Linezolid	Easy	S	2	100.0	563	99.5	48	100.0	232	100.0	39	97.4	1	100.0	1	100.0	**886**	**99.5**
Teicoplanin	Easy	S	1	100.0	509	98.6	76	97.4	132	97.7	112	100.0	1	100.0	1	100.0	**832**	**98.6**
Vancomycin	Easy	R	2	50.0*	487	99.4	78	100.0	150	96.0	170	97.1	1	100.0	4	100.0	**892**	**98.3**
Total			**8**	**87.5**	**2 575**	**98.0**	**304**	**98.0**	**1 095**	**98.5**	**634**	**98.1**	**6**	**100.0**	**29**	**100.0**	**4 651**	**98.1**

n: number of reporting laboratories;
'-': no data;
shaded cells: Below the threshold of satisfactory concordance (80%).

** n<5 laboratories reported concordant results.*
*** 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, or added to the latest EUCAST clinical breakpoint table.*
Percentages may not total 100% due to rounding. Most relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening. For enterococci, absence of high-level aminoglycoside resistance (HLAR) was registered as 'S' and presence of HLAR was registered as 'R'.

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

Inclusion of the strain in multiple EARS-Net EQA exercises

The strain **'2024 EARS-Net 3' (*Escherichia coli*)** was the same strain as strain '2022 EARS-Net 2' (*E. coli*) and strain '2023 EARS-Net 1' (*E. coli*) from the 2022 and 2023 EARS-Net EQAs, respectively. These were the most challenging strains for participating laboratories in those years and therefore it was decided to include the exact same *E. coli* strain in the 2024 EARS-Net EQA [2,3].

To ensure harmonisation between expected results included in the 2024 EQA exercise, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA exercise. The expected results were essentially in agreement with those obtained and described in the 2023 and 2022 EARS-Net EQA exercises. However, there were three differences between the 'expected results' in the different years. The variation observed between the expected results in 2022, 2023 and 2024 was within the acceptable method variation (+/- 1 dilution) and is likely to be due to the complex genetic resistance mechanisms harboured by the strain, as well as cumulative small variations in the material used for testing. Firstly, the consensus for piperacillin-tazobactam (const. 4) was MIC=16/4 mg/L for both the 2024 and the 2023 EARS-Net EQAs, and therefore it was interpreted as 'Resistant', whereas for the 2022 EARS-Net EQA the expected result was MIC=8/4 mg/L and the interpretation was 'Susceptible, standard dosing regimen'. Secondly, the obtained consensus for amikacin was MIC=8 mg/L for both the 2024 and the 2023 EARS-Net EQAs, and therefore it was interpreted as 'Susceptible, standard dosing regimen', whereas for the 2022 EARS-Net EQA the expected result was MIC>8 mg/L and the interpretation was 'Resistant'. Finally, the expected result for cefepime in the 2024 EARS-Net EQA was MIC=2 mg/L with the interpretation '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 difficulty of obtaining concordant AST results for this strain.

When the results of the three EARS-Net EQAs are compared, including the results submitted by all participating laboratories, for all antimicrobial agents excluding amikacin and piperacillin-tazobactam, the largest change in performance observed was the decrease in MEs for cefepime, from 20% of the participating laboratories in 2022 to 17% in 2023 and 2024.

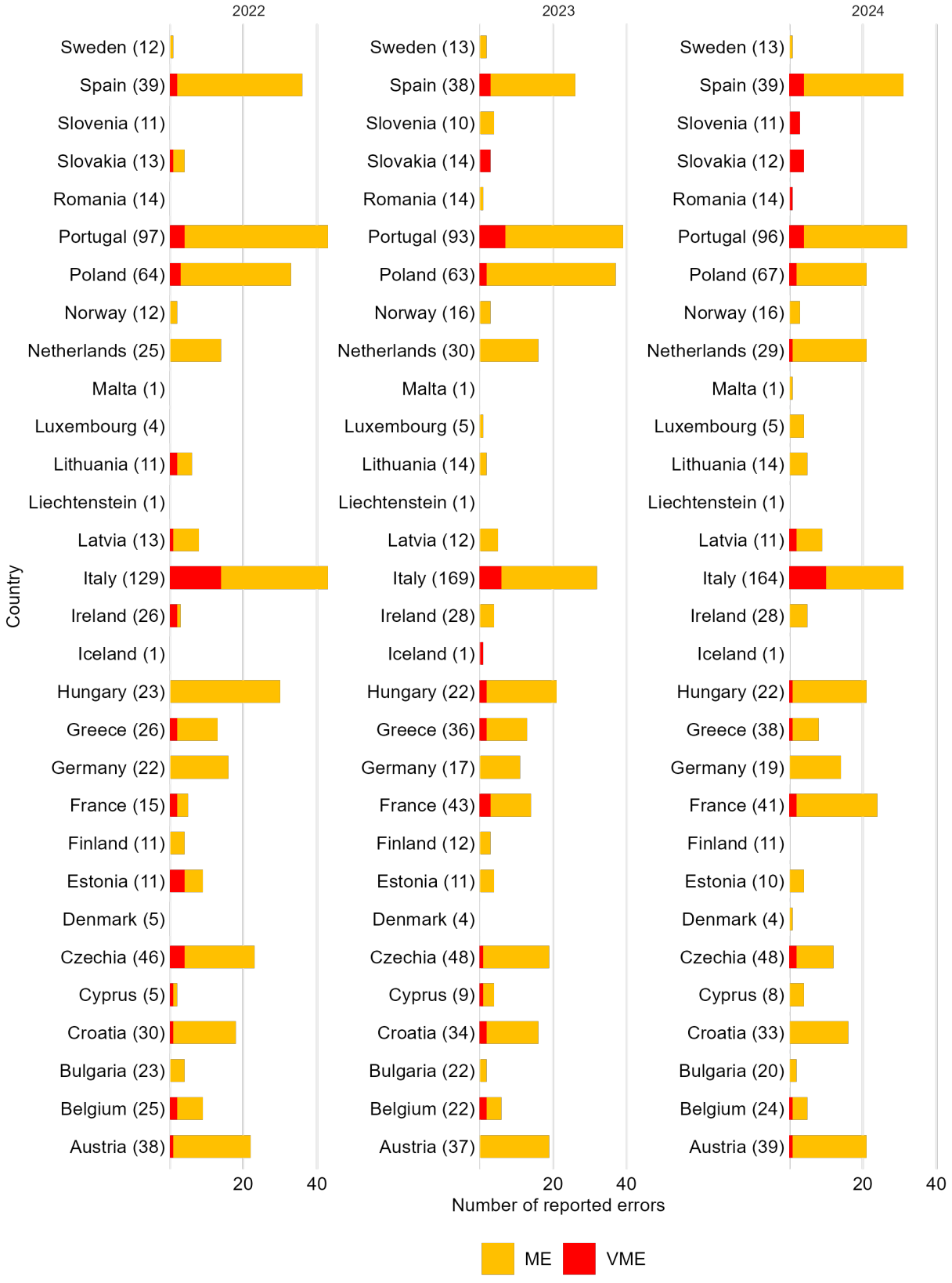
In 2022, 60.5% of participating laboratories reported a correct AST interpretation for piperacillin-tazobactam (expected interpretation as S). In 2023, with a new expected interpretation of R, this proportion decreased to 40.6%. In 2024, with an expected interpretation of R (i.e. equal to the previous year but different from 2022), the proportion of correct submitted interpretations was practically maintained, at 39.9%.

In 2022, 64.0% of participating laboratories reported a correct AST interpretation for amikacin (expected interpretation as R). In 2023, with a new expected interpretation as S, this proportion decreased to 29.2%. In 2024, with an expected interpretation of R (i.e. equal to the previous year but different from 2022), the proportion of correct submitted interpretations increased slightly to 34.0%.

In 2022 and 2023, 79.6% and 83.4% of participating laboratories, respectively, reported a correct AST interpretation for cefepime (expected interpretation as S). In 2024, with a new expected interpretation of I, this proportion was maintained at 83.3%. The proportions are similar because in all three years only VMEs would yield deviations from the expected results.

At the EU/EEA level, 885 laboratories submitted interpretation of AST results for a minimum two years, and 427 laboratories reported results with VME/ME for at least one year (the results on piperacillin-tazobactam (const. 4) and amikacin were excluded). When comparing results between the 2022, 2023 and 2024 EARS-Net EQA exercises, there was little variability in the results for this strain (excluding the results obtained for amikacin and piperacillin-tazobactam) (Figure 6).

Figure 6. Reported errors (%) of interpretation for AST results* for the same strain (i.e. '2022 EARS-Net 2, '2023 EARS-Net 1' and '2024 EARS-Net') for laboratories providing results for at least two of these (n=885), by country



**excluding piperacillin-tazobactam and amikacin because the expected interpretation for these antimicrobials was not the same in the three EARS-Net EQA exercises. The number in brackets is the number of laboratories providing AST results for the specific year; VME - very major error; ME - major error.*

Results for the strain in the 2024 EARS-Net EQA

The strain **'2024 EARS-Net 3' (*Escherichia coli*)** was described as being obtained from a patient with a bloodstream infection. This strain was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin and tobramycin. The strain was susceptible to ertapenem, imipenem, meropenem, amikacin, gentamicin, tigecycline and colistin, and the expected MIC values for ceftazidime and cefepime were in the I range (Table 4). The level of difficulty was considered to be 'difficult' for piperacillin-tazobactam, cefepime, ceftazidime and amikacin since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'. The strain harboured two beta-lactamase genes that contributed to the complex beta-lactam resistance profile: *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{CTX-M-15}}$. The strain also harboured genes and chromosomal point mutations associated with resistance towards aminoglycosides and fluoroquinolones.

Interpretation of AST results for the *E. coli* strain were analysed for the 907 laboratories with correct species identification (Table 9). In total, 19.2% of the laboratories (n=174) would have sent the strain to a reference or other laboratory for further testing. In total, 14 777 tests were performed, and 13 373 reported interpretations were correct. Therefore the reported interpretations were in 'very good' concordance with expected results (90.5%) (Table 16). MEs were observed for 5.6% (n=830) and VMEs for 3.9% (n=574) of the reported interpretations (Figure 7).

The following methods were applied: automated systems (56.3%), disk/tablet diffusion (26.3%), broth microdilution (10.4%), gradient test (6.5%), other (0.4%), agar dilution (0.2%), macro broth dilution (tubes) (0%) (Table 11). Overall, most methods achieved a 'very good' level of concordance with the expected results (>90% of concordance). The exceptions were automated systems (88.8%) and macro broth dilution (80.0%) which achieved a 'good' concordance (Table 16).

VMEs were observed for nine of the 11 antimicrobials with an expected interpretation of R: amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin (Figure 7). VMEs for piperacillin-tazobactam corresponded to 60.1% of all submitted interpretations for that antimicrobial agent and were reported for almost all methods, except agar dilution. For the other antimicrobials, VMEs represented <2% of all submitted interpretations for those antimicrobial agents. The only antimicrobials with an expected interpretation of R for which no VMEs were reported were ampicillin and amoxicillin (Table 16).

A high proportion of MEs was observed for amikacin (66.6% of submitted results) cefepime (16.7%) and ceftazidime (12.6%) and these were reported when using the automated systems, broth microdilution, disk/tablet diffusion and gradient test. Lower proportions of MEs were observed for tigecycline (3.1%), and for the remaining antimicrobial agents there were very low proportions of MEs (Figure 7, Table 16).

Discussion

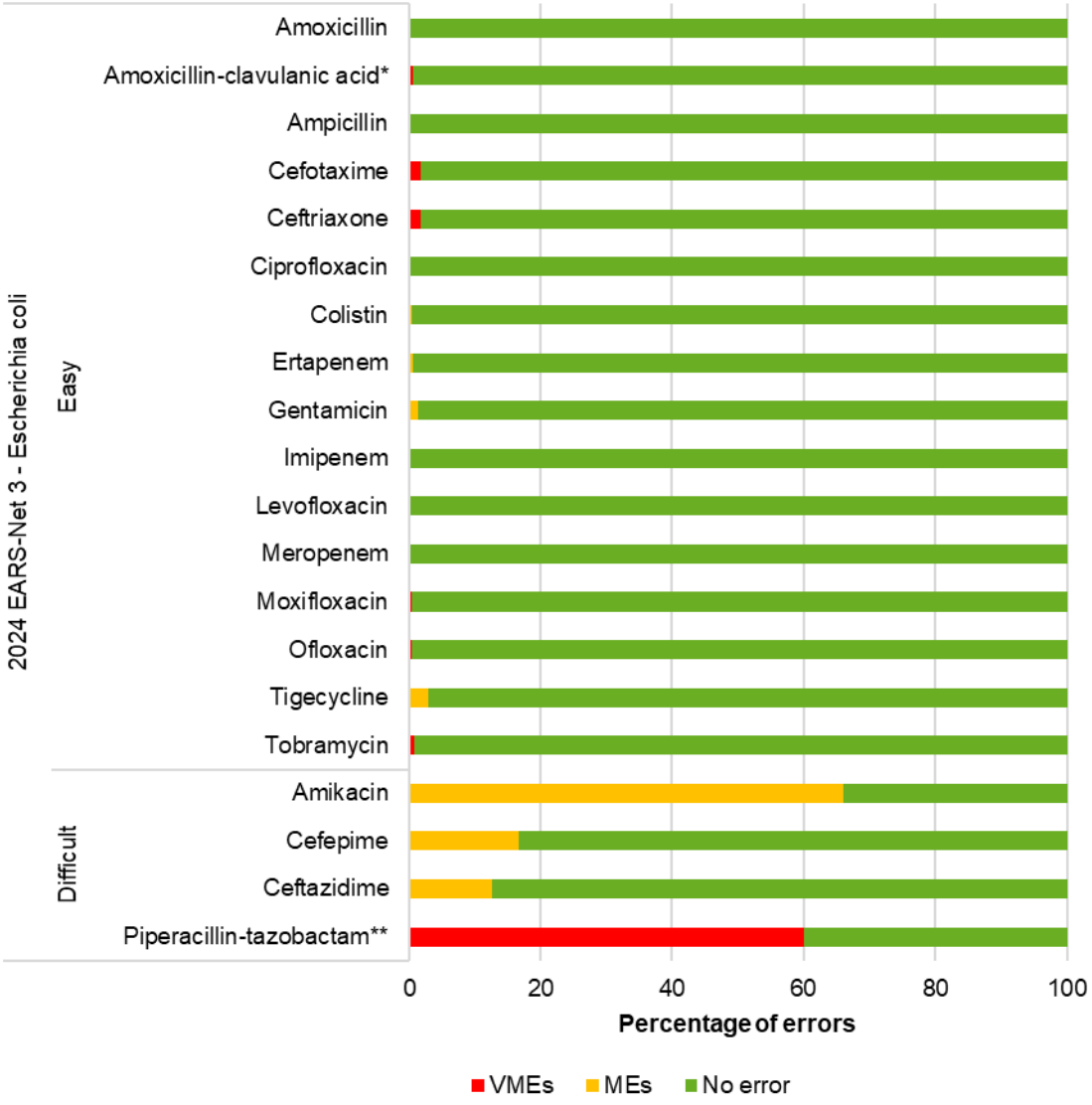
Strain **'2024 EARS-Net 3' (*Escherichia coli*)** was susceptible to amikacin, but concordance of results for this antimicrobial agent was poor (34.0%) and did not reach a 'satisfactory' level. The deviations corresponded to MEs (S → R) and were observed for most methods, except agar dilution that was only applied once. The method with best performance among the commonly applied methods was broth microdilution (71.4%). The expected MIC result (MIC = 8 mg/L) was very close to the clinical breakpoints (S ≤ 8 mg/L and R > 8 mg/L). Therefore the prediction of this AST profile was considered difficult and the observed deviations might be attributable to the inherent method variability, since the expected MIC value corresponds to a borderline concentration, increasing the likelihood of misclassification. The strain was also included in the 2023 EARS-Net EQA, where the expected MIC and respective interpretation were the same as for 2024. In 2023, 29.2% of participants reported a correct interpretation, therefore there was a small improvement from 2023 to 2024. The strain had also been included in the 2022 EARS-Net EQA, where the expected MIC for amikacin was MIC > 8 mg/L, with an interpretation of 'Resistant'. In 2022, 64.0% of participating laboratories reported a correct AST interpretation (R) for amikacin. The variation observed between the expected results in 2022 and in 2023−2024 is within the acceptable method variation (+/- 1 dilution) and it is probably due to cumulative small variations in the material used for testing. These results indicate that in the EU/EEA resistance to amikacin in *E. coli* isolates, and more generally resistance to an antimicrobial agent close to the clinical breakpoint, may be mis-reported, and this reporting may be influenced by the methods and materials used in different settings.

The same situation was observed with piperacillin-tazobactam, for which results did not reach a satisfactory level (39.9%). The deviations corresponded to VMEs (R → S) and were observed in particular for the automated systems, broth microdilution and gradient test. The determination of the expected MIC value (MIC = 16/4 mg/L) was considered to be 'difficult' due to the closeness to the clinical breakpoints (S ≤ 8 mg/L and R > 8 mg/L), which means that even the acceptable inherent method variability of plus or minus one dilution could lead to a misclassification of piperacillin-tazobactam susceptibility, or resistance, for this strain. Furthermore, the differential expression of the *bla*$_{\text{OXA-1}}$ gene harboured by the strain could exacerbate the deviations. As for amikacin, the expected result for piperacillin-tazobactam varied between years. In 2023, the expected MIC value and interpretation were the same as for 2024, and 40.6% of participating laboratories reported a correct interpretation. Therefore the results for 2024 and 2023 were similar, not better or worse. In 2022, the expected result was MIC=8/4 mg/L with an interpretation of 'Susceptible, standard dosing regimen' and 60.5% of participating laboratories reported a correct AST interpretation for piperacillin-tazobactam (S). These results indicate that, in *E. coli* isolates, resistance to piperacillin-tazobactam may be anomalously reported in the EU/EEA, and influenced by the methods and materials in use.

Suboptimal results were also observed for cefepime (which reached a 'satisfactory' level with 83.3% of concordance) and ceftazidime (with a 'good' level with 87.4% concordance). The deviations corresponded to MEs (I → R) and were observed for most methods. The worst performance was observed for the disk or tablet diffusion method (67.5% to 80.6% concordance), which is frequently used by the laboratories for AST of these antimicrobial agents (23.8% - 24.1%). The expected MIC values for cefepime and ceftazidime (MIC = 2 mg/L for both antimicrobials) were very close to the clinical breakpoints (S ≤ 1 mg/L and R > 4 mg/L, for both antimicrobial agents), which were also classified as 'difficult'. Furthermore, variations in results for these cephalosporins can also be derived from the differential expression of the *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{OXA-1}}$ genes that were harboured by the strain. The expected results for ceftazidime did not vary in the 2022–2024 EARS-Net EQAs, and the proportions of correct results submitted by participants were very similar between 2024 (87.4%) and 2023 (87.5%), and slightly better when compared to 2022 (83.7%). The expected results for cefepime had a slight variation between years, because in 2023 and 2022 the expected MIC was 1 mg/L with the interpretation 'S' whereas in 2024 the expected MIC was 2 mg/L with an interpretation 'I'. However, this variation did not change the type of errors that could be observed for cefepime: MEs (S/I → R). As for ceftazidime, the proportions of correct results submitted by participants were very similar between 2024 (83.3%) and 2023 (83.4%) and slightly better for those years than for 2022 (79.6%). These results may indicate that, for *E. coli*, resistance to these agents is overestimated in the EU/EEA. However, when compared with results from the 2022 EARS-Net EQA exercise, the results in the 2023 and 2024 EARS-Net EQA exercises show an improvement for these two cephalosporins.

For strain '2024 EARS-Net 3', the concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

Figure 7. Reported interpretation of AST results for strain '2024 EARS-Net 3' (*Escherichia coli*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).

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

Table 16. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2024 EARS-Net 3' (*Escherichia coli*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty****	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Difficult	S	1	100.0	480	28.8	91	71.4	205	28.3	35	40.0	-	-	3	33.3	**815**	**34.0**
Amoxicillin	Easy	R	1	100.0	146	100.0	22	100.0	69	100.0	196	100.0	-	-	12	100.0	**446**	**100.0**
Amoxicillin-clavulanic acid**	Easy	R	2	100.0	562	99.5	47	97.9	248	99.2	24	100.0	-	-	1	100.0	**884**	**99.3**
Ampicillin	Easy	R	2	100.0	513	100.0	53	100.0	241	100.0	30	100.0	-	-	2	100.0	**841**	**100.0**
Cefepime	Difficult	I	2	100.0	500	87.4	66	90.9	191	67.5	43	93.0	-	-	2	100.0	**804**	**83.3**
Cefotaxime	Easy	R	3	66.7*	519	99.2	60	95.0	211	97.6	28	92.9	-	-	3	100.0	**824**	**98.2**
Ceftazidime	Difficult	I	3	100.0	547	88.3	80	96.3	216	80.6	49	91.8	-	-	2	100.0	**897**	**87.4**
Ceftriaxone	Easy	R	2	50.0*	234	98.7	30	96.7	212	99.5	120	95.8	-	-	4	100.0	**602**	**98.2**
Ciprofloxacin	Easy	R	2	100.0	583	99.8	72	100.0	233	100.0	11	100.0	-	-	2	100.0	**903**	**99.9**
Colistin	Easy	S	-	-	259	99.6	412	99.5	3	100.0	12	100.0	4	100.0	2	100.0	**692**	**99.6**
Ertapenem	Easy	S	2	100.0	498	99.6	66	100.0	209	98.6	29	100.0	-	-	2	100.0	**806**	**99.4**
Gentamicin	Easy	S	2	100.0	572	99.0	70	97.1	218	98.2	19	94.7	-	-	2	100.0	**883**	**98.5**
Imipenem	Easy	S	1	100.0	470	99.6	56	100.0	217	100.0	40	100.0	-	-	2	100.0	**786**	**99.7**
Levofloxacin	Easy	R	2	100.0	369	99.5	54	100.0	235	100.0	51	100.0	-	-	1	100.0	**712**	**99.7**
Meropenem	Easy	S	2	100.0	562	100.0	80	100.0	222	99.5	20	100.0	-	-	2	100.0	**888**	**99.9**
Moxifloxacin	Easy	R	3	100.0	118	99.2	16	100.0	210	99.5	72	100.0	-	-	3	100.0	**422**	**99.5**
Ofloxacin	Easy	R	1	100.0	55	100.0	11	100.0	167	99.4	27	100.0	-	-	3	100.0	**264**	**99.6**
Piperacillin-tazobactam***	Difficult	R	3	100.0	513	19.1	86	34.9	229	80.3	48	75.0	1	0.0	2	50.0	**882**	**39.9**
Tigecycline	Easy	S	-	-	386	95.6	96	99.0	128	100.0	76	96.1	-	-	1	100.0	**687**	**96.9**
Tobramycin	Easy	R	1	100.0	431	99.3	64	100.0	216	99.1	25	96.0	-	-	2	100.0	**739**	**99.2**
Total			**35**	**94.3**	**8 317**	**88.8**	**1 532**	**93.4**	**3 880**	**91.9**	**955**	**94.6**	**5**	**80.0**	**53**	**94.3**	**14 777**	**90.5**

n: number of reporting laboratories;
'-': no data;
shaded cells: Below the threshold of satisfactory concordance (80%).

** n<5 laboratories reported concordant results.*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
**** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam. Percentages may not total 100% due to rounding.*
*****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, or added to the latest EUCAST clinical breakpoint table.*
Percentages may not total 100% due to rounding. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

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

The *P. aeruginosa* EQA strain ('2024 EARS-Net 4') was described as being obtained from a patient with bloodstream infection. This strain was resistant to piperacillin, ceftazidime, imipenem, ciprofloxacin and levofloxacin. The strain was susceptible to amikacin, tobramycin and colistin, and the expected MIC values for piperacillin-tazobactam, cefepime and meropenem were in the I range (Table 5). The level of difficulty was considered to be 'difficult' for piperacillin, piperacillin-tazobactam, cefepime, ceftazidime and meropenem since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'. The strain harboured a chromosomal point mutation that contributed to the complex beta-lactam resistance profile, specifically *oprD* W339STOP. The strain also harboured a gene and chromosomal point mutation that confer fluoroquinolone resistance (*crpP* and *gyrA* T83I).

Interpretation of AST results for the *P. aeruginosa* strain were analysed for the 902 laboratories with correct species identification (Table 9). In total, 22.2% of the laboratories (n=200) would have sent the strain to a reference or other laboratory for further testing. In total, 8 700 tests were performed, and 7 038 reported interpretations were correct. Therefore the reported interpretations were in 'good' concordance with expected results (80.9%) (Table 17). MEs were observed for 5.9% (n=516) and VMEs were observed for 13.2% (n=1 146) of the reported interpretations (Figure 8).

The following methods were applied: automated systems (57.2%), disk/tablet diffusion (24.7%), broth microdilution (13.1%), gradient test (4.6%), other (0.3%), agar dilution (0.2%), macro broth dilution (tubes) (0.1%) (Table 11). Overall, most methods achieved a 'good' level of concordance with the expected results (>80% of concordance) as a minimum. The exceptions were disk and table diffusion (76.6%), gradient test (61.5%) and 'other methods' (68.2%) which achieved less than 'satisfactory' concordance (Table 17).

VMEs were observed for all five antimicrobials with an expected interpretation of R: piperacillin, ceftazidime, imipenem, ciprofloxacin and levofloxacin (Figure 8). By far the most VMEs were observed for ceftazidime (93.8% of all submitted interpretations for that antimicrobial agent) and piperacillin (77.1%) and were reported for all methods. For the other antimicrobial agents, VMEs represented <2.5% of all submitted interpretations (Table 17).

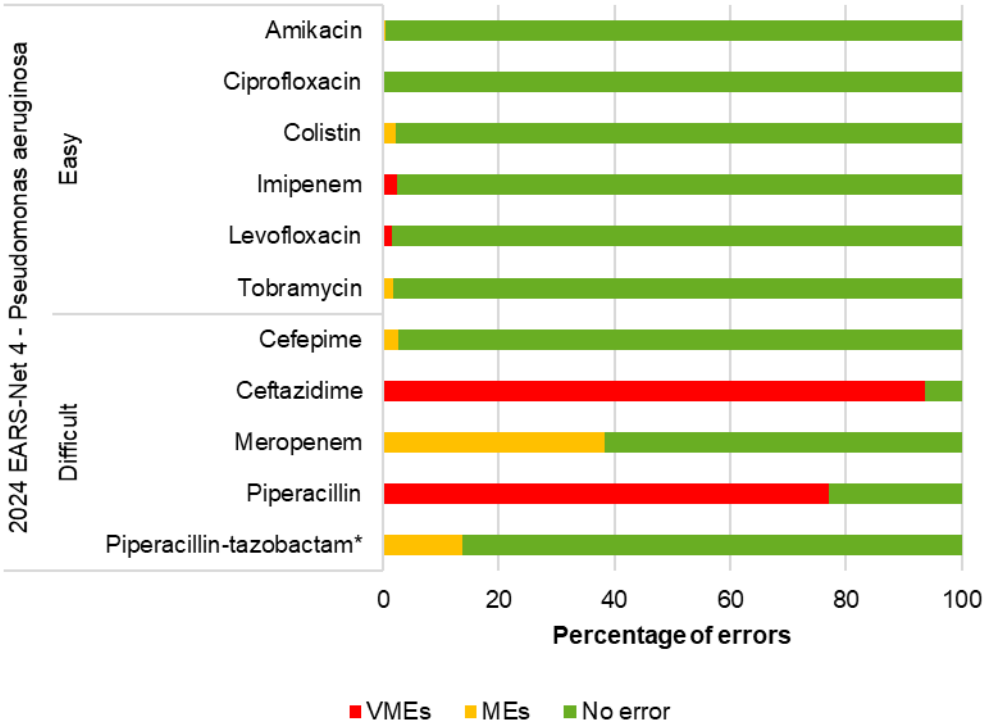
A high proportion of MEs was observed for meropenem (38.2% of submitted results) and piperacillin-tazobactam (13.8%) and these were reported for all or almost all methods. For the remaining antimicrobial agents there were very low proportions of MEs (Figure 8, Table 17).

Discussion

Strain **'2024 EARS-Net 4' (*Pseudomonas aeruginosa*)** was resistant to ceftazidime and piperacillin but prediction of these profiles was very problematic and did not reach satisfactory levels (6.2% and 22.9% of correct interpretations, respectively). The deviations corresponded to VMEs (R → S/I) and were observed for all methods. Results for meropenem were also not satisfactory, with 61.8% of concordance, and results for piperacillin-tazobactam only reached a 'good' level of concordance (86.2%). These were MEs (I → R) and there was especially poor performance of the gradient test and disk/tablet diffusion methods. The determination of AST results for all these antimicrobials was considered to be 'difficult', and many of the deviations observed might be attributable to the inherent method variability. Importantly, the strain harbours a chromosomal point mutation affecting the expression of porins that contributes to a complex and potentially variable AMR profile towards carbapenems. Databases of genetic determinants of AMR for *Pseudomonas* spp. remain incomplete and therefore it is not known whether the strain could harbour further mechanisms contributing to the overall difficult susceptibility profile of other beta-lactam agents. These results might indicate anomalous reporting of beta-lactam resistance in *P. aeruginosa* in the EU/EEA, especially for bacterial isolates harbouring complex genetic mechanisms of AMR.

For strain '2024 EARS-Net 4', the concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

Figure 8. Reported interpretation of AST results for strain '2024 EARS-Net 4' (*Pseudomonas aeruginosa*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).

** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 17. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2024 EARS-Net 4' (*Pseudomonas aeruginosa*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty***	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	S	1	100.0	523	99.4	81	98.8	229	99.6	17	100.0	-	-	2	100.0	**853**	**99.4**
Cefepime	Difficult	I	2	100.0	530	98.7	61	95.1	215	95.3	23	91.3	-	-	2	100.0	**833**	**97.4**
Ceftazidime	Difficult	R	2	0.0*	551	2.9	77	9.1	232	12.9	33	9.1	-	-	2	0.0*	**897**	**6.2**
Ciprofloxacin	Easy	R	2	100.0	562	99.8	75	100.0	246	100.0	13	100.0	-	-	2	100.0	**900**	**99.9**
Colistin	Easy	S	-	-	259	94.6	424	99.5	4	100.0	12	100.0	4	100.0	2	100.0	**705**	**97.7**
Imipenem	Easy	R	2	100.0	487	98.6	67	97.0	211	95.7	60	96.7	-	-	2	100.0	**829**	**97.6**
Levofloxacin	Easy	R	2	100.0	390	97.9	57	100.0	223	98.7	67	100.0	-	-	1	100.0	**740**	**98.5**
Meropenem	Difficult	I	2	50.0*	485	86.2	85	71.8	212	22.6	93	16.1	-	-	2	0.0*	**879**	**61.8**
Piperacillin	Difficult	R	2	50.0*	156	17.3	55	16.4	106	34.9	32	21.9	-	-	3	0.0*	**354**	**22.9**
Piperacillin-tazobactam**	Difficult	I	3	100.0	531	90.0	94	87.2	228	81.1	32	53.1	1	100.0	2	100.0	**891**	**86.2**
Tobramycin	Easy	S	3	100.0	500	98.2	60	96.7	239	99.2	15	93.3	-	-	2	100.0	**819**	**98.3**
Total			**21**	**81.0**	**4 974**	**83.3**	**1 136**	**85.7**	**2 145**	**76.6**	**397**	**61.5**	**5**	**100.0**	**22**	**68.2**	**8 700**	**80.9**

n: number of reporting laboratories;
'-': no data; shaded cells: Below the threshold of satisfactory concordance (80%).

** n<5 laboratories reported concordant results.*
*** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam. Percentages may not total 100% due to rounding.*
**** 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, or added to the latest EUCAST clinical breakpoint table.*
Percentages may not total 100% due to rounding. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

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

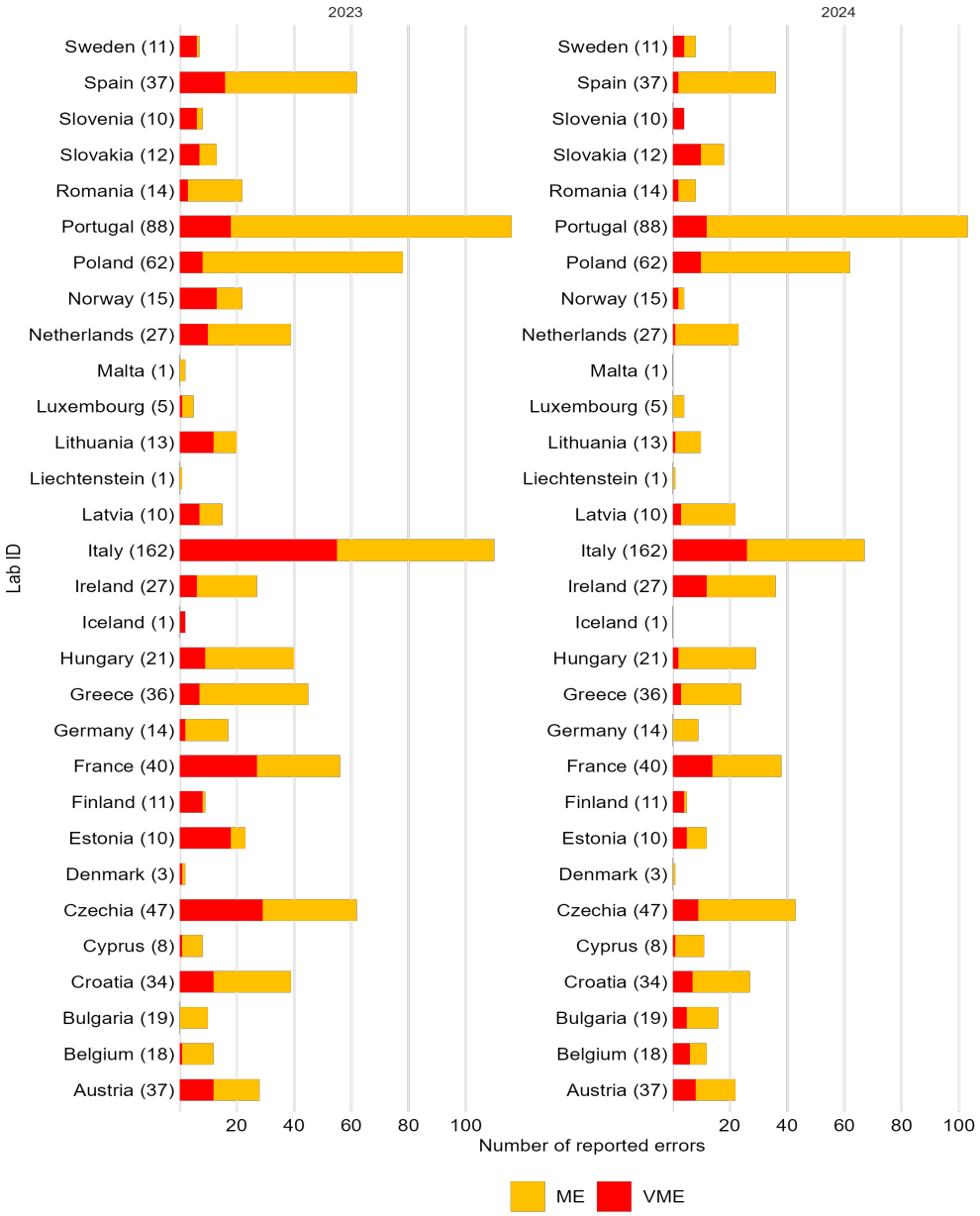
Inclusion of the strain in multiple EARS-Net EQA exercises

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

When the results submitted by all participating laboratories were considered, the highest variation was the decrease in ME for amikacin, from 33% of the participating laboratories in 2023 to 29% in 2024.

At the EU/EEA level, 794 laboratories submitted interpretation of AST results both years, and 609 laboratories reported results with VME/ME at least one year. When comparing results between the 2023 and 2024 EARS-Net EQA exercises, there was little variability for this strain (Figure 9).

Figure 9. Reported errors (%) of interpretation for AST results for the same strain (i.e. '2024 EARS-Net 5' and '2023 EARS-Net 2') for laboratories providing results in both 2024 and 2023 (n=794), by country



The number in brackets is the number of laboratories providing AST results for both 2023 and 2024.
VME - very major error; ME - major error.

Results for the strain in the 2024 EARS-Net EQA

The strain **'2024 EARS-Net 5' (*Klebsiella pneumoniae*)** was described as being obtained from a patient with a bloodstream infection. This strain was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin and tobramycin. The strain was susceptible to meropenem, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, amikacin and colistin, and the expected MIC values for cefepime and imipenem were in the I range (Table 6). The level of difficulty was considered to be 'difficult' for cefepime, cefotaxime, imipenem, meropenem, ofloxacin and gentamicin since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered to be 'easy'. The strain harboured three beta-lactamase genes that contributed to the complex beta-lactam resistance profile, specifically *bla*$_{\text{VEB-1}}$, *bla*$_{\text{SHV-11}}$ (or a similar *bla*$_{\text{SHV}}$ variant) and *bla*$_{\text{OXA-10}}$. The strain also harboured genes associated with aminoglycoside resistance.

Interpretation of AST results for the *K. pneumoniae* strain were analysed for the 906 laboratories with correct species identification (Table 9). In total, 49.0 % of the laboratories (n=444) would have sent the strain to a reference or other laboratory for further testing. In total, 12 812 tests were performed, and 12 071 reported interpretations were correct. Therefore the reported interpretations were in 'excellent' concordance with expected results (94.2%) (Table 18). MEs were observed for 4.5% (n=571) and VMEs for 1.3% (n=170) of the reported interpretations (Figure 10).

The following methods were applied: automated systems (55.6%), disk/tablet diffusion (26.3%), broth microdilution (11.1%), gradient test (6.4%), other (0.3%), agar dilution (0.2%), macro broth dilution (tubes) (0.05%) (Table 12). All methods achieved a 'very good' level of concordance with the expected results (>90% of concordance) as a minimum (Table 18).

VMEs were observed for all eight antimicrobials with an expected interpretation of R: amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin, piperacillin-tazobactam and tobramycin (Figure 10). VMEs in ceftriaxone (6.7% of all submitted interpretations for that antimicrobial), ertapenem (4.4%), gentamicin (4.4%) and cefotaxime (3.5%) and piperacillin-tazobactam (3.5%) were mainly reported when using automated systems, broth microdilution, disk/tablet diffusion, and gradient tests. For the other antimicrobial agents, VMEs represented <2% of all submitted interpretations (Table 18).

A high proportion of MEs was observed for amikacin (28.5% of submitted results), cefepime (17.0%) and imipenem (13.6%) and these were reported when using the automated systems, broth microdilution, disk/tablet diffusion, and gradient tests. Lower proportions of MEs were observed for meropenem (6.4%) and ofloxacin (3.8%). For the remaining antimicrobial agents there were very low proportions of MEs (Figure 10, Table 18).

Discussion

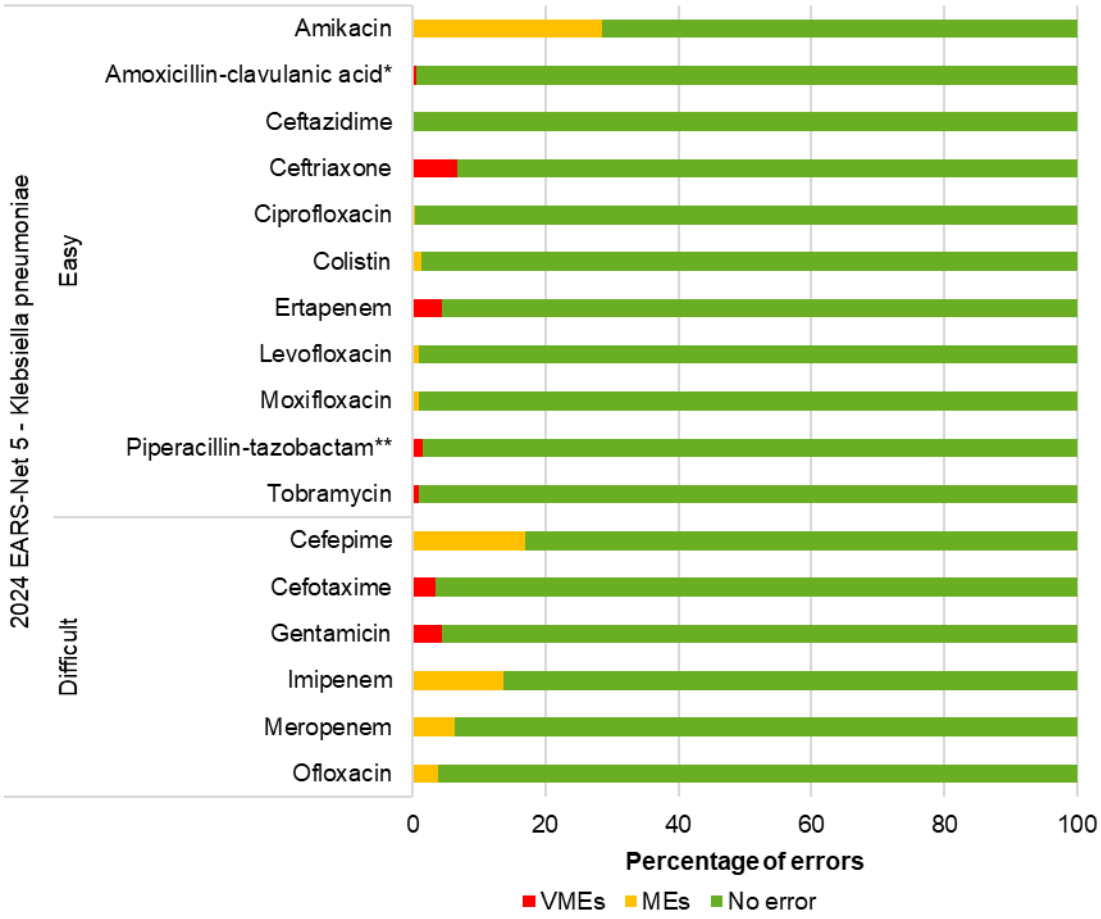
Strain **'2024 EARS-Net 5' (*Klebsiella pneumoniae*)** was susceptible to amikacin but prediction of this profile was problematic and concordance did not reach a satisfactory level (71.5%). These deviations corresponded to MEs (S → R) and were observed for all the frequently applied methods, with a significantly lower proportion of errors for broth microdilution. The expected MIC = 4 mg/L was considered to be an 'easy' determination, because it was not close to the clinical breakpoints and the acceptable method variation of +/- 1 dilution would yield the same classification (S). Therefore, the natural methodological variability should not be a justification for these deviations, which may potentially be justified by variations in the methods and/or the material used for testing [7-9]. The strain was also included in the 2023 EARS-Net EQA, where the expected MIC and respective interpretation for amikacin were the same as for 2024. In 2023, 66.7% of participants reported a correct interpretation, therefore there was a small improvement from 2023 to 2024.

Less than ideal proportions of concordance were also observed for cefepime ('satisfactory' results with 83.0% of concordance) and imipenem ('good' results with 86.4% of concordance). These deviations corresponded to MEs (I → R) and were more prevalent when using automated systems, broth microdilution and disk/tablet diffusion. The expected MIC results for cefepime (MIC = 2 mg/L) and imipenem (MIC = 4 mg/L) were very close to the clinical breakpoints (i.e. S ≤ 1 mg/L and R > 4 mg/L for cefepime, and S ≤ 2 mg/L and R > 4 mg/L for imipenem). Therefore the prediction of these AST profiles was considered difficult and the observed deviations can be attributed to the inherent method variability, since the expected MIC values correspond to borderline concentrations, thereby increasing the likelihood of misclassification. Furthermore, variations in results for these beta-lactam agents can also be derived from the differential expression of the *bla*$_{\text{VEB-1}}$ and *bla*$_{\text{OXA-10}}$ genes that were harboured by the strain. The expected results for cefepime did not vary in the 2023–2024 EARS-Net EQAs, and the proportions of correct results submitted by participants were very similar between 2023 (78.9%) and 2024 (83.0%). The expected results for imipenem varied slightly between years, because in 2023 the expected MIC was 2 mg/L with the interpretation 'S', whereas in 2024 the expected MIC was 4 mg/L with an interpretation 'I'. However, this variation did not change the type of errors that could be observed for imipenem, which were MEs (S/I → R). As for cefepime, the proportions of correct results submitted by participants improved slightly between 2023 (82.5%) and 2024 (86.4%).

The results described may be an indication that resistance to these agents in *K. pneumoniae* isolates is overestimated in the EU/EEA, especially for aminoglycosides.

For strain '2024 EARS-Net 5', the concordance of results for the remaining antimicrobial agents was 'very good' (>90% to <95%) or 'excellent' (≥95%).

Figure 10. Reported interpretation of AST results for strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).

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

Table 18. Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2024 EARS-Net 5' (*Klebsiella pneumoniae*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty****	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/table diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	S	1	100.0	495	69.5	90	88.9	213	68.1	30	73.3	-	-	2	100.0	**831**	**71.5**
Amoxicillin-clavulanic acid**	Easy	R	2	100.0	557	99.5	51	100.0	252	99.2	20	100.0	-	-	2	100.0	**884**	**99.4**
Cefepime	Difficult	I	2	100.0	508	87.4	69	85.5	192	67.2	37	97.3	-	-	2	100.0	**810**	**83.0**
Cefotaxime	Difficult	R	3	100.0	509	98.8	66	95.5	206	94.2	31	74.2	-	-	3	100.0	**818**	**96.5**
Ceftazidime	Easy	R	3	100.0	572	99.8	76	100.0	233	100.0	13	100.0	-	-	2	100.0	**899**	**99.9**
Ceftriaxone	Easy	R	2	100.0	241	97.5	30	96.7	221	94.6	117	81.2	-	-	2	100.0	**613**	**93.3**
Ciprofloxacin	Easy	S	2	100.0	576	99.7	78	100.0	234	99.1	10	100.0	-	-	2	100.0	**902**	**99.6**
Colistin	Easy	S	1	100.0	257	97.7	425	99.3	5	100.0	12	100.0	5	100.0	2	100.0	**707**	**98.7**
Ertapenem	Easy	R	2	100.0	471	97.2	73	97.3	191	96.3	65	80.0	-	-	2	100.0	**804**	**95.6**
Gentamicin	Difficult	R	2	50.0*	575	99.1	76	71.1	209	95.2	24	95.8	-	-	2	100.0	**888**	**95.6**
Imipenem	Difficult	I	1	100.0	403	81.1	69	87.0	177	92.1	124	94.4	-	-	3	100.0	**777**	**86.4**
Levofloxacin	Easy	S	2	100.0	357	99.4	48	95.8	239	99.2	56	98.2	-	-	1	100.0	**703**	**99.0**
Meropenem	Difficult	S	1	100.0	477	94.3	96	94.8	178	92.1	136	92.6	-	-	2	50.0*	**890**	**93.6**
Moxifloxacin	Easy	S	3	100.0	101	100.0	16	100.0	211	98.6	70	98.6	-	-	2	100.0	**403**	**99.0**
Ofloxacin	Difficult	S	1	100.0	35	97.1	12	100.0	161	95.0	25	100.0	-	-	2	100.0	**236**	**96.2**
Piperacillin-tazobactam***	Easy	R	2	100.0	560	98.2	82	98.8	234	99.1	16	100.0	1	100.0	2	100.0	**897**	**98.6**
Tobramycin	Easy	R	1	100.0	432	99.5	69	97.1	218	98.6	28	100.0	-	-	2	100.0	**750**	**99.1**
Total			**31**	**96.8**	**7 126**	**94.7**	**1 426**	**95.1**	**3 374**	**93.4**	**814**	**91.2**	**6**	**100.0**	**35**	**97.1**	**12 812**	**94.2**

n: number of reporting laboratories;
'-': no data;
shaded cells: Below the threshold of satisfactory concordance (80%).

** n<5 laboratories reported concordant results.*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
**** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
***** 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, or added to the latest EUCAST clinical breakpoint table.*
Percentages may not total 100% due to rounding. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

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

The *S. aureus* EQA strain ('2024 EARS-Net 6') was described as being obtained from a patient with a bloodstream infection. This strain was resistant to oxacillin and susceptible to cefoxitin, norfloxacin, vancomycin, linezolid, daptomycin and rifampicin. The expected MIC values for ciprofloxacin and levofloxacin were in the I range (Table 7). The level of difficulty was considered to be 'difficult' for cefoxitin and ciprofloxacin since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'. No known genetic mechanisms of resistance were detected.

Interpretation of AST results for the *S. aureus* strain were analysed for the 899 laboratories with correct species identification (Table 9). In total, 24.5% of the laboratories (n=220) would have sent the strain to a reference or other laboratory for further testing. In total, 6 533 tests were performed, and 6 215 reported interpretations were correct. Therefore the reported interpretations were in 'excellent' concordance with expected results (95.1%). MEs were observed for 1.9% (n=127) and VMEs for 2.9% (n=191) of the reported interpretations (Figure 11).

The following methods were applied: automated systems (54.5%), disk/tablet diffusion (28.8%), gradient test (9.4%), broth microdilution (6.6%), other (0.4%) and agar dilution (0.3%) (Table 12). Overall, most methods achieved a 'good' level of concordance with the expected results (>85% of concordance) as a minimum. The exception was 'other methods' (60.0%) which achieved less than 'satisfactory' concordance (Table 19).

VMEs were observed to the only antimicrobial with an expected interpretation of R: oxacillin (Figure 11). These VMEs represented 27.2% of all submitted interpretations for the antimicrobial agent and were reported for all methods (Table 19).

A high proportion of MEs was observed for cefoxitin (13.4% of submitted results) and these were reported for all methods. For the remaining antimicrobial agents there were no or very low proportions of MEs (Figure 11, Table 19).

Discussion

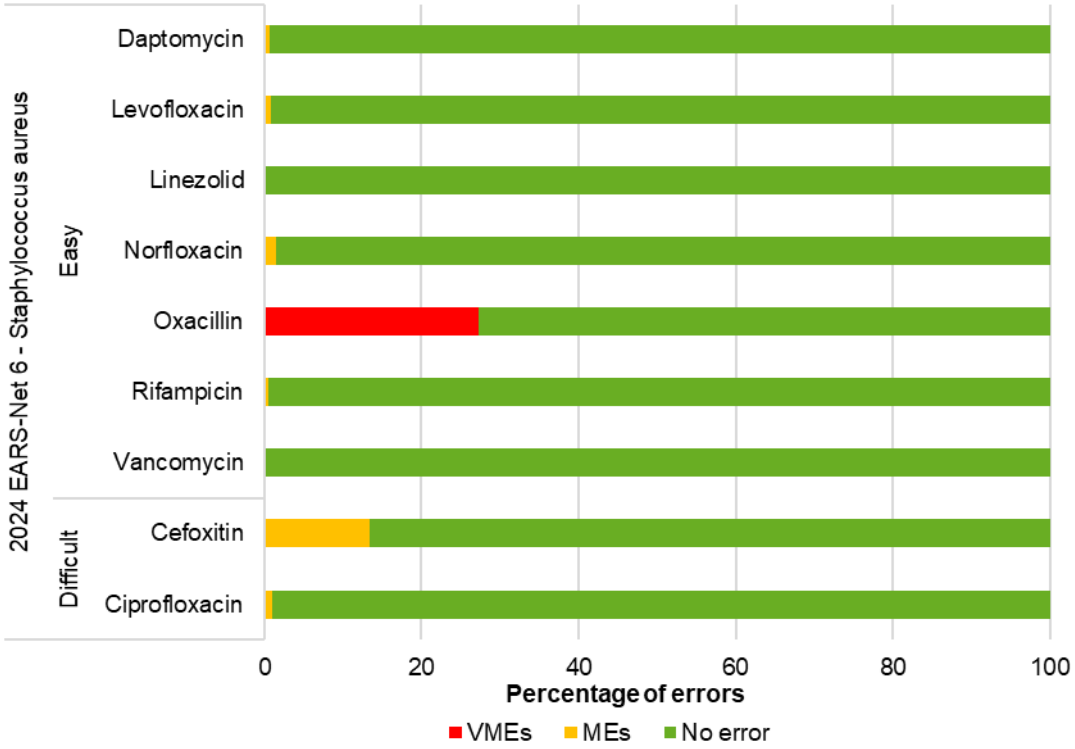
Strain **'2024 EARS-Net 6' (*Staphylococcus aureus*)** was resistant to oxacillin, but concordance of results for this antimicrobial agent did not reach a satisfactory level (72.8%). These deviations corresponded to VMEs (R → S) and were observed for all methods, with a significantly lower proportion of errors for the automated systems. The expected MIC = 8 mg/L was considered to be an 'easy' determination, because it was not close to the clinical breakpoints and the acceptable method variation of +/- 1 dilution would yield the same classification (R). Therefore, the natural methodological variability should not be a justification for these deviations. A possible explanation is that laboratories could have missed the specific recommendations for AST of oxacillin in *Staphylococcus* spp., which require special adjustments to the conditions and broth used for broth microdilution. A likely justification for some of these deviations is that some laboratories might have inferred oxacillin susceptibility from cefoxitin susceptibility. The strain is classified as a borderline oxacillin resistant *S. aureus* (BORSA) with a categorisation of 'S' towards cefoxitin accompanied by a categorisation of 'R' towards oxacillin. Systematic screening of BORSA isolates is not recommended by EUCAST, therefore the AST of both antimicrobials simultaneously might not be part of routine procedures at the participating laboratories. In this case, the laboratories are still complying with EUCAST guidelines and do not necessarily need to review their routine procedures. However, for the purpose of the EARS-Net EQA, laboratories are asked to only report AST results from tests that they actually performed for the exercise, and to avoid using results from certain antimicrobials to predict results for other agents.

Problematic results were also observed for cefoxitin ('good' results with 86.6% concordance). These deviations corresponded to MEs (S → R) and were observed for the automated systems and broth microdilution in particular. The recommended method for AST of cefoxitin is disk diffusion and the expected zone diameter (27 mm) was not close to the clinical breakpoints. Therefore using the disk diffusion method should provide accurate results for the determination of susceptibility towards cefoxitin, and this is illustrated by the high concordance of results observed for that method (95.2%). However, if using MIC methods, such as broth microdilution and automated systems, the expected MIC result (MIC = 4 mg/L, as determined by consensus of the standard broth microdilution results obtained by the three reference laboratories) was very close to the clinical breakpoints (R > 4 mg/L). Therefore the prediction of cefoxitin susceptibility, especially using MIC methods, was considered difficult and the observed deviations could be attributable to the inherent method variability.

The *S. aureus* strain was relatively challenging for an EQA, but its results might indicate over-reporting of methicillin resistance in *S. aureus* in the EU/EEA, especially if inadequate AST methodologies are applied. As described in EUCAST guidelines, disk diffusion is more reliable than other methods for detection of *S. aureus* penicillinase producers, therefore it is the method recommended to ensure specificity of MRSA reporting. The use of other methods might lead to inaccurate conclusions, as illustrated in this EARS-Net EQA where the AST results for oxacillin reported using an automated system had just 79.4% concordance. Other results indicate some non-compliance with current EUCAST guidelines, specifically the use of MIC methods for AST of cefoxitin. For cefoxitin, although there is no specific note in the EUCAST clinical breakpoint table discouraging the use of other methods in *S. aureus*, it is explicitly stated that disk diffusion reliably predicts methicillin resistance.

For strain '2024 EARS-Net 6', the concordance of results for the remaining antimicrobial agents was 'excellent'.

Figure 11. Reported interpretation of AST results for strain '2024 EARS-Net 6' (*Staphylococcus aureus*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; M E: major error; NA: not appliable (e.g. no data).

Table 19. Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2024 EARS-Net 6' (*Staphylococcus aureus*), by antimicrobial agent and AST method

Antimicrobial agent	Level of difficulty**	Expected interpretation	Agar dilution		Automated systems		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cefoxitin	Difficult	S	9	88.9	159	61.0	30	76.7	521	95.2	10	80.0	-	-	3	66.7	**732**	**86.6**
Ciprofloxacin	Difficult	I	1	100.0	338	99.1	36	100.0	264	98.5	70	100.0	-	-	3	100.0	**712**	**99.0**
Daptomycin	Easy	S	-	-	505	99.6	62	96.8	3	100.0	133	99.2	-	-	1	100.0	**704**	**99.3**
Levofloxacin	Easy	I	2	100.0	461	99.3	36	100.0	221	98.2	54	100.0	-	-	1	100.0	**775**	**99.1**
Linezolid	Easy	S	2	100.0	582	100.0	55	100.0	228	100.0	19	100.0	-	-	2	100.0	**888**	**100.0**
Norfloxacin	Easy	S	2	100.0	14	92.9	4	75.0	314	99.4	5	100.0	-	-	1	0.0	**340**	**98.5**
Oxacillin	Easy	R	1	0.0*	485	79.4	50	62.0	58	67.2	97	54.6	-	-	10	20.0	**701**	**72.8**
Rifampicin	Easy	S	3	66.7*	463	99.8	43	100.0	265	99.2	35	100.0	-	-	2	100.0	**811**	**99.5**
Vancomycin	Easy	S	1	100.0	553	99.8	116	100.0	6	100.0	192	100.0	-	-	2	100.0	**870**	**99.9**
Total			**21**	**85.7**	**3 560**	**95.1**	**432**	**93.3**	**1 880**	**97.0**	**615**	**92.4**	**-**	**-**	**25**	**60.0**	**6 533**	**95.1**

n: number of reporting laboratories; '-': no data; shaded cells: Below the threshold of satisfactory concordance (80%).

** n<5 laboratories reported concordant results.*
***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, or added to the latest EUCAST clinical breakpoint table.*
Percentages may not total 100% due to rounding. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

Feedback survey of participating laboratories

A link to the feedback survey was shared with all contacts in the participating laboratories via email on 10 October 2024, with a deadline to reply by 31 October 2024. The survey questions can be found in Annex 2. In total, 192 laboratories provided feedback (21.1% of the 912 laboratories submitting results), similar to the response rate in 2023 (21.5%) [3]. Of these, 120 (62.5%) reported having taken corrective action based on their EQA results. The main actions taken were re-testing of isolate(s), verification of reagents, evaluation of the procedures, review of standard operating procedures and updating/validating methods. For 37 (19.3%) of the 192 laboratories, all EQA analytical test results conformed to expected results and no further action was taken.

Ninety-eight (51.0%) laboratories replied that they would use the results as documentation for accreditation and/or licensing purposes. This is a little higher than in 2023 (48.1%).

Overall, 171 (88.6%) laboratories were satisfied with the individual evaluation report. This is a small decrease from 2023 (92.5%). Sixteen laboratories provided additional comments, and the majority of the comments were related to their own results. Twenty-one laboratories provided suggestions for improvement of the next EQA. Some laboratories asked for additional information, such as the AMR genes detected in EQA strains. Information on AMR genes had been uploaded to the website when the evaluation reports were released, and all participants were informed via email.

Some laboratories indicated that they would appreciate receiving information on the results obtained by other laboratories and the inclusion of stratification by AST methodology, to enable comparisons. Comparison between laboratories is already included in national summary reports shared with each national EARS-Net EQA coordinator. Other laboratories suggested that the EQA feedback reports might include multi-annual trends in AST results. Comparison of trends over time will be presented in a multiannual report covering four years.

4. Summary and discussion

Participation

As in recent years, all 30 EU/EEA countries participated in the 2024 EARS-Net EQA exercise [2–4]. The 2024 EARS-Net EQA had the most laboratories participating of any EARS-Net EQA to date, and a high percentage of those invited (912 of 983 laboratories; 93.1%) submitted results for validation. This percentage is similar to previous EARS-Net EQA exercises in 2021–2023, for which 90.2–92.2% laboratories submitted results [2–4].

Comparisons between EARS-Net EQA results from different years should only be made with great caution, as the strains included were different in terms of their species and level of difficulty for AST of the antimicrobial agents. Moreover, the EARS-Net EQA was affected by the response to the COVID-19 pandemic in the EU/EEA, as countries reallocated laboratory resources to cope. In 2020, ECDC did not initiate an EARS-Net EQA exercise, and, in 2021, only 642 laboratories registered to participate and 592 submitted results [4].

Speciation and overall AST results

As in previous years, species identification was a component of the EQA exercise in 2024 and the species identification results submitted were in 'excellent' concordance (98.5 to 99.5%) with the expected results for each of the six EQA strains. There was no additional information collected from two laboratories that reported the species incorrectly for all six strains, to determine whether this was a clerical or true error. The laboratories are included in the country reports that were sent to national teams.

The distribution of AST methods used in the 2024 EARS-Net EQA exercise was similar to that observed in previous years, as 55.8% of submitted results were obtained using automated systems (50.3% to 54.7% in previous years), 26.0% using disk or tablet diffusion (27.3% to 39.8% in previous years), and 17.6% using MIC methods including broth microdilution and gradient tests (8.3% to 19.3% in previous years) [2,3,4]. Future EARS-Net EQA may revisit the categories of AST methodology that can be reported, such as permitting 'disk diffusion' and 'tablet diffusion' to be reported separately, rather than in one combined category.

In 2024, 'excellent' concordance was only observed for macro broth dilution (96.3%), and 'very good' concordance using MIC methods including broth microdilution and gradient tests (92.1%), disk/tablet diffusion (91.9%), agar dilution (91.5%), and automated systems (91.5%). More generally, these results indicate that the methods applied by laboratories in Europe are robust and accurate for the species and antimicrobial agents included in this EARS-Net EQA exercise.

Concordance of AST results at national level for the 2024 EARS-Net EQA exercise were not as good as for the 2023 EQA exercise [2]. In 2024, no countries achieved an 'excellent' level of concordance, whereas in 2023, 17 countries achieved an 'excellent' level of concordance. Twenty-eight countries achieved a 'very good' level of concordance in 2024, compared to 13 countries in 2023. Two countries achieved a 'good' level of concordance in 2024.

At laboratory level, almost three quarters of the participating laboratories achieved either a 'very good' (n=527, 57.9%) or 'excellent' (n=140, 15.4%) level of concordance, including two laboratories that reported every interpretation correctly, and almost a quarter of the laboratories (n=213; 23.4%) achieved a 'good' level of concordance. Otherwise, 0.4% laboratories (n=4 in four countries) were below the 'satisfactory' level, and 2.5% laboratories (n=23 in nine countries) achieved a 'satisfactory' level of concordance (i.e. results that could be improved). This indicates that, overall, the participating laboratories are able to produce reliable AST results from clinical samples, complying with the most recent EUCAST guidelines and breakpoints.

Overall, the concordance of all submitted AST interpretations with the expected results was 'very good', as 91.7% of all submitted interpretations were correct. The majority of the AST interpretations for the 71 included strain-antimicrobial agent combinations had 'excellent' concordance with the expected results (n=53; 74.6% of the combinations). This is slightly lower than the percentage of 'excellent' results in EARS-Net EQA exercises from 2023 (78.4%), 2022 (79.3%), and 2021 (80.2%) [2-4]. Otherwise, in the 2024 EQA, four combinations (5.6%) had a 'very good' level of concordance, while 'good' and 'satisfactory' levels of concordance were observed for four (5.6%) and three (4.2%) strain-antimicrobial agent combinations, respectively.

There were also seven (9.9%) strain-antimicrobial combinations that did not achieve a 'satisfactory' level of concordance (≤80%). The lowest level of concordance was observed for the *P. aeruginosa* strain ('2024 EARS-Net EQA 4'), for which only 6.2% of the results submitted for ceftazidime were correct. The same strain also had non-satisfactory levels of concordance for piperacillin (22.9%) and meropenem (61.8%). The other non-satisfactory results were for the *E. coli* strain ('2024 EARS-Net EQA 3') for amikacin (34.0%) and piperacillin-tazobactam (39.9%), for the *K. pneumoniae* strain ('2024 EARS-Net EQA 5') for amikacin (71.5%), and for the *S. aureus* strain ('2024 EARS-Net EQA 6') for oxacillin (72.8%).

Strain '2024 EARS-Net 2' (*E. faecium*) was the strain with the best overall concordance of results, with 'excellent' concordance (98.1%). This is probably related to the relative level of difficulty of AST interpretation for its six included antimicrobial agents, which were deemed to be 'easy'.

The observation that errors were very prevalent for strain-antimicrobial agent combinations classified as 'difficult' (with expected AST results near the clinical breakpoints) may be due to the inherent and acceptable variability of laboratory methods. However, it also suggests that some participants do not always strictly adhere to the most recent EUCAST guidelines.

Results from the feedback survey showed that participants use the results from EARS-Net EQA exercises to identify and implement corrective action for their routine AST procedures, and potentially also for accreditation or licensing purposes.

Common issues identified in results reported by laboratories during this EQA exercise

In previous EARS-Net EQA exercises, 2023 [2], 2022 [3] and 2021 [4], the determination and interpretation of AST results had issues for the following:

- *E. coli* with I and R results for fluoroquinolones (2021)
- *E. coli* with S result for gentamicin (2021)
- *E. coli* with S and R results for amikacin (2022, 2023)
- *E. coli* with R result for tigecycline (2021)
- *E. coli* with S, I and R results for carbapenems (2021)
- *E. coli* with I and R results for ceftazidime (2021, 2022, 2023)
- *E. coli* with S result for cefepime (2022, 2023)
- *E. coli* with S and R results for piperacillin-tazobactam (2022, 2023)
- *E. coli* with S result for amoxicillin-clavulanic acid (2021)
- *K. pneumoniae* with I and S results for imipenem and meropenem (2021, 2023)
- *K. pneumoniae* with R and S results for cefepime (2021, 2023)
- *K. pneumoniae* with S result for amikacin (2023)
- *P. aeruginosa* with I result for levofloxacin (2022)
- *A. baumannii* with R results for tobramycin and gentamicin (2022)
- *A. baumannii* with R result for amikacin (2023)
- Prediction of not-HLAR profile for *E. faecium* (2023)
- Other issues related to species not included in the 2024 EARS-Net EQA (*Streptococcus pneumoniae*).

The laboratories participating in the 2024 EARS-Net EQA exercise reported issues for several of the same species-antimicrobial combinations that were problematic in previous EQA exercises, specifically:

- *E. coli* with S result for amikacin
- *E. coli* with R result for piperacillin-tazobactam
- *E. coli* with I result for cefepime
- *E. coli* with I result for ceftazidime
- *K. pneumoniae* with S result for amikacin
- *K. pneumoniae* with I result for cefepime
- *K. pneumoniae* with I result for imipenem
- *A. baumannii* with R result for tobramycin.

Furthermore, prediction of a negative HLAR profile from gentamicin results remained slightly problematic for *E. faecium* which was also observed in previous EQAs, but it is suggested that these results reflect a misinterpretation of the EQA protocol rather than problems with the methods applied by the laboratories.

New issues were observed in the 2024 EARS-Net EQA that had not been problematic in past EARS-Net EQAs, specifically:

- *S. aureus* with BORSA profile (S result for cefoxitin and R result for oxacillin)
- *P. aeruginosa* with I result for meropenem
- *P. aeruginosa* with R result for ceftazidime
- *P. aeruginosa* with R result for piperacillin
- *P. aeruginosa* with I result for piperacillin-tazobactam.

The results from the 2024 EARS-Net EQA did not highlight any systematic underperformance of a certain AST method when compared to other reported methods, and the deviations were generally distributed throughout all of the methods applied. From the commonly applied methods, the gradient test was the one with the lowest concordance of submitted results, even though it achieved 90.7% of accurate results. However, there were three situations where a specific method seemed to influence the percentage of correct results:

- The use of disk/tablet diffusion for AST of cephalosporins in *E. coli* had worse performance than other methods.
- The use of MIC methods was not adequate for prediction of cefoxitin susceptibility in *S. aureus.*
- The gradient test performed particularly badly for prediction of AST results of beta-lactam agents in P. *aeruginosa.*

Overall, results of the 2024 EARS-Net EQA exercise did not show systematic overestimation or underestimation of AMR in the EU/EEA, with deviations being distributed across both types of errors (MEs and VMEs). However, they indicate that there are still difficulties and that there has been a lack of improvement in the prediction of AST profiles for beta-lactam antimicrobials in *E. coli* and *K. pneumoniae*. The results also support a continuing trend across species of difficulties in predicting AST results for aminoglycosides. The results reveal that AST of *S. aureus* and *P. aeruginosa* bacterial isolates with difficult or unexpected resistance profiles is problematic.

5. Conclusions

The species identification results submitted (with 99.2% correct) strongly suggest that the species data reported to EARS-Net are accurate overall.

The AST interpretations submitted also imply that AST data reported to EARS-Net are mostly accurate, although MEs were observed for 4.1% and VMEs for 4.2% of the reported interpretations. Both MEs and VMEs suggest the possibility for sub-optimal treatment outcomes, albeit in a small percentage of bloodstream infections. The MEs and VMEs detected in this EARS-Net EQA exercise included strain-antimicrobial agent combinations that were classified as 'easy' (with expected AST results far from the clinical breakpoints). This suggests that some participating laboratories did not always strictly adhere to the most recent EUCAST guidelines.

For specific species, certain antimicrobial agents or groups presented higher percentages of deviations, namely beta-lactam agents in *P. aeruginosa*, piperacillin-tazobactam and certain cephalosporins in *E. coli*, cefepime and imipenem in *K. pneumoniae*, oxacillin and cefoxitin in *S. aureus*, and aminoglycosides in *E. coli*, *K. pneumoniae* and *A. baumannii*. Some of these problematic species-antimicrobial combinations had been observed in previous EQA exercises. These problematic cases highlight an opportunity for improvement at EU/EEA level.

The findings may also indicate that AMR is reported heterogeneously in the EU/EEA. The VMEs (R → S/I) showed a tendency to under-report resistance to piperacillin-tazobactam in *E. coli*, resistance to ceftazidime and piperacillin in *P. aeruginosa* and resistance to tobramycin in *A. baumannii*. Conversely, the MEs (S → R or I → R) indicate a trend of over-reporting of resistance to amikacin, cefepime and ceftazidime in *E. coli*, resistance to amikacin, cefepime and imipenem in *K. pneumoniae*, and resistance to meropenem and piperacillin-tazobactam in *P. aeruginosa*. The results observed for *S. aureus* point to potential over-reporting of MRSA profiles in the EU/EEA, revealing that the methods applied for AST, especially of cefoxitin, have a noticeable impact on those results.

The 2024 EARS-Net EQA exercise also revealed a continued tendency to incorrectly report high-level aminoglycoside resistance in enterococci, however this was probably due to misinterpretation of the EQA protocol. These results do not seem to indicate anomalous reporting of resistance to aminoglycosides in *E. faecium* in the EU/EEA, nor do they illustrate problems with the methods applied by the laboratories.

One frequent justification for the submission of unexpected results was the inherent method variability of plus or minus one dilution in MIC methods, especially when the expected MIC values corresponded to borderline concentrations very close to the clinical breakpoints, which increased the likelihood of misclassification.

Some of the strains harboured known genetic mechanisms associated with resistance to certain antimicrobial agents or groups. Although genotypic characterisation of the strains was outside the scope of this exercise, it is conceivable that laboratories could screen isolates for AMR genetic determinants during their routine procedures. Therefore, when considering both phenotypic and genotypic data, the final reporting of results could present lower proportions of deviations. For example, detection of genes encoding extended-spectrum beta-lactamases in the *E. coli* strain would be likely to promote increased attention in interpretation of AST results for cephalosporins and other beta-lactams, or even prompt confirmatory AST using other methods. However, one possible consequence of detecting AMR genes or mutations is the tendency to further over-report resistant or decreased susceptibility profiles.

The analysis of the overall performance of the different AST methods showed few differences between methods, except for slightly poorer performance in gradient tests overall. Specific shortcomings were observed in AST of *E. coli* for cephalosporins when using the disk/tablet diffusion method, as well as when using gradient tests for AST of beta-lactam agents in *P. aeruginosa*, and with MIC methods for AST of cefoxitin in *S. aureus*.

In conclusion, there is no exclusive pattern of over- or under-reporting of decreased susceptibility profiles in the EU/EEA.

6. Recommendations

Participating laboratories observing errors in their EQA exercise results should review their AST methods and reporting practices and confirm that the AST protocols in use are in accordance with the latest EUCAST recommendations and guidelines, and that the most current breakpoints are applied.

Furthermore, results from this EQA exercise indicate that some inaccuracy, through both under- and overestimation of AMR percentages, may occur in Europe. Although additional data from genotypic analyses of AMR genes or chromosomal point mutations of the EQA strains might help explain some of the errors reported by the participating laboratories, the focus of this EQA exercise was phenotypic testing. Overall, AST guidance, and surveillance and control efforts should consider the specific deviations observed for each specific antimicrobial agent or group, with particular attention to AST results close to current breakpoints. This is particularly relevant for the low performance of AST for aminoglycosides across species; the problems with determining AMR profiles for beta-lactam antimicrobials in *Enterobacterales* and *P. aeruginosa*, and the difficulty of detecting the BORSA profile for *S. aureus*.

Laboratories that participate in the EARS-Net surveillance scheme should review their individual performance in this EQA exercise and revisit all areas where they did not achieve the intended results. It would be advisable for laboratories that reported errors in AST results that were not common in this EQA exercise to review their methodologies and procedures, as suggested below.

- Strengthening awareness and potentially seeking advice regarding AST and reading of results for the problematic species-antimicrobial combinations detected in the EARS-Net EQA exercises.
- Revising criteria for performing and reading results for aminoglycosides susceptibility testing, since the variability in the AST results for aminoglycosides may have been due to differences in media composition.
- Revising criteria for the performance and reading of results for species-antimicrobial agent combinations that may be associated with differential expression of AMR genes, such as for β-lactam antimicrobials where the AST result obtained is close to a breakpoint, or where the results for antimicrobial agents within the same subclass are different – for example, performing screening tests to detect the genes encoding for extended-spectrum β-lactamases, AmpC enzymes or carbapenemases [10-12]. Results from these screening tests should not influence the quantitative or qualitative results obtained in previous susceptibility testing runs, but can provide valuable additional information for the reporting of AST results and guide re-testing strategies.
- Opting to use the recommended AST methods for each species-antimicrobial agent combination being tested and confirming that the AST protocols in use are in accordance with the latest EUCAST recommendations and guidelines (including the general or specific recommendations regarding the performance, interpretation and evaluation of AST for certain species-antimicrobial agent combinations). It is also important to ensure that adequate control strains are being used and their results monitored to guarantee reliability of results.
- Becoming familiar with EUCAST recommendations regarding AST results within the ATU or results near the clinical breakpoints.
- Ensuring that the relevant quality management systems and control measures are in place, including but not limited to the monitoring of AST results over time, to allow detection of random and systematic deviations.
- Strengthening awareness of method variability when applying the different AST methods, especially those showing lower percentages of concordance in this EQA exercise and previous EQA exercises.
- Seeking advice from national stakeholders, such as National Reference Laboratories, National Antibiotic Committees, and national public health institutes, to ensure attainment of compliance with national and international guidelines.

Continued regular participation in the annual EQA exercise by the laboratories reporting to EARS-Net supports the evaluation and review of their performance in species identification and AST for clinical practice. It will also enable the identification and monitoring of those species-antimicrobial agent combinations that may be problematic when performing AST and for which improvement is possible, facilitating the correct interpretation of AST results reported to EARS-Net.

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Annex 1. List of participating countries

Table A1. Number of laboratories receiving material and submitting results for the 2024 EARS-Net EQA exercise

EU/EEA country	Number of laboratories receiving material for the EQA exercise	Number of laboratories submitting data		Number of laboratories included in the analysis of AST results	
	N	N	%	N	%
Austria	39	39	100.0	39	100.0
Belgium	28	28	100.0	28	100.0
Bulgaria	24	20	83.3	20	100.0
Croatia*	37	36	97.3	35	97.2
Cyprus*	10	9	90.0	8	88.9
Czechia	49	48	98.0	48	100.0
Denmark	6	4	66.7	4	100.0
Estonia	11	10	90.9	10	100.0
Finland	12	11	91.7	11	100.0
France	58	49	84.5	49	100.0
Germany	22	22	100.0	22	100.0
Greece	44	42	95.5	42	100.0
Hungary	24	23	95.8	23	100.0
Iceland	1	1	100.0	1	100.0
Ireland	34	31	91.2	31	100.0
Italy	208	191	91.8	191	100.0
Latvia	14	12	85.7	12	100.0
Liechtenstein	1	1	100.0	1	100.0
Lithuania	15	14	93.3	14	100.0
Luxembourg	5	5	100.0	5	100.0
Malta	1	1	100.0	1	100.0
Netherlands	34	30	88.2	30	100.0
Norway	17	16	94.1	16	100.0
Poland	70	69	98.6	69	100.0
Portugal	107	99	92.5	99	100.0
Romania	22	20	90.9	20	100.0
Slovakia	14	12	85.7	12	100.0
Slovenia	11	11	100.0	11	100.0
Spain	43	40	93.0	40	100.0
Sweden	19	18	94.7	18	100.0
Total	**980**	**912**	**93.1**	**910**	**99.8**

** One laboratory was excluded from the antimicrobial susceptibility testing evaluation because all species identifications were incorrect.*

Annex 2. Feedback survey questionnaire

EARS-Net EQA 2024 feedback survey

Fields marked with * are mandatory.

Disclaimer

The European Commission is not responsible for the content of questionnaires created using the EUSurvey service - it remains the sole responsibility of the form creator and manager. The use of EUSurvey service does not imply a recommendation or endorsement, by the European Commission, of the views expressed within them.

Dear Participant,
Recently you have participated in an ECDC external quality assessment exercise. To ensure maximum benefit we hereby invite you to answer this short survey. Please note ECDC will receive all your responses anonymised.

Fields marked with * are mandatory.

* **Question 1:** Regarding any of your analytical test results that did not conform to the expected results, can you specify which corrective action(s), if any, was/were taken (e.g. review and adjust SOPs, verify reagents)?

- ☐ Not applicable: all our EQA analytical test results conformed to expected results.
- ☐ No corrective actions for non-conformities were taken.
- ☐ Yes, corrective actions were taken.

Please specify which corrective actions were taken.

* **Question 2:** Are results of this EQA exercise to be used as documentation for accreditation and/or licensing purposes for the method(s) used in your laboratory?

- ☐ Yes.
- ☐ No.
- ☐ Not applicable.

Please specify.

* **Question 3:** Were you satisfied with the EQA report of results specific to your laboratory?

- ☐ Yes.
- ☐ No.

If no, please specify.

Question 4: Do you have any suggestions that would make the EQA scheme more useful?

Question 5: Do you have any suggestions to improve the next EARS-Net EQA exercise?

On behalf of the ECDC Antimicrobial Resistance and Healthcare-Associated Infections Disease Programme and the Technical University of Denmark (DTU), many thanks for your participation in this EQA exercise and follow-up survey. The anonymised results will be summarised in the final EQA exercise report and aggregated to monitor the Member States' benefits from all EQA exercises commissioned each year by ECDC.

Submit

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