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Phenotypic External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net), 2025

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Contents

Tables

Figures

Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
DTU Food	Technical University of Denmark, National Food Institute
EURGen-Net	European Antimicrobial Resistance Genes Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EQA	External quality assessment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	'Susceptible, increased exposure'
ME	Major error
MIC	Minimum inhibitory concentration
NRL	National Reference Laboratory
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 National Reference Laboratories (NRLs) participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net). It includes a short conclusion on the capacities of the participating laboratories, and recommendations for improvement. In 2025, 29 of the 31 eligible countries participated in the EURGen-Net EQA exercise.

This was the first phenotypic AST EQA exercise conducted by the EURL-PH-AMR for NRLs within EURGen-Net, which aimed to assess (i) the quality of species identification by participating laboratories, (ii) assess the accuracy of the qualitative AST results reported by participating laboratories and (iii) evaluate the overall comparability of routinely collected AST results between laboratories and countries.

Participating NRLs were requested to identify the species of four bacterial strains belonging to previously defined priority pathogens [1] and to submit AST results for a list of clinically relevant antimicrobials adapted from the EURL-PH-AMR Laboratory Testing strategy [2], using the AST methods that they apply routinely.

The 2025 EURGen-Net EQA consisted of a panel of four strains comprising 67 strain-antimicrobial combinations: *Escherichia coli* (22 antimicrobials), *Klebsiella pneumoniae* (20), *Pseudomonas aeruginosa* (15), and *Acinetobacter baumannii* (10).

On 8 October 2025, the four strains were distributed to 32 NRLs in 29 countries. An EQA webtool was opened to receive submission of results between 9 October and 10 November 2025. Results were submitted by all 32 laboratories.

Overall, 30 laboratories (93.8%) followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2025 guidelines v15.0, one (3.1%) followed EUCAST v13.0, and one (3.1%) followed national guidelines based on EUCAST (Comité de l'Antibiogramme de la Société Française de Microbiologie guideline).

Concordance of species and AST interpretations with expected results was defined as 'excellent' (≥95% of interpretations in concordance with expected results), 'very good' (>90% to <95%), or 'good' (>85 to ≤90%).

Species identification of the four EQA strains was correct in 30/32 laboratories (93.8%). The remaining two laboratories reported incorrect species identification for all four strains due to a data entry error.

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 EUCAST Clinical Breakpoints Tables v15.0 [3], applying the EUCAST categories 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R).

The 2025 EURGen-Net EQA exercise scoring system was the same as the one applied to recent EARS-Net EQAs [4]. 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' results, 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, 1,715 AST interpretations were evaluated, reported by 30 laboratories. Overall, the submitted AST interpretations were in 'excellent' concordance with the expected results, with 1,677 (97.8%) being correct. MEs and VMEs were observed for 11 (0.6%) and 27 (1.6%) interpretations, respectively.

A total of 67 strain-antimicrobial combinations were included in the 2025 EURGen-Net EQA exercise. Most combinations showed 'excellent' concordance with the expected results (n=59, 88.1%). 'Very good' concordance was achieved for one combination, and 'good' concordance for four combinations. The remaining three combinations were below the threshold for 'good' concordance.

At laboratory level, 28 of 30 laboratories (93.3%) showed 'excellent' concordance, and two (6.7%) had 'very good' concordance.

Among the 1,715 evaluated AST results, all the most frequently reported AST methods showed 'excellent' concordance with the expected results. Broth microdilution was the most commonly used (54.2% of all tests, 98.7% correct), followed by disk or tablet diffusion (33.5%, 97.2% correct), automated systems (5.8%, 95.0% correct), and gradient test (5.8%, 96% correct).

In total, 38 errors were recorded. Most were associated with AST determinations classified as 'difficult' (n=31, 81.6% of all errors). Cefiderocol was the antimicrobial with the highest number of recorded errors (n=15, 39.5% of all errors), and these were recorded in three of the four strains, corresponding to the three in which cefiderocol AST was classified as 'difficult'. Other challenging AST determinations included aztreonam in the *P. aeruginosa* strain, with seven recorded MEs, and carbapenems and carbapenem-beta-lactamase inhibitor combinations,

particularly ertapenem, meropenem, and meropenem-vaborbactam. Although VMEs were more frequent than MEs, the number of observed deviations was limited, and the findings do not support a strong conclusion regarding an overall tendency to overestimate or underestimate AMR among the participating laboratories.

Laboratories should ensure that their AST protocols, interpretation criteria, and reporting procedures are in accordance with the latest EUCAST recommendations and guidelines, including use of the most recent EUCAST breakpoints, to support clinical practice and AMR surveillance reporting.

AMR surveillance schemes and control activities and organisations involved in developing AST guidelines should note the specific deviations observed in this EQA exercise, particularly for technically challenging determinations such as cefiderocol, aminoglycosides, and some carbapenem-related tests, as well as the small number of errors linked to incorrect application of breakpoints or data entry.

Summary of results for each EQA strain

Strain **'2025 EURGen-Net 1' (*Escherichia coli*)** was correctly identified by 30/32 (93.8%) of the laboratories. Out of 22 antimicrobials included in the EQA for this strain, two AST determinations were classified as 'difficult' (Annex 1).

Overall, the AST interpretations reported for the strain showed 'excellent' concordance with the expected results, with 553/558 (99.1%) being concordant. No MEs were recorded but 5 VMEs (0.9%) were observed among the reported interpretations.

Most VMEs were observed for ertapenem (n=3, 11.1% of all results reported for that antimicrobial), which was classified as 'difficult'. Most ertapenem tests were performed by broth microdilution (16/27, 59.3%), followed by disk diffusion (8/27, 29.6%), and all three ertapenem VMEs were reported from broth microdilution. The reported minimum inhibitory concentration (MIC) values among ertapenem VMEs were one dilution away from the expected value, therefore within the acceptable method variability and justified by the 'difficult' classification of this determination.

The remaining two VMEs in this strain were observed for levofloxacin (n=1) and piperacillin-tazobactam (n=1), both classified as 'easy'. In both cases, the VME was reported from an automated system. Automated systems were used in two tests for each antimicrobial, yielding one VME and one concordant AST interpretation in each case. As both antimicrobials were classified as 'easy', these deviations were not expected to be affected by inherent method variability and are more suggestive of random or systematic errors in laboratory procedures. In both cases the reported MIC value was not consistent with the submitted interpretation, and both errors originated from the same laboratory, indicating incorrect application of breakpoints or errors in data entry.

Strain **'2025 EURGen-Net 2' (*Klebsiella pneumoniae*)** was correctly identified by 30/32 (93.8%) of the laboratories. Out of 20 antimicrobials included in the EQA for this strain, three AST determinations were classified as 'difficult' (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results, with 500/515 (97.1%) being concordant. MEs and VMEs were observed for two (0.4%) and 13 (2.5%) of the reported interpretations, respectively.

A high proportion of errors was observed among AST determinations classified as 'difficult'. Cefiderocol accounted for the highest number of errors (n=5, 22.7% of all results reported for that antimicrobial). Most laboratories adhered to current EUCAST recommendations by using disk/tablet diffusion for cefiderocol, which showed the highest concordance for this antimicrobial (93.8%), whereas broth microdilution showed lower concordance (40.0%). As cefiderocol AST was classified as 'difficult', with the expected zone diameter located less than four millimetres from the area of technical uncertainty (ATU), these deviations are likely attributable to inherent method variability. Moreover, cefiderocol AST results are known to vary substantially according to the method and materials used [5,6].

Other 'difficult' tests producing VMEs were amikacin (n=3, 10.7%) and meropenem-vaborbactam (n=3, 14.3%). For amikacin, two VMEs were reported from broth microdilution and one from an automated system. The reported broth microdilution results were close to the breakpoint and are therefore likely attributable to inherent method variability. However, the reported automated system result was associated with a MIC value that should have been interpreted as R, indicating incorrect application of breakpoints or an error in data entry. For meropenem-vaborbactam, the methods accounting for most tests showed 100% concordance, whereas all three VMEs were reported from gradient tests. This suggests that the observed VMEs may have been influenced by the specific method instead of the inherent difficulty of the determination.

This was also the strain with the highest number of errors among AST determinations classified as 'easy' (n=4), each by a different laboratory. These errors were observed for meropenem, colistin, and gentamicin. They were reported from broth microdilution (two VMEs for meropenem and one ME for colistin) and disk/tablet diffusion (one ME for gentamicin). As these were expected to be 'easy' tests, the errors are suggestive of random or systematic errors in laboratory procedures.

Strain **'2025 EURGen-Net 3' (*Pseudomonas aeruginosa*)** was correctly identified by 30/32 (93.8%) of the laboratories. Out of 15 antimicrobials included in the EQA for this strain, two AST determinations were classified as 'difficult' (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results, with 372/387 (96.1%) being concordant. MEs and VMEs were observed for seven (1.8%) and eight (2.1%) of the reported interpretations, respectively. All errors were associated with tests classified as 'difficult'.

All eight VMEs were recorded for cefiderocol (36.4% of all results reported for that antimicrobial). Seven originated from disk/tablet diffusion, which showed 56.2% concordance for this antimicrobial, and one originated from broth microdilution, which showed 75.0% concordance. Most laboratories adhered to current EUCAST recommendations by using disk/tablet diffusion for cefiderocol, which accounted for 16 of the 22 cefiderocol AST determinations (72.7%). As cefiderocol AST was classified as 'difficult', with the expected zone diameter within the ATU, these deviations are likely attributable to inherent method variability. Moreover, cefiderocol AST results are known to vary substantially according to the method and materials used [5,6].

The seven MEs recorded for aztreonam (30.4% of all results reported for that antimicrobial) were distributed across all methods except broth microdilution, which was the most frequently reported method for this antimicrobial and showed 100% concordance. Five of the aztreonam errors originated from disk/tablet diffusion, one from automated systems, and one from gradient test. This suggests that the observed MEs may have been influenced more by specific testing methods than by the inherent difficulty of the determination. The error recorded from gradient test was associated with a correct MIC value but an incorrect submitted interpretation, suggesting incorrect application of breakpoints or an error in data entry.

Strain **'2025 EURGen-Net 4' (*Acinetobacter baumannii*)** was correctly identified by 30/32 (93.8%) of the laboratories. Only one of the ten AST determinations was classified as 'difficult' (Annex 1).

Overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results, with 252/255 (98.8%) being concordant. MEs and VMEs were observed for two (0.8%) and one (0.4%) of the reported interpretations, respectively.

Cefiderocol was the only antimicrobial classified as 'difficult'. For this antimicrobial, MEs were recorded from two laboratories (13.3% of all results reported for that antimicrobial), both from disk/tablet diffusion, which was also the method used for most cefiderocol tests (13/15, 86.7%). As cefiderocol AST was classified as 'difficult', with the expected zone diameter located less than four millimetres from the breakpoint, these deviations are likely attributable to inherent method variability. Moreover, cefiderocol AST results are known to vary substantially according to the method and materials used [5,6].

The remaining error was a VME for meropenem, which was classified as 'easy'. Errors in AST determinations classified as 'easy' are not generally expected to reflect inherent method variability and are more usually suggestive of random or systematic errors in laboratory procedures. In this case, the reported MIC was not consistent with the submitted interpretation, suggesting incorrect application of breakpoints or an error in data entry.

1. Introduction

The European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net), coordinated by the European Centre for Disease Prevention and Control (ECDC), supports genomic-based surveillance of multidrug-resistant bacteria of public health importance in Europe. Since 2025, performance assessment activities for participating laboratories have been conducted under the European Union Reference Laboratory for Public Health on Antimicrobial Resistance (EURL-PH-AMR) through both a phenotypic external quality assessment (EQA) scheme and a genomic proficiency testing (PT) scheme. Earlier EQA activities within the network were conducted as part of the EURGen-RefLabCap project [1]. This report presents and summarises the results of the 2025 phenotypic EQA for antimicrobial susceptibility testing (AST).

The 2025 EURGen-Net EQA exercise was organised by the National Food Institute, Technical University of Denmark (DTU Food), in its role as a consortium partner of the EURL-PH-AMR.

The 2025 EURGen-Net EQA exercise aimed to:

- assess the quality of species identification by participating national reference 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 across countries.

2. Study design and methods

Antimicrobial susceptibility testing, and selected antimicrobials

The 2025 EURGen-Net EQA protocol [7] 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 organism-antimicrobial combinations included in this EQA are adapted from the EURL-PH-AMR Laboratory testing strategy [2]. The 2025 EURGen-Net EQA is provided to national reference laboratories (NRLs) in countries participating in the EURGen-Net for the microorganisms included in the EURGen-Net surveillance.

Characteristics of the EQA strains and interpretation of AST results

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

The European Committee on Antimicrobial Susceptibility Testing (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 EURGen-Net EQA the following adaptations were made to the EUCAST reporting recommendations, and the same instructions were provided to participants in the EQA protocol [7] 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 *E. coli*, it was assumed that fosfomycin 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'.
- Results from one antimicrobial were not used to infer results for other antimicrobials belonging to the same class, and instead all AST determinations were interpreted 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 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 an additional reference laboratory: the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory (Växjö, Sweden).

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

The 2025 EURGen-Net EQA included 67 strain-antimicrobial combinations: 36 with an expected interpretation of 'R', two combinations with expected interpretation 'I', and 29 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, eight determinations were considered 'Difficult (five 'R', one 'I' and two 'S') and 59 determinations were considered 'Easy' (31 'R', one 'I' and 27 'S').

Procedure for participating laboratories

The 2025 EURGen-Net EQA protocol [7] specified that participating laboratories should identify the species of four bacterial strains and subsequently perform AST in accordance with EUCAST recommendations [3]. If the species identification was incorrect, the corresponding AST results were not evaluated.

Identification of eligible laboratories

All NRLs in countries participating in EURGen-Net surveillance were eligible to participate in this EQA exercise. In total, 32 NRLs representing 29 countries took part, with three countries each represented by two NRLs. Since 2019, eligibility for participation in the EURGen-Net EQA has been restricted to laboratories performing AST in accordance with EUCAST guidelines.

Distribution of EQA strains to laboratories

On 8 October 2025, DTU Food shipped the EQA parcels to each of the participating laboratories, using a single shipping (courier) company. Shipments followed International Air Transport Association (IATA) regulations (UN3373, biological substances category B). The individual packages (double-pack containers, class UN 6.2) were labelled with the address of the respective participating laboratory.

Each package contained:

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

Instructions for submitting EQA results

The 2025 EURGen-Net EQA protocol, test forms, and a guideline on how to access the password-protected webtool for submission of results were distributed through a centralised system with standardised email dissemination to all participating laboratories. The dedicated password-protected EURGen-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 EURGen-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); and
- The manufacturers of the materials and reagents employed.

The deadline for submission of results was 10 November 2025. One week prior to the submission deadline, a reminder email was sent to all enrolled laboratories. Submission of results remained accessible until 19 November 2025 to allow receipt of results from one laboratory that experienced delayed delivery of the EQA material. 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 four EQA strains. On 15 December 2025, laboratories were informed that the certificates were available for download. Laboratories could access their own certificate via the password protected webtool.

Participants were also encouraged to complete an electronic feedback survey, accessible via a link distributed by automated email, with support the continuous improvement of future EQA exercises. The evaluation questions are provided in Annex 2.

Evaluation of reported EQA results

Scoring antimicrobial susceptibility results

The participants were asked to report AST results, i.e. MIC or zone diameter values, and their 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 [7] 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.

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 or diameter 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 1). The same scoring system was also applied in the recent EARS-Net EQA exercises provided by DTU Food [4].

Table 1 2025 EURGen-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, no overall score was calculated for individual laboratories, as such aggregation may not support a fair comparison across laboratories. For example, a laboratory reporting all AST interpretations correctly for a limited number of strain-antimicrobial combinations could receive the same overall score as a laboratory that tested a larger number of combinations but reported some errors. Therefore, as stated in the EQA protocol [7], laboratories are recommended to analyse their scores separately for each strain-antimicrobial combination.

Categories for concordance of the submitted results

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%; and
- 'Good': >85 to ≤90%.

Reporting EQA results

Laboratories that submitted results received a laboratory evaluation report for each submitted sample. Only laboratories that followed EUCAST guidelines were included in the analysis presented in this report.

Contacts from each participating laboratory were notified through a centralised system with standardised email dissemination when their evaluation reports became available for download from the password-protected webtool using their login credentials. An overview of the expected results was also distributed through this system. Access to evaluation reports was restricted to the respective laboratory.

Participating laboratories were identified by unique codes, known only to the respective laboratory and the EQA provider.

ECDC received the anonymised database containing all submitted results.

3. Results

Participation

In 2025, 29 of the 31 eligible countries participated in the EURGen-Net EQA exercise. In most countries, one NRL enrolled in the exercise, while in three countries two NRLs enrolled, giving a total of 32 participating laboratories. All 32 laboratories submitted results.

Adherence to the EUCAST guideline is mandatory for participation in the EURGen-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 EURGen-Net EQA). Most laboratories (30/32, 93.7%) followed EUCAST v15.0, one (3.1%) followed EUCAST v13.0, and one (3.1%) followed the EUCAST-based CA-SFM guideline (Comité de l'Antibiogramme de la Société Française de Microbiologie).

All 32 participating laboratories submitted AST interpretation for all four strains and were therefore eligible to receive a certificate.

Species identification results

All 32 laboratories submitted species identification for the four strains included in the panel. For each strain, 30/32 (93.8%) of the laboratories reported correct species identification. Therefore, the overall concordance between the submitted and expected species identifications was 'very good' (>90 to ≤95%).

All incorrect species identifications were reported by two laboratories, both of which reported incorrect identifications for all four strains due to a data entry error. These two laboratories were excluded from all further analyses presented in this report. An overview of the species identification results for the four strains, and the corresponding number of laboratories reporting the correct identification, is provided in Table 2.

Table 2 Number and percentage of correct species identifications in the 2025 EURGen-Net EQA exercise

Strain ID	Expected species	No. of identifications	No. of correct species identifications	Percentage of laboratories reporting correct species identification
2025 EURGen-Net 1	*Escherichia coli*	32	30	93.8
2025 EURGen-Net 2	*Klebsiella pneumoniae*	32	30	93.8
2025 EURGen-Net 3	*Pseudomonas aeruginosa*	32	30	93.8
2025 EURGen-Net 4	*Acinetobacter baumannii*	32	30	93.8
Total		**128**	**120**	**93.8**

Antimicrobial susceptibility testing results

If the 32 participating laboratories had reported AST interpretation data with correct species identification for every strain-antimicrobial combination included in this EQA exercise, a total of 2,144 results would have been generated. In practice, the 30 laboratories that reported correct species identification submitted 1,715 AST interpretations, corresponding to 80.0% of the theoretical maximum.

Overall, the reported AST interpretations showed 'excellent' concordance with the expected results, with 97.8% (1,677/1,715) interpreted correctly. MEs and VMEs were observed in 0.6% (n=11) and 1.6% (n=27) of the reported interpretations, respectively (Table 3).

Table 3 Overview of antimicrobial susceptibility testing (AST) results reported by National Reference Laboratories participating in the 2025 EURGen-Net EQA exercise

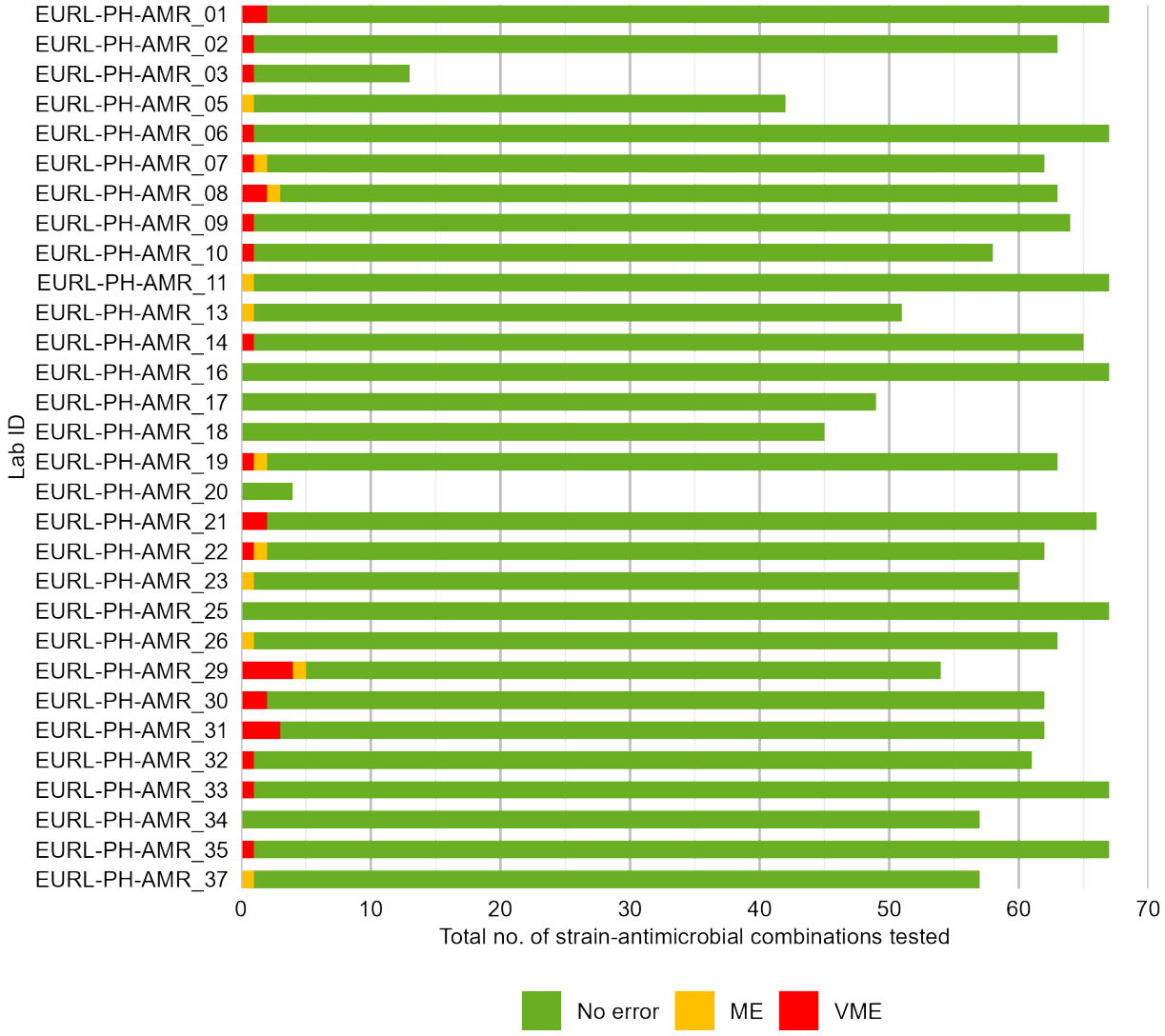
Strain ID	Species and expected AST results for antimicrobials included in the panel*	AST results			
		Reported AST results (N)	Correct AST interpretations (N(%))	Major errors (N (%))	Very major errors (N (%))
2025 EURGen-Net 1	*Escherichia coli* S: AMK, ATM, AZA, FDC, CTX, CAZ, CZA, CZT, COL, FOS, GEN, IMP, IMR, MEM, MEV, TGC, TOB, SXT R: CIP, LVX, ETP, TZP	558	553 (99.1%)	0 (0.0%)	5 (0.9%)
2025 EURGen-Net 2	*Klebsiella pneumoniae* S: AZA, COL, GEN, SXT R: AMK, ATM, FDC, CTX, CAZ, CZA, CZT, CIP, ETP, IMP, IMR, LVX, MEM, MEV, TZP, TOB	515	500 (97.1%)	2 (0.4%)	13 (2.5%)
2025 EURGen-Net 3	*Pseudomonas aeruginosa* S: ATM, COL R: AMK, FDC, CAZ, CZA, CZT, CIP, IMP, IMR, LVX, MEM, MEV, TZP, TOB	387	372 (96.1%)	7 (1.8%)	8 (2.1%)
2025 EURGen-Net 4	*Acinetobacter baumannii* S: AMK, FDC, GEN, LVX, TOB, SXT I: CIP R: COL, IMP, MEM	255	252 (98.8%)	2 (0.8%)	1 (0.4%)
Total		1,715	1,677 (97.8%)	11 (0.6%)	27 (1.6%)

** 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 EURGen-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'; all AST were performed individually, and results from one antimicrobial were not used for interpretation of other antimicrobials belonging to the same class.*
AST: Antimicrobial susceptibility testing; S: Susceptible, standard dosing regimen; I: Susceptible, increased exposure; R: Resistant; NA: Not applicable; AMK: Amikacin; ATM: Aztreonam; AZA: Aztreonam-avibactam; CAZ: Ceftazidime; CIP: Ciprofloxacin; COL: Colistin; CTX: Cefotaxime; CZA: Ceftazidime-avibactam; CZT: Ceftolozane-tazobactam; ETP: Ertapenem; FDC: Cefiderocol; FOS: Fosfomycin; GEN: Gentamicin; IMP: Imipenem; IMR: Imipenem-relebactam; LVX: Levofloxacin; MEM: Meropenem; MEV: Meropenem-vaborbactam; SXT: Trimethoprim-sulfamethoxazole; TGC: Tigecycline; TOB: Tobramycin; TZP: Piperacillin-tazobactam.

Among the 30 participating laboratories with correct species identification, six reported 100% concordant results, although it should be noted that one of the laboratories (EURL-PH-AMR_20) submitted only four AST results, all for meropenem, one for each strain. The highest number of VMEs were recorded for EURL-PH-AMR_29 (n=4), EURL-PH-AMR_31 (n=3), and EURL-PH-AMR_8, EURL-PH-AMR_21, and EURL-PH-AMR_30 (n=2 each). In addition, one VME was observed in each of nine laboratories. MEs were recorded in ten laboratories, with a maximum of one ME per laboratory. The highest total number of errors recorded in a single laboratory was five (EURL-PH-AMR_29), of which two were associated with tests classified as 'difficult' and three with tests classified as 'easy' (Figure 1).

Overall, 28 laboratories (93.3%) achieved 'excellent' concordance and two (6.7%) achieved 'very good' concordance.

Figure 1 Number of errors among the reported interpretation of AST results, by laboratory, 2025 EURGen-Net EQA exercise

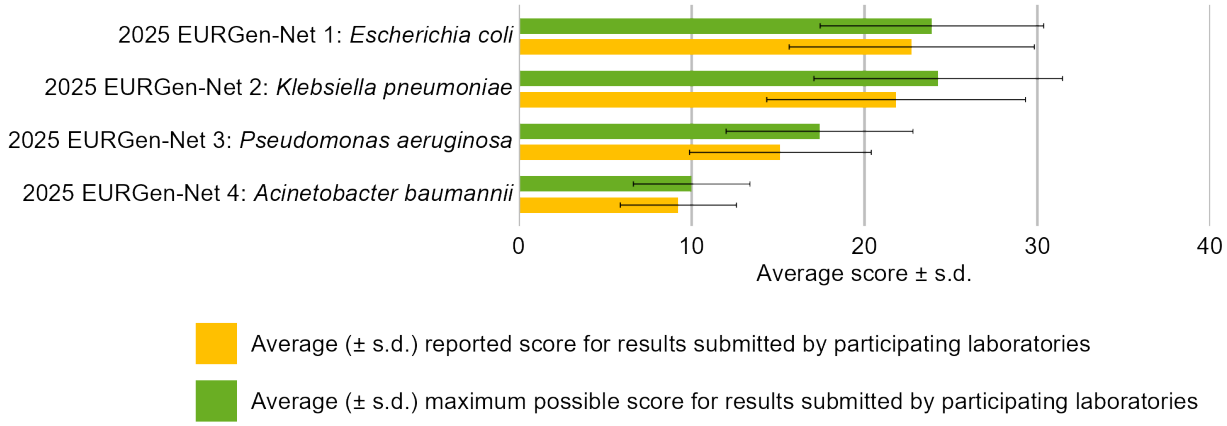


AST: antimicrobial susceptibility testing

In the 2025 EURGen-Net EQA, each laboratory could report an interpretation for 67 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 four strains, was 91 (59 points from the 'Easy' determinations plus 32 points from the 'Difficult' determinations).

As expected, the participating laboratories did not report results for every strain-antimicrobial combination. Consequently, the maximum possible score for each laboratory was lower, because it depended on the number of reported results. In the 2025 EURGen-Net EQA exercise, the average score actually achieved by the 30 participating laboratories was 68.9 ± 21. Figure 2 presents the averages of maximum possible score and the reported scores for the participating laboratories, by strain.

Figure 2 Average maximum possible score, and average total scores, for the AST results reported by participating laboratories, by EQA strain, 2025 EURGen-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 broth microdilution (54.2%), followed by disk or tablet diffusion (33.5%), automated system (5.8%), gradient test (5.8%), macro broth dilution (0.5%), other tests (0.2%) and agar dilution (0.06%). (Table 4).

All methods except those reported under the category 'other' showed overall 'excellent' concordance (≥95%). The lower concordance for 'other' methods was due to a single VME among the three reported results. All three 'other' method entries were reported for cefiderocol AST determinations and were submitted by different laboratories (Table 4). These corresponded to one in-house iron-depleted cation-adjusted Mueller-Hinton medium (which could theoretically have been reported under 'broth microdilution') and two instances of one commercial product. The single VME within 'other' was recorded for the commercial product.

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

Method	2025 EURGen-Net 1 *Escherichia coli*			2025 EURGen-Net 2 *Klebsiella pneumoniae*			2025 EURGen-Net 3 *Pseudomonas aeruginosa*			2025 EURGen-Net 4 *Acinetobacter baumannii*			Total		
	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	1	0.2	100.0	0	-	-	0	-	-	0	-	-	1	0.06	**100.0**
Automated system	32	5.7	93.8	30	5.8	96.7	22	5.7	95.5	16	6.3	93.8	100	5.8	**95.0**
Broth microdilution	306	54.8	99.0	284	55.1	97.2	209	54.0	99.5	131	51.4	100.0	930	54.2	**98.7**
Disk/Tablet diffusion	180	32.3	100.0	170	33.0	98.8	130	33.6	90.8	94	36.9	97.9	574	33.5	**97.2**
Gradient test	39	7.0	100.0	30	5.8	90.0	24	6.2	95.8	6	2.4	100.0	99	5.8	**96.0**
Macro broth dilution	0	-	-	0	-	-	0	-	-	8	3.1	100.0	8	0.5	**100.0**
Other	0	-	-	1	0.2	-	2	0.5	100.0	0	-	-	3	0.2	**66.7**
Total	**558**	**100.0**	**99.1**	**515**	**100.0**	**97.1**	**387**	**100.0**	**96.1**	**255**	**100.0**	**98.8**	**1,715**	**100.0**	**97.8**

Percentages might not total 100% due to rounding.

Strain '2025 EURGen-Net 1' (*Escherichia coli*)

Strain **'2025 EURGen-Net 1' (*Escherichia coli*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to four of the 22 antimicrobials included in the EQA: ciprofloxacin, ertapenem, levofloxacin, and piperacillin-tazobactam. It was susceptible to 18 antimicrobials: amikacin, aztreonam, aztreonam-avibactam, cefiderocol, cefotaxime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, colistin, fosfomycin, gentamicin, imipenem, imipenem-relebactam, meropenem, meropenem-vaborbactam, tigecycline, tobramycin, trimethoprim-sulfamethoxazole (Annex 1).

Two of the antimicrobials included for this strain were classified as 'difficult' AST determinations: ciprofloxacin and ertapenem, both with expected MIC values less than two dilutions away from the clinical breakpoints. For the remaining antimicrobials, the level of difficulty was considered 'easy'.

Interpretation of AST results for the *E. coli* strain were analysed for the 30 laboratories that reported correct species identification (Table 2).

Overall, 26 laboratories (86.7%) showed full concordance with the expected interpretations. A further two laboratories (6.7%) achieved 'excellent' concordance, although not full concordance. Of the remaining laboratories, one (3.3%) achieved 'very good' concordance and one (3.3%) achieved 'good' concordance.

The theoretical maximum number of results was 660 (30 laboratories × 22 antimicrobials). In total, 558 included AST interpretations of which 553 were correct (Table 3). The reported interpretations were therefore in 'excellent' concordance with the expected results (99.1%) (Table 5). No MEs were observed, whereas five VMEs (0.9%) were recorded (Figure 3).

Broth microdilution was the most frequently reported method (54.9%), followed by disk/tablet diffusion (32.2%), gradient test (7%), automated system (5.7%), and agar dilution (0.2%) (Table 4).

All methods except automated systems showed 'excellent' concordance with the expected results (≥95% concordance): broth microdilution (99.0%), disk/tablet diffusion (100%), gradient tests (100%), and agar dilution (100%). Automated systems showed 'very good' concordance (93.8%) (Table 5).

VMEs were observed for three out of the four antimicrobials with an expected interpretation of R: ertapenem, levofloxacin, and piperacillin-tazobactam (Figure 3, Table 5Table 5 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EURGen-Net 1' (*Escherichia coli*), by antimicrobial and AST method).

- Three VMEs were recorded for ertapenem, corresponding to 11.1% of all submitted interpretations for that antimicrobial, and all were from broth microdilution tests. The reported MIC values (all three MIC=0.5 mg/L) were one dilution away from the expected value (MIC>0.5 mg/L).
- One VME was recorded for levofloxacin (4.3%) from an automated system. The reported MIC (MIC>2 mg/L) was not concordant with the submitted interpretation (S), based on the current breakpoint (R>1 mg/L).
- One VMEs was recorded for piperacillin-tazobactam (3.4%), also from an automated system. The reported MIC (MIC=64 mg/L) was not concordant with the submitted interpretation (S), based on the current breakpoint (R>8 mg/L).

Discussion

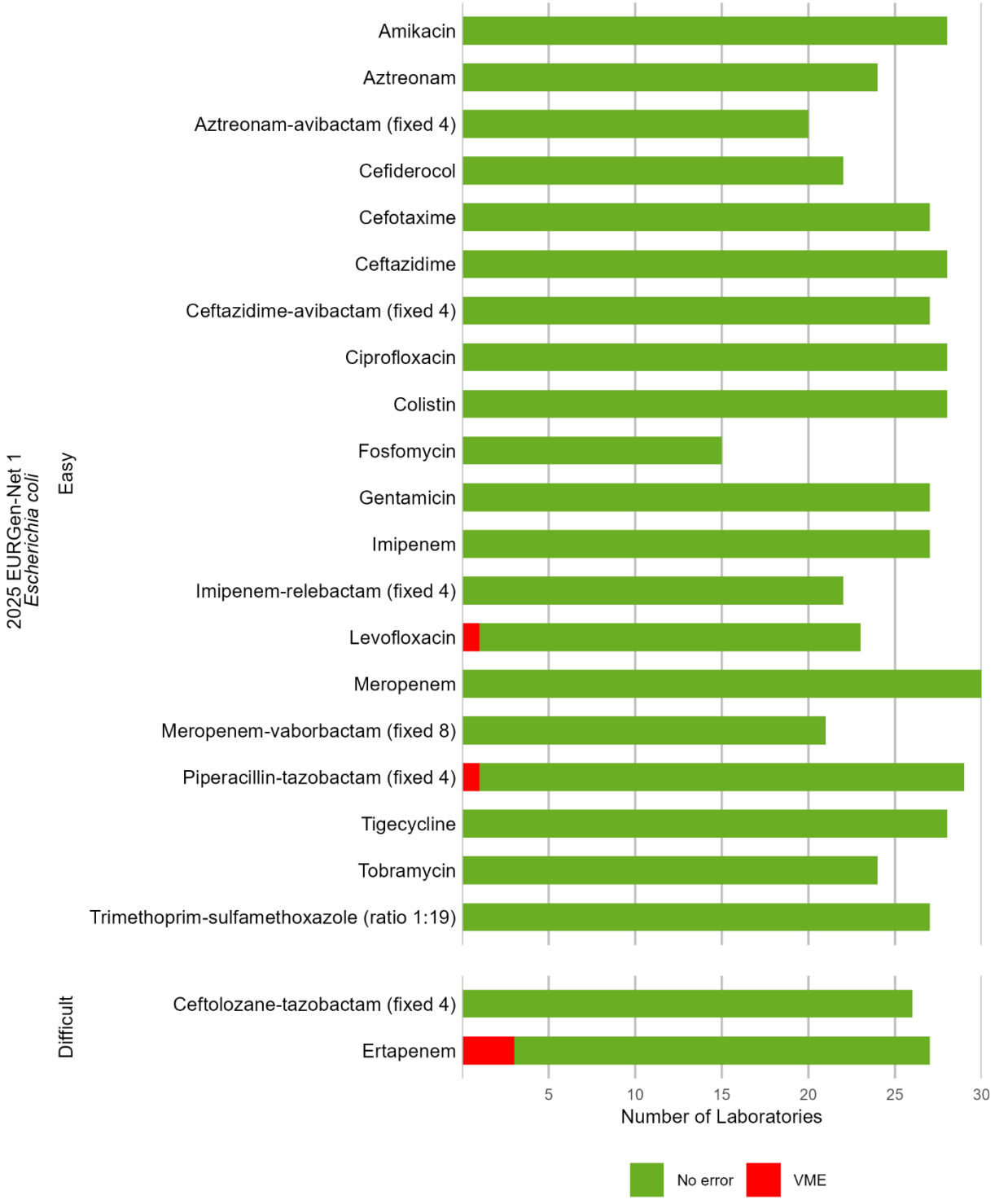
For strain **'2025 EURGen-Net 1' (*Escherichia coli*)** the reported AST interpretations showed very high concordance with the expected results, with only five errors observed overall, all VME. Three of these VMEs were recorded for ertapenem, which was classified as 'difficult'. All three were identified in broth microdilution results, which was the most frequently used method for ertapenem, consistent with current EUCAST recommendations. Given the 'difficult' classification of this determination, these deviations are attributable to the inherent variability of the method since the borderline expected MIC value increased the likelihood of misclassification. Moreover, the observed one-dilution difference on MIC values falls within the accepted method variability.

The remaining two VMEs were recorded for levofloxacin and piperacillin-tazobactam, both classified as 'easy'. These determinations were not expected to be affected by inherent method variability and are more likely attributable to systematic or random errors in the participating laboratories' procedures. As both errors were associated with the use of an automated system, which produced one concordant and one non-concordant result for each antimicrobial, the findings could suggest that the method may have contributed to the deviations. However, in both cases the reported MIC value was not consistent with the submitted interpretation, and both errors originated from the same laboratory, indicating errors in breakpoint interpretation or data entry. These interpretation errors were reported by the same laboratory that also submitted discordant interpretations for strains '2025 EURGen-Net 2' and '2025 EURGen-Net 4', which further supports this explanation.

Overall, three of the four most frequently applied methods, broth microdilution, disk/tablet diffusion, and gradient test, achieved 'excellent' concordance with the expected results (99.0% to 100%). Results obtained using automated systems showed lower concordance (93.8%) and were associated to the two VMEs observed in 'easy'

AST determinations. The single test performed using agar dilution (n=1) was also concordant with the expected result (Table 5).

Figure 3 Reported interpretation of AST results for strain '2025 EURGen-Net 1' (*Escherichia coli*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.
Fixed concentration (mg/L) of beta-lactamase inhibitors shown in parentheses.

Table 5 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EURGen-Net 1' (*Escherichia coli*), by antimicrobial and AST method

Antimicrobial	Difficulty*	Expected interpretation**	Agar dilution		Automated system		Broth microdilution		Disk/Tablet diffusion		Gradient test		Total	
			n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	S	-	-	2	100.0	16	100.0	10	100	-	-	**28**	**100.0**
Aztreonam	Easy	S	-	-	2	100.0	14	100.0	8	100	-	-	**24**	**100.0**
Aztreonam-avibactam (fixed 4)***	Easy	S	-	-	-	-	5	100.0	4	100	11	100	**20**	**100.0**
Cefiderocol	Easy	S	-	-	-	-	5	100.0	17	100	-	-	**22**	**100.0**
Cefotaxime	Easy	S	-	-	1	100.0	12	100.0	13	100	1	100	**27**	**100.0**
Ceftazidime	Easy	S	-	-	2	100.0	15	100.0	11	100	-	-	**28**	**100.0**
Ceftazidime-avibactam (fixed 4)***	Easy	S	-	-	2	100.0	17	100.0	7	100	1	100	**27**	**100.0**
Ceftolozane-tazobactam (fixed 4)***	Difficult	S	-	-	1	100.0	19	100.0	5	100	1	100	**26**	**100.0**
Ciprofloxacin	Easy	R	-	-	2	100.0	15	100.0	11	100	-	-	**28**	**100.0**
Colistin	Easy	S	-	-	1	100.0	26	100.0	1	100	-	-	**28**	**100.0**
Ertapenem	Difficult	R	-	-	2	100.0	16	81.2	8	100	1	100	**27**	**88.9**
Fosfomycin	Easy	S	1	100	-	-	4	100.0	8	100	2	100	**15**	**100.0**
Gentamicin	Easy	S	-	-	2	100.0	15	100.0	10	100	-	-	**27**	**100.0**
Imipenem	Easy	S	-	-	2	100.0	17	100.0	8	100	-	-	**27**	**100.0**
Imipenem-relebactam (fixed 4)***	Easy	S	-	-	1	100.0	13	100.0	3	100	5	100	**22**	**100.0**
Levofloxacin	Easy	R	-	-	2	50.0	10	100.0	8	100	3	100	**23**	**95.7**
Meropenem	Easy	S	-	-	2	100.0	19	100.0	8	100	1	100	**30**	**100.0**
Meropenem-vaborbactam (fixed 8)***	Easy	S	-	-	1	100.0	11	100.0	3	100	6	100	**21**	**100.0**
Piperacillin-tazobactam (fixed 4)***	Easy	R	-	-	2	50.0	16	100.0	10	100	1	100	**29**	**96.6**
Tigecycline	Easy	S	-	-	1	100.0	18	100.0	5	100	4	100	**28**	**100.0**
Tobramycin	Easy	S	-	-	2	100.0	10	100.0	10	100	2	100	**24**	**100.0**
Trimethoprim-sulfamethoxazole (ratio 1:19)	Easy	S	-	-	2	100.0	13	100.0	12	100	-	-	**27**	**100.0**
Total			**1**	**100**	**32**	**93.8**	**306**	**99.0**	**180**	**100**	**39**	**100**	**558**	**99.1**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of good concordance (≤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'.*

**** Fixed concentration (mg/L) of the beta-lactamase inhibitor shown in parentheses.*

Percentages might not total 100% due to rounding.

Strain '2025 EURGen-Net 2' (*Klebsiella pneumoniae*)

Strain **'2025 EURGen-Net 2' (*Klebsiella pneumoniae*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to 16 of the 20 antimicrobials included in the EQA: amikacin, aztreonam, cefiderocol, cefotaxime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, ertapenem, imipenem, imipenem-relebactam, levofloxacin, meropenem, meropenem-vaborbactam, piperacillin-tazobactam, and tobramycin (Annex 1).

Three of the antimicrobials included for this strain were classified as 'difficult' AST determinations: amikacin, cefiderocol, and meropenem-vaborbactam, all with expected MIC values less than two dilutions away from the clinical breakpoints or expected zone diameters located less than four millimetres from the ATU (cefiderocol). For the remaining antimicrobials, the level of difficulty was considered 'easy'.

Interpretation of AST results for the *K. pneumoniae* strain were analysed for the 30 laboratories that reported correct species identification (Table 2).

Overall, 16 laboratories (53.3%) showed full concordance with the expected interpretations. A further four laboratories (13.3%) achieved 'excellent' concordance. Of the remaining laboratories, eight (26.7%) achieved 'very good' concordance, one (3.3%) achieved 'good' concordance, and one (3.3%) was below level of the 'good' concordance (75%).

The theoretical maximum number of results for this strain was 600 (30 laboratories × 20 antimicrobials). In total, 515 included AST interpretations of which 500 were correct (Table 3). The reported interpretations were therefore in 'excellent' concordance with the expected results (97.1%) (Table 6). MEs were observed for two (0.4%) of the interpretations and VMEs for 13 (2.5%) (Figure 4).

Broth microdilution was the most frequently reported method (54.9%), followed by disk/tablet diffusion (33.0%), gradient test (5.8%), automated system (5.8%), and other methods (0.1%) (Table 4).

All methods except gradient test and those reported as 'other' showed 'excellent' concordance with the expected results (≥95% concordance): broth microdilution (97.2%), disk/tablet diffusion (98.8%), and automated systems (96.7%). Gradient test showed 'good' concordance (90.0%). The only test reported under the 'other' category used a commercial product and the result was not concordant (Table 6).

VMEs were observed for four out of the 16 antimicrobials with an expected interpretation of R: amikacin, cefiderocol, meropenem, and meropenem-vaborbactam (Figure 4, Table 6).

- Five VMEs were recorded for cefiderocol, corresponding to 22.7% of all submitted interpretations for that antimicrobial. These involved broth microdilution (n=3), disk/tablet diffusion (n=1), and other methods (commercial product) (n=1). The MIC values reported using broth microdilution, two MIC=1 mg/L and one MIC=2 mg/L, were two and one dilutions away from the breakpoint (R>2 mg/L) respectively, whereas the MIC value reported using the commercial product, MIC=2 mg/L, was one dilution away from the breakpoint.
- Three VMEs were recorded for amikacin (10.7%) from broth microdilution (n=2) and automated system (n=1). The reported MIC values from broth microdilution (two MIC=8 mg/L) were one dilution away from the expected value (MIC=16 mg/L). The reported MIC from the automated system (MIC=16 mg/L) corresponded to the expected value, but the submitted interpretation (S) was not correct when applying current breakpoints (R>8 mg/L).
- Three VMEs were recorded for meropenem-vaborbactam (14.3%), all from gradient test. All reported MICs were one or two dilutions away from the expected value.
- Two VMEs were recorded for meropenem (6.7%), both from broth microdilution tests. The reported MICs (MIC=8 mg/L) were at least two dilutions away from the expected value (>16 mg/L). Meropenem was the only antimicrobial with VMEs classified as an 'easy' AST determination.

Two MEs were recorded for this strain, one for colistin and one for gentamicin, both classified as 'easy' AST determinations. The reported MIC for colistin (MIC=4 mg/L) was at least three dilutions away from the expected value (≤0.5 mg/L), and the reported zone diameter for gentamicin (16 mm) was exactly 1 mm away from the breakpoint (R<17 mm).

Discussion

For strain **'2025 EURGen-Net 2' (*Klebsiella pneumoniae*)**, most observed errors were associated with AST determinations classified as 'difficult'.

Cefiderocol accounted for the highest number of errors. Most laboratories adhered to current EUCAST recommendations by using disk/tablet diffusion for cefiderocol, which showed the highest concordance for this antimicrobial (93.8%), whereas broth microdilution showed lower concordance (40%). As cefiderocol AST was classified as 'difficult', with the expected zone diameter located less than four millimetres from the ATU, these deviations are likely attributable to inherent method variability. Moreover, cefiderocol AST results are known to vary substantially according to the method and materials used [5,6]. The only result reported using the commercial product was not concordant, but due to the limited number of tests (n=1) this should be interpreted with caution.

The laboratory that reported using the commercial product for cefiderocol used disk diffusion for this antimicrobial in the other strains. This may suggest that the commercial product was intended as a confirmatory method in this instance, although this cannot be determined from the available data.

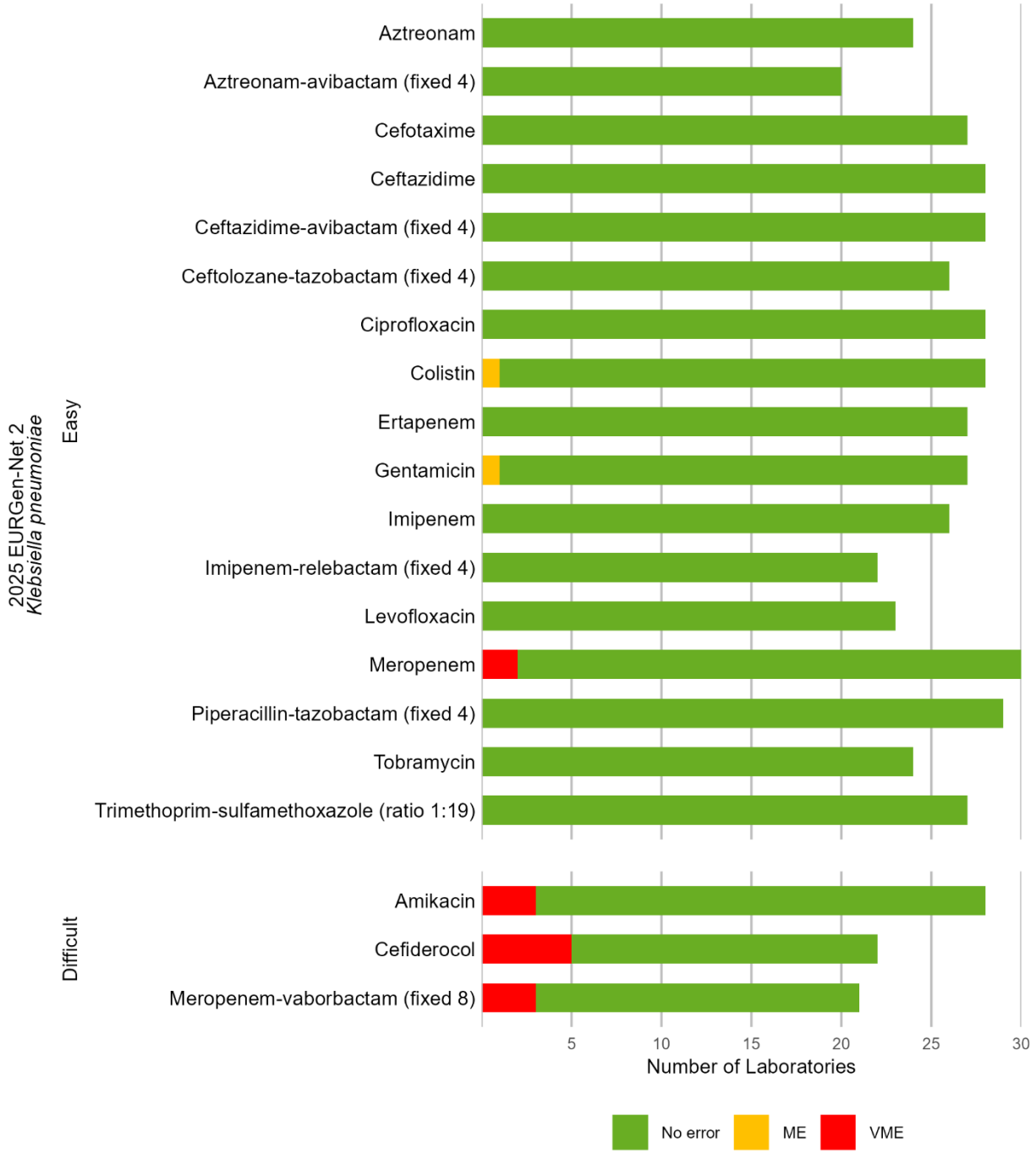
Other 'difficult' tests producing VMEs were amikacin and meropenem-vaborbactam. For amikacin, the two VMEs recorded from broth microdilution involved MIC values close to the expected value are therefore likely attributable to the inherent variability of AST methods. Moreover, the observed one-dilution difference on MIC values falls within the accepted method variability. AST results for aminoglycosides such as amikacin are known to vary depending on the method and materials used [8]. However, the VME recorded from the automated system was associated with an MIC that should have been interpreted as R, suggesting an error in breakpoint interpretation or data entry. This interpretation error was observed in the same laboratory that also submitted discordant interpretations for strains '2025 EURGen-Net 1' and '2025 EURGen-Net 4', which further supports this explanation.

For meropenem-vaborbactam, the methods accounting for most tests showed 100% concordance, whereas all recorded errors originated from gradient tests. This suggests that the VMEs observed for this antimicrobial may have been influenced more by the specific method of choice than by the inherent difficulty of the test, for the participating laboratories.

This was the strain with the highest number of errors among AST determinations classified as 'easy' (n=4), each by a different laboratory. These errors were observed for meropenem, colistin, and gentamicin. They were recorded from broth microdilution (two VMEs for meropenem and one ME for colistin) and disk/tablet diffusion (one ME for gentamicin). These deviations suggest systematic or random errors in laboratory procedures.

Overall, broth microdilution, disk/tablet diffusion, and automated systems achieved 'excellent' concordance with the expected results, whereas gradient tests showed the lowest concordance (90.0%), alongside 'other' methods (commercial product). The poorer performance of gradient tests was driven by meropenem-vaborbactam, for which all three VMEs were recorded using this method.

Figure 4 Reported interpretation of AST results for strain '2025 EURGen-Net 2' (*Klebsiella pneumoniae*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.
Fixed concentration (mg/L) of beta-lactamase inhibitors shown in parentheses.

Table 6 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EURGen-Net 2' (*Klebsiella pneumoniae*), by antimicrobial and AST method

Antimicrobial	Difficulty*	Expected interpretation**	Automated system		Broth microdilution		Disk/Tablet diffusion		Gradient test		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Difficult	R	1	0.0	15	86.7	11	100.0	1	100	-	-	**28**	**89.3**
Aztreonam	Easy	R	2	100.0	14	100.0	8	100.0	-	-	-	-	**24**	**100.0**
Aztreonam-avibactam (fixed 4)***	Easy	S	-	-	6	100.0	4	100.0	10	100	-	-	**20**	**100.0**
Cefiderocol	Difficult	R	-	-	5	40.0	16	93.8	-	-	1	0.0	**22**	**77.3**
Cefotaxime	Easy	R	1	100.0	13	100.0	13	100.0	-	-	-	-	**27**	**100.0**
Ceftazidime	Easy	R	2	100.0	15	100.0	11	100.0	-	-	-	-	**28**	**100.0**
Ceftazidime-avibactam (fixed 4)***	Easy	R	2	100.0	17	100.0	8	100.0	1	100	-	-	**28**	**100.0**
Ceftolozane-tazobactam (fixed 4)***	Easy	R	1	100.0	18	100.0	6	100.0	1	100	-	-	**26**	**100.0**
Ciprofloxacin	Easy	R	2	100.0	15	100.0	11	100.0	-	-	-	-	**28**	**100.0**
Colistin	Easy	S	1	100.0	26	96.2	1	100.0	-	-	-	-	**28**	**96.4**
Ertapenem	Easy	R	2	100.0	16	100.0	8	100.0	1	100	-	-	**27**	**100.0**
Gentamicin	Easy	S	2	100.0	14	100.0	11	90.9	-	-	-	-	**27**	**96.3**
Imipenem	Easy	R	2	100.0	18	100.0	6	100.0	-	-	-	-	**26**	**100.0**
Imipenem-relebactam (fixed 4)***	Easy	R	1	100.0	13	100.0	3	100.0	5	100	-	-	**22**	**100.0**
Levofloxacin	Easy	R	2	100.0	10	100.0	8	100.0	3	100	-	-	**23**	**100.0**
Meropenem	Easy	R	2	100.0	19	89.5	8	100.0	1	100	-	-	**30**	**93.3**
Meropenem-vaborbactam (fixed 8)***	Difficult	R	1	100.0	11	100.0	4	100.0	5	40	-	-	**21**	**85.7**
Piperacillin-tazobactam (fixed 4)***	Easy	R	2	100.0	14	100.0	12	100.0	1	100	-	-	**29**	**100.0**
Tobramycin	Easy	R	2	100.0	11	100.0	10	100.0	1	100	-	-	**24**	**100.0**
Trimethoprim-sulfamethoxazole (ratio 1:19)	Easy	S	2	100.0	14	100.0	11	100.0	-	-	-	-	**27**	**100.0**
Total			**30**	**96.7**	**284**	**97.2**	**170**	**98.8**	**30**	**90**	**1**	**0.0**	**515**	**97.1**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of good concordance (⩽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'.*

**** Fixed concentration (mg/L) of the beta-lactamase inhibitor shown in parentheses.*

Percentages might not total 100% due to rounding.

Strain '2025 EURGen-Net 3' (*Pseudomonas aeruginosa*)

Strain **'2025 EURGen-Net 3' (*Pseudomonas aeruginosa*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to 13 of the 15 antimicrobials included in the EQA: amikacin, cefiderocol, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ciprofloxacin, imipenem, imipenem-relebactam, levofloxacin, meropenem, meropenem-vaborbactam, piperacillin-tazobactam, and tobramycin (Annex 1).

Two of the antimicrobials included for this strain were classified as 'difficult' AST determinations: aztreonam and cefiderocol, with expected MIC values less than two dilutions away from the clinical breakpoints (aztreonam) or expected zone diameter within the ATU (cefiderocol). For the remaining antimicrobials, the level of difficulty was considered 'easy'.

Interpretation of AST results for the *P. aeruginosa* strain were analysed for the 30 laboratories that reported correct species identification (Table 2).

Overall, 17 laboratories (56.7%) showed full concordance with the expected interpretations. Eleven laboratories (36.7%) achieved 'very good' concordance, and two (6.7%) achieved 'good' concordance.

The theoretical maximum number of results was 450 (30 laboratories × 15 antimicrobials). In total, 387 submitted AST interpretations of which 372 were correct (Table 3). The reported interpretations were therefore in 'excellent' concordance with the expected results (96.1%) (Table 7). MEs were observed for seven (1.8%) of the interpretations and VMEs for eight (2.1%) (Figure 5).

Broth microdilution was the most frequently reported method (54.0%), followed by disk/tablet diffusion (33.6%), gradient test (6.2%), automated system (5.7%), and other methods (0.5%) (Table 4).

All methods except disk/tablet diffusion showed 'excellent' concordance with the expected results (≥95% concordance): broth microdilution (99.5%), gradient test (95.8%), and automated systems (95.5%). Disk/tablet diffusion showed 'very good' concordance (90.8%). The two tests reported under the 'other' category were fully concordant with the expected results and involved the use of a commercial product and a 'home-made iron-depleted cation-adjusted Mueller-Hinton broth' (Table 7).

Eight VMEs were observed for one out of the 13 antimicrobials with an expected interpretation of R: cefiderocol (Figure 5, Table 7). These VMEs corresponded to 36.4% of all submitted interpretations for that antimicrobial and involved disk/tablet diffusion (n=7) and broth microdilution (n=1). The reported zone diameters in the seven VMEs from disk/table diffusion ranged between 22-25 mm, i.e. 1-4 mm away from the expected value (≤21 mm).

In addition, seven MEs were recorded for aztreonam, also classified as a 'difficult' AST determination. These MEs corresponded to 30.4% of all submitted interpretations for that antimicrobial. Five of them resulted from disk/tablet diffusion, which showed a concordance of 37.5%, while the other two resulted from automated systems (n=1) and gradient test (n=1). All laboratories reporting an error for aztreonam by disk/tablet diffusion (n=5) reported zone diameters 2-6 mm below the breakpoint (R<18 mm). The remaining two MEs, one from the gradient test and one from the automated system, were associated to MIC values of 16 mg/L and >16 mg/L, respectively. The result obtained with gradient test (MIC=16 mg/L) corresponded to the expected value, but the submitted interpretation was not correct according to the current breakpoint (R>16 mg/L).

Participating laboratories did not record any errors for the antimicrobials classified as 'easy' AST determinations in this strain.

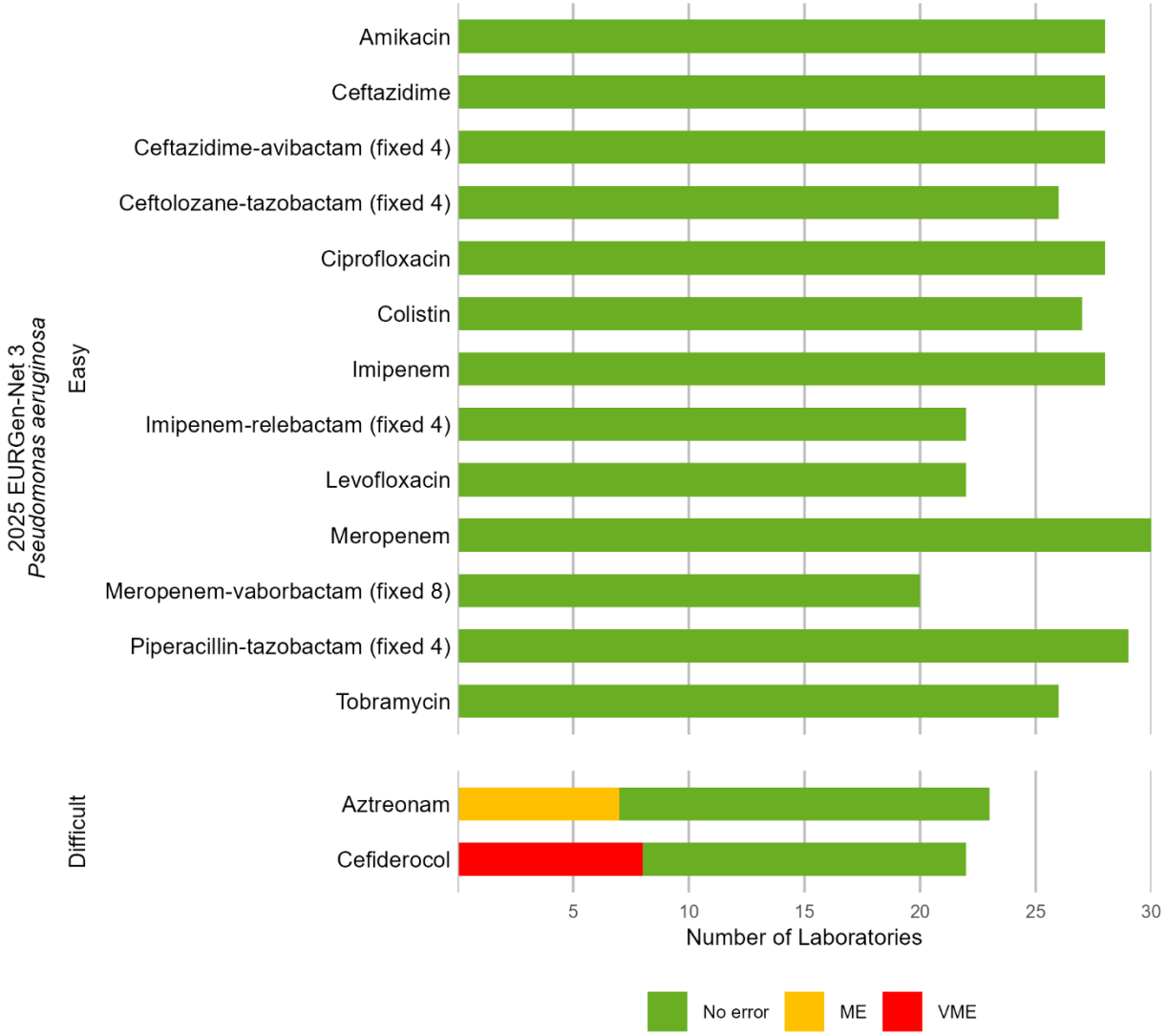
Discussion

For strain **'2025 EURGen-Net 3' (*Pseudomonas aeruginosa*)** all observed errors were recorded for the two AST determinations classified as 'difficult': cefiderocol and aztreonam. For cefiderocol, most laboratories adhered to current EUCAST recommendations by using disk/tablet diffusion. However, disk/tablet diffusion concordance was not satisfactory (56.2%), which is consistent with the 'difficult' classification of this antimicrobial. Specifically, the observed VMEs reported zone diameters close to the expected value and therefore mainly justified by the inherent method variability. Although the four laboratories that used broth microdilution for cefiderocol achieved higher concordance (75.0%), this result was based on only four tests. Moreover, cefiderocol AST results are known to vary substantially according to the method and materials used [5,6]. The two results reported under 'other' methods were both concordant, but due to the limited number of tests (n=2) this should be interpreted with caution.

Aztreonam was also classified as 'difficult'. However, broth microdilution, which was the most frequently reported method for this antimicrobial, showed 100% concordance, whereas all recorded errors originated from other methods, predominantly disk/tablet diffusion. This suggests that the MEs observed for aztreonam may have been influenced more by specific testing methods than by the inherent difficulty of the determination. The error obtained with the automated system could potentially be within the accepted method variability (reported MIC >16 mg/L). Notably, the error recorded using gradient test was not related to the MIC value obtained, but to the interpretation submitted, suggesting an error in breakpoint interpretation or data entry. Unlike the discordant interpretations observed in the other EQA strains, this error was observed in a different laboratory.

No errors were recorded for any of the antimicrobials classified as 'easy' for this strain. Overall, broth microdilution, automated systems, gradient tests, and 'other' methods achieved 'excellent' concordance with the expected results, whereas disk/tablet diffusion showed 'very good' concordance.

Figure 5 Reported interpretation of AST results for strain '2025 EURGen-Net 3' (*Pseudomonas aeruginosa*) by antimicrobial and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error.
Fixed concentration (mg/L) of beta-lactamase inhibitors shown in parentheses.

Table 7 Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2025 EURGen-Net 3' (*Pseudomonas aeruginosa*), by antimicrobial and AST method

Antimicrobial	Difficulty*	Expected interpretation**	Automated system		Broth microdilution		Disk/Tablet diffusion		Gradient test		Other		Total	
			n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	R	2	100.0	14	100.0	12	100.0	-	-	-	-	**28**	**100.0**
Aztreonam	Difficult	I	2	50.0	12	100.0	8	37.5	1	0.0	-	-	**23**	**69.6**
Cefiderocol	Difficult	R	-	-	4	75.0	16	56.2	-	-	2	100	**22**	**63.6**
Ceftazidime	Easy	R	1	100.0	14	100.0	12	100.0	1	100.0	-	-	**28**	**100.0**
Ceftazidime-avibactam (fixed 4)***	Easy	R	1	100.0	16	100.0	8	100.0	3	100.0	-	-	**28**	**100.0**
Ceftolozane-tazobactam (fixed 4)[a]	Easy	R	1	100.0	17	100.0	6	100.0	2	100.0	-	-	**26**	**100.0**
Ciprofloxacin	Easy	R	2	100.0	14	100.0	12	100.0	-	-	-	-	**28**	**100.0**
Colistin	Easy	S	1	100.0	26	100.0	-	-	-	-	-	-	**27**	**100.0**
Imipenem	Easy	R	2	100.0	17	100.0	9	100.0	-	-	-	-	**28**	**100.0**
Imipenem-relebactam (fixed 4)***	Easy	R	1	100.0	13	100.0	3	100.0	5	100.0	-	-	**22**	**100.0**
Levofloxacin	Easy	R	2	100.0	7	100.0	10	100.0	3	100.0	-	-	**22**	**100.0**
Meropenem	Easy	R	2	100.0	19	100.0	8	100.0	1	100.0	-	-	**30**	**100.0**
Meropenem-vaborbactam (fixed 8)***	Easy	R	1	100.0	10	100.0	3	100.0	6	100.0	-	-	**20**	**100.0**
Piperacillin-tazobactam (fixed 4)***	Easy	R	2	100.0	15	100.0	11	100.0	1	100.0	-	-	**29**	**100.0**
Tobramycin	Easy	R	2	100.0	11	100.0	12	100.0	1	100.0	-	-	**26**	**100.0**
Total			**22**	**95.5**	**209**	**99.5**	**130**	**90.8**	**24**	**95.8**	**2**	**100**	**387**	**96.1**

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of good concordance (⩽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'.*

**** Fixed concentration (mg/L) of the beta-lactamase inhibitor shown in parentheses.*

Percentages might not total 100% due to rounding.

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

Strain **'2025 EURGen-Net 4' (*Acinetobacter baumannii*)** was described as being obtained from a patient with bloodstream infection. This strain was resistant to three of the ten antimicrobials included in the EQA: colistin, imipenem, and meropenem (Annex 1).

One antimicrobial was classified as a 'difficult' AST determination: cefiderocol, with an expected zone diameter located less than four millimetres from the breakpoint. For the remaining antimicrobials, the level of difficulty was considered 'easy'.

Interpretation of AST results for the *A. baumannii* strain were analysed for the 30 laboratories that reported correct species identification (Table 2).

Overall, 27 laboratories (90.0%) showed full concordance with the expected interpretations, and three laboratories (10.0%) achieved 'good' concordance.

The theoretical maximum number of results was 300 (30 laboratories × 10 antimicrobials). In total, 255 included AST interpretations of which 252 were correct (Table 3). The reported interpretations were therefore in 'excellent' concordance with the expected results (98.8%) (Table 8). MEs were observed for two (0.8%) of the interpretations and VMEs for one (0.4%) (Figure 6).

Broth microdilution was the most frequently reported method (51.4%), followed by disk/tablet diffusion (36.9%), automated system (6.3%), gradient test (2.4%), and macro broth dilution (3.1%) (Table 4).

All methods except automated systems showed 'excellent' concordance with the expected results (≥95% concordance): broth microdilution (100%), disk/tablet diffusion (97.9%), macro broth dilution (100%), and gradient test (100%). Automated systems showed 'very good' concordance (93.8%) (Table 8).

Of the three antimicrobials with an expected interpretation of R, one VME was observed for meropenem (Figure 6, Table 8). The reported MIC (>8 mg/L) was not consistent with the submitted interpretation (S) and involved the use of an automated system. Meropenem AST in this strain was classified as an 'easy' determination.

In addition, two MEs were recorded for cefiderocol, the only antimicrobial classified as 'difficult' in this strain. Both MEs resulted from disk/tablet diffusion, which was used for most AST determinations for this antimicrobial (n=13, 86.7%) and showed a concordance of 86.7%. The reported zone diameters (14 mm and 16 mm) were more than 6 mm and 4mm away from the expected value (>20 mm), respectively.

Discussion

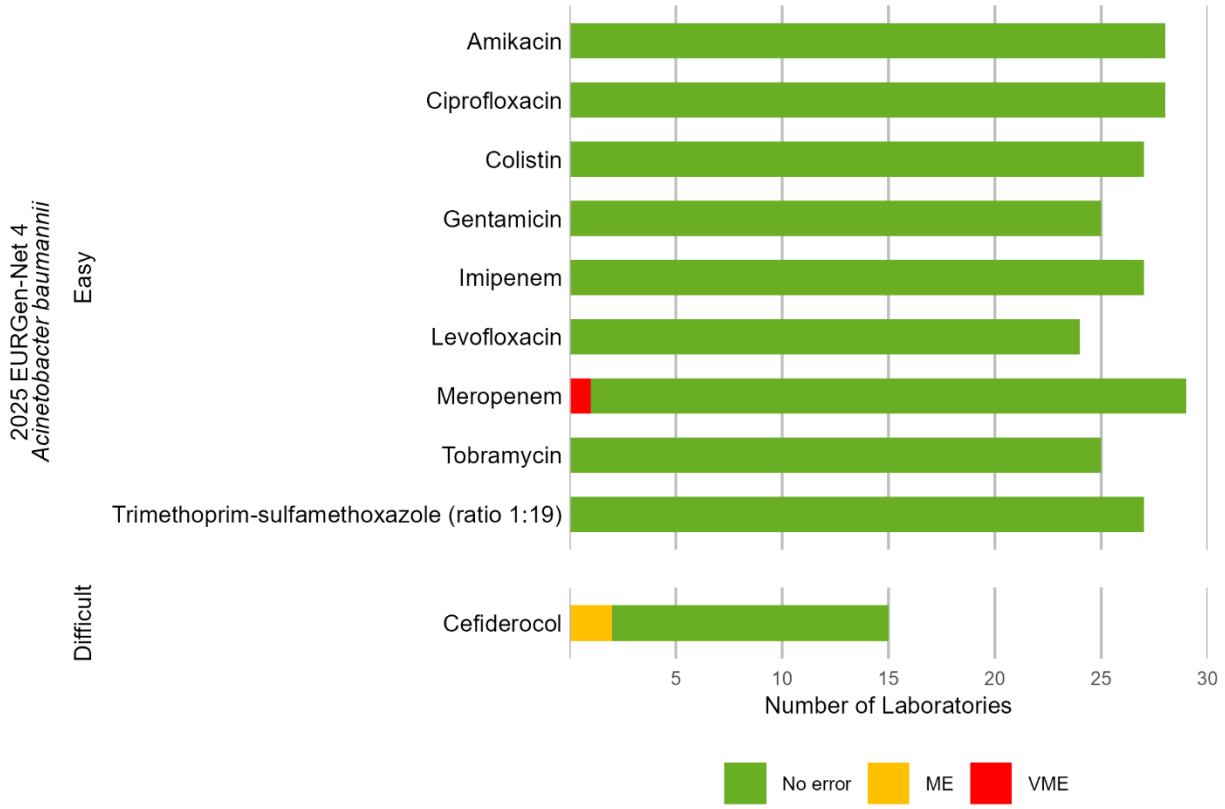
For strain **'2025 EURGen-Net 4' (*Acinetobacter baumannii*)** the reported AST interpretations showed very high concordance with the expected results, with only three errors observed overall. This strain had the highest proportion of laboratories reporting 100% concordant results, although it also included the smallest antimicrobial panel in the EQA.

One VME was recorded for meropenem, which was classified as an 'easy' AST determination. Errors in AST determinations classified as 'easy' are not generally expected to reflect inherent method variability and are more usually suggestive of random or systematic errors in laboratory procedures. In this case, the reported MIC (>8 mg/L) was not consistent with the submitted interpretation (S), suggesting incorrect interpretation of the MIC value or an error in data entry. This interpretation error was observed in the same laboratory that also submitted discordant interpretations for strains '2025 EURGen-Net 1' and '2025 EURGen-Net 2', which further supports this explanation.

The remaining two errors were MEs recorded for cefiderocol, the only antimicrobial classified as 'difficult' in this strain. Both resulted from disk/tablet diffusion, which was used for most AST determinations for this antimicrobial, in line with current EUCAST recommendations. As cefiderocol AST was classified as 'difficult', with an expected value close to the breakpoint, such errors could be due to the inherent method variability. However, the reported diameters were more than 4 mm away from the expected value, which could point to certain systematic or random errors in laboratory procedures.

Overall, broth microdilution, disk/tablet diffusion, macro broth dilution, and gradient test achieved 'excellent' concordance with the expected results, whereas automated systems showed 'very good' concordance (Table 8).

Figure 6 Reported interpretation of AST results for strain '2025 EURGen-Net 4' (*Acinetobacter baumannii*) 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 EURGen-Net 4' (*Acinetobacter baumannii*), by antimicrobial and AST method

Antimicrobial	Difficulty*	Expected interpretation**	Automated system		Broth microdilution		Disk/Tablet diffusion		Gradient test		Macro broth dilution		Total	
			n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	Easy	S	2	100.0	14	100	11	100.0	-	-	1	100	28	100.0
Cefiderocol	Difficult	S	-	-	2	100	13	84.6	-	-	-	-	15	86.7
Ciprofloxacin	Easy	I	2	100.0	13	100	11	100.0	1	100	1	100	28	100.0
Colistin	Easy	R	-	-	26	100	-	-	-	-	1	100	27	100.0
Gentamicin	Easy	S	2	100.0	12	100	10	100.0	-	-	1	100	25	100.0
Imipenem	Easy	R	2	100.0	16	100	8	100.0	-	-	1	100	27	100.0
Levofloxacin	Easy	S	2	100.0	9	100	10	100.0	3	100	-	-	24	100.0
Meropenem	Easy	R	2	50.0	17	100	8	100.0	1	100	1	100	29	96.6
Tobramycin	Easy	S	2	100.0	10	100	11	100.0	1	100	1	100	25	100.0
Trimethoprim-sulfamethoxazole (ratio 1:19)	Easy	S	2	100.0	12	100	12	100.0	-	-	1	100	27	100.0
Total			**16**	**93.8**	**131**	**100**	**94**	**97.9**	**6**	**100**	**8**	**100**	255	98.8

n: number of reporting laboratories; '-': no data; Shading of cells containing percentages: dark blue: below the threshold of good concordance (⩽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 participating laboratories after completion of the 2025 EURGen-Net EQA exercise, on 11 December 2025. In total, five of the 32 laboratories that submitted results provided feedback, corresponding to a response rate of 15.6%. This very low response rate limits interpretation of the findings and does not allow robust conclusions regarding the views of participating laboratories as a whole.

Of the five responding laboratories, three reported having taken corrective actions for results that did not conform to the expected results, whereas two reported that no action was needed because all analytical test results conformed to the expected results. The actions described included re-evaluation of submitted results and the submission process, revision of tools and methodologies, and use of the EQA results to support internal quality improvement. Four laboratories reported that the results would be used for accreditation, licensing, or related quality purposes.

No suggestions for improvement of the next phenotypic EQA exercise were received.

4. Summary and discussion

Participation

A total of 29 of the 31 eligible countries participated in the 2025 EURGen-Net EQA exercise, corresponding to 32 participating laboratories. All laboratories that enrolled submitted results. Given that the participating institutions are NRLs, this high level of participation was expected.

Species identification and overall AST results

Across all four strains, submitted species identification results were in 'very good' concordance with the expected results, with correct species identification reported by 30 of 32 laboratories (93.8%). All incorrect species identifications were reported by the same two laboratories and resulted from data entry errors. As both laboratories had incorrect species for all four strains, they were excluded from all AST analyses.

The submitted AST interpretations from the 30 laboratories with correct species identification were in 'excellent' concordance with the expected results, with 1,677 correct interpretations out of 1,715 submitted AST interpretations (97.8%). At strain level, concordance with the expected results was also 'excellent' for all four individual EQA strains, ranging from 96.1% to 99.1%. Overall, 28 of 30 laboratories achieved 'excellent' concordance and the remaining two achieved 'very good' concordance. Six laboratories reported fully concordant AST interpretations for all submitted results. It should, however, be noted that one of these laboratories (EURL-PH-AMR_20) submitted only four AST interpretations, one for each strain, which was the minimum criterium required to receive a certificate of participation.

These results indicate that the participating NRLs were generally able to produce highly reliable species identification and AST interpretations for the strains included in the 2025 EURGen-Net EQA. This overall performance was expected, given the role of these laboratories within national AMR surveillance systems.

Most errors within the submitted AST interpretations were associated with AST determinations classified as 'difficult'. Of the 38 total errors observed in this EQA, 31 (81.6%) were recorded for strain-antimicrobial combinations classified as 'difficult', whereas seven (18.4%) were recorded for combinations classified as 'easy'. This is consistent with the expected effect of borderline MIC values or zone diameters close to clinical breakpoints or ATUs, which increase the likelihood of misclassification due to the inherent variability of AST methods.

Most errors were VMEs (n=27, 71,1%), where the expected interpretation was R but the submitted result was S or I. These VMEs were distributed across nine strain-antimicrobial combinations. Eleven MEs (28.9%) were observed across four combinations, where the expected interpretation was S or I but the submitted result was R. Overall, the errors did not indicate a systematic tendency either to overestimate or to underestimate resistance. Rather, they were concentrated in a limited number of antimicrobial determinations, many of which were technically challenging.

Cefiderocol was the antimicrobial with the highest number of recorded errors, accounting for 15 of the 38 errors observed (39.5%). These were observed in three of the four strains, corresponding to the three in which cefiderocol AST was classified as 'difficult'. This supports the view that cefiderocol AST is one of the most challenging tests in Gram-negative bacteria, as reflected by recent EUCAST Warnings (https://www.eucast.org/bacteria/methodology-and-instructions/warnings/). As many of the deviations were associated with values close to the ATU or breakpoints, they were likely attributable to inherent method variability. Cefiderocol AST results are also known to vary according to the method and materials used [5,6].

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

The most frequently applied methods in the 2025 EURGen-Net EQA were broth microdilution and disk/tablet diffusion, accounting for 54.2% and 33.5% of all submitted results, respectively. These were also the methods with the highest absolute number of recorded errors, which is expected in light of their much higher frequency of use. Overall concordance was 'excellent' for both broth microdilution (98.7%) and disk/tablet diffusion (97.2%).

Disk/tablet diffusion accounted for 16 of the 38 identified errors (42.1%). This was driven mainly by the use of that method for AST of cefiderocol, for which the AST determination was considered 'difficult' in three of the four EQA strains ('2025 EURGen-Net 2', '2025 EURGen-Net 3', and '2025 EURGen-Net 4'). AST of aztreonam in strain '2025 EURGen-Net 3' further contributed to discrepancies within that method. In the case of cefiderocol, this pattern should be interpreted with caution because disk/tablet diffusion is the EUCAST-recommended method and was also the method used by most laboratories. The lower concordance observed for disk/tablet diffusion was therefore largely driven by the difficulty of these specific determinations rather than by poor overall performance of the method.

Broth microdilution accounted for 12 of the 38 recorded errors (31.6%). Four of these were associated with cefiderocol, for which broth microdilution is not the EUCAST-recommended method. Additional errors were observed for ertapenem in strain '2025 EURGen-Net 1', meropenem and colistin in strain '2025 EURGen-Net 2', and

amikacin in strain '2025 EURGen-Net 2'. In strain '2025 EURGen-Net 1', the lower concordance of broth microdilution for ertapenem is likely related to the 'difficult' classification of this determination, with MIC values close to the breakpoint. For amikacin in strain '2025 EURGen-Net 2', the lower concordance of broth microdilution is in line with the known variability of AST results for aminoglycosides, which may depend on the methods and materials used [8].

Automated systems (n=100, 5.8% of all submitted results) showed the lowest overall concordance among the commonly applied methods, while nevertheless achieving 'excellent' concordance (95.0%). Gradient test (n=99, 5.8% of all submitted results) also achieved 'excellent' concordance overall (96.0%).

In general, errors were distributed across laboratories and antimicrobials, and no broad pattern of systematic underperformance by a specific laboratory or method was observed. The main patterns were restricted to specific strain-antimicrobial-method combinations, particularly cefiderocol across strains, meropenem-vaborbactam by gradient test in strain '2025 EURGen-Net 2', and aztreonam by methods other than broth microdilution in strain '2025 EURGen-Net 3'.

A notable exception was laboratory EURL-PH-AMR_29, where the highest total number of errors was observed (n=5). Four of these, all associated with automated systems and recorded across three of the four EQA strains, involved discordance between the reported MIC value and the submitted interpretation. As the reported MIC values should have yielded the correct interpretation according to EUCAST breakpoints, these deviations are more suggestive of laboratory-specific issues, such as incorrect breakpoint interpretation or data entry, than of technical difficulties with the execution of the method. A similar pattern was observed once more in the dataset, in laboratory EURL-PH-AMR_07, which reported an aztreonam result by gradient test for strain '2025 EURGen-Net 3' with a correct MIC value but an incorrect submitted interpretation.

Particular findings of note

Some strain-antimicrobial-method combinations showed lower concordance and merit specific attention:

- In strain '2025 EURGen-Net 1' (*E. coli*), broth microdilution for ertapenem showed lower concordance, consistent with the 'difficult' classification of this determination, while automated systems showed low concordance for levofloxacin and piperacillin-tazobactam, both associated with discordant interpretation of the reported MIC values by one laboratory.
- In strain '2025 EURGen-Net 2' (*K. pneumoniae*), lower concordance was observed for automated systems and broth microdilution for amikacin, broth microdilution for meropenem and gradient test for meropenem-vaborbactam. All other methods performed worse than disk/tablet diffusion for cefiderocol, supporting current EUCAST recommendations to test the antimicrobial with disk diffusion.
- In strain '2025 EURGen-Net 3' (*P. aeruginosa*), all methods except broth microdilution produced errors for aztreonam, suggesting that broth microdilution may provide more robust results for this antimicrobial in *P. aeruginosa*.
- In strain '2025 EURGen-Net 4' (*A. baumannii*), the single meropenem VME reported by automated system was associated with discordant interpretation of the reported MIC value by one laboratory.
- In strains '2025 EURGen-Net 3' and '2025 EURGen-Net 4', cefiderocol again showed lower concordance, particularly for disk/tablet diffusion, reflecting the technical difficulty of these determinations.

Overall, the results of the 2025 EURGen-Net EQA indicate that AST data generated by participating NRLs are highly reliable. The observed deviations were concentrated mainly in technically challenging determinations and in a limited number of method-antimicrobial combinations. At the same time, several errors were associated not with the measured MIC or zone diameter itself, but with the S/I/R interpretation assigned to the reported value, indicating that continued attention should be given not only to method selection and technical performance, but also to breakpoint application and data entry procedures.

5. Conclusions

The results of the first phenotypic EQA offered by the EURL-PH-AMR to the EURGen-Net indicate that AST data generated by participating NRLs are highly reliable.

The submitted species identification results suggest that, overall, species identification in the NRLs is accurate, with correct species identification reported by 30 of 32 laboratories (93.8%). All incorrect species identifications were reported by the same two laboratories, both for all four strains. The EURL-PH-AMR confirmed that these deviations were due to an error in data entry, after detailed analysis of results and direct discussion with the two laboratories.

The submitted AST interpretations also indicate that AST data generated by participating NRLs are highly reliable. Overall, 97.8% of the 1,715 submitted AST interpretations were concordant with the expected results, and concordance was 'excellent' for all four strains. These findings are in line with the expected high level of performance of NRLs.

Most observed errors were associated with AST determinations classified as 'difficult', with 31 (81.6%) of the 38 total errors falling into this category. This supports the impact of borderline MIC values and zone diameters close to clinical breakpoints or ATUs as a source of misclassification, due to the inherent variability of AST methods. At the same time, seven errors were observed in determinations classified as 'easy', indicating that not all deviations can be explained by methodological variability alone.

Although VMEs were more frequent than MEs in this EQA, the number of observed deviations was limited, and the findings do not support a strong conclusion regarding an overall tendency to under-report or over-report resistance.

Cefiderocol was the antimicrobial with the highest number of recorded errors, accounting for 15 (39.5%) of the 38 observed errors. Errors were recorded for cefiderocol in three of the four strains, corresponding to the three in which this AST determination was classified as 'difficult'. This supports that cefiderocol is one of the most challenging antimicrobials for AST in Gram-negative bacteria.

Overall differences between AST methods were small, and all commonly applied methods achieved 'excellent' concordance. The methods with the highest absolute number of errors, broth microdilution and disk/tablet diffusion, were also by far the most frequently used. Lower concordance was mainly restricted to specific strain-antimicrobial-method combinations, including cefiderocol by non-recommended methods, meropenem-vaborbactam by gradient test, and aztreonam in *P. aeruginosa* by methods other than broth microdilution.

Several deviations were associated not with the measured MIC or zone diameter itself, but with the interpretation assigned to the reported value. This was particularly evident in one laboratory that reported multiple discordant interpretations across strains using automated systems, and in one additional laboratory reporting discordant interpretation for aztreonam result using gradient test. These findings suggest that, in addition to method choice and technical performance, continued attention should be given to careful application of EUCAST breakpoints and to result entry procedures.

6. Recommendations for EQA participants

Given the overall high performance observed in this EQA, the recommendations arising from these findings are primarily focused on laboratory practice, method selection, breakpoint application, and result review procedures.

Participating laboratories should review their individual performance in this EQA exercise and revisit all areas where they did not achieve the expected results. In general, it is recommended to:

- Use the EUCAST-recommended AST method for each species-antimicrobial combination tested;
- Ensure that AST protocols, interpretation criteria, and reporting procedures are aligned with the most recent EUCAST guidance;
- Strengthen awareness of AST results close to clinical breakpoints or ATUs, and of the expected variability of methods in these situations;
- Ensure that adequate internal quality management and control procedures are in place, including but not limited to monitoring AST results over time, to allow control of random or systematic deviations;
- Ensure that reported S/I/R categories are consistent with the measured MIC or zone diameter and the applicable EUCAST breakpoint;
- Continue regular participation in EURGen-Net EQA exercises, to support monitoring of performance over time and identification of strain-antimicrobial combinations for which further improvement is needed.

The results from this EQA exercise indicate that some inaccuracies may still occur, including in NRL settings. Particular findings of note included:

- Frequent errors in AST determinations classified as 'difficult';
- Recurrent difficulties in AST of cefiderocol across Gram-negative species, especially when laboratories deviate from the methods recommended by EUCAST;
- Lower concordance for specific carbapenem and carbapenem-beta-lactamase inhibitor determinations, including ertapenem and meropenem-vaborbactam;
- Lower concordance for amikacin in *K. pneumoniae*;
- Lower concordance for aztreonam in *P. aeruginosa* when tested by methods other than broth microdilution;
- A small number of deviations related not to the measured MIC or zone diameter itself, but to the interpretation assigned to the reported value.

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

- 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.
- Revising criteria for performing and reading results for aminoglycosides susceptibility testing, since the variability in the AST results for aminoglycosides may have been influenced by differences in media composition.
- Strengthening awareness and potentially seeking advice regarding AST and reading of results of carbapenems.
- Review the use of gradient test for determinations in which lower concordance was observed, particularly meropenem-vaborbactam and aztreonam;
- Review internal procedures for assigning S/I/R categories to measured MIC or zone diameter values, in order to reduce errors related to incorrect application of breakpoints or data entry;
- Pay particular attention to technically challenging determinations and seek clarification when needed regarding interpretation of results close to breakpoints or ATUs.

Continued participation of NRLs in annual EQA exercises remains important to support high-quality AST reporting and to identify determinations for which further improvement is still needed.

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

Table 9 EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EURGen-Net 1' (*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]
	S ≤	R >	ATU	S ≥	R <	ATU			
Amikacin (systemic infections)	8	8		18	18		Easy	≤4	S
Aztreonam	1	4		26	21		Easy	≤1	S
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	0.06/4	S
Cefiderocol	2	2		23	23	21-23	Easy	≥28 mm	S
Cefotaxime (indications other than meningitis)	1	2		20	17		Easy	≤0.5	S
Ceftazidime	1	4		22	19		Easy	≤0.5	S
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	≤0.25/4	S
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Difficult	2/4	S
Ciprofloxacin (indications other than meningitis)	0.25	0.5	0.5	25	22	22-24	Easy	>8	R
Colistin	2	2		Note	Note		Easy	0.5	S
Ertapenem	0.5	0.5		23	23		Difficult	>0.5	R
Fosfomycin iv (infections originating from the urinary tract)	8	8		24	24		Easy	≤4	S
Gentamicin (systemic infections)	2	2		17	17		Easy	≤1	S
Imipenem	2	4		22	19		Easy	≤1	S
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	≤0.25/4	S
Levofloxacin	0.5	1		23	19		Easy	>8	R
Meropenem (indications other than meningitis)	2	8		22	16		Easy	0.5	S
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Easy	0.25/8	S
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64/4	R
Tigecycline, *E. coli* and *C. koseri*	0.5	0.5		18	18		Easy	0.25	S
Tobramycin (systemic infections)	2	2		16	16		Easy	1	S
Trimethoprim-sulfamethoxazole (ratio 1:19)	2	4		14	11		Easy	≤0.5/9.5	S

MALDI-TOF by DTU: Escherichia coli (score 2.35). MLST: ST-648 (Achtmann), ST-472 / ST-662 / ST-721 (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'.

Table 10 EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EURGen-Net 2' (*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]
	S ≤	R >	ATU	S ≥	R <	ATU			
Amikacin (systemic infections)	8	8		18	18		Difficult	16	R
Aztreonam	1	4		26	21		Easy	>64	R
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	0.25/4	S
Cefiderocol	2	2		23	23	21-23	Difficult	≤20 mm	R
Cefotaxime (indications other than meningitis)	1	2		20	17		Easy	>8	R
Ceftazidime	1	4		22	19		Easy	>32	R
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	>16/4	R
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Easy	>8/4	R
Ciprofloxacin (indications other than meningitis)	0.25	0.5	0.5	25	22	22-24	Easy	>8	R
Colistin	2	2		Note	Note		Easy	≤0.5	S
Ertapenem	0.5	0.5		23	23		Easy	>4	R
Gentamicin (systemic infections)	2	2		17	17		Easy	≤1	S
Imipenem	2	4		22	19		Easy	>16	R
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	>8/4	R
Levofloxacin	0.5	1		23	19		Easy	>8	R
Meropenem (indications other than meningitis)	2	8		22	16		Easy	>16	R
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Difficult	>8/8	R
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64/4	R
Tobramycin (systemic infections)	2	2		16	16		Easy	16	R
Trimethoprim-sulfamethoxazole (ratio 1:19)	2	4		14	11		Easy	≤0.5/9.5	S

MALDI-TOF by DTU: Klebsiella pneumoniae (score 2.27). MLST: ST-147.

[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'.

Table 11 EUCAST clinical breakpoints for *Pseudomonas aeruginosa* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EURGen-Net 3' (*P. aeruginosa*), 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]
	S ≤	R >	ATU	S ≥	R <	ATU			
Amikacin (systemic infections)	16	16		15	15		Easy	>32	R
Aztreonam	0.001	16		50	18		Difficult	16	I
Cefiderocol	2	2		22	22	20-21	Difficult	≤21 mm	R
Ceftazidime	0.001	8		50	17		Easy	>32	R
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		17	17	16-17	Easy	>16/4	R
Ceftolozane-tazobactam (fixed 4 mg/L)	4	4		23	23		Easy	>8/4	R
Ciprofloxacin	0.001	0.5		50	26		Easy	>8	R
Colistin	4	4		Note	Note		Easy	≤2	S
Imipenem	0.001	4		50	20		Easy	>16	R
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22		Easy	>8/4	R
Levofloxacin	0.001	2		50	18		Easy	>8	R
Meropenem (indications other than meningitis)	2	8		20	14		Easy	>32	R
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		14	14		Easy	>16/8	R
Piperacillin-tazobactam (fixed 4)	0.001	16		50	18	18-19	Easy	>64/4	R
Tobramycin (systemic infections)	2	2		18	18		Easy	>16	R

MALDI-TOF by DTU: Pseudomonas aeruginosa (score 2.46). MLST: ST-773.

[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'.

Table 12 EUCAST clinical breakpoints, expected AST results for *Acinetobacter baumannii* and the level of difficulty in interpretation and expected interpretations for strain '2025 EURGen-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]
	S ≤	R >	ATU	S ≥	R <	ATU			
Amikacin (systemic infections)	8	8		19	19		Easy	4	S
Cefiderocol	Note (S WT<=0.5; NWT impaired clinical response 1-2)	Note (R>2)		Note (S WT>=21; NWT impaired clinical response 20-17)	Note (R<17)		Difficult	≥21 mm	S
Ciprofloxacin	0.001	1		50	21		Easy	0.12	I
Colistin	2	2		Note	Note		Easy	>8	R
Gentamicin (systemic infections)	4	4		17	17		Easy	1	S
Imipenem	2	4		24	21		Easy	>16	R
Levofloxacin	0.5	1		23	20		Easy	≤0.12	S
Meropenem (indications other than meningitis)	2	8		21	15		Easy	>32	R
Tobramycin (systemic infections)	4	4		17	17		Easy	1	S
Trimethoprim-sulfamethoxazole (ratio 1:19)	2	4		14	11		Easy	≤0.5/9.5	S

MALDI-TOF by DTU: Acinetobacter baumannii (score 2.43). MLST: ST-1591 (Oxford), ST-329 (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'.

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Annex 2. Feedback Survey Questionnaire

EURGen-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 the ECDC EURGen-Net phenotypic EQA.
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 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 actions for non-conformities were taken.
- ☐ Yes, actions were taken.

Please specify which 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 EURGen-Net phenotypic 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