EURL-PH-AMR

EU Reference Laboratory for Public Health on Antimicrobial Resistance in Bacteria

GA 101194806

Deliverable number: D7.2

Deliverable name: Summary report on EARS-Net EQA 2025

Funded by the European Union

Document overview

Deliverable number	D7.2
Deliverable name	Summary report on EARS-Net EQA 2025
Work package number / name	WP7
Task number / name	T7.1 Conducting phenotypic AMR EQA for local clinical laboratories (EARS-Net)
Due date	M13
Reviewed and agreed by ECDC	10 March 2026
Revision (requested by the Commission)	No revisions, 22 May 2026
Dissemination level	Public
Authors	Birgitte Helwigh and Ana Rita Rebelo
Lead Beneficiary	DTU Food
Project website	https://www.eurl-ph-amr.dk/

This report was produced by Technical University of Denmark, National Food Institute (DTU Food), in their capacity as a consortium member of the European Union Reference Laboratory for Public Health on Antimicrobial Resistance in Bacteria (EURL-PH-AMR) under EU Grant Agreement Number 101194806.

Authors
Birgitte Helwigh and Ana Rita Rebelo, National Food Institute, Technical University of Denmark.

Acknowledgements
The authors would like to acknowledge the role of the European Centre for Disease Prevention and Control (ECDC) in coordinating the European Union Reference Laboratory (EURL) network.

The authors further thank Pete Kinross (ECDC) for technical advice and peer-review support during the development of this report.

The authors acknowledge René S. Hendriksen for his support.

The authors also acknowledge Statens Serum Institut (SSI) and EUCAST Development Laboratory (EDL) for their support.

The authors further wish to express their sincere appreciation to the technical staff in the Research Group for Global Capacity Building, National Food Institute, Technical University of Denmark, for their essential contributions to the work supporting this report.

Suggested citation
European Union Reference Laboratory for Public Health on AMR (EURL-PH-AMR). External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2025. Denmark: EURL-PH-AMR; 2026.

ISBN: 978-87-7586-080-7

Provider of the EARS-Net EQA:
Research group for Global Capacity Building
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby, Denmark

EARS-Net EQA Coordinator: Birgitte Helwigh
earsnet-eqa@food.dtu.dk
1. edition, 2026

Contents

Tables

Figures

Abbreviations

AMR	Antimicrobial resistance
ARG	Antimicrobial resistance genes
AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
CLSI	Clinical and Laboratory Standards Institute
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
EQA	External quality assessment
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	'Susceptible, increased exposure'
ME	Major error
MIC	Minimum inhibitory concentration
PM	Point mutation (chromosomal)
R	'Resistant'
S	'Susceptible, standard dosing regimen'
s.d.	Standard deviation
VME	Very major error

Executive summary

This report describes the results of the 2025 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 European Union/European Economic Area (EU/EEA) countries were eligible to participate if they were participants in EU4Health (n=29, i.e. all except Liechtenstein). In total, all 29 eligible EU/EEA countries participated in this EARS-Net EQA exercise.

The 2025 EARS-Net EQA exercise aimed to assess the quality of species identification by participating laboratories; assess the accuracy of the qualitative AST results reported by participating laboratories; and evaluate the overall comparability of routinely collected AST results between laboratories and EU/EEA countries.

In each annual EARS-Net EQA exercise, eligible laboratories are identified by National EARS-Net EQA Coordinators, designated by the Coordinating Competent Body in each EU/EEA country. Participating laboratories are requested to identify the species of six bacterial strains and to submit AST results for the antimicrobials included in EARS-Net surveillance, using the AST methods that they apply routinely.

In 2025, the panel of six EQA strains consisted of *Acinetobacter baumannii* (n=2)*, Escherichia coli, Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* (

Table 1). The strain '2025 EARS-Net 6' (*S. pneumoniae*) exhibited reduced viability compared to the other EQA strains, which is not uncommon for *S. pneumoniae* strains. Therefore, the instructions included in the EQA package for each participating laboratory strongly encouraged the recipient laboratory to process the sample labelled '2025 EARS-Net 6' immediately upon receipt.

On 3 June 2025, the six strains were distributed via the National EARS-Net EQA Coordinators to 977 laboratories in 29 EU/EEA countries. An EQA webtool was opened to receive submission of results between 4 June and 10 August 2025. Results were submitted by 895 laboratories (91.6%).

Overall, 762 laboratories (85.1%) followed EUCAST v15.0, 94 (10.5%) followed previous versions of EUCAST guidelines, and 38 (4.3%) followed national guidelines based on EUCAST (Comité de l'Antibiogramme de la Société Française de Microbiologie guideline or NordicAST guideline). One laboratory (0.1%) reported using CLSI and was excluded, and thus EQA results were evaluated for 894 laboratories (91.5%).

As in previous EARS-Net EQA exercises [2-5], concordance of species and AST interpretations with expected AST 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.

In total, 4 917 (99.7%) of the 4 932 evaluated species identification results were correct. By mid-July 2025, 23 countries had reported that at least one laboratory was unable to revive the strain '2025 EARS-Net 6' (*S. pneumoniae*). Therefore, the EQA coordination team decided that laboratories that did not report results for this strain would still be eligible to receive certificates of participation, as long as they submitted AST interpretations for the remaining five strains. Consequently, the number of laboratories submitting results for this strain (n=490 laboratories) was lower when compared to the other strains included in this EQA (mean: 888 labs). Nonetheless, there was 'excellent' concordance between the reported and expected species results, for all six strains, ranging from 98.8% to 100%.

The interpretation of AST results was evaluated only for samples with correctly identified species. The evaluation was conducted according to the clinical breakpoints in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints Tables v15.0 [7], applying the EUCAST categories 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R).

The 2025 EARS-Net EQA exercise used the same scoring system as in the 2023 and 2024 EARS-Net EQAs to evaluate reported interpretations of AST results. It incorporated an assessment of both the 'level of difficulty' and the 'severity of error' for each strain-antimicrobial combination. The level of difficulty was classified as either 'easy' or 'difficult', reflecting the likelihood of obtaining an incorrect AST result. The system assigned higher scores to 'difficult' results than 'easy' ones, while penalising errors in 'easy' results more severely. The severity of error was categorised into three levels: very major error (VME), major error (ME), and no error. A VME corresponded to false susceptibility: the expected result was R, but the reported result was S or I. A ME corresponded to false resistance: the expected result was S or I, but the reported result was R. The scoring system penalised VMEs more heavily for 'easy' results than for 'difficult' results and did not penalise MEs when the test was considered 'difficult'.

In total, 56 924 interpretations of AST results were evaluated, reported by 894 laboratories. Overall, the submitted AST interpretations were in 'very good' concordance with the expected results, with 94.4% (n=53 742) being correct. Otherwise, MEs and VMEs were observed for 2.6% and 3.0% of interpretations, respectively.

A total of 86 strain-antimicrobial combinations were tested for antimicrobial susceptibility in the 2025 EARS-Net EQA exercise. The majority of the combinations had results in 'excellent' concordance with the expected results (n=68 or 79.1% of the combinations). A 'very good' concordance was achieved for two combinations, 'good' concordance was achieved for six, and 'satisfactory' concordance was achieved for three. The remaining seven combinations were under the threshold for 'satisfactory' concordance.

At country level, 14 countries achieved 'excellent' concordance with expected results and 15 countries achieved 'very good' concordance.

At laboratory level, 52.7% (n=471) of the laboratories showed 'excellent' concordance, 39.1% (n=350) had 'very good' concordance, 6.9% (n=62) had 'good' concordance, 0.7% (n=6) had 'satisfactory' concordance and 0.6% (n=5) were below 'satisfactory' concordance.

Among the 56 924 evaluated AST results, the most frequently reported AST methods generally showed 'very good' concordance with the expected results. The automated system was most commonly used (53.0% of all tests with 94.9% correct results, with 'very good' concordance), followed by disk or tablet diffusion (27.6% of all tests with 95.6% correct results, with 'excellent' concordance), broth microdilution (11.0% of all tests and 92.6% correct, with 'very good' concordance) and gradient test (8.2% of all tests and 89.7% correct, with 'good' concordance). As in previous EARS-Net EQAs, disk diffusion and tablet diffusion were grouped together; future EARS-Net EQAs may permit reporting them separately.

Overall, the 2025 EARS-Net EQA exercise results did not show a systematic overestimation or underestimation of AMR in the EU/EEA, with deviations being distributed across both types of errors (MEs and VMEs), and country-level results having very similar percentages of MEs and VMEs. However, these results show that there are still inconsistent results between participating laboratories, as shown below in this Executive Summary, within under the subheading 'Summary of results for each EQA strain'. There was no improvement in the prediction of AST

profiles for carbapenems for the *E. coli* and *K. pneumoniae* EQA strains since the previous EARS-Net EQAs. Moreover, the combinations of carbapenems and beta-lactamase inhibitors recently added to European surveillance also appear to pose a challenge for the laboratories, as well as cefiderocol. The results support a continuing trend across species of difficulties in predicting AST results for aminoglycosides and show that AST of azithromycin for *S. pneumoniae* remains technically challenging. Determination of the AST profile for colistin in *A. baumannii* and the AST of levofloxacin for *K. pneumoniae* were challenging for participants, which had not been observed in previous EQAs.

There were situations where a specific method seemed to influence the percentage of correct results. Results from this EQA confirm EUCAST recommendations to use specific method for certain antimicrobials, namely disk diffusion for cefiderocol, norfloxacin and oxacillin, and broth microdilution for colistin. Specific shortcomings were observed for some methods: overall, gradient test had the worst performance for AST of colistin; gradient test had particularly poor performance for AST of azithromycin for *S. pneumoniae*; broth microdilution and gradient test had the worst performance for AST of carbapenems and their combinations with beta-lactamase inhibitors, for Enterobacterales; and automated system had particularly bad performance for AST of aztreonam-avibactam in Enterobacterales.

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, to ensure optional standard clinical practice and AMR surveillance reporting activities.

AMR surveillance and control activities, and organisations that develop guidelines for AST, should note the specific deviations in AST results observed for each species and antimicrobial or class during this EQA exercise in 894 clinical laboratories across all EU/EEA countries.

Table 1 Overview of species identification results and antimicrobial susceptibility testing (AST) results reported by clinical laboratories participating in the 2025 EARS-Net EQA exercise

Strain ID	Species and expected AST results for tested antimicrobials*	Species identification		AST results			
		Reported species identifications (N)	Correct species identification (N(%))	Reported AST results (N)	Correct AST interpretations (N(%))	Major errors (N (%))	Very major errors (N (%))
2025 EARS-Net 1	*Klebsiella pneumoniae* S: AZA, COL, GEN, MEV I: LVX R: AMC, AMK, CAZ, CIP, CRO, CTX, CZA, CZT, ETP, FDC, FEP, IMR, IPM, MEM, MFX, OFX, TOB, TZP	886	883 (99.7%)	15 589	14 127 (90.6%)	949 (6.1%)	513 (3.3%)
2025 EARS-Net 2	*Acinetobacter baumannii* S: COL, GEN, TOB R: AMK, CIP, FDC, IPM, LVX, MEM	888	884 (99.5%)	6 824	6 128 (89.8%)	140 (2.1%)	556 (8.1%)
2025 EARS-Net 3	*Staphylococcus aureus* S: DAP, LNZ, NOR, RIF, VAN I: CIP, LVX R: FOX, OXA	890	890 (100%)	6 585	6 506 (98.8%)	73 (1.1%)	6 (0.1%)
2025 EARS-Net 4	*Acinetobacter baumannii* I: FDC R: AMK, CIP, COL, GEN, IPM, LVX, MEM, TOB	887	886 (99.9%)	6 812	6 549 (96.1%)	129 (1.9%)	134 (2.0%)
2025 EARS-Net 5	*Escherichia coli* S: AMK, AZA, COL, TGC R: AMC, AMP, AMX, CAZ, CIP, CRO, CTX, CZA, CZT, ETP, FDC, FEP, GEN, IMR, IPM, LVX, MEM, MEV, MFX, OFX, TOB, TZP	891	890 (99.9%)	17 607	17 033 (96.7%)	62 (0.4%)	512 (2.9%)
2025 EARS-Net 6	*Streptococcus pneumoniae* S: AZM, CLR, CRO, CTX, ERY, MFX, NOR, PEN, OXA I: LVX	490	484 (98.8%)	3 507	3 399 (96.9%)	108 (3.1%)	NA
Total		**4 932**	**4 917 (99.7%)**	**56 924**	**53 742 (94.4%)**	**1 461 (2.6%)**	**1 721 (3.0%)**

** All samples were considered to be obtained from patients with bloodstream infections. The expected SIR results were generated using EUCAST breakpoint tables v15.0. For describing the expected results of the strains included in the 2025 EARS-Net EQA, the following adaptations were made to the EUCAST reporting recommendations: 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 antimicrobials; for Enterobacterales it was assumed that penicillins would be administered intravenously; for cefiderocol in A. baumannii, wild-type isolates were registered as 'S', non-wild type isolates which may be associated with impaired clinical response were registered as 'I' and likely resistant isolates were registered as 'R'; breakpoints were applied for screening antimicrobials regardless of their status as 'screen only'; results from screening antimicrobials were not used for interpretation of other antimicrobials belonging to the same class and instead all AST were performed individually.*
AST: antimicrobial susceptibility testing; S: susceptible, standard dosing regimen; I: susceptible, increased exposure; R: resistant; NA: Not applicable; AMC: Amoxicillin-clavulanic acid; AMK: Amikacin; AMP: Ampicillin; AMX: Amoxicillin; AZA: Aztreonam-avibactam; AZM: Azithromycin; CAZ: Ceftazidime; CIP: Ciprofloxacin; CLR: Clarithromycin; COL: Colistin; CRO: Ceftriaxone; CTX: Cefotaxime; CZA: Ceftazidime-avibactam; CZT: Ceftolozane-tazobactam; DAP: Daptomycin; ERY: Erythromycin; ETP: Ertapenem; FDC: Cefiderocol; FEP: Cefepime; FOX: Cefoxitin; GEN: Gentamicin; IMR: Imipenem-relebactam; IPM: Imipenem; LNZ: Linezolid; LVX: Levofloxacin; MEM: Meropenem; MEV: Meropenem-vaborbactam; MFX: Moxifloxacin; NOR: Norfloxacin; OFX: Ofloxacin; OXA: Oxacillin; PEN: Benzylpenicillin; PIP: Piperacillin; RIF: Rifampicin; TEC: Teicoplanin; TGC: Tigecycline; TOB: Tobramycin; TZP: Piperacillin-tazobactam; VAN: Vancomycin.

Summary of results for each EQA strain

Strain **'2025 EARS-Net 1' (*Klebsiella pneumoniae*)** was correctly identified by 99.7% of the laboratories. Out of 23 antimicrobials included in the EQA for the strain, seven AST determinations were expected to be difficult, the most of any included strain in 2025 (Annex 1).

Overall, the AST interpretations reported for the strain were in 'very good' concordance with expected results (90.6%). MEs and VMEs were observed for 6.1% and 3.3% of the reported interpretations, respectively (

Table 1). However, the worst overall results among all strain-antimicrobial combinations included in the 2025 EARS-Net EQA were for this strain. These were the prediction of susceptibility towards meropenem-vaborbactam (76.7% of MEs) and towards levofloxacin (71.1% of MEs). These AST determinations were classified as 'difficult', and so it 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 or +/-3 mm) would lead to an incorrect AST interpretation. In both cases, the errors were reported across most methods but were especially frequent among those reported to be from automated systems and disk/tablet diffusion.

VMEs were observed for imipenem (13.0%), imipenem-relebactam (13.5%) and meropenem (13.0%), with higher proportions of errors associated with broth microdilution and gradient test. Characterisation of resistance to cefiderocol was also challenging (21.6% of VMEs) with problematic results for all methods except disk/tablet diffusion. VMEs were also reported for amikacin (10.2%) especially when using broth microdilution. All, except for imipenem-relebactam, were classified as being 'difficult' determinations and therefore the errors are likely attributable to the inherent method variability since the closeness of the expected MIC or diameter values to a breakpoint increased the likelihood of misclassification. Also, AST results for carbapenems [8,9], aminoglycosides [10,11] and cefiderocol [12,13] are known to be affected by the choice of testing methods and/or material, which may also partially explain the suboptimal observed results.

The determination of AST results for imipenem-relebactam was considered 'easy'. Therefore, the observed errors should not be associated with the inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures, potentially exacerbated by the choice of AST method or specific materials used for AST.

Overall, determination of susceptibility to aztreonam-avibactam and of resistance to ciprofloxacin were excellent (96.9% and 97.6% of concordance with expected results, respectively). However, the results reported to be from automated systems had poor performance for aztreonam-avibactam (64.3%) and the gradient test showed poor performance for ciprofloxacin (84.2%).

Strain **'2025 EARS-Net 2' (*Acinetobacter baumannii*)** was correctly identified by 99.5% of the participating laboratories. Out of nine antimicrobials included in the EQA for the strain, three AST determinations were expected to be difficult. This was the second most 'difficult' interpretations of the included EQA strains (Annex 1).

Overall, the AST interpretations reported for the strain were in 'good' concordance with expected results (89.8%). MEs and VMEs were observed for 2.1% and 8.1% of the reported interpretations, respectively (

Table 1).

A high proportion of VMEs was observed for cefiderocol (9.6%) and these were distributed across methods, with a better performance for disk/tablet diffusion. That determination was classified as 'difficult' as the expected zone diameter was less than four millimetres away from the ATU, therefore the deviations are likely attributable to the inherent method variability since the borderline expected zone diameter increased the likelihood of misclassification. Moreover, results from AST of cefiderocol are known to be highly variable depending on the method and materials [12,13].

Prediction of resistance to amikacin was problematic (58.1% of VMEs, mainly observed for the automated system), as well as prediction of susceptibility to gentamicin (12.8% of MEs, mainly observed for broth microdilution and disk/tablet diffusion). These deviations can be attributed to the inherent method variability, since the determinations were classified as 'difficult' because results within the acceptable variation range (+/-1 dilution or +/-3 mm) would lead to an incorrect AST interpretation. Additionally, results from AST of aminoglycosides are known to be variable depending on the method and materials [14].

Strain **'2025 EARS-Net 3' (*Staphylococcus aureus*)** was correctly identified by 100% of the laboratories. Only one of the nine AST determinations were expected to be difficult (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (98.8%). MEs and VMEs were observed for 1.1% and 0.1% of the reported interpretations, respectively (

Table 1).

There were no systematic methodological issues identified in the submitted AST results for this strain. However, automated system showed worse performance for AST of norfloxacin (81.3%), when compared with disk/tablet diffusion (98.2%).

Strain **'2025 EARS-Net 4' (*Acinetobacter baumannii*)** was correctly identified by 99.9% of the laboratories. Only one of the nine AST determinations were expected to be difficult (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (96.1%). MEs and VMEs were observed for 1.9% and 2.0% of the reported interpretations, respectively (

Table 1).

Prediction of resistance to colistin was problematic with a high proportion of VMEs (17.0%). These deviations are likely attributable to systematic or random errors in the participating laboratories' procedures since the determination was considered 'easy'. They were distributed across methods, with a better performance for broth microdilution and the worst performance for gradient test.

A high proportion of MEs was observed for cefiderocol (35.7%). That determination was classified as 'difficult' as the expected zone diameter was less than four millimetres away from the ATU, therefore the deviations are likely attributable to the inherent method variability since the borderline expected zone diameter increased the likelihood of misclassification. Moreover, results from AST of cefiderocol are known to be highly variable depending on the method and materials [12,13]. However, the concordance of AST results for disk/tablet diffusion (59.9%) was not higher than for other methods, which was not in agreement with results obtained for cefiderocol in other strains included in this EQA. Additional analysis of the quantitative results submitted by the participants for cefiderocol revealed a tendency of assigning the wrong S/I/R categories to diffusion results (and in lesser extent to dilution results). This was potentially due to conflicts between EUCAST guidelines (which do not contain clinical breakpoints for cefiderocol in the species but instead include information in the Notes), when compared with the instructions for the EQA (where participants must use S/I/R categories for several situations that are not necessarily categorised as S/I/R during routine laboratory practice). However, this misattribution of S/I/R categories was not observed in the results for cefiderocol in the other *A. baumannii* strain nor for the Enterobacterales strains, for which the expected result was R. This suggests that participants did not struggle with the concept of using S/I/R categories to report results of cefiderocol in *A. baumannii* for the purpose of this EQA. Instead, participants may not strictly follow instructions presented within the Notes sections of the EUCAST guidelines.

Strain **'2025 EARS-Net 5' (*Escherichia coli*)** was correctly identified by 99.9% of the laboratories. None of the 26 AST determinations were expected to be difficult (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (96.7%). MEs and VMEs were observed for 0.4% and 2.9% of the reported interpretations, respectively (

Table 1).

VMEs were observed for imipenem (8.7%), imipenem-relebactam (12.3%), meropenem (15.7%) and meropenem-vaborbactam (29.5%), with higher proportions of errors associated with broth microdilution and gradient test. Characterisation of resistance to cefiderocol was also challenging (16.2% of VMEs) with problematic results across all methods except disk/tablet diffusion.

All expected result were classified as 'easy' since the expected MICs or diameters were not close to the clinical breakpoints, and therefore incorrect results are unlikely to be due to inherent method variability. The deviations are likely attributable to systematic or random errors in the participating laboratories' procedures, potentially exacerbated by variations in the methods and/or material used for testing, which are known to affect AST results for carbapenems, aminoglycosides and cefiderocol.

Overall, determination of susceptibility to amikacin and aztreonam-avibactam were excellent (96.1% and 96.0% of concordance with expected results, respectively). However, the gradient test showed poor performance for amikacin (76.9%) and the automated system showed poor performance for aztreonam-avibactam (71.4%).

Strain **'2025 EARS-Net 6' (*Streptococcus pneumoniae*)** was correctly identified by 98.8% of the laboratories.

None of the 10 AST determinations were expected to be difficult (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (96.9%). MEs were observed for 3.1% of the reported interpretations, and no VMEs could be observed as the strain was susceptible towards all included antimicrobials (

Table 1).

Prediction of susceptibility to azithromycin was technically challenging with a high proportion of MEs (26.7%). These deviations are likely attributable to systematic or random errors in the participating laboratories' procedures since the determination was considered 'easy'. They also appear to be associated to a specific methodology, since errors were more prevalent for gradient test when compared with other methods.

Overall, determination of susceptibility to oxacillin was excellent (98.1%), but automated system (85.7%) had worse performance than disk/tablet diffusion (98.3%).

1. Introduction

From 2000 to 2009, an annual external quality assessment (EQA) exercise for antimicrobial susceptibility testing (AST) was conducted for clinical laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS). In 2010, this activity was transferred to the European Centre for disease Prevention and Control (ECDC) as the European Antimicrobial Resistance Surveillance Network (EARS-Net). This report presents and summarises the results of the 2025 EARS-Net EQA performance by participating laboratories.

In 2025, the EARS-Net EQA exercise was organised by the National Food Institute, Technical University of Denmark (DTU Food), in their capacity as a consortium member of the European Union Reference Laboratory (EURL) for Public Health on Antimicrobial Resistance in Bacteria (EURL-PH-AMR). In 2021-2024, DTU Food organised the EARS-Net EQA on the behalf of ECDC under a framework contract.

The 2025 EARS-Net EQA exercise aimed to:

- Assess the quality of species identification by participating laboratories;
- Assess the accuracy of the qualitative AST results reported by participating laboratories; and
- 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 antimicrobials

The 2025 EARS-Net EQA protocol [6] specified that participating laboratories should perform AST according to their routine procedures, using methods such as broth microdilution, agar dilution, use of automated systems, disk or tablet diffusion, gradient tests, or other methods.

The antimicrobials included in this EQA exercise corresponded to the panel of species–antimicrobial combinations under surveillance by EARS-Net [1].

When performing their standard practices, clinical laboratories in the EU/EEA are generally unlikely to conduct AST for every species-antimicrobial combination that can be reported to EARS-Net. For example, many will use the services of reference laboratories for selected isolates or specific antimicrobials. This consideration is discussed in further detail in the section 'Evaluation of EQA result'.

Characteristics of the EQA strains and interpretation of AST results

In the 2025 EQA exercise, participating laboratories were instructed to consider all six samples as if they had been obtained from patients with bloodstream infections.

The EUCAST Clinical Breakpoints Tables v15.0 [7] were used for the interpretation of AST results. 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.

For describing the expected results of the strains included in the 2025 EARS-Net EQA the following adaptations were made to the EUCAST reporting recommendations, and the same instructions were provided to participants in the EQA protocol [6] and test forms available in the EQA website:

- Breakpoints based on ECOFF values (i.e. breakpoints in brackets) were used for interpretation of results when no other relevant EUCAST clinical breakpoints existed, under the assumption that the antimicrobials would be administered in combination with other antimicrobials.
- For Enterobacterales, it was assumed that penicillins would be administered intravenously.
- For cefiderocol in *A. baumannii*, wild-type isolates were categorised as 'S', non-wild type isolates which may be associated with impaired clinical response as 'I', and likely resistant isolates as 'R'.
- Breakpoints for screening antimicrobials were applied irrespective of their 'screen only' status.
- Results from screening antimicrobials were not used to infer results for other antimicrobials belonging to the same class, and instead all AST were performed individually.

This permitted classification of the expected AST results into three categories: susceptible, standard dosing regimen (S), susceptible, increased exposure (I), and resistant (R).

The EQA protocol and test forms furthermore presented reporting instructions for other species included in EARS-Net surveillance, but that were not part of the 2026 EARS-Net EQA.

The expected results were determined based on consensus AST results obtained by DTU Food using broth microdilution and/or disk diffusion, and results from confirmatory testing provided by two other reference laboratories: 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 profiles were then compared with whole-genome sequencing (WGS) data on acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs), generated at DTU Food using the bioinformatics tools ResFinder v4.6.0, AMRFinderPlus v4.0.19, and CARD RGI v6.0.4 (Annex 1).

Following the preparation of the agar swab cultures/charcoal swabs for shipment to participants, MIC determinations were performed again at DTU Food to confirm that the vials contained the correct strains with the expected AST results.

The 2025 EARS-Net EQA included 86 strain-antimicrobial combinations: 56 with an expected interpretation of 'R', five combinations with expected interpretation 'I', and 25 with expected 'S'. The AST determinations were categorised as either 'Easy' or 'Difficult', as described in the section 'Scoring antimicrobial susceptibility results'. In this EQA, 12 determinations were considered 'Difficult (seven 'R', two 'I' and three 'S') and 74 determinations were considered 'Easy' (49 'R', three 'I' and 22 'S').

Procedure for participating laboratories

The 2025 EARS-Net EQA protocol [6] specified that participating laboratories should identify the species of six bacterial strains and subsequently perform AST in accordance with EUCAST recommendations [7] on species included in EARS-Net surveillance. If the species identification was incorrect, the corresponding AST results were not evaluated.

Identification of eligible laboratories

All European Union/European Economic Area (EU/EEA) countries were eligible to participate if they were participants in EU4Health (n=29, i.e. all except Liechtenstein). In total, all 29 eligible EU/EEA countries participated in this EARS-Net EQA exercise. Each participating country designated a National EARS-Net EQA Coordinator for the 2025 EARS-Net EQA exercise. The National EARS-Net EQA Coordinators were requested to provide a list of laboratories eligible for participation, and these laboratories subsequently received an information letter. Since 2019, only laboratories that perform AST in accordance with EUCAST guidelines have been eligible to participate in the EARS-Net EQA exercise.

Distribution of EQA strains to laboratories

On 3 June 2025, DTU Food shipped all of EQA parcels, to each of the National EARS-Net EQA Coordinator, using a single shipping (courier) company. The shipments were following International Air Transport Association (IATA) regulations (UN3373, biological substances category B). Each shipment contained individual laboratory packages for national distribution. The individual packages (double-pack containers, class UN 6.2) were labelled with the address of the respective participating laboratory.

Each package contained:

- Six swabs (five Copan Transystem™ and one Stuarts transport medium) each holding a pure culture of one of the six EQA strains; and
- A cover letter with safety instructions and guidance on how to process the swabs upon arrival at a laboratory.

The strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*) exhibited reduced viability compared to the other EQA strains. Participating laboratories were therefore strongly advised to process the Stuarts transport medium sample labelled '2025 EARS-Net 6' immediately upon receipt.

Instructions for submitting EQA results

The 2025 EARS-Net EQA protocol, test forms and a guide on how to access the password-protected webtool for submission of results were available on the EARS-Net EQA website (https://antimicrobialresistance.dk/ears-net-EQA.aspx). The dedicated password-protected EARS-Net EQA webtool, developed and hosted by DTU Food, enabled participating laboratories to submit EQA results for evaluation using a personal login and password.

The EARS-Net EQA protocol specified that participants should report AST results, including minimum inhibitory concentration (MIC) or zone diameter values along with their respective categorisation as S, I, or R, based on the most recent EUCAST clinical breakpoints (v15.0).

Participants were also asked to provide information regarding:

- The guideline used for interpretation;
- The AST method applied (e.g. agar dilution, automated system, broth microdilution, disk or tablet diffusion, gradient test, macro broth dilution, or other);
- The manufacturers of the materials and reagents employed; and
- Whether the strain would be sent to a reference laboratory for further testing.

The deadline for submission of results was 4 August 2025. One week prior to the submission deadline, a reminder email was sent to all enrolled laboratories. At submission deadline, another email was sent to the enrolled laboratories,

extending it until 10 August 2025. Upon submission, an automated email was sent to all designated contacts from the respective laboratory, containing a report of the submitted results.

Laboratories received a certificate for participation if they had submitted AST interpretations for the five EQA strains '2025 EARS-Net 1' to '2025 EARS-Net 5'. Submitting results for strain '2025 EARS-Net 6' was not required to receive a certificate, as it had exhibited reduced viability compared to the other EQA strains, and 23 countries had reported that at least one laboratory was unable to revive the strain by mid-July 2025. This was communicated to participating laboratories in October 2025 when they were informed that the certificates were available for download. Laboratories could access their own certificate via the password protected webtool, while National EARS-Net EQA Coordinators received copies of all certificates issued for the laboratories in their respective countries.

Participants were also encouraged to complete an electronic feedback survey, accessible via a link distributed by email, with support the continuous improvement of future EQA exercises. The evaluation questions were provided by ECDC (Annex 3).

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 corresponding categorisation as S, I or R, following the specific EQA reporting instructions including the adaptations to EUCAST reporting guidelines (as described in Section 2 and in the EQA protocol [6] and test forms). Only the qualitative 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 corresponding interpretations of AST results were not evaluated for that strain.

The 2025 EARS-Net EQA protocol defined the scoring system used to evaluate submitted results (

Table 2). Scores were assigned for each strain-antimicrobial combination based on two criteria: the 'level of difficulty' and the 'severity of error' for the submitted AST interpretation.

Level of Difficulty

The level of difficulty reflected the likelihood of misclassification of the AST interpretation and was categorised as either 'easy' or 'difficult'.

The 'Difficult' level included cases where:

- An AST result with a one-fold difference in dilution from the expected MIC value, or with less than four millimetres difference from the expected zone diameter, would result in a different S/I/R interpretation; and/or
- The expected MIC value fell within the area of technical uncertainty (ATU); and/or
- The EUCAST clinical breakpoint had been recently changed or introduced in the latest version of the EUCAST clinical breakpoint tables.

All other situations were categorised as 'Easy'.

Severity of Error

Errors were classified into three categories: very major error (VME), major error (ME), and no error. Both VMEs and MEs were penalized in the scoring system.

- A VME corresponded to false susceptibility: the expected result was R, but the reported result was S or I.
- A ME corresponded to false resistance: the expected result was S or I, but the reported result was R.

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.

The score assigned to each result reflected both the level of difficulty and the severity of error (Table 2). The same scoring system was also applied in the 2023 and 2024 EARS-Net EQA exercises.

Table 2 2025 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

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

Total scores

This report presents the scores of results for all participating laboratories, by EQA strain. However, the total score per laboratory was not calculated, because there are scenarios in which it is not appropriate to compare laboratories according to that aggregation. For example, a laboratory that performed excellently, reporting every AST interpretation correctly for a limited number of strain-antimicrobial combinations, could achieve the same total score as a laboratory that tested a larger number of combinations with some incorrect interpretations. Therefore, as stated in the EQA protocol [6], it is recommended that laboratories analyse their scores for each strain-antimicrobial combination individually.

The National EARS-Net EQA Coordinators received the raw data containing the scores for all laboratories in their respective countries, enabling national-level analyses that consider local and subnational contexts.

It has been a persistent challenge, across all EARS-Net EQA exercises, to define a minimum set of species–antimicrobial combinations that participating laboratories should report, that is both relevant and appropriate for all (sub-)national settings, in all EU/EEA countries. Consequently, as the EARS-Net EQA methodology is designed to support assessment of EARS-Net surveillance data quality, all species-antimicrobial combination that can be reported to EARS-Net are included in the EQA exercise, and laboratories are not penalised for not reporting individual AST interpretations.

Categories for concordance of the submitted results

As in previous EARS-Net EQA exercises [2–5], the concordance of submitted species identification and AST interpretations with the expected results was categorised as follows:

- 'Excellent': ≥95% of interpretations in concordance with expected results);
- 'Very good': >90% to <95%;
- 'Good': >85 to ≤90%; and
- 'Satisfactory': >80 to ≤85%, representing results with the scope for improvement.

Reporting EQA results

Laboratories that submitted results received a laboratory evaluation report for each submitted sample. Laboratories that followed EUCAST guidelines were included in the analysis for this report and the national summary reports.

Contacts from each participating laboratory were notified via email when their evaluation reports became available for download through the password-protected webtool using their personal login and password. An overview of the expected results was made available for download on the EARS-Net EQA website. Contacts could only access the evaluation reports from 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 describing 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 unique codes, known only to the respective 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 2025, all 29 eligible EU/EEA countries participated in the EARS-Net EQA exercise. In total, 977 laboratories were identified by the National EARS-Net EQA Coordinators as eligible to participate and received an information letter. All laboratories enrolled in the exercise.

Overall, 895 laboratories (91.6%) submitted results, representing all 29 participating EU/EEA countries (Figure 1, Annex 2). Eighty-two enrolled laboratories (8.4%) from 19 countries did not submit results, a proportion comparable to previous years (2024: 6.9%, 2023: 8.4%, 2022: 9.8%).

The laboratories reported their institutional designation as follows: 30 national laboratories, 102 regional, 750 local, and 13 categorised as 'other'.

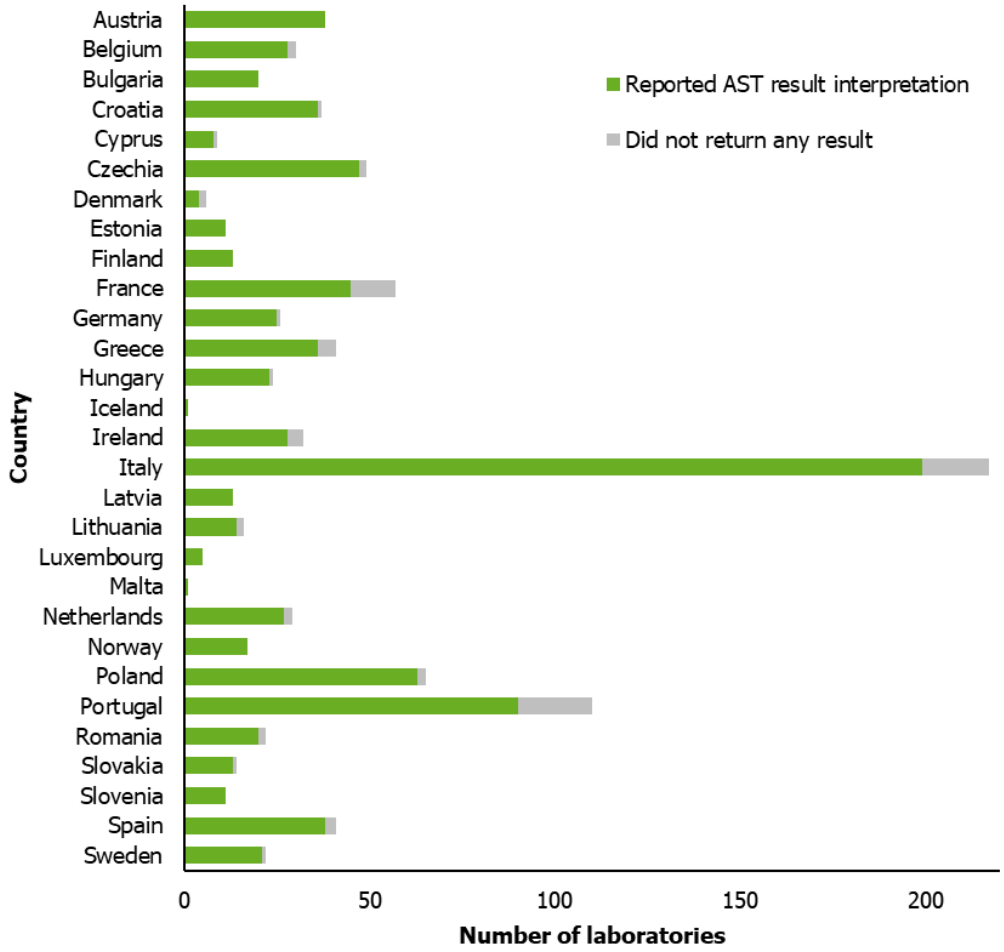
Adherence to the EUCAST guideline is mandatory for participation in the EARS-Net EQA. The guideline is updated annually in the beginning of the year, and laboratories are expected to apply the latest version when analysing results (v15.0 for the 2025 EARS-Net EQA). Overall, 762 laboratories (85.1%) followed EUCAST v15.0, 57 (6.4%) followed EUCAST v14.0, 13 (1.5%) followed EUCAST v13.0, 24 (2.7%) followed another EUCAST version, 36 (4.0%) followed the EUCAST-based CA-SFM guideline (Comité de l'Antibiogramme de la Société Française de Microbiologie), and 2 (0.2%) followed the EUCAST-based NordicAST guideline. One laboratory (0.1%) reported using the CLSI guidelines, therefore data submitted by this laboratory were excluded, as only laboratories that perform AST in accordance with EUCAST guidelines are eligible to participate.

Nine laboratories (three from Czechia, four from Italy, one from Netherlands and one from Romania) entered data into the webtool but did not complete the submission process. Consequently, their data could not be validated and were therefore excluded from the dataset.

In total, results were evaluated for 894 laboratories, corresponding to 91.5% of all laboratories that received the EQA strains. Of these, 876 laboratories (98.0%) submitted AST result interpretations for the five strains required to receive a certificate.

Altogether, the 894 included laboratories submitted EQA results for 5 013 strains, of which 4 932 (98.4%) results included the laboratory's interpretation of AST results. Submitting AST interpretations was a prerequisite for inclusion in the analyses presented in this report, and submissions lacking AST interpretations were excluded from further analysis. In total, 556 laboratories (62.2%) successfully submitted AST interpretations for all six strains, i.e. including strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*), despite the change to the EQA process, during the EQA reporting period, to make the reporting of this strain non-mandatory.

Figure 1 Number of laboratories receiving EQA material and returning interpretation of AST results based on EUCAST guidelines, by country, 2025 EARS-Net EQA exercise



AST: antimicrobial susceptibility testing.

Species identification results

In total, 894 laboratories submitted species identification results and AST interpretations for 4 932 strains, of which 4 917 strains (99.7%) were correct. Therefore the overall concordance between the submitted and expected results for species identification was 'excellent' (≥95%).

An overview of the species identification for the six strains and the corresponding number of laboratories reporting the correct identification is provided in Table 3. There was 'excellent' concordance between the submitted and expected species identification for all six EQA strains. The lowest concordance was reported for strain '2025 EARS-Net 1' (*Streptococcus pneumoniae*) (98.8%).

Table 3 Number and percentage of correct species identifications in the 2025 EARS-Net EQA exercise

Strain ID	Expected species	No. of identifications	No. of correct species identifications	Percentage of laboratories reporting correct species identification
2025 EARS-Net 1	*Klebsiella pneumoniae*	886	883	99.7
2025 EARS-Net 2	*Acinetobacter baumannii*	888	884	99.5
2025 EARS-Net 3	*Staphylococcus aureus*	890	890	100.0
2025 EARS-Net 4	*Acinetobacter baumannii*	887	886	99.9
2025 EARS-Net 5	*Escherichia coli*	891	890	99.9
2025 EARS-Net 6	*Streptococcus pneumoniae*	490	484	98.8
Total		**4 932**	**4 917**	**99.7**

Antimicrobial susceptibility testing results

If the 894 participating laboratories had reported AST interpretation data for every strain-antimicrobial combination included in this EQA exercise, for each strain with correct species identification, a total of 72 229 results would have been generated. Ultimately, the 894 laboratories submitted 56 924 AST interpretations, corresponding to 78.8% of the theoretical maximum.

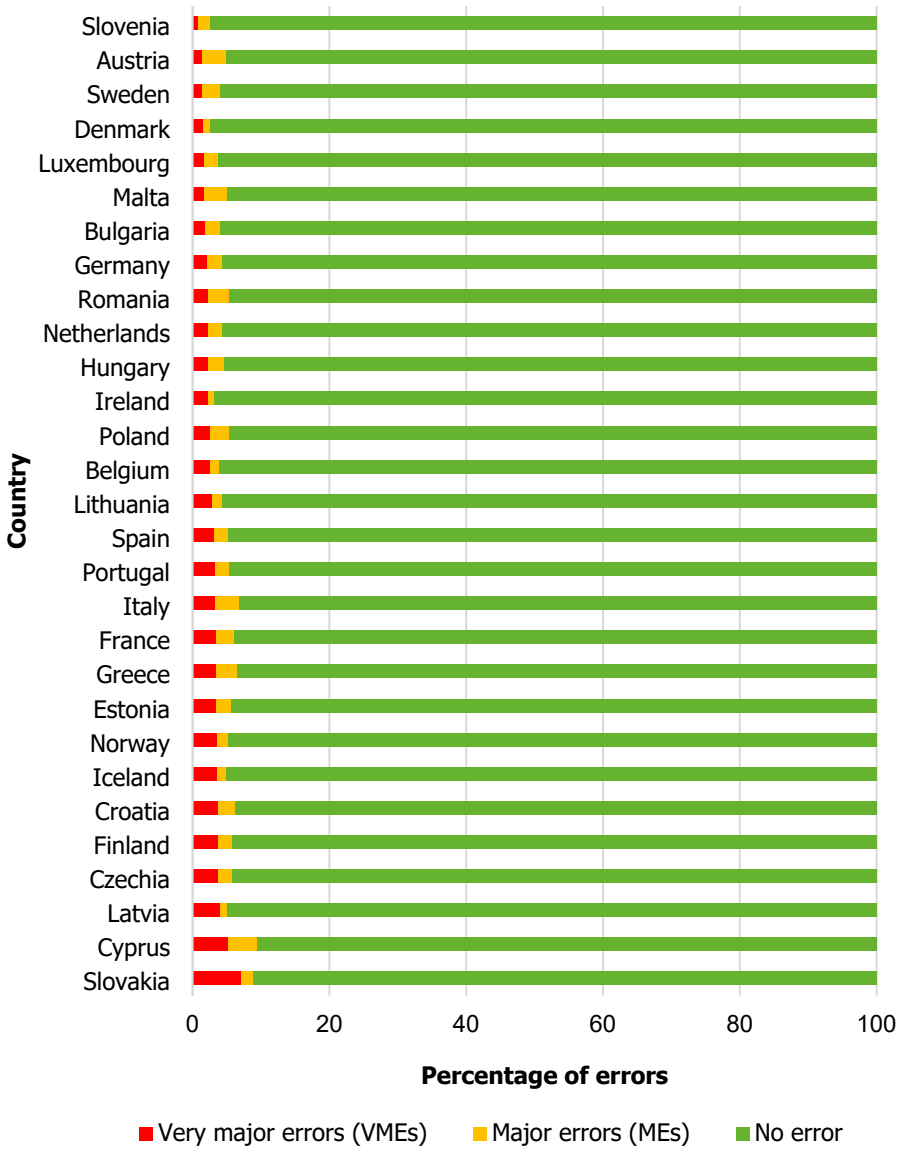
Overall, the interpretations were in 'very good' concordance with expected results, as 94.4% (n=53 742) of the 56 924 reported interpretations were correct. MEs were observed for 2.6% (n=1 461) of the interpretations and VMEs were observed for 3.0% (n=1 721).

At country level, concordance with the expected AST interpretation ranged from 90.6% ('very good') to 97.4% ('excellent'). In total, 14 countries achieved an 'excellent' concordance, while 15 countries achieved a 'very good' concordance.

Among the 29 participating countries, the three countries with the fewest VMEs (Austria, Slovenia and Sweden) had ≤1.5% VMEs, and the three with the most (Cyprus, Latvia and Slovakia) had ≥4.0% VMEs. Similarly, the three with the least MEs had ≤1.1% MEs, and the three with the most had ≥3.4% MEs. The range of MEs was 0.8% to 4.2%, and the range of VMEs was 0.8% to 7.1% (Figure 2).

At the laboratory level, 471 of the laboratories (52.7%) achieved 'excellent' concordance, 350 (39.1%) achieved 'very good' concordance and 62 (6.9%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 6 laboratories (0.7%), while 5 (0.6%) performed below the threshold for 'satisfactory' concordance.

Figure 2 Percentage of errors among the reported interpretation of AST results, by country, 2025 EARS-Net EQA exercise, sorted by country according to the proportion of AST results representing very major errors

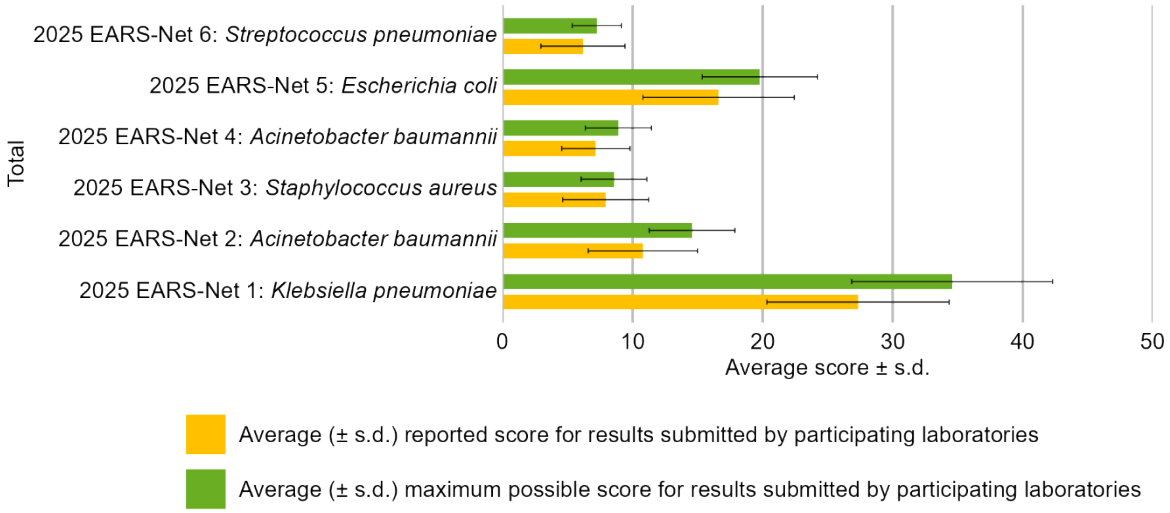


AST: antimicrobial susceptibility testing

In the 2025 EARS-Net EQA, each laboratory could report an interpretation for 86 different strain-antimicrobial combinations. According to the EQA scoring system, the maximum possible score that a laboratory could achieve, if correct AST results were submitted for all strain-antimicrobial combinations for all six strains, was 122 (74 points from the 'Easy' determinations plus 48 points from the 'Difficult' determinations).

If every participating laboratory had reported correct AST interpretations for all the results that they submitted, the average of maximum possible score per laboratory would have been 89.6 ± 20.4. As expected, the participating laboratories did not report results for every strain-antimicrobial combination or for every strain. Consequently, the maximum possible score for each laboratory was lower, because it depended on the number of reported results. In the 2025 EARS-Net EQA exercise, the average score actually achieved by the 894 participating laboratories was 72.6 ± 18.7. Figure 3 presents the averages of maximum possible score and the reported scores for the participating laboratories, by strain.

Figure 3 Average maximum possible score, and average total scores, for the AST results reported by participating laboratories, by EQA strain, 2025 EARS-Net EQA exercise



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

Table 4 presents the distribution of the methods used per strain and the percentage of correct AST interpretations for each method and each strain. The most commonly used method was automated system (53.0%), followed by disk or tablet diffusion (27.6%), and MIC methods including broth microdilution (11.0%) and gradient test (8.2%) (Table 5).

Excellent concordance was observed for disk/tablet diffusion (95.6%), while 'very good' concordance was observed for automated system (94.9%), macro broth dilution (93.1%), broth microdilution (92.6%) and agar dilution (90.3%). The gradient test demonstrated 'good' concordance (89.7%) (Table 5).

Table 4 Overview of methods used for determination of AST results for the six EQA strains

Methods	2025 EARS-Net 1 *Klebsiella pneumoniae*			2025 EARS-Net 2 *Acinetobacter baumannii*			2025 EARS-Net 3 *Staphylococcus aureus*			2025 EARS-Net 4 *Acinetobacter baumannii*			2025 EARS-Net 5 *Escherichia coli*			2025 EARS-Net 6 *Streptococcus pneumoniae*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	4	0.03	75.0	12	0.2	91.7	3	0.05	100.0	4	0.06	100.0	4	0.02	75.0	4	0.1	100.0
Automated system	8 332	53.4	91.7	3 558	52.1	87.6	3 632	55.2	98.4	3 574	52.5	98.1	9 508	54.0	97.5	1 544	44.0	97.6
Broth microdilution	1 749	11.2	89.3	1 050	15.4	92.6	403	6.1	99.3	1 064	15.6	92.7	1 856	10.5	94.1	145	4.1	95.9
Disk/tablet diffusion	4 196	26.9	91.8	1 904	27.9	92.3	1 950	29.6	99.2	1 917	28.1	94.5	4 719	26.8	98.4	1 051	30.0	99.0
Gradient test	1 290	8.3	82.0	294	4.3	90.1	586	8.9	99.7	247	3.6	96.4	1 501	8.5	89.8	734	20.9	92.5
Macro broth dilution	8	0.05	87.5	2	0.03	100.0	1	0.02	100.0	2	0.03	50.0	8	0.05	100.0	8	0.2	100.0
Other	10	0.06	90.0	4	0.06	75.0	10	0.2	100.0	4	0.06	75.0	11	0.06	81.8	21	0.6	100.0
Total	**15 589**	**100.0**	**90.6**	**6 824**	**100.0**	**89.8**	**6 585**	**100.0**	**98.8**	**6 812**	**100.0**	**96.1**	**17 607**	**100.0**	**96.7**	**3 507**	**100.0**	**96.9**

Percentages might not total 100% due to rounding.

Table 5 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	31	0.05	90.3
Automated system	30 148	53.0	94.9
Broth microdilution	6 267	11.0	92.6
Disk/tablet diffusion	15 737	27.6	95.6
Gradient test	4 652	8.2	89.7
Macro broth dilution	29	0.05	93.1
Other	60	0.1	91.7
Total	**56 924**	**100.0**	**94.4**

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 1' (*Klebsiella pneumoniae*)

The strain **'2025 EARS-Net 1' (*Klebsiella pneumoniae*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to 18 of the 23 antimicrobials included in the EQA: amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, ertapenem, imipenem, meropenem, imipenem-relebactam, ciprofloxacin, moxifloxacin, ofloxacin, amikacin and tobramycin. It was susceptible to four antimicrobials: gentamicin, colistin, meropenem-vaborbactam and aztreonam-avibactam. The expected MIC values fell within the 'I' range for one antimicrobial: levofloxacin (Annex 1).

The level of difficulty was classified as 'difficult' for seven of the antimicrobials: amikacin, ciprofloxacin, levofloxacin, imipenem, meropenem, meropenem-vaborbactam and cefiderocol. For the first six of these antimicrobials, the expected MIC values were less than two dilutions away from the clinical breakpoints, while for cefiderocol the expected zone diameter was less than four millimetres away from the ATU. For the remaining 16 antimicrobials the level of difficulty was considered 'easy'.

The strain harboured the $bla_{\text{NDM-1}}$ gene which confers extended resistance towards beta-lactam antimicrobials, including carbapenems, as well as other beta-lactamases ($bla_{\text{CTX-M-15}}$, $bla_{\text{DHA-1}}$). Moreover, it carried aminoglycoside resistance genes (*aac(6')-Ib* and *aph(3')-Vi)* and fluoroquinolone resistance genes (*qnrS1*, *qnrB4*).

Interpretation of AST results for the *K. pneumoniae* strain were analysed for the 883 laboratories that reported correct species identification (Table 3). In total, 54.7% of the laboratories (n=483) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 139 laboratories (15.7%) were in full concordance with the expected interpretations. A total of 72 laboratories (8.2%) achieved 'excellent' concordance, 362 (41.0%) achieved 'very good', and 191 (21.6%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 91 laboratories (10.3%) while 28 (3.2%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 23 antimicrobials, corresponding to a maximum of 20 309 possible submissions. In total, 15 589 tests were performed, of which 14 127 interpretations were correct. Thus, the reported interpretations were in 'very good' concordance with expected results (90.6%) (Table 6). MEs were observed for 6.1% (n=949) of the interpretations, and VMEs for 3.3% (n=513) (Figure 4).

The following AST methods were applied: automated system (53.4%), disk/tablet diffusion (26.9%), broth microdilution (11.2%), gradient test (8.3%), macro broth dilution (0.05%), agar dilution (0.03%), other (0.06%) (Table 4).

Automated system and disk/tablet diffusion achieved a 'very good' concordance with the expected results (>90% of concordance). Broth microdilution (89.3%), macro broth dilution (87.5%) and 'other methods' (90.0%) achieved a 'good' concordance, while gradient test (82.0%) achieved a 'satisfactory' concordance, and agar dilution (75.0%) was under the threshold for 'satisfactory' concordance (Table 6).

VMEs were observed for 17 out of the 18 antimicrobials with an expected interpretation of R: amikacin, amoxicillin-clavulanic acid, cefepime, cefiderocol, cefotaxime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, ertapenem, imipenem, imipenem-relebactam, meropenem, moxifloxacin, ofloxacin, piperacillin-tazobactam, tobramycin (Figure 4, Table 6):

- VMEs in amikacin (10.2% of all submitted interpretations for that antimicrobial) were reported across all methods, with a higher proportion of errors for broth microdilution.
- VMEs in imipenem (13.0%) were reported across all methods, with a higher proportion of errors for broth microdilution and gradient test.
- VMEs in meropenem (13.0%) were reported across all methods, with a higher proportion of errors for broth microdilution and gradient test.
- VMEs in cefiderocol (21.6%) were reported across all methods, with a lower proportion of errors for disk diffusion.
- VMEs in imipenem-relebactam (13.5%) were reported across most methods, with a higher proportion of errors for gradient test.
- For the other antimicrobials, VMEs represented <5% of the submitted interpretations, and in some cases these deviations were more prevalent for certain methods, specifically: gradient test for ciprofloxacin.

A high proportion of MEs was observed for meropenem-vaborbactam (76.7%) and levofloxacin (71.1%). In both cases, errors were reported across most methods but were especially frequent with automated system and disk/tablet diffusion. Low proportions of MEs were observed for other antimicrobials (Figure 4, Table 6).

Discussion

For strain **'2025 EARS-Net 1' (*Klebsiella pneumoniae*)** the majority of errors appeared to be associated with the level of difficulty of the respective AST determinations. The highest proportions of VMEs and MEs were observed for antimicrobials for which the expected MIC values were less than two dilutions away from the clinical

breakpoints and therefore the determinations were considered 'difficult', specifically: amikacin, imipenem, meropenem, levofloxacin and meropenem-vaborbactam. Therefore, these deviations are likely attributable to the inherent method variability since the borderline expected MIC values increased the likelihood of misclassification. The deviations may also represent, or have been exacerbated by, variations in the methods and/or material used for testing [8-11].

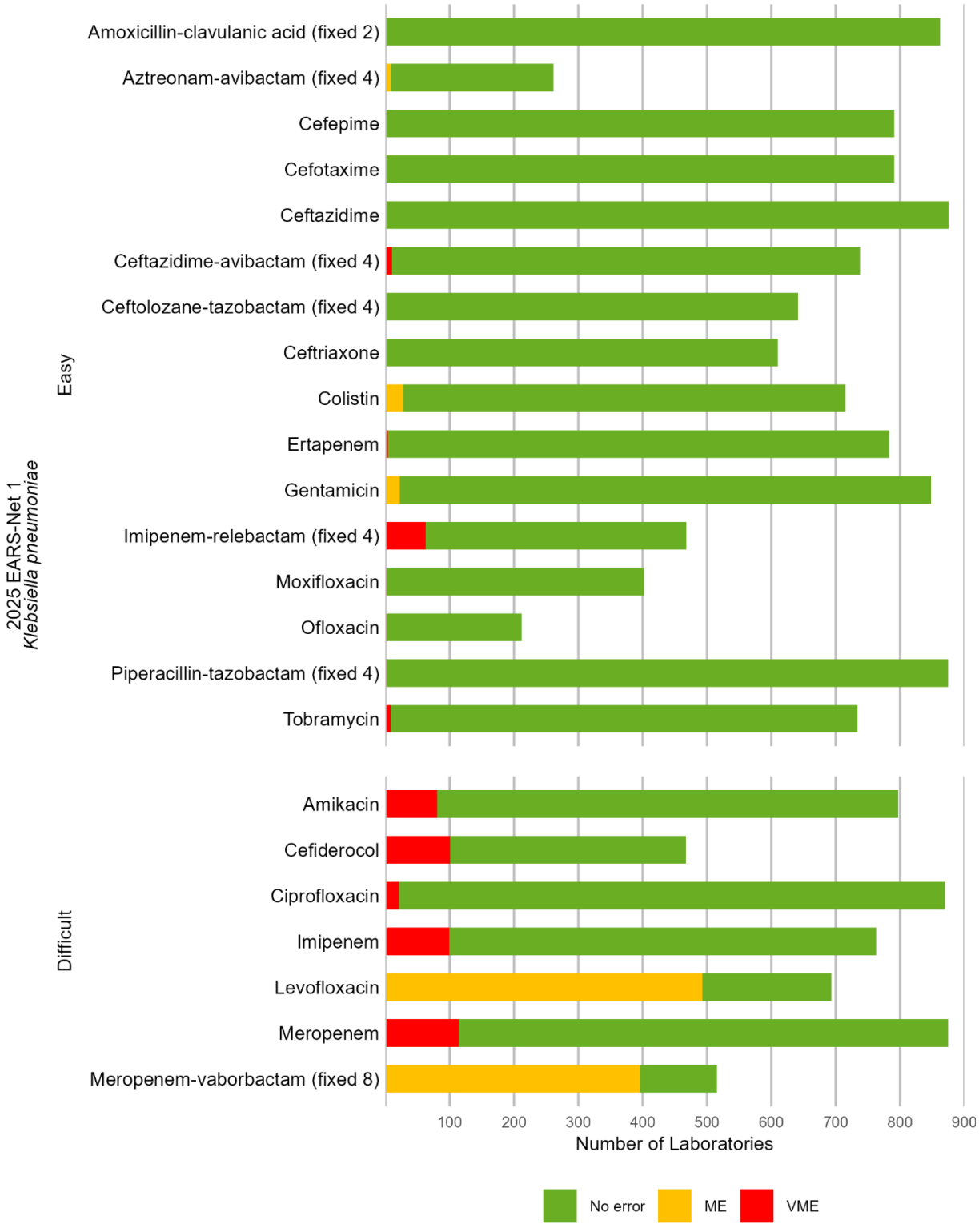
The expected result for cefiderocol was also considered 'difficult' as the expected zone diameter was less than four millimetres away from the ATU. In addition, results from AST of cefiderocol are known to be highly variable, depending on the testing method and materials used [12,13]. Disk diffusion is the method currently recommended by EUCAST for AST of cefiderocol, and the results for this *K. pneumoniae* strain confirm that it was the method achieving the highest concordance for this antimicrobial.

The determination of AST results for imipenem-relebactam was considered 'easy'. Therefore, the observed errors should not be associated with inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures. The discrepancies appeared to be especially associated with the gradient test, for which 39.8% of results were incorrect.

Overall, three of the four most frequently applied methods (automated system, broth microdilution and disk/tablet diffusion) achieved 'good' to 'very good' concordance with expected results (89.3% to 91.8%). Results obtained using gradient test showed a lower concordance (82.0%). In most cases, deviations were distributed across multiple methods. However, some methods exhibited lower performance for specific antimicrobials:

- Automated system for aztreonam-avibactam, meropenem-vaborbactam, cefiderocol and levofloxacin;
- Disk/tablet diffusion for meropenem-vaborbactam and levofloxacin;
- Broth microdilution for imipenem, meropenem, meropenem-vaborbactam, cefiderocol and amikacin; and
- Gradient test for imipenem, meropenem, imipenem-relebactam, cefiderocol, ciprofloxacin and levofloxacin.

Figure 4 Reported interpretation of AST results for strain '2025 EARS-Net 1' (*Klebsiella pneumoniae*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.

Table 6 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EARS-Net 1' (*Klebsiella pneumoniae*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Difficult	R	-	-	464	90.5	87	64.4	214	97.7	32	96.9	-	-	-	-	**797**	**89.8**
Amoxicillin-clavulanic acid (fixed 2 mg/L)	Easy	R	-	-	548	99.8	44	100.0	255	100.0	15	100.0	-	-	-	-	**862**	**99.9**
Aztreonam-avibactam (fixed 4 mg/L)	Easy	S	-	-	14	64.3	15	93.3	48	97.9	183	98.9	-	-	1	100.0	**261**	**96.6**
Cefepime	Easy	R	-	-	510	99.8	63	100.0	208	100.0	9	100.0	1	100.0	-	-	**791**	**99.9**
Cefiderocol	Difficult	R	3	66.7	26	50.0	80	70.0	323	89.5	34	17.6	-	-	1	0.0	**467**	**78.4**
Cefotaxime	Easy	R	-	-	494	100.0	59	100.0	213	100.0	25	96.0	-	-	-	-	**791**	**99.9**
Ceftazidime	Easy	R	-	-	561	99.8	68	100.0	238	100.0	9	100.0	-	-	-	-	**876**	**99.9**
Ceftazidime-avibactam (fixed 4 mg/L)	Easy	R	-	-	345	99.1	90	96.7	181	98.3	122	99.2	-	-	-	-	**738**	**98.6**
Ceftolozane-tazobactam (fixed 4 mg/L)	Easy	R	-	-	342	99.7	90	100.0	119	100.0	91	100.0	-	-	-	-	**642**	**99.8**
Ceftriaxone	Easy	R	-	-	275	100.0	27	100.0	211	100.0	94	100.0	1	100.0	2	100.0	**610**	**100.0**
Ciprofloxacin	Difficult	R	-	-	552	98.9	71	97.2	227	95.6	19	84.2	-	-	1	100.0	**870**	**97.6**
Colistin	Easy	S	1	100.0	249	94.8	443	97.1	9	88.9	8	87.5	3	100.0	2	100.0	**715**	**96.1**
Ertapenem	Easy	R	-	-	464	99.8	63	96.8	211	99.5	44	100.0	1	100.0	-	-	**783**	**99.5**
Gentamicin	Easy	S	-	-	549	98.0	62	98.4	215	95.8	22	90.9	-	-	-	-	**848**	**97.3**
Imipenem	Difficult	R	-	-	423	95.0	79	65.8	187	84.5	74	70.3	-	-	-	-	**763**	**87.0**
Imipenem-relebactam (fixed 4 mg/L)	Easy	R	-	-	220	98.2	54	85.2	79	92.4	113	60.2	-	-	2	100.0	**468**	**86.5**
Levofloxacin	Difficult	I	-	-	367	28.3	35	60.0	225	22.2	66	37.9	-	-	-	-	**693**	**28.9**
Meropenem	Difficult	R	-	-	513	94.7	91	63.7	194	86.6	76	64.5	1	0.0	-	-	**875**	**87.0**
Meropenem-vaborbactam (fixed 8 mg/L)	Difficult	S	-	-	297	9.1	55	49.1	44	4.5	119	52.9	-	-	1	100.0	**516**	**23.3**
Moxifloxacin	Easy	R	-	-	116	99.1	17	100.0	197	100.0	72	98.6	-	-	-	-	**402**	**99.5**
Ofloxacin	Easy	R	-	-	24	100.0	8	100.0	146	98.6	34	100.0	-	-	-	-	**212**	**99.1**
Piperacillin-tazobactam (fixed 4 mg/L)	Easy	R	-	-	554	99.8	77	100.0	234	99.6	10	100.0	-	-	-	-	**875**	**99.8**
Tobramycin	Easy	R	-	-	425	98.6	71	98.6	218	99.5	19	94.7	1	100.0	-	-	**734**	**98.8**
Total			**4**	**75.0**	**8332**	**91.7**	**1749**	**89.3**	**4196**	**91.8**	**1290**	**82.0**	**8**	**87.5**	**10**	**90.0**	**15589**	**90.6**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 2' (*Acinetobacter baumannii*)

The strain **'2025 EARS-Net 2' (*Acinetobacter baumannii*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to six of the nine antimicrobials included in the EQA: cefiderocol, imipenem, meropenem, ciprofloxacin, levofloxacin and amikacin. It was susceptible to three antimicrobials: gentamicin, tobramycin and colistin (Annex 1).

The level of difficulty was classified as 'difficult' for three of the antimicrobials: amikacin, gentamicin and cefiderocol. For amikacin and gentamicin, the expected MIC values were less than two dilutions away from the clinical breakpoints, while for cefiderocol the expected zone diameter was less than four millimetres away from the recommended screening cut-off of 17 mm. For the remaining six antimicrobials the level of difficulty was considered 'easy'.

The strain harboured the *bla*$_{\text{NDM-1}}$ gene which confers extended resistance towards beta-lactam antimicrobials, including carbapenems, as well as aminoglycoside resistance genes (*aph(3')-Vi)* and chromosomal mutations associated with fluoroquinolone resistance.

Interpretation of AST results for the *A. baumannii* strain were analysed for the 884 laboratories that reported correct species identification (Table 3). In total, 46.8% of the laboratories (n=414) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 291 laboratories (32.9%) were in full concordance with the expected interpretations. A total of 431 (48.8%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 56 laboratories (16.3%) while 106 (12.0%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 9 antimicrobials, corresponding to a maximum of 7 956 possible submissions. In total, 6 824 tests were performed, of which 6 128 reported interpretations were correct. Thus, the reported interpretations were in 'good' concordance with expected results (89.8%) (Table 7). MEs were observed for 2.1% (n=140) of the interpretations and VMEs for 8.1% (n=556) (Figure 5).

The following AST methods were applied: automated system (52.1%), disk/tablet diffusion (27.9%), broth microdilution (15.4%), gradient test (4.3%), agar dilution (0.2%), macro broth dilution (0.03%), other (0.06%) (Table 4).

Overall, most methods achieved, as a minimum, a 'very good' concordance with the expected results (>90% of concordance). The exceptions were automated system (87.6%), which achieved a 'good' concordance, and 'other methods' (75.0%) which were under the threshold for 'satisfactory' concordance (Table 7).

VMEs were observed for four of the six antimicrobials with an expected interpretation of R: amikacin, cefiderocol, imipenem, meropenem (Figure 5, Table 7):

- VMEs in amikacin (58.1% of all submitted interpretations for that antimicrobial) were reported across all methods, with higher proportions for automated system.
- VMEs in cefiderocol (9.6%) were reported across most methods, with a lower proportion of errors for disk diffusion.
- For imipenem and meropenem, VMEs represented <5% of the submitted interpretations, and in some cases these deviations were more prevalent for certain methods, specifically: automated system for meropenem.

A high proportion of MEs was observed for gentamicin (12.8%), and these were distributed through most methods but more prevalent for broth microdilution and disk/tablet diffusion. Low proportions of MEs were observed for other antimicrobials (Figure 5, Table 7).

Discussion

For strain **'2025 EARS-Net 2' (*Acinetobacter baumannii*)** the majority of errors appeared to be associated with the level of difficulty of the respective AST determinations. The highest proportions of VMEs and MEs were observed for antimicrobials for which the expected MIC values were less than two dilutions away from the clinical breakpoints and therefore the determinations were considered 'difficult', specifically: amikacin and gentamicin. Therefore, these deviations are likely attributable to the inherent method variability since the borderline expected MIC values increased the likelihood of misclassification. The deviations may also represent, or have been exacerbated by, variations in the methods and/or material used for testing [14].

The expected result for cefiderocol was also considered 'difficult' as the expected zone diameter was less than four millimetres away from the recommended screening cut-off of 17 mm. Moreover, results from AST of cefiderocol are known to be highly variable depending on the method and materials [12,13]. Disk diffusion is the method currently recommended by EUCAST for AST of cefiderocol, and the results for this *A. baumannii* strain confirm that it was the method achieving the highest concordance for this antimicrobial.

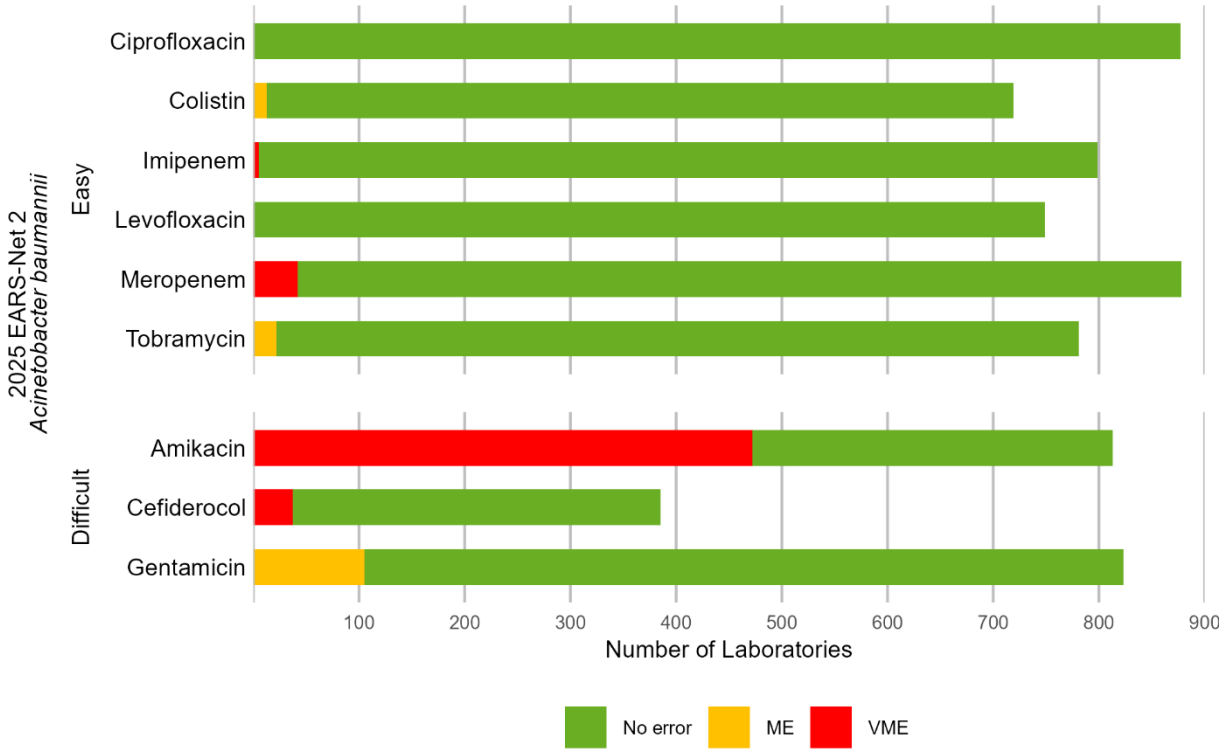
Due to the problematic reporting of S/I/R interpretations for cefiderocol detected for strain '2025 EARS-Net 4' (*A. baumannii)*, the quantitative results submitted by participants for strain '2025 EARS-Net 2' were investigated

further as well. Minor issues were observed which did not impact previous conclusions regarding AST of cefiderocol for this strain.

Overall, the four most frequently applied methods (automated system, broth microdilution, disk/tablet diffusion and gradient test) showed 'good' or 'very good' concordance with expected results, ranging from 87.6% to 92.6%. In most cases, deviations were distributed across multiple methods; however, some methods exhibited lower performance for specific antimicrobials:

- Automated system for amikacin, meropenem and cefiderocol;
- Disk/tablet diffusion for gentamicin;
- Broth microdilution for amikacin, gentamicin and cefiderocol; and
- Gradient test for amikacin and cefiderocol.

Figure 5 Reported interpretation of AST results for strain '2025 EARS-Net 2' (*Acinetobacter baumannii*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.

Table 7 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EARS-Net 2' (*Acinetobacter baumannii*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Difficult	R	2	50.0	448	28.1	96	54.2	221	62.4	45	53.3	-	-	1	0.0	**813**	**41.9**
Cefiderocol	Difficult	R	3	100.0	15	73.3	71	83.1	273	93.8	22	81.8	-	-	1	100.0	**385**	**90.4**
Ciprofloxacin	Easy	R	1	100.0	539	100.0	75	100.0	249	100.0	12	100.0	-	-	1	100.0	**877**	**100.0**
Colistin	Easy	S	1	100.0	249	96.8	447	98.9	10	100.0	9	100.0	2	100.0	1	100.0	**719**	**98.2**
Gentamicin	Difficult	S	1	100.0	477	89.3	76	82.9	228	82.9	41	95.1	-	-	-	-	**823**	**87.2**
Imipenem	Easy	R	1	100.0	456	99.6	78	100.0	217	99.1	47	97.9	-	-	-	-	**799**	**99.4**
Levofloxacin	Easy	R	1	100.0	415	100.0	44	100.0	240	100.0	49	100.0	-	-	-	-	**749**	**100.0**
Meropenem	Easy	R	1	100.0	520	91.9	86	100.0	224	100.0	47	100.0	-	-	-	-	**878**	**95.2**
Tobramycin	Easy	S	1	100.0	439	97.5	77	94.8	242	97.5	22	95.5	-	-	-	-	**781**	**97.2**
Total			**12**	**91.7**	**3558**	**87.6**	**1050**	**92.6**	**1904**	**92.3**	**294**	**90.1**	**2**	**100.0**	**4**	**75.0**	**6824**	**89.8**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 3' (*Staphylococcus aureus*)

The strain **'2025 EARS-Net 3' (*Staphylococcus aureus*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to two of the nine antimicrobials included in the EQA: oxacillin and cefoxitin. It was susceptible to five antimicrobials: norfloxacin, vancomycin, linezolid, daptomycin and rifampicin. The expected MIC values fell within the 'I' range for two antimicrobials: ciprofloxacin and levofloxacin (Annex 1).

The level of difficulty was classified as 'difficult' for one of the antimicrobials: norfloxacin, as the expected zone diameter was less than four millimetres away from the breakpoint. For the remaining eight antimicrobials the level of difficulty was considered 'easy'.

The strain harboured the *mecA* gene which confers methicillin resistance and therefore yields results in the 'R' category for oxacillin and cefoxitin.

Interpretation of AST results for the *S. aureus* strain were analysed for the 890 laboratories that reported correct species identification (Table 3). In total, 26.9% of the laboratories (n=239) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 842 laboratories (94.6%) were in full concordance with the expected interpretations. A total of 24 (2.7%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 7 laboratories (0.8%) while 17 (1.9%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 9 antimicrobials, corresponding to a maximum of 8 010 possible submissions. In total, 6 585 tests were performed, of which 6 506 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (98.8%) (Table 8). MEs were observed for 1.1% (n=73) of the interpretations and VMEs for 0.1% (n=6) (Figure 6).

The following AST methods were applied: automated system (55.2%), disk/tablet diffusion (29.6%), gradient test (8.9%), broth microdilution (6.1%), agar dilution (0.05%), macro broth dilution (0.02%), other (0.2%) (Table 4).

All methods achieved 'excellent' concordance with the expected results (>95% of concordance) (Table 8).

VMEs were observed for both antimicrobials with an expected interpretation of R: cefoxitin, oxacillin (Figure 6, Table 8):

- These VMEs represented <5% of the submitted interpretations and were not associated with any specific method.

Low proportions of MEs were observed for some antimicrobials (Figure 6, Table 8). In some cases, these deviations were more prevalent for certain methods, specifically: automated system for norfloxacin.

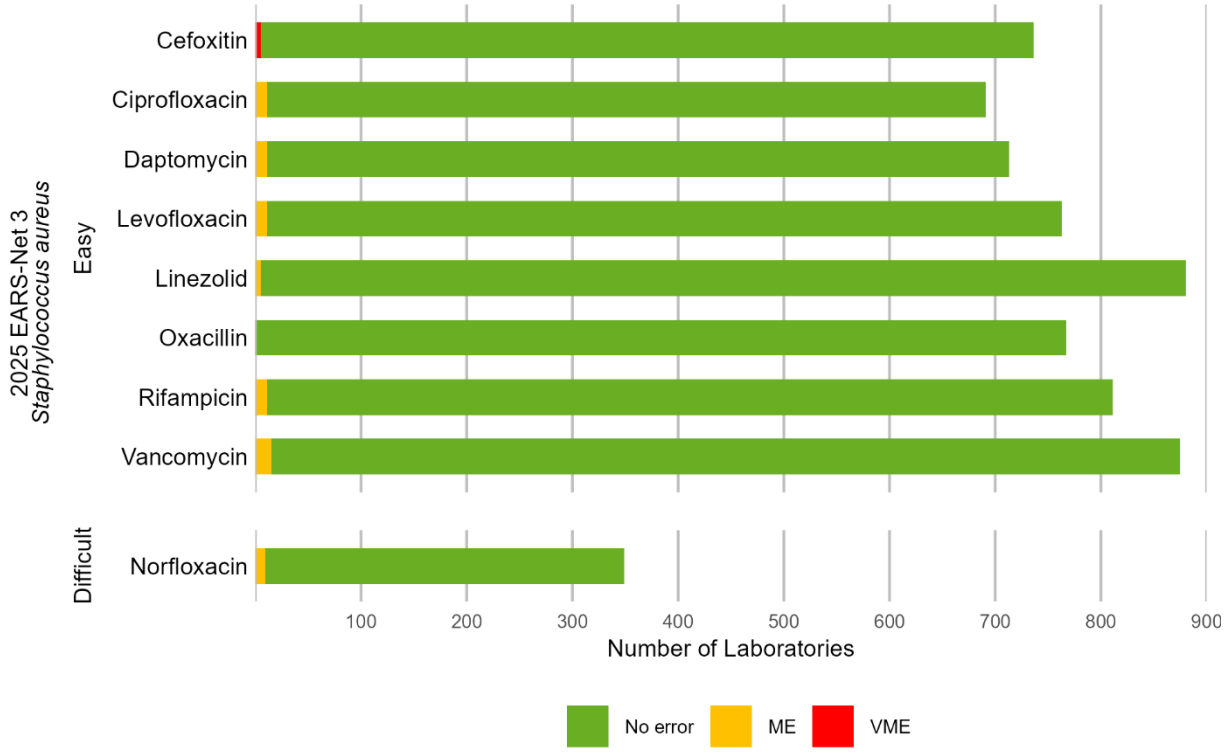
Discussion

For strain **'2025 EARS-Net 3' (*Staphylococcus aureus*)** only few errors were reported, and the majority of errors occurred for AST determinations classified as 'easy'. Therefore, the observed errors should not be associated with the inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures.

The expected result for norfloxacin was considered 'difficult' as the expected zone diameter was less than four millimetres away from the breakpoint. Therefore, these deviations are likely attributable to the inherent method variability since the borderline expected zone diameter increased the likelihood of misclassification. Disk diffusion is the method currently recommended by EUCAST for AST of norfloxacin, and the results for the *S. aureus* strain confirm that it achieved the highest concordance among the methods commonly applied for the AST of that antimicrobial (disk/tablet diffusion and automated system).

No other major deviations or method-specific issues were identified for this strain.

Figure 6 Reported interpretation of AST results for strain '2025 EARS-Net 3' (*Staphylococcus aureus*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.

Table 8 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EARS-Net 3' (*Staphylococcus aureus*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Cefoxitin	Easy	R	1	100.0	186	97.8	24	100.0	522	99.8	3	100.0	-	-	-	-	**736**	**99.3**
Ciprofloxacin	Easy	I	-	-	305	97.7	33	100.0	276	98.9	75	98.7	1	100.0	1	100.0	**691**	**98.4**
Daptomycin	Easy	S	-	-	503	98.0	66	98.5	4	100.0	138	100.0	-	-	2	100.0	**713**	**98.5**
Levofloxacin	Easy	I	-	-	473	97.7	32	100.0	206	100.0	51	100.0	-	-	1	100.0	**763**	**98.6**
Linezolid	Easy	S	-	-	578	99.3	49	100.0	234	99.6	19	100.0	-	-	-	-	**880**	**99.4**
Norfloxacin	Difficult	S	-	-	16	81.3	2	100.0	331	98.2	-	-	-	-	-	-	**349**	**97.4**
Oxacillin	Easy	R	-	-	563	99.8	42	100.0	86	100.0	71	100.0	-	-	5	100.0	**767**	**99.9**
Rifampicin	Easy	S	-	-	464	98.5	42	100.0	280	98.6	25	100.0	-	-	-	-	**811**	**98.6**
Vancomycin	Easy	S	2	100.0	544	97.8	113	98.2	11	100.0	204	99.5	-	-	1	100.0	**875**	**98.3**
Total			**3**	**100.0**	**3632**	**98.4**	**403**	**99.3**	**1950**	**99.2**	**586**	**99.7**	**1**	**100.0**	**10**	**100.0**	**6585**	**98.8**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 4' (*Acinetobacter baumannii*)

The strain **'2025 EARS-Net 4' (*Acinetobacter baumannii*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to eight of the nine antimicrobials included in the EQA: imipenem, meropenem, gentamicin, amikacin, tobramycin, ciprofloxacin, levofloxacin and colistin. The expected MIC values fell within the 'I' range for one antimicrobial: cefiderocol (Annex 1).

The level of difficulty was classified as 'difficult' for one of the antimicrobials: cefiderocol, as the expected zone diameter was less than four millimetres away from the recommended screening cut-offs of 17 mm and 21 mm. For the remaining eight antimicrobials, the level of difficulty was considered 'easy'.

The strain harboured the *bla*$_{OXA-23}$ gene which confers extended resistance towards beta-lactam antimicrobials, including carbapenems, as well as aminoglycoside resistance genes (*armA*, *aph(3')-VI*a) and chromosomal mutations associated with fluoroquinolone resistance. No currently known genetic determinants of colistin resistance were detected with the bioinformatics tools.

Interpretation of AST results for the *A. baumannii* strain were analysed for the 886 laboratories that reported correct species identification (Table 3). In total, 53.7% of the laboratories (n=476) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 648 laboratories (73.2%) were in full concordance with the expected interpretations. A total of 205 (23.1%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 13 laboratories (1.5%) while 20 (2.3%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 9 antimicrobials, corresponding to a maximum of 7 974 possible submissions. In total, 6 812 tests were performed, of which 6 549 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (96.1%) (Table 9). MEs were observed for 1.9% (n=129) of the interpretations and VMEs were observed for 2.0% (n=134) (Figure 7).

The following AST methods were applied: automated system (52.5%), disk/tablet diffusion (28.1%), broth microdilution (15.6%), gradient test (3.6%), agar dilution (0.06%), macro broth dilution (0.03%), other (0.06%) (Table 4).

Overall, most methods achieved, as a minimum, a 'very good' concordance with the expected results (>90% of concordance). The exceptions were macro broth dilution (50.0%) and 'other methods' (75.0%), which were under the threshold for 'satisfactory' concordance (Table 9).

VMEs were observed for four of the eight antimicrobials with an expected interpretation of R: amikacin, colistin, gentamicin, tobramycin (Figure 7, Table 9):

- VMEs in colistin (17.0% of all submitted interpretations for that antimicrobial) were reported across most methods with a lower proportion of errors for broth microdilution.
- For the other antimicrobials, VMEs represented <5% of the submitted interpretations and were not associated with any specific method.

A high proportion of MEs was observed for cefiderocol (35.7%), reported across most methods, but especially prevalent with automated system and disk/tablet diffusion. Low proportions of MEs were observed for other antimicrobials (Figure 7, Table 9).

Discussion

For strain **'2025 EARS-Net 4' (*Acinetobacter baumannii*)** many of the errors appeared to be associated with the level of difficulty of the respective AST determinations. The highest proportion of MEs was observed for cefiderocol, and that determination was classified as 'difficult' as the expected zone diameter was less than four millimetres away from the ATU. Therefore, these deviations are likely attributable to the inherent method variability since the borderline expected zone diameter increased the likelihood of misclassification. Moreover, results from AST of cefiderocol are known to be highly variable depending on the method and materials [12,13].

Disk diffusion is the method currently recommended by EUCAST for AST of cefiderocol, and results from other strains included in this EQA confirm that it is overall the method achieving the highest concordance for this antimicrobial. However, the results for this *A. baumannii* strain did not show better performance of disk diffusion when compared with other methods. To investigate this discrepancy, the quantitative results submitted by the participants were investigated further. This revealed a tendency of assigning the wrong S/I/R categories to diffusion results (and in lesser extent to dilution results):

- From the 111 results submitted with agar dilution (n=2), automated system (n=12), broth microdilution (n=76) and gradient test (n=21), 29 were submitted with an interpretation of R. The quantitative results submitted for those 29 cases show that five cases should have been classified as I, as the MICs were between 1 and 2 mg/L. According to EUCAST clinical breakpoint tables v15.0, isolates with MIC values 1-2 mg/L have acquired mechanisms which may result in impaired clinical response. All five cases were observed when using broth microdilution, which would bring the concordance of that method to 80.3%.
- From the 247 results submitted with disk/tablet diffusion, 99 were submitted with an interpretation of R. The quantitative results submitted for those 99 cases show that six cases should have been classified as S, as the zone diameters were ≥21 mm, and 38 should have been classified as I, as the zone diameters

were between 17 and 20 mm. According to EUCAST clinical breakpoint tables v15.0, isolates with zone diameters ≥21 mm are mostly devoid of resistant mechanisms and isolates with zone diameters 17-20 mm have acquired mechanisms which may result in impaired clinical response. Re-classification of those 44 cases as S or I would bring the concordance of the method to 77.7%.

Misclassification of the MICs or zone diameters could be due to conflicts between EUCAST guidelines (which do not contain clinical breakpoints for cefiderocol in the species but instead include information in the Notes), when compared with the instructions for the EQA (where participants must use S/I/R categories for several situations that are not necessarily categorised as S/I/R during routine laboratory practice). However, this misattribution of S/I/R categories was not observed in the results for cefiderocol in the other *A. baumannii* strain nor for any of the Enterobacterales strains, for which the expected result was R, and therefore relatively more straightforward to categorise. This suggests that participants did not struggle with the concept of using S/I/R categories to report results of cefiderocol in *A. baumannii* for the purpose of this EQA. Instead, participants may not strictly comply with information presented in the Notes sections of the EUCAST guidelines.

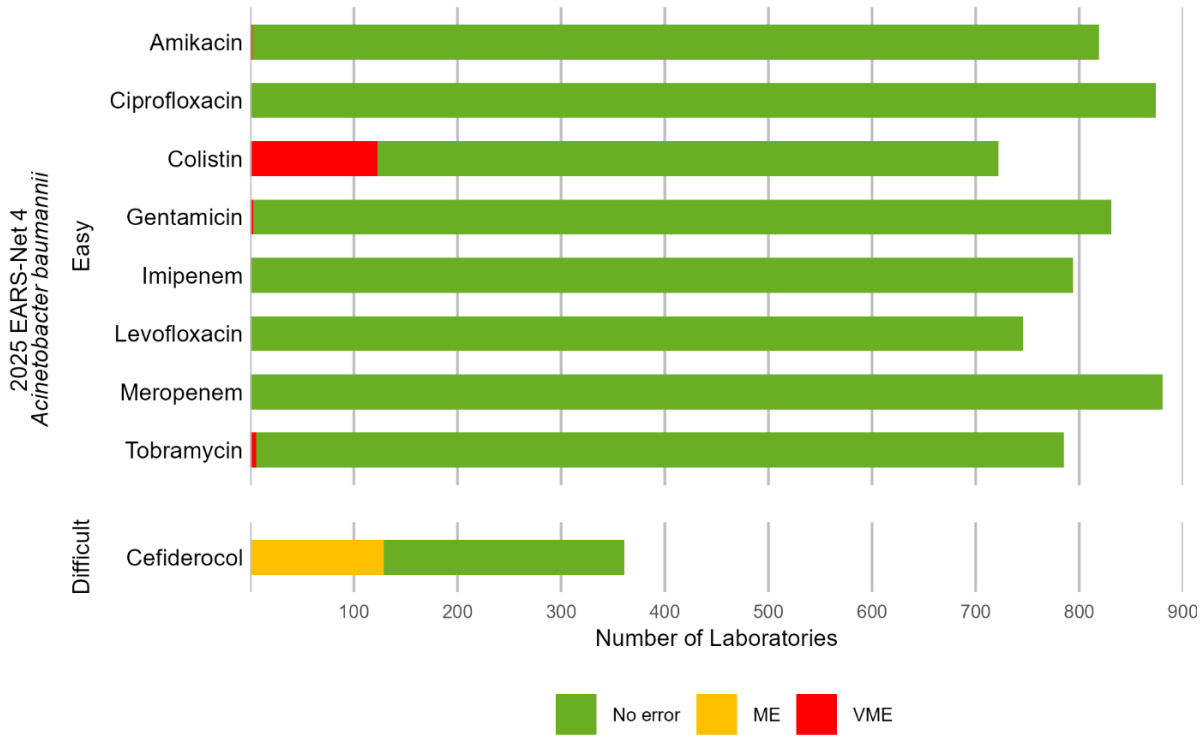
The quantitative results submitted for cefiderocol show that disk diffusion did not perform markedly worse than broth microdilution nor gradient test for AST of the antimicrobial, and that automated system remained the method with worst concordance from the frequently used methods. In conclusion, these results support adherence to EUCAST recommendations for AST of cefiderocol.

The determination of AST results for colistin was classified as 'easy'. Therefore, the observed errors should not be associated with the inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures. These deviations were distributed across most methods, with lower proportion of errors observed for broth microdilution. Broth microdilution is the method currently recommended by EUCAST for AST of colistin, and the results for this *A. baumannii* strain confirm that it achieved the highest concordance among all methods.

Overall, the four frequently applied methods (automated system, broth microdilution, disk/tablet diffusion and gradient test) achieved at least 'very good' concordance with expected results ranging from 92.7% to 98.1%. In most cases, deviations were distributed across multiple methods; however, some methods exhibited lower performance for specific antimicrobials:

- Automated system for cefiderocol and colistin;
- Disk/tablet diffusion for cefiderocol, partially due to incorrect interpretation of quantitative results;
- Broth microdilution for cefiderocol, partially due to incorrect interpretation of quantitative results; and
- Gradient test for colistin.

Figure 7 Reported interpretation of AST results for strain '2025 EARS-Net 4' (*Acinetobacter baumannii*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.

Table 9 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EARS-Net 4' (*Acinetobacter baumannii*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	R	1	100.0	465	99.8	92	100.0	239	99.6	22	100.0	-	-	-	-	**819**	**99.8**
Cefiderocol	Difficult	I	2	100.0	12	58.3	76	73.7	247	59.9	21	81.0	-	-	3	66.7	**361**	**64.3**
Ciprofloxacin	Easy	R	-	-	536	100.0	77	100.0	250	100.0	11	100.0	-	-	-	-	**874**	**100.0**
Colistin	Easy	R	-	-	242	75.2	460	87.8	5	80.0	12	58.3	2	50.0	1	100.0	**722**	**83.0**
Gentamicin	Easy	R	-	-	497	99.6	77	100.0	235	99.6	22	100.0	-	-	-	-	**831**	**99.6**
Imipenem	Easy	R	-	-	448	100.0	75	100.0	224	100.0	47	100.0	-	-	-	-	**794**	**100.0**
Levofloxacin	Easy	R	-	-	414	100.0	43	100.0	237	100.0	52	100.0	-	-	-	-	**746**	**100.0**
Meropenem	Easy	R	-	-	520	100.0	87	100.0	229	100.0	44	100.0	-	-	-	-	**880**	**100.0**
Tobramycin	Easy	R	1	100.0	440	99.8	77	97.4	251	98.8	16	100.0	-	-	-	-	**785**	**99.2**
Total			**4**	**100.0**	**3574**	**98.1**	**1064**	**92.7**	**1917**	**94.5**	**247**	**96.4**	**2**	**50.0**	**4**	**75.0**	**6812**	**96.1**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 5' (*Escherichia coli*)

The strain **'2025 EARS-Net 5' (*Escherichia coli*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to 22 of the 26 antimicrobials included in the EQA: ampicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, ertapenem, imipenem, meropenem, imipenem-relebactam, meropenem-vaborbactam, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, gentamicin and tobramycin. It was susceptible to four antimicrobials: amikacin, colistin, tigecycline and aztreonam-avibactam (Annex 1).

The level of difficulty was classified as 'easy' for all the antimicrobials.

The strain harboured the *bla*$_{\text{NDM-5}}$ gene which confers extended resistance towards beta-lactam antimicrobials, including carbapenems, as well as other beta-lactamases (*bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{OXA-1}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{CMY-2}}$). Moreover, it carried aminoglycoside resistance genes (*aac(6')-Ib-cr* and *aac(3)-Iid*) and multiple mechanisms conferring fluoroquinolone resistance (*aac(6')-Ib-cr*, *qnrS1* and several chromosomal mutations).

Interpretation of AST results for the *E. coli* strain were analysed for the 890 laboratories that reported correct species identification (Table 3). In total, 55.6 % of the laboratories (n=495) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 497 laboratories (55.8%) were in full concordance with the expected interpretations. A total of 165 (18.5%) achieved 'excellent concordance, 168 (18.9%) achieved 'very good', and 33 (3.7%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 21 laboratories (2.4%) while 6 (0.7%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 26 antimicrobials corresponding to a maximum of 23 140 possible submissions. In total, 17 607 tests were performed, of which 17 033 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (96.7%) (Table 10). MEs were observed for 0.4% (n=62) of the interpretations and VMEs for 2.9% (n=512) (Figure 8).

The following AST methods were applied: automated system (54.0%), disk/tablet diffusion (26.8%), broth microdilution (10.5%), gradient test (8.5%), agar dilution (0.02%), macro broth dilution (0.05%), other (0.06%) (Table 4).

Automated system, disk/tablet diffusion and macro broth dilution achieved 'excellent' concordance with the expected results (>95% of concordance). Broth microdilution (94.1%) achieved 'very good' concordance and gradient test (89.8%) achieved a 'good' concordance. 'Other methods' (81.8%) achieved 'satisfactory' concordance and agar dilution (75.0%) was under the threshold for 'satisfactory' concordance (Table 10).

VMEs were observed for 12 of the 22 antimicrobials with an expected interpretation of R: cefepime, cefiderocol, cefotaxime, ceftazidime-avibactam, ertapenem, gentamicin, imipenem, imipenem-relebactam, levofloxacin, meropenem, meropenem-vaborbactam, tobramycin (Figure 8, Table 10):

- VMEs in imipenem (8.7% of all submitted interpretations for that antimicrobial) were reported across all methods with a higher proportion of errors for broth microdilution and gradient test.
- VMEs in imipenem-relebactam (12.3%) were reported across most methods with a higher proportion of errors for broth microdilution and gradient test.
- VMEs in meropenem (15.7%) were reported across all methods with a higher proportion of errors for automated system, broth microdilution and gradient test.
- VMEs in meropenem-vaborbactam (29.5%) were reported across all methods with a higher proportion of errors for broth microdilution gradient test.
- VMEs in cefiderocol (16.2%) were reported across all methods with a lower proportion of errors for disk/tablet diffusion.
- For the other antimicrobials, VMEs represented <5% of the submitted interpretations and were not associated with any specific method.

Low proportions of MEs were observed for some antimicrobials (Figure 8, Table 10). In some cases, these deviations were more prevalent for certain methods, specifically: gradient test for amikacin, automated system for aztreonam-avibactam.

Discussion

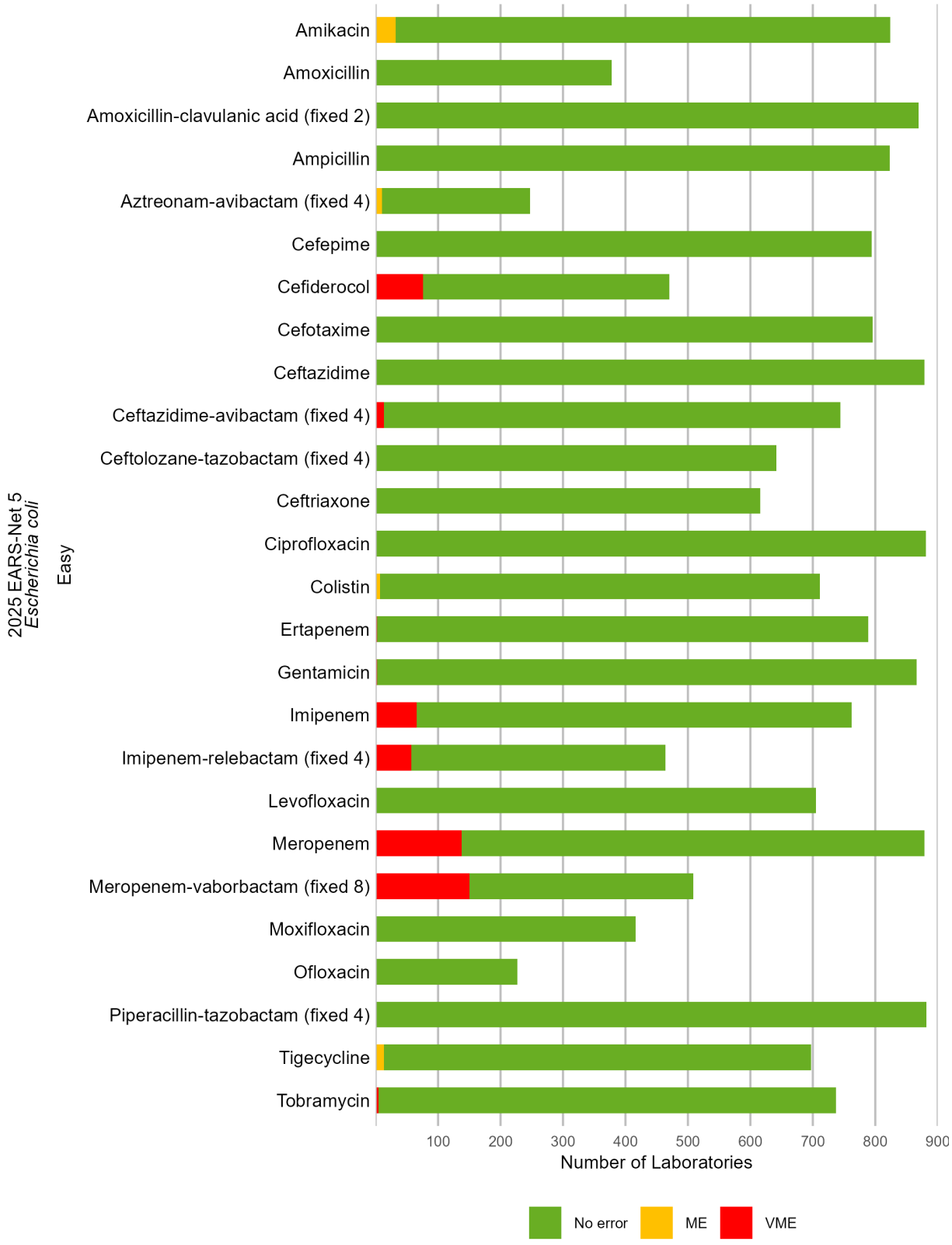
For strain **'2025 EARS-Net 5' (*Escherichia coli*)** all AST determinations were classified as 'easy'. Therefore, the observed errors should not be associated with the inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures.

For carbapenems, cefiderocol and aminoglycosides, the deviations may also reflect, or have been exacerbated by, variations in the methods and/or material used for testing [8-13]. Disk diffusion is the method currently recommended by EUCAST for AST of cefiderocol, and the results for this *E. coli* strain confirm that it achieved the highest concordance for that antimicrobial among the methods applied for this antimicrobial.

Overall, three out of four most frequently applied methods (automated system, broth microdilution and disk/tablet diffusion) achieved at least a 'very good' concordance with expected results (from 94.1% to 98.4%). Results obtained using the gradient test showed a relatively lower concordance (89.8%). In most cases, deviations were distributed across multiple methods; however, some methods exhibited lower performance for specific antimicrobials:

- Automated system for aztreonam-avibactam, cefiderocol, meropenem and meropenem-vaborbactam;
- Disk/tablet diffusion for aztreonam-avibactam;
- Broth microdilution for cefiderocol, imipenem, imipenem-relebactam, meropenem and meropenem-vaborbactam; and
- Gradient test for amikacin, cefiderocol, imipenem, imipenem-relebactam, meropenem and meropenem-vaborbactam.

Figure 8 Reported interpretation of AST results for strain '2025 EARS-Net 5' (*Escherichia coli*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.

Table 10 Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2025 EARS-Net 5' (*Escherichia coli*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	S	-	-	505	98.4	81	93.8	212	93.9	26	76.9	-	-	-	-	**824**	**96.1**
Amoxicillin	Easy	R	-	-	117	100.0	12	100.0	89	100.0	157	100.0	-	-	3	100.0	**378**	**100.0**
Amoxicillin-clavulanic acid (fixed 2 mg/L)	Easy	R	-	-	557	100.0	41	100.0	254	100.0	17	100.0	-	-	-	-	**869**	**100.0**
Ampicillin	Easy	R	-	-	508	100.0	45	100.0	250	100.0	19	100.0	-	-	1	100.0	**823**	**100.0**
Aztreonam-avibactam (fixed 4 mg/L)	Easy	S	-	-	7	71.4	17	100.0	41	85.4	180	99.4	-	-	2	50.0	**247**	**96.0**
Cefepime	Easy	R	-	-	512	100.0	61	100.0	213	99.5	8	100.0	-	-	-	-	**794**	**99.9**
Cefiderocol	Easy	R	1	0.0	22	68.2	85	64.7	328	96.0	33	27.3	-	-	1	0.0	**470**	**83.8**
Cefotaxime	Easy	R	-	-	494	100.0	58	98.3	218	100.0	26	100.0	-	-	-	-	**796**	**99.9**
Ceftazidime	Easy	R	-	-	563	100.0	67	100.0	239	100.0	9	100.0	1	100.0	-	-	**879**	**100.0**
Ceftazidime-avibactam (fixed 4 mg/L)	Easy	R	-	-	353	99.2	88	96.6	179	96.6	124	99.2	-	-	-	-	**744**	**98.3**
Ceftolozane-tazobactam (fixed 4 mg/L)	Easy	R	-	-	346	100.0	86	100.0	120	100.0	90	100.0	-	-	-	-	**642**	**100.0**
Ceftriaxone	Easy	R	-	-	279	100.0	25	100.0	217	100.0	94	100.0	-	-	1	100.0	**616**	**100.0**
Ciprofloxacin	Easy	R	2	100.0	569	100.0	67	100.0	236	100.0	7	100.0	-	-	-	-	**881**	**100.0**
Colistin	Easy	S	-	-	257	99.2	429	98.8	12	100.0	8	100.0	4	100.0	1	100.0	**711**	**99.0**
Ertapenem	Easy	R	-	-	479	99.8	59	98.3	204	100.0	46	100.0	1	100.0	-	-	**789**	**99.7**
Gentamicin	Easy	R	-	-	569	99.6	62	100.0	224	100.0	11	100.0	-	-	-	-	**866**	**99.8**
Imipenem	Easy	R	-	-	426	94.1	73	76.7	195	93.3	68	83.8	-	-	-	-	**762**	**91.3**
Imipenem-relebactam (fixed 4 mg/L)	Easy	R	-	-	215	96.3	54	81.5	74	95.9	119	69.7	1	100.0	1	100.0	**464**	**87.7**
Levofloxacin	Easy	R	-	-	390	99.7	38	100.0	228	100.0	49	100.0	-	-	-	-	**705**	**99.9**
Meropenem	Easy	R	-	-	505	82.2	84	77.4	209	93.8	80	80.0	1	100.0	-	-	**879**	**84.3**
Meropenem-vaborbactam (fixed 8 mg/L)	Easy	R	-	-	287	75.6	53	67.9	44	88.6	125	53.6	-	-	-	-	**509**	**70.5**
Moxifloxacin	Easy	R	-	-	127	100.0	16	100.0	203	100.0	70	100.0	-	-	-	-	**416**	**100.0**
Ofloxacin	Easy	R	1	100.0	40	100.0	6	100.0	145	100.0	34	100.0	-	-	1	100.0	**227**	**100.0**
Piperacillin-tazobactam (fixed 4 mg/L)	Easy	R	-	-	561	100.0	80	100.0	233	100.0	8	100.0	-	-	-	-	**882**	**100.0**
Tigecycline	Easy	S	-	-	385	97.1	103	99.0	136	99.3	73	100.0	-	-	-	-	**697**	**98.1**
Tobramycin	Easy	R	-	-	435	99.3	66	100.0	216	99.1	20	100.0	-	-	-	-	**737**	**99.3**
Total			**4**	**75.0**	**9508**	**97.5**	**1856**	**94.1**	**4719**	**98.4**	**1501**	**89.8**	**8**	**100.0**	**11**	**81.8**	**17607**	**96.7**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*)

Strain **'2025 EARS-Net 6' (*Streptococcus pneumoniae*)** was described as being obtained from a patient with bloodstream infection. This strain was susceptible to nine of the 10 antimicrobials included in the EQA: penicillin, oxacillin, cefotaxime, ceftriaxone, erythromycin, azithromycin, clarithromycin, moxifloxacin and norfloxacin. The expected MIC values fell within the 'I' range for one antimicrobial: levofloxacin (Annex 1).

The level of difficulty was considered 'easy' for all antimicrobials.

The strain did not harbour any known genetic mechanisms of resistance.

The strain showed reduced viability compared to the other EQA strains. Participants were therefore strongly encouraged to process this strain immediately upon receipt. However, several laboratories were unable to revive the sample. Consequently, the number of laboratories submitting results for this strain was lower when compared to the other strains included in this EQA.

Interpretation of AST results for the *S. pneumoniae* strain were analysed for the 484 laboratories that reported correct species identification (Table 3). In total, 40.1% of the laboratories (n=194) reported that they would have sent the strain to a reference or other laboratory for further testing.

Overall, 405 laboratories (83.7%) were in full concordance with the expected interpretations. A total of 18 (3.7%) achieved 'very good' concordance and 38 (7.9%) achieved 'good' concordance. 'Satisfactory' concordance was achieved by 11 laboratories (2.3%) while 12 (2.5%) performed below the threshold for 'satisfactory' concordance.

Each laboratory could submit results from 10 antimicrobials, corresponding to a maximum of 4 840 possible submissions. In total, 3 507 tests were performed, of which 3 399 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (96.9%). MEs were observed for 3.1% (n=108) of the reported interpretations (Figure 9). It was not possible to obtain VMEs for this strain.

The following AST methods were applied: automated system (44.0%), disk/tablet diffusion (30.0%), gradient test (20.9%), broth microdilution (4.1%), macro broth dilution (0.2%), agar dilution (0.1%), other (0.6%) (Table 4).

Most methods achieved 'excellent' concordance with the expected results (>95% of concordance). The exception was gradient test (92.5%), which achieved a 'very good' concordance (Table 11).

It was not possible to observe VMEs for this strain, as all antimicrobials had an expected result of S or I (Figure 9, Table 11).

A high proportion of MEs was observed for azithromycin (26.7% of submitted results), reported across most methods but especially prevalent with gradient test. Low proportions of MEs were observed for other antimicrobials (Figure 9, Table 11).

Discussion

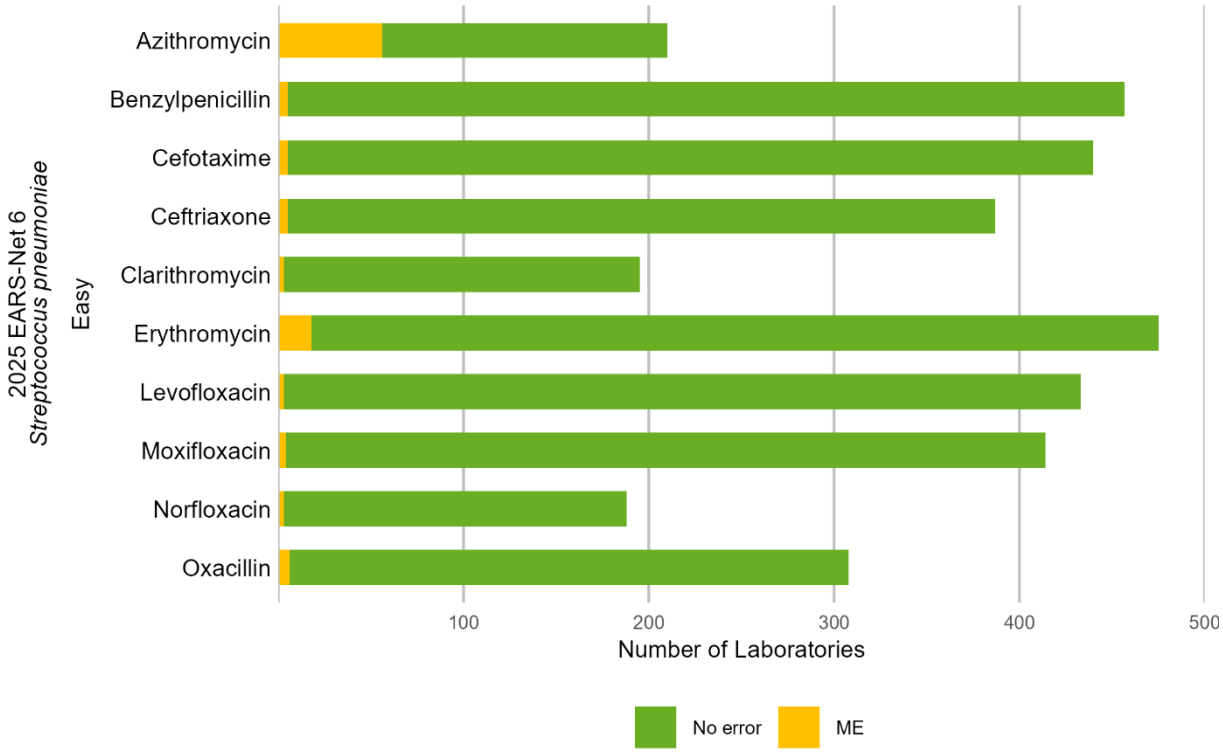
For strain **'2025 EARS-Net 6' (*Streptococcus pneumoniae*)** all AST determinations were classified as 'easy'. Therefore, the observed errors should not be associated with the inherent method variability. Instead, they are likely attributable to systematic or random errors in the participating laboratories' procedures.

Overall, three out of four frequently applied methods (automated system, broth microdilution and disk/tablet diffusion) achieved an 'excellent' concordance with expected results (>95%). Results obtained using the gradient test showed a slightly lower proportion of concordance (92.5%). In most cases, deviations were distributed across multiple methods; however, some methods exhibited lower performance for specific antimicrobials:

- Gradient test for azithromycin; and
- Automated system for oxacillin, whereas the other more frequently applied methods of disk/tablet diffusion and gradient test showed excellent concordance.

Disk diffusion is the method currently recommended by EUCAST for AST of oxacillin, and the results for the *S. pneumoniae* strain confirm that it achieved very high concordance when compared with automated system. Gradient test also achieved excellent concordance for this antimicrobial, however comparison must be cautious due to the large difference in number of tests performed with disk diffusion against other methods.

Figure 9 Reported interpretation of AST results for strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; M E: major error.

Table 11 Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*), by antimicrobial and AST method

Antimicrobial	Level of difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Azithromycin	Easy	S	-	-	49	95.9	12	91.7	35	94.3	104	51.0	1	100.0	9	100.0	**210**	**73.3**
Benzylpenicillin	Easy	S	1	100.0	223	98.2	30	96.7	42	100.0	158	100.0	1	100.0	2	100.0	**457**	**98.9**
Cefotaxime	Easy	S	-	-	246	98.4	21	95.2	25	100.0	146	100.0	1	100.0	1	100.0	**440**	**98.9**
Ceftriaxone	Easy	S	-	-	207	98.6	18	94.4	27	100.0	133	99.2	1	100.0	1	100.0	**387**	**98.7**
Clarithromycin	Easy	S	-	-	48	100.0	15	100.0	39	97.4	86	97.7	1	100.0	6	100.0	**195**	**98.5**
Erythromycin	Easy	S	1	100.0	256	93.4	20	95.0	176	100.0	21	100.0	1	100.0	-	-	**475**	**96.2**
Levofloxacin	Easy	I	-	-	254	98.8	15	100.0	117	100.0	45	100.0	1	100.0	1	100.0	**433**	**99.3**
Moxifloxacin	Easy	S	-	-	248	98.8	13	92.3	122	100.0	29	100.0	1	100.0	1	100.0	**414**	**99.0**
Norfloxacin	Easy	S	1	100.0	6	100.0	-	-	180	98.9	1	0.0	-	-	-	-	**188**	**98.4**
Oxacillin	Easy	S	1	100.0	7	85.7	1	100.0	288	98.3	11	100.0	-	-	-	-	**308**	**98.1**
Total			**4**	**100.0**	**1544**	**97.6**	**145**	**95.9**	**1051**	**99.0**	**734**	**92.5**	**8**	**100.0**	**21**	**100.0**	**3507**	**96.9**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of satisfactory concordance (<80%); light blue: satisfactory concordance (>80 to ⩽85%); light green: good concordance (>85 to ⩽90%); intermediate green: very good concordance (>90% to <95%); dark green: excellent concordance (⩾95%).

** 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. The remaining situations are 'Easy'.*

*** Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

Percentages might not total 100% due to rounding.

Feedback survey of participating laboratories

A link to the feedback survey was shared with all contacts in the participating laboratories via email on 18 November 2025, with a deadline to reply by 10 December 2025. The survey questions can be found in Annex 3.

In total, 199 laboratories provided feedback (22.2% of the 895 laboratories submitting results), similar to the response rate in 2024 (21.1%) [2]. Of these, 95 (47.7%) reported having taken corrective action based on their EQA results. The main actions taken were re-testing of isolate(s), reviewing standard operating procedures or other laboratory protocols, validating and/or updating the AST methods, verifying reagents, contacting and/or changing suppliers of materials or equipment. 50 laboratories (25.1%) replied that all EQA analytical test results conformed to expected results and no further action was taken.

There were 105 laboratories (52.8%) that replied that they would use the results as documentation for accreditation and/or licensing purposes. This is similar to the proportion reported in 2024 (51.0%).

Overall, 180 laboratories (90.5%) were satisfied with the individual evaluation report. This is an increase from 2024 (88.6%). Moreover, 21 laboratories provided additional comments, and the majority of the comments were related to their own results.

There were 31 laboratories that provided suggestions for improvement of the next EQA:

- Some laboratories asked for additional information, such as lists of antimicrobials included in the EQA, guidelines for reporting results for each antimicrobial, description of the methods used by EQA providers to define expected results, descriptions of the types of errors and respective scores, and the AMR genes detected in EQA strains. Generally, the requested types of information were available to participants in the EARS-Net EQA protocol and/or the test forms for each species, as well as within the individual evaluation reports or documents with expected results uploaded to the EQA website.
- Some laboratories expressed 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 and detailed AST results stratified by method are 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 4 years.
- Some laboratories expressed dissatisfaction with the EQA period (i.e. summer).
- Some laboratories provided specific wishes for future strains to be included in the EQA.

4. Summary and discussion

Participation

A total of 29 out of 29 eligible EU/EEA countries participated in the 2025 EARS-Net EQA exercise. The number of invited laboratories for the 2025 EARS-Net EQA (n=977) was similar to the previous year (n=980). A high proportion of invited laboratories (n=895; 91.6%) submitted results for validation, consistent with previous EARS-Net EQA exercises conducted in 2021–2024, during which 90.2–93.1% of laboratories submitted results [2–5].

Comparison between EARS-Net EQA results across different years should be made with great caution, as the included strains are different in terms of their species, the included antimicrobials and level of difficulty of each AST determination. In addition, in 2020–2021, the response to the EARS-Net EQA was affected by the COVID-19 pandemic, as many countries reallocated laboratory resources to pandemic-related activities. In 2020, ECDC did not initiate an EARS-Net EQA exercise, and, in 2021, only 642 laboratories registered to participate and 592 submitted results [5].

Speciation and overall AST results

In the 2025 EQA exercise, the submitted species identification results were in 'excellent' concordance with the expected results, as correct species identification was reported for 4 917 of 4 932 submissions (99.7%). This is comparable to the results obtained in 2022-2024 EARS-Net EQAs (95.7%-99.2%) [2-4]. At the strain level, concordance with the expected results was also 'excellent' for each of the six EQA strains (98.8% to 100%). Similar results were observed in the 2022-2024 EQAs (97.7%-99.4%), with the exception of one strain in 2022 (80.1%) [4].

The submitted interpretations of AST results were in 'very good' concordance with expected results, with 53 742 correct interpretations out of 56 924 submissions (94.4%). In the 2021-2024 EARS-Net EQAs, the concordance of AST interpretation was also 'very good', ranging from 91.7% to 94.7%) [2-5]. At strain level, concordance with the expected results ranged from 'good' to 'excellent' (89.8% to 98.8%). Similar variation was observed in the 2021-2024 EQAs (88.0%-99.0%), with the exception of one strain in 2024 that showed lower concordance (80.9%) [2].

By country, concordance with the expected interpretation of AST results ranged from 90.6% ('very good') to 97.4% ('excellent'). In total, 14 countries achieved 'excellent' concordance, and 15 countries achieved 'very good' concordance. This represents an improvement compared with 2024, when no countries achieved 'excellent' concordance, 28 countries achieved a 'very good' concordance, and two countries achieved only 'good' concordance [2]. The results from the 2025 EARS-Net EQA are more consistent with those observed in 2023, when 17 countries achieved an 'excellent' concordance, and 13 countries achieved a 'very good' concordance [3].

Results at laboratory level indicate that, overall, the participating laboratories are able to produce reliable AST interpretations from clinical samples, suggesting that they are following the most recent EUCAST guidelines and breakpoints.

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

The lowest percentages of concordance were observed for the *K. pneumoniae* strain '2025 EARS-Net EQA 1', with only 23.3% of correct results submitted for meropenem-vaborbactam, 28.9% for levofloxacin, and 78.4% for cefiderocol. Other non-satisfactory results were observed for the two *A. baumannii* strains: '2025 EARS-Net 2' with 41.9% of correct results for amikacin, and '2025 EARS-Net 4' with 64.3% of correct results for cefiderocol. Two additional combinations did not achieve the satisfactory threshold: meropenem-vaborbactam in *E. coli* '2025 EARS-Net 5' (70.5%) and azithromycin in *S. pneumoniae* '2025 EARS-Net 6' (73.3%).

Strain *S. aureus* '2025 EARS-Net 3' showed the best overall concordance, with 'excellent' agreement (98.8%). This is likely due to the lower number of included antimicrobials and their relative ease of determination, as most were classified as 'easy'.

The distribution of AST methods used in the 2025 EARS-Net EQA exercise was similar to that observed in previous years. A total of 53.0% of submitted results were obtained using automated systems (compared with 51.4% to 55.8% in previous years), 27.6% using disk or tablet diffusion (26.0% to 28.2% in previous years), 11.0% using broth microdilution (9.5% to 11.4% in previous years) and 8.2% using gradient test (4.9% to 9.7% in previous years) [2-5].

In 2025, 'excellent' concordance was observed only for disk/tablet diffusion (95.6%), while 'very good' concordance was achieved for automated system (94.9%), macro broth dilution (93.1%), broth microdilution (92.6%), and agar dilution (90.3%). The gradient test had 'good' concordance (89.7%). Overall, these results indicate that AST methods applied by European laboratories are robust and accurate for the species and antimicrobials included in the 2025 EARS-Net EQA exercise.

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 for accreditation or licensing purposes.

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

In the 2021-2024 EARS-Net EQA exercises [2-5], the determination and interpretation of AST results showed problematic outcomes for several bacteria and antimicrobials. The laboratories participating in the 2025 EARS-Net EQA exercise reported issues for several of the same species-antimicrobial combinations that had been problematic in previous EQA exercises. Importantly, new issues were observed in the 2025 EARS-Net EQA that had not been problematic in previous EARS-Net EQA exercises, or for antimicrobials included for the first time in this EQA (Annex 4).

The results of the 2025 EARS-Net EQA exercise show that there are still difficulties and that there has been a lack of improvement regarding the prediction of AST profiles for beta-lactam antimicrobials, especially carbapenems, in *E. coli* and *K. pneumoniae*. Moreover, the antimicrobial agents recently added to European surveillance also appear to pose a challenge for the laboratories, i.e., the combinations of carbapenems and beta-lactamase inhibitors, and cefiderocol.

The results support a continuing phenomenon, observed across species, of difficulties in predicting AST results for aminoglycosides.

The results show that AST of azithromycin for *S. pneumoniae* strains remains technically challenging, as observed in 2022.

For the first time since the 2021 EARS-Net EQA exercise, determination of the AST profile for colistin was challenging for participants.

Overall, results of the 2025 EARS-Net EQA exercise did not show a systematic overestimation or underestimation of AMR in the EU/EEA, with deviations being distributed across both types of errors (MEs and VMEs).

The results from the 2025 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 across all of the methods applied. From the commonly applied methods the gradient test was the one with the lowest concordance of submitted results, nevertheless reaching 89.7% of accurate results. However, there were situations where a specific method seemed to influence the percentage of correct results:

- Disk diffusion was the most accurate method for AST of cefiderocol in the Gram-negative species.
- Broth microdilution was the most accurate method for AST of colistin in the Gram-negative species, and overall, gradient test had the worst performance.
- Automated systems, followed by disk diffusion, were the most accurate methods for AST of carbapenems and their combinations with beta-lactamase inhibitors, for Enterobacterales. For meropenem and meropenem-vaborbactam in the *E. coli* strain, disk/tablet diffusion was more accurate than automated system.
- Conversely, broth microdilution and gradient test had the worst performance for AST of carbapenems and their combinations with beta-lactamase inhibitors, for Enterobacterales. The exception was meropenem-vaborbactam in *K. pneumoniae* where those were the most accurate methods, when compared with automated system and disk/tablet diffusion.
- Automated systems had particularly bad performance for AST of aztreonam-avibactam in Enterobacterales.
- Automated systems had worse performance than disk diffusion for norfloxacin (*S. aureus*) and oxacillin (*S. pneumoniae*).
- Gradient test had particularly poor performance for AST of azithromycin for *S. pneumoniae*.

5. Conclusions

The submitted species identification results strongly suggest that overall, the species data reported to EARS-Net are accurate, as there were 99.7% correct species identification results submitted in this EQA.

The submitted AST interpretations also imply that AST data reported to EARS-Net are mostly accurate, although MEs were observed for 2.6% and VMEs for 3.0% of the reported interpretations. Both MEs and VMEs suggest the possibility for sub-optimal treatment outcomes, albeit in a small percentage of bloodstream infections. One frequent justification for the submission of incorrect results was the inherent method variability of plus or minus one dilution in dilution methods, and plus or minus three millimetres in diffusion methods, especially when the AST determinations were classified as 'difficult' because the expected values corresponded to borderline concentrations or diameters very close to the clinical breakpoints, which increased the likelihood of misclassification. However, the MEs and VMEs detected in this EARS-Net EQA exercise also included strain-antimicrobial 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.

In specific cases, certain antimicrobials or classes presented higher percentages of deviations, namely:

- Carbapenems and their combinations with beta-lactamase inhibitors in Enterobacterales.
- Cefiderocol, across Gram-negative species.
- Aminoglycosides, especially amikacin and gentamicin, across Gram-negative species.
- Levofloxacin in *K. pneumoniae*.
- Colistin in *A. baumannii*.
- Azithromycin in *S. pneumoniae*.

Some of these problematic species-antimicrobial combinations had been observed in previous EQA exercises. These technically challenging cases highlight an opportunity for improvement at EU/EEA level. The findings may also indicate that AMR is heterogeneously reported in the EU/EEA. The VMEs showed a tendency to under-report resistance to carbapenems and their combinations with beta-lactamase inhibitors in Enterobacterales. Likewise, resistance of Gram-negative pathogens towards cefiderocol and amikacin may be under-reported, as well as resistance of *A. baumannii* to colistin. Inversely, the MEs indicate a trend of over-reporting of resistance to gentamicin in *A. baumannii*, levofloxacin in *K. pneumoniae*, and azithromycin in *S. pneumoniae*.

The analysis of the overall performance of the different AST methods showed few differences between methods, except for a slightly poorer performance of gradient tests. Results from this EQA confirm EUCAST recommendations to use specific method for certain antimicrobials, namely disk diffusion for cefiderocol, norfloxacin and oxacillin, and broth microdilution for colistin. Specific shortcomings were observed for some methods:

- Overall, gradient test had the worst performance for AST of colistin.
- Broth microdilution and gradient test had the worst performance for AST of carbapenems and their combinations with beta-lactamase inhibitors, for Enterobacterales.
- Automated system had particularly bad performance for AST of aztreonam-avibactam in Enterobacterales.
- Gradient test had particularly poor performance for AST of azithromycin for *S. pneumoniae*.

The 2025 EARS-Net EQA exercise also revealed a tendency of assigning the wrong S/I/R categories to diffusion results (and in lesser extent to dilution results) during AST of cefiderocol in one of the *A. baumannii* strains, where the expected interpretation was I. This was potentially due to conflicts between EUCAST guidelines (which do not contain clinical breakpoints for cefiderocol in the species but instead include information in the Notes), when compared with the instructions for the EQA (where participants must use S/I/R categories for several situations that are not necessarily categorised as S/I/R during routine laboratory practice). However, this misattribution of S/I/R categories was not observed in the results for cefiderocol in the other *A. baumannii* strain nor for the Enterobacterales strains, for which the expected result was R. This suggests that participants did not struggle with the concept of using S/I/R categories to report results of cefiderocol in *A. baumannii* for the purpose of this EQA. Instead, participants may not strictly comply with information presented in the Notes sections of the EUCAST guidelines.

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

6. Recommendations

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. In general, it is recommended to:

- Opting to use the EUCAST-recommended AST methods for each species-antimicrobial combination being tested.
- Confirming that the AST protocols in use for each method 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 combinations).
- Becoming familiar with EUCAST recommendations regarding AST results within the ATU or results near the clinical breakpoints.
- 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.
- 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.
- Ensuring that adequate control strains are being used and monitored to guarantee reliability of results.
- Ensuring that the relevant quality management systems and control measures are in place, including but not limited to monitoring AST results over time, to allow the control of random deviations and the detection of systematic deviations.
- 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.

The results from this EQA exercise indicate that some inaccuracy, through both under- and overestimation of AMR percentages, may occur in Europe. Consequently, AST guidance, and surveillance and control efforts should consider the specific inaccuracies observed during this EQA, for each specific antimicrobial or class, most particularly regarding inaccurate AST interpretations for results close to current breakpoints. In that regard, there were findings of note, including:

- Challenges for AST of aminoglycosides across species.
- Problems with determining AMR profiles for beta-lactam antimicrobials (especially carbapenems) in Enterobacterales.
- Difficulties in the AST of colistin and cefiderocol, clearly confirming that the EUCAST-recommended methods were the ones with better performance.
- Poor performance of gradient test for AST of azithromycin in *S. pneumoniae*.
- Poor performance of automated systems for AST of aztreonam-avibactam, norfloxacin and oxacillin.

Participating laboratories observing errors in their results should assess those errors to evaluate their methodologies and procedures, and consider the suggested corrective actions:

- Strengthening awareness and potentially seeking advice regarding AST and reading of results for macrolides.
- Strengthening awareness and potentially seeking advice regarding AST and reading of results for carbapenems and for the more recent beta-lactam/beta-lactamase inhibitor combinations.
- 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.
- Ensuring compliance with the most recent EUCAST recommendations and warnings regarding AST of cefiderocol, including applying the disk diffusion method and being aware of possible variation in results depending on the media and disks used for AST. Additionally, strengthening awareness and potentially seeking advice regarding the interpretation of AST results for cefiderocol.
- Ensuring compliance with the most recent EUCAST recommendations and warnings regarding AST of colistin, including applying the broth microdilution method.

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 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.

References

1. European Centre for Disease Prevention and Control (ECDC). Antimicrobial resistance (AMR) reporting protocol 2025 European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2024. Available at: https://www.ecdc.europa.eu/en/publications-data/reporting-protocol-antimicrobial-resistance-amr
2. European Centre for Disease Prevention and Control (ECDC). External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2024. Stockholm: ECDC; 2025. Available at: https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-external-quality-assessment-laboratories-ears-net-2024
3. European Centre for Disease Prevention and Control (ECDC). External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2023. Stockholm: ECDC; 2024. Available at: https://www.ecdc.europa.eu/en/publications-data/external-quality-assessment-eqa-performance-laboratories-participating-european-1
4. European Centre for Disease Prevention and Control (ECDC). External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2022. Stockholm: ECDC; 2023. Available at: https://www.ecdc.europa.eu/en/publications-data/external-quality-assessment-eqa-performance-laboratories-participating-european-0
5. European Centre for Disease Prevention and Control (ECDC). External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2021. Stockholm: ECDC; 2022. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/antibiotic-resistance-external-quality-assessment-laboratories-EARS-Net-2021.pdf
6. Technical University of Denmark (DTU). Protocol for the External Quality Assessment (EQA) exercise for laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2025. Available at: https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net
7. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical Breakpoints Tables v15.0. EUCAST; 2025. Available at: https://www.eucast.org/clinical_breakpoints
8. Haldorsen B, Giske CG, Hansen DS, Helgason KO, Kahlmeter G, Löhr IH, et al. Performance of the EUCAST disc diffusion method and two MIC methods in detection of Enterobacteriaceae with reduced susceptibility to meropenem: the NordicAST CPE study. J Antimicrob Chemother. 2018;73(10):2738-2747. doi: 10.1093/jac/dky276.
9. Cimen C, Siemer P, Sattler J, Voss A, Berends MS, Hamprecht A. Performance of disk diffusion, gradient test, and VITEK 2 for carbapenem susceptibility testing in OXA-48-like carbapenemase-producing Enterobacterales: a comparative study. J Clin Microbiol. 2025;63(5):e0189324. doi: 10.1128/jcm.01893-24.
10. Zhao Z, Lan F, Liu M, Chen W, Huang L, Lin Q, et al. Evaluation of automated systems for aminoglycosides and fluoroquinolones susceptibility testing for Carbapenem-resistant Enterobacteriaceae. Antimicrob Resist Infect Control 2017;6:77. doi: 10.1186/s13756-017-0235-7.
11. Cayci YT, Ulker KH, Birinci A. Evaluation of three different methods for susceptibility testing of gentamicin in carbapenem resistant Enterobacterales. Infez Med. 2021;29(4):568-573. doi: 10.53854/liim-2904-10.
12. Bonnin RA, Emeraud C, Jousset AB, Naas T, Dortet L. Comparison of disk diffusion, MIC test strip and broth microdilution methods for cefiderocol susceptibility testing on carbapenem-resistant Enterobacterales. Clin Microbiol Infect. 2022;28(8):1156e1-e5. doi: 10.1016/j.cmi.2022.04.013.
13. DeMarco MG, Field AM, Donohue LE, Cox HL, Kidd TS, Barry KE, et al. Impact of media brand on cefiderocol disk diffusion results. J Clin Microbiol. 2025;63(5):e01648-24. doi: 10.1128/jcm.01648-24.
14. Akers KS, Chaney C, Barsoumian A, Beckius M, Zera W, Yu X, et al. Aminoglycoside resistance and susceptibility testing errors in *Acinetobacter baumannii-calcoaceticus* complex. J Clin Microbiol. 2010;48(4):1132-8. doi: 10.1128/JCM.02006-09.

Annex 1. Expected results for the 2025 EARS-Net EQA

Table 12. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 1' (*K. pneumoniae),* by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]			EUCAST zone diameter breakpoints (mm) [a]			Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	ATU	S ≥	R <	ATU				
Amikacin	8	8		18	18		Difficult	16	R	*aac(6')-Ib, aph(3')-VI*
Amoxicillin-clavulanic acid iv (fixed 2 mg/L)	8	8		19	19	19-20	Easy	>64	R	bla_{NDM-1}, bla_{DHA-1}
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	0.06	S	(Absent from databases)
Cefepime	1	4		27	24		Easy	>16	R	bla_{NDM-1}, $bla_{CTX-M-15}$
Cefiderocol	2	2		23	23	21-23	Difficult	18 mm	R	(Absent from databases)
Cefotaxime	1	2		20	17		Easy	>8	R	bla_{NDM-1}, bla_{DHA-1}, $bla_{CTX-M-15}$
Ceftazidime	1	4		22	19		Easy	>16	R	bla_{NDM-1}, bla_{DHA-1}, $bla_{CTX-M-15}$
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	>16	R	bla_{NDM-1}
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Easy	>8	R	(Absent from databases)
Ceftriaxone	1	2		27	24		Easy	>4	R	$bla_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Difficult	1	R	*qnrS1, qnrB4*
Colistin	2	2		Note	Note		Easy	0.5	S	None
Ertapenem	0.5	0.5		23	23		Easy	>4	R	bla_{NDM-1}
Gentamicin	2	2		17	17		Easy	0.5	S	None
Imipenem	2	4		22	19		Difficult	8	R	bla_{NDM-1}
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	8	R	(Absent from databases)
Levofloxacin	0.5	1		23	19		Difficult	1	I	*qnrS1, qnrB4*
Meropenem	2	8		22	16		Difficult	16	R	bla_{NDM-1}
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Difficult	8	S	(Absent from databases)
Moxifloxacin	0.25	0.25		22	22		Easy	2	R	*qnrS1, qnrB4*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]			EUCAST zone diameter breakpoints (mm) [a]			Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	ATU	S ≥	R <	ATU				
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	*qnrS1, qnrB4*
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64	R	bla_{NDM-1}, bla_{DHA-1}
Tobramycin	2	2		16	16		Easy	>8	R	*aac(6')-Ib*

MALDI-TOF by DTU: Klebsiella pneumoniae (score 2,41). MLST: ST-391.

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

[c] *For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For cefiderocol the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines or EUCAST warnings recommend a disk diffusion test instead of broth microdilution.*

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: blaTEM-1, blaOXA-9, blaSHV-11 (intrinsic), sul1, dfra14, aadA1, aph(6)-Id, aph(3'')-Ib, mph(A), oqxA (intrinsic), oqxB (intrinsic), fosA6 (intrinsic), acrR mutations potentially associated with decreased susceptibility towards fluoroquinolones (P161R, G164A, F172S, R173G, L195V, F197I, K201M), ompK36 mutations potentially associated with decreased susceptibility towards cephalosporins (N49S, L59V, L191S, F207W, D224E, L228V, E232R, T254S), ompK36 mutations potentially associated with decreased susceptibility towards carbapenems (A217S, N218H), ompK37 mutations potentially associated with decreased susceptibility towards carbapenems (I70M, I128M).*

Table 13. EUCAST clinical breakpoints for *Acinetobacter baumannii* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 2' (*A. baumannii*), by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]		EUCAST zone diameter breakpoints (mm) [a]		Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Difficult	16	R	*aph(3')-VI*
Cefiderocol	Note	Note	Note	Note	Difficult	14 mm	R	(Absent from databases)
Ciprofloxacin	0.001	1	50	21	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin	2	2	Note	Note	Easy	<=0.5	S	None
Gentamicin	4	4	17	17	Difficult	4	S	None
Imipenem	2	4	24	21	Easy	>8	R	bla_{NDM-1}
Levofloxacin	0.5	1	23	20	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	>16	R	bla_{NDM-1}
Tobramycin	4	4	17	17	Easy	1	S	None

MALDI-TOF by DTU: Acinetobacter baumannii (score 2,41). MLST: ST-1089 (Oxford) / ST-85 (Pasteur).

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

[c] *For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For cefiderocol the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines or EUCAST warnings recommend a disk diffusion test instead of broth microdilution.*

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: msr(E), mph(E), sul2, ant(3'')-IIa, blaOXA-94 (OXA-51-like, intrinsic), blaADC-25 (intrinsic).*

Table 14. EUCAST clinical breakpoints for *Staphylococcus aureus* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 3' (*S. aureus*), by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]		EUCAST zone diameter breakpoints (mm) [a]		Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	S ≥	R <				
Cefoxitin	Note	Note	22	22	Easy	11 mm	R	*mecA*
Ciprofloxacin	0.001	2	50	17	Easy	<=0.5	I	None
Daptomycin	1	1	Note	Note	Easy	<=0.25	S	None
Levofloxacin	0.001	1	50	22	Easy	<=0.25	I	None
Linezolid	4	4	21	21	Easy	<=2	S	None
Norfloxacin	-	-	17	17	Difficult	20 mm	S	None
Oxacillin	Note	Note	Note	Note	Easy	>8	R	*mecA*
Rifampicin	0.06	0.06	26	26	Easy	<=0.08	S	None
Vancomycin	2	2	Note	Note	Easy	<=1	S	None

MALDI-TOF by DTU: Staphylococcus aureus (score 2,51). MLST: ST-398.

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

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

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: blaZ, tet(M), dfrG, str, murA mutations potentially associated with decreased susceptibility towards fosfomycin (D278E, E291D), glpT mutations potentially associated with decreased susceptibility towards fosfomycin (F3I, A100V).*

Table 15. EUCAST clinical breakpoints, expected AST results for *Acinetobacter baumannii* and the level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 4' (*A. baumannii*), by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]		EUCAST zone diameter breakpoints (mm) [a]		Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Easy	>32	R	*armA, aph(3')-Via*
Cefiderocol	Note	Note	Note	Note	Difficult	19 mm	I	(Absent from databases)
Ciprofloxacin	0.001	1	50	21	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin	2	2	Note	Note	Easy	>4	R	None
Gentamicin	4	4	17	17	Easy	>16	R	*armA, aph(3')-Via*
Imipenem	2	4	24	21	Easy	>8	R	bla_{OXA-23}
Levofloxacin	0.5	1	23	20	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	>16	R	bla_{OXA-23}
Tobramycin	4	4	17	17	Easy	>8	R	*armA*

MALDI-TOF by DTU: Acinetobacter baumannii (score 2,36). MLST: ST-1089 or ST-451 (Oxford; multiple gdhB hits) / ST-2 (Pasteur).

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

[c] *For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For cefiderocol the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines or EUCAST warnings recommend a disk diffusion test instead of broth microdilution.*

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: msr(E), mph(E), sul2, tet(B), aph(6)-Id, aph(3')-Ia, aph(3'')-Ib, ant(3'')-IIa, blaTEM-1, blaOXA-66 (OXA-51-like, intrinsic), blaADC-25 (intrinsic), ftsI A515V potentially associated with decreased susceptibility towards carbapenems, pmrC R125P potentially associated with decreased susceptibility towards colistin.*

Table 16. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 5' (*E. coli*), by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]			EUCAST zone diameter breakpoints (mm) [a]			Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	ATU	R <	S ≤	ATU				
Amikacin	8	8		18	18		Easy	4	S	*aac(6')-Ib-cr*
Amoxicillin	8	8		Note	Note		Easy	>32	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{CMY-2}$
Amoxicillin-clavulanic acid (fixed 2 mg/L)	8	8		19	19	19-20	Easy	>32	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{CMY-2}$
Ampicillin	8	8		14	14		Easy	>32	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{CMY-2}$
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	2	S	(Absent from databases)
Cefepime	1	4		27	24		Easy	>16	R	*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$
Cefiderocol	2	2		23	23	21-23	Easy	16 mm	R	(Absent from databases)
Cefotaxime	1	2		20	17		Easy	>8	R	*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{CMY-2}$
Ceftazidime	1	4		22	19		Easy	>16	R	*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{CMY-2}$
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	>16	R	*bla*$_{NDM-5}$
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Easy	>8	R	(Absent from databases)
Ceftriaxone	1	2		27	24		Easy	>4	R	*bla*$_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	>4	R	*qnrS1, aac(6')-Ib-cr, gyrA* S83L, *gyrA* D87N, *parC* S80I, *parE* S458A
Colistin	2	2		Note	Note		Easy	<=0.5	S	None
Ertapenem	0.5	0.5		23	23		Easy	>4	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Gentamicin	2	2		17	17		Easy	>16	R	*aac(3)-Iid*
Imipenem	2	4		22	19		Easy	>8	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	>4	R	(Absent from databases)
Levofloxacin	0.5	1		23	19		Easy	>4	R	*qnrS1, aac(6')-Ib-cr, gyrA* S83L, *gyrA* D87N, *parC* S80I, *parE* S458A
Meropenem	2	8		22	16		Easy	>16	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Easy	>16	R	(Absent from databases)
Moxifloxacin	0.25	0.25		22	22		Easy	>8	R	q*nrS1, aac(6')-Ib-cr, gyrA* S83L, *gyrA* D87N, *parC* S80I, *parE* S458A

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]			EUCAST zone diameter breakpoints (mm) [a]			Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	ATU	R <	S ≤	ATU				
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	*qnrS1, aac(6')-Ib-cr, gyrA* S83L, *gyrA* D87N, *parC* S80I, *parE* S458A
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64	R	bla_{NDM-5}, bla_{OXA-1}, $bla_{OXA-181}$, bla_{CMY-2}
Tigecycline	0.5	0.5		18	18		Easy	<=0.25	S	None
Tobramycin	2	2		16	16		Easy	>8	R	*aac(6')-Ib-cr*

MALDI-TOF by DTU: Escherichia coli (score 2,49). MLST: ST-410 (Achtman).

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

[c] *For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For cefiderocol the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines or EUCAST warnings recommend a disk diffusion test instead of broth microdilution.*

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: blaTEM-1, aph(6)-Id, aph(3")-Ib, aadA2, aadA5, mph(A), catB3, sul1, sul2, tet(B), dfrA12, dfrA17, ompC R195L potentially associated with decreased susceptibility towards carbapenems, glpT E448K potentially associated with decreased susceptibility towards fosfomycin, ftsI N337NYRIN potentially associated with decreased susceptibility towards aztreonam and cephalosporins.*

Table 17. EUCAST clinical breakpoints for *Streptococcus pneumoniae* and the expected MIC value, level of difficulty in interpretation and interpretation for strain '2025 EARS-Net 6' (*S. pneumoniae*), by antimicrobial

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L) [a]		EUCAST zone diameter breakpoints (mm) [a]		Level of difficulty [b]	Expected result [c]	Expected interpretation [d]	Genetic determinants of AMR [e]
	S ≤	R >	S ≥	R <				
Azithromycin	0.25	0.25	Note	Note	Easy	<=0.06	S	None
Benzylpenicillin	0.06	1	Note	Note	Easy	0.015	S	None
Cefotaxime	0.5	2	Note	Note	Easy	<=0.03	S	None
Ceftriaxone	0.5	2	Note	Note	Easy	<=0.03	S	None
Clarithromycin	0.25	0.25	Note	Note	Easy	<=0.03	S	None
Erythromycin	0.25	0.25	22	22	Easy	0.03	S	None
Levofloxacin	0.001	2	50	16	Easy	1	I	None
Moxifloxacin	0.5	0.5	22	22	Easy	0.125	S	None
Norfloxacin	-	-	10	10	Easy	20 mm	S	None
Oxacillin	-	-	20	20	Easy	27 mm	S	None

MALDI-TOF by DTU: Streptococcus pneumoniae (score 2,25). MLST: ST-53.

[a] *EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables.*

[b] *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. The remaining situations are 'Easy'.*

[c] *For most antimicrobials the expected value corresponds to the MIC expressed in 'mg/L'. For oxacillin and norfloxacin the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines or EUCAST warnings recommend a disk diffusion test instead of broth microdilution.*

[d] *Generated using EUCAST Clinical Breakpoint Tables v15.0 (valid from 01-01-2025), and the adjustments to the EUCAST reporting guidelines described in Section 2 of this report 'Characteristics of the EQA strains and interpretation of AST results'.*

[e] *Additional determinants of AMR: None.*

Annex 2. List of participating countries

Table 18. Number of laboratories receiving material and submitting results for the 2025 EARS-Net EQA exercise

EU/EEA country	Number of laboratories receiving EQA material	Number of laboratories submitting data		Number of laboratories included in the analysis of AST results	
	N	N	%	N	%
Austria	38	38	100.0	38	100.0
Belgium	30	28	93.3	28	100.0
Bulgaria	20	20	100.0	20	100.0
Croatia	37	36	97.3	36	100.0
Cyprus	9	8	88.9	8	100.0
Czechia	49	47	95.9	47	100.0
Denmark	6	4	66.7	4	100.0
Estonia	11	11	100.0	11	100.0
Finland	13	13	100.0	13	100.0
France	57	45	78.9	45	100.0
Germany	26	25	96.2	25	100.0
Greece	41	36	87.8	36	100.0
Hungary	24	23	95.8	23	100.0
Iceland	1	1	100.0	1	100.0
Ireland	32	28	87.5	28	100.0
Italy	217	199	91.7	199	100.0
Latvia	13	13	100.0	13	100.0
Lithuania	16	14	87.5	14	100.0
Luxembourg	5	5	100.0	5	100.0
Malta	1	1	100.0	1	100.0
Netherlands	29	27	93.1	27	100.0
Norway	17	17	100.0	17	100.0
Poland	65	63	96.9	63	100.0
Portugal*	110	90	81.8	89	98.9
Romania	22	20	90.9	20	100.0
Slovakia	14	13	92.9	13	100.0
Slovenia	11	11	100.0	11	100.0
Spain	41	38	92.7	38	100.0
Sweden	22	21	95.5	21	100.0
Total	**977**	**895**	**91.6**	**894**	**99.9**

** One laboratory was excluded from the analysis for this EQA because they reported using CLSI guidelines.*

Annex 3. Feedback Survey Questionnaire

EARS-Net EQA 2025 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 European Union Reference Laboratory for Public Health on Antimicrobial Resistance in Bacteria (EURL-PH-AMR), 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

Annex 4. Problematic AST determinations in the 2021-2025 EARS-Net EQAs

Table 19. Problematic AST determinations detected in the 2021-2025 EARS-Net EQAs

Species	2021 [5]	2022 [4]	2023 [3]	2024 [2]	2025
E. coli	Gentamicin, S				
		Amikacin, R	Amikacin, S	Amikacin, S	
	Tigecycline, R				
	Fluoroquinolones, I and R				
	Imipenem, S and R				Imipenem, R
	Meropenem, S and I				Meropenem, R
	Ceftazidime, R	Ceftazidime, I	Ceftazidime, I	Ceftazidime, I	
		Cefepime, S	Cefepime, S	Cefepime, I	
		Piperacillin-tazobactam, S	Piperacillin-tazobactam, R	Piperacillin-tazobactam, R	
	Amoxicillin-clavulanic acid, S				
					Cefiderocol, R
					Imipenem-relebactam, R
					Meropenem-vaborbactam, R
K. pneumoniae	Imipenem, I and S		Imipenem, S	Imipenem, I	Imipenem, R
	Meropenem, S				Meropenem, R
	Cefepime, R		Cefepime, S	Cefepime, I	
			Amikacin, S	Amikacin, S	Amikacin, R
					Levofloxacin, I
					Cefiderocol, R
					Imipenem-relebactam, R
					Meropenem-vaborbactam, S
A. baumannii		Tobramycin, R		Tobramycin, R	
		Gentamicin, R			Gentamicin, S
			Amikacin, R		Amikacin, R
					Colistin, R
					Cefiderocol, I and R
P. aeruginosa		Levofloxacin, I			
				Meropenem, I	

Species	2021 [5]	2022 [4]	2023 [3]	2024 [2]	2025
				Ceftazidime, R	
				Piperacillin, R	
				Piperacillin-tazobactam, I	
S. aureus				BORSA profile	
S. pneumoniae		Azithromycin, S			Azithromycin, S
		Cephalosporins, S (meningitis)			
		Penicillin, R (meningitis)			
E. faecium* and *E. faecalis			Gentamicin, not-HLAR	Gentamicin, not-HLAR	

S: Susceptible, standard dosing regimen; I: Susceptible, increased exposure; R: Resistant; BORSA: Borderline oxacillin-resistant S. aureus (cefoxitin-S and oxacillin-R); HLAR: High-level aminoglycoside resistance.
Shaded cells: Species-antimicrobial combination not included in that EARS-Net EQAs
Grey text: Problematic AST determinations for EQA strains that were included in multiple EARS-Net EQAs, therefore the observations were expected when including the strains in subsequent EQAs.