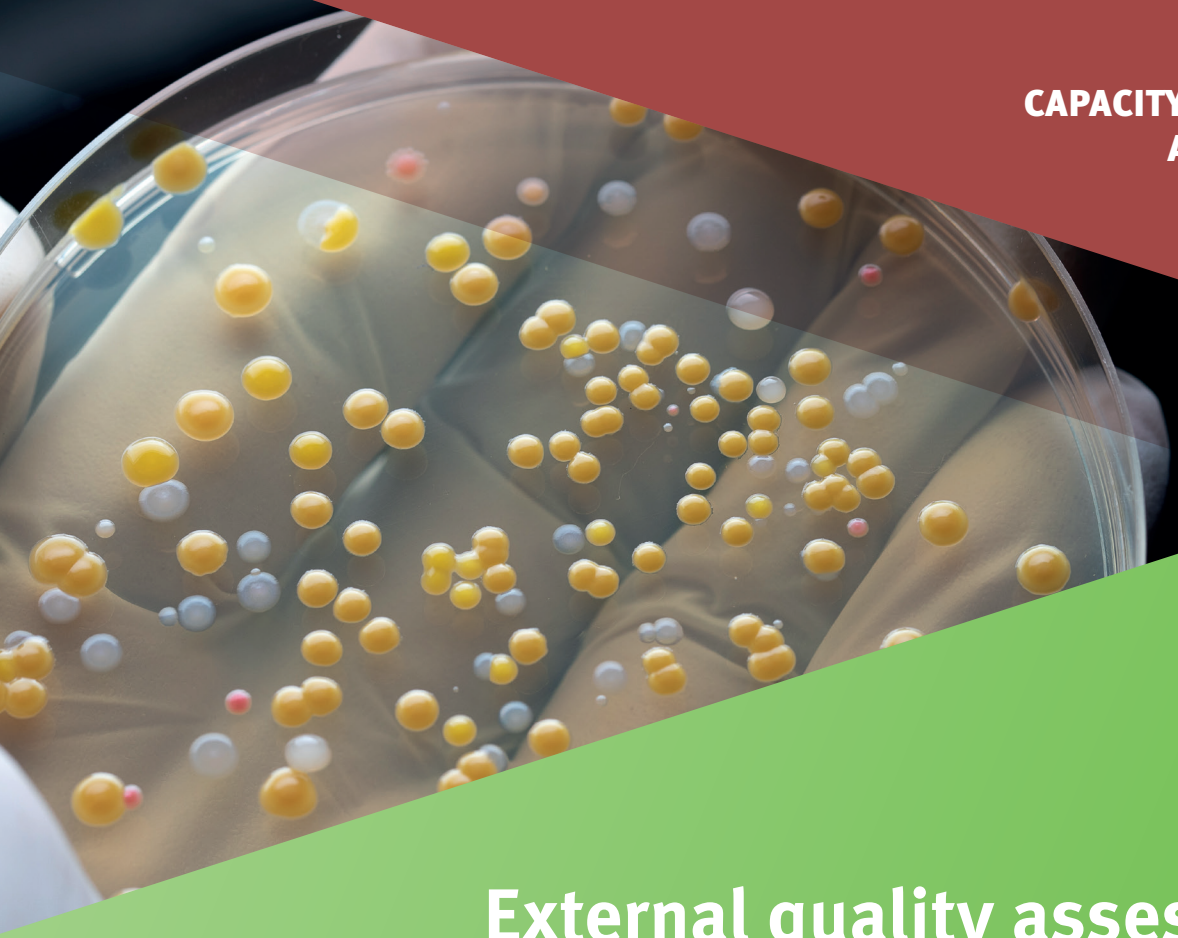

CAPACITY/CAPABILITY ASSESSMENT

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

2023

ECDC CAPACITY/CAPABILITY ASSESSMENT

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Abbreviations

AMR	Antimicrobial resistance
ARG	Antimicrobial resistance genes
AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
CLSI	Clinical and Laboratory Standards Institute
DTU Food	Technical University of Denmark, National Food Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EARSS	European Antimicrobial Resistance Surveillance System
ECDC	European Centre for Disease Prevention and Control
EQA	External quality assessment
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	'Susceptible, increased exposure'
ME	Major error
MIC	Minimum inhibitory concentration
PM	Point mutation (chromosomal)
R	'Resistant'
S	'Susceptible, standard dosing regimen'
std	Standard deviation
VME	Very major error

Executive summary

This report describes the results of the 2023 external quality assessment (EQA) exercise for antimicrobial susceptibility testing (AST) by clinical laboratories that participate in the European Antimicrobial Resistance Surveillance Network (EARS-Net). It includes a short conclusion on the capacities of the participating laboratories, and recommendations for improvement. All 30 European Union/European Economic Area (EU/EEA) countries participated in this EARS-Net EQA exercise.

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

In 2023, the panel of six EQA strains consisted of *Escherichia coli*, *Klebsiella pneumoniae* (two strains), *Enterococcus faecalis*, *Enterococcus faecium* and *Acinetobacter baumannii* (Table 1). The *E. coli* strain, and one of the two *K. pneumoniae* strains had been included in previous EARS-Net EQA exercises. The *E. coli* strain ('2023 EARS-Net 1') was the most challenging strain in the 2022 EQA panel (strain '2022 EARS-Net 2') – i.e. the strain with the most incorrect results. The *K. pneumoniae* strain ('2023 EARS-Net 4') was the strain with the highest concordance of AST results in the 2021 EQA exercise (strain 'EARS-Net KPN 21.1'). It was included in the 2023 EQA panel to facilitate comparison of the performance of AST methods with the more challenging *K. pneumoniae* strain in the 2023 panel (strain '2023 EARS-Net 2').

On 12 June 2023, the six strains were distributed via the National EARS-Net EQA Coordinators to 951 laboratories in all 30 EU/EEA countries. An EQA webpage was opened to receive submission of results between 14 June and 11 August 2023. As in previous EARS-Net EQA exercises [2-4], concordance of species and AST interpretations with the expected results was defined as 'excellent' (≥95% of interpretations in concordance with expected results), 'very good' (>90% to <95%), 'good' (>85 to ≤90%) or 'satisfactory' (>80 to ≤85%).

Results were submitted by 871 laboratories and two were excluded from the analysis because they did not submit data on AST interpretation. Species identification was evaluated for 869 laboratories, and 5 150 (99.1%) of the 5 197 reported species were correct. There was 'excellent' concordance for each of the six strains (98.8 to 99.3% concordance). Four laboratories reported the wrong species for all submitted strains.

Interpretation of AST results was only evaluated if the species had been correctly identified. The evaluation was performed according to the clinical breakpoints in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints Tables v13.0 [6], with the EUCAST categories 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R).

In the 2023 EARS-Net EQA exercise, the scoring system for the evaluation of interpreted results included an assessment of the 'level of difficulty' and the 'severity of error' of the submitted AST result for each species-antimicrobial agent combination. The scoring system was similar to that applied in the 2022 EARS-Net EQA exercise, with the exception that missing results did not generate a negative score.

The 'level of difficulty' had two levels ('easy' and 'difficult'), reflecting the magnitude of the risk of getting the AST result wrong. 'Easy' results were those with expected AST results far from the breakpoint, where the categorisation was obvious. Conversely, 'difficult' results were those close to the breakpoint or inside the area of technical uncertainty (ATU), or those for which breakpoints had been recently changed or added. Consequently, the scoring system allocated a higher score to 'difficult' results than 'easy' results, and penalised errors for 'easy' results more severely than errors for 'difficult' results.

The severity of error was divided into three levels: very major error (VME), which indicated reporting false susceptibility (i.e. reporting S or I, instead of R); major error (ME), which indicated reporting false resistance (i.e. reporting R, instead of S or I) and no error. The scoring system penalised VMEs more severely for 'easy' results than for 'difficult' results and did not penalise MEs if the test was considered 'difficult'.

The reported interpretations of AST results were evaluated for 865 laboratories (excluding the two laboratories that did not submit data on AST interpretation and the four laboratories that reported the wrong species for all submitted strains).

Among the 53 272 evaluated AST results, the most frequently reported methods for AST had very good or excellent concordance with the expected result (Table 13). These were automated systems (53.9% of all tests; of which 94.2% were correct), followed by disk or tablet diffusion (27.3% of all tests, of which 95.2% were correct) and minimum inhibitory concentration (MIC) methods, including broth microdilution (10.7% tests, of which 95.6% were correct).

Overall, the submitted AST interpretations were in 'very good' concordance with the expected results, with 94.7% (50 441 out of 53 272) being correct. Otherwise, MEs and VMEs were observed for 3.2% and 2.2% of interpretations, respectively. At country level, 17 countries (Austria, Belgium, Bulgaria, Croatia, Cyprus, Denmark, Finland, Iceland, Italy, Liechtenstein, Luxembourg, Malta, Norway, Poland, Slovakia, Slovenia, and Sweden) achieved an 'excellent' level of concordance with the expected interpretation of AST results, and 13 countries (Latvia, Czechia, Estonia, France, Germany, Greece, Hungary, Ireland, Lithuania, Netherlands, Portugal, Romania, and Spain) achieved a 'very good' level concordance. At laboratory level, 50.7% (n=439) of the laboratories achieved an 'excellent' level of concordance, 39.8% (n=345) achieved a 'very good' level of concordance, 7.7% (n=67) achieved a 'good' level of concordance, 1.2% (n=10) achieved a 'satisfactory' level, and 0.6% (n=5) were below the 'satisfactory' level.

There were 74 species-antimicrobial agent combinations tested for antimicrobial susceptibility in the 2023 EARS-Net EQA exercise, and the vast majority had results in 'excellent' concordance with the expected results (n=58 or 78.4% of the combinations). A 'very good' level of concordance was achieved for eight combinations (10.8%). The species-antimicrobial agent combination with the lowest level of concordance was amikacin for the *E. coli* strain, with only 29.2% of correct interpretations and deviations that were MEs (S → R) reported for all of the frequently used methods. Low concordance was also observed for AST of other antimicrobial agents for the *E. coli* strain (piperacillin-tazobactam with 40.6% of concordance, cefepime with 83.4%, ceftazidime with 87.5%), for one of the *K. pneumoniae* strains (strain '2023 EARS-Net 2') (amikacin with 66.7%, cefepime with 78.9% and imipenem with 82.5%), and the *A. baumannii* strain (amikacin with 70.0%). All remaining species-antimicrobial agent combinations achieved at least a 'very good' concordance (>90%).

Table 1. Overview of species identification results and antimicrobial susceptibility testing (AST) results reported by clinical laboratories participating in the 2023 EARS-Net EQA exercise

Strain ID	Species and expected AST results for tested antimicrobial agents*	Species identification		AST results			
		Labs reporting species (N)	Labs reporting correct species (N(%))	Reported AST results (N)	Correct AST interpretations (N(%))	Major errors (N (%))	Very major errors (N (%))
2023 EARS-Net 1	*Escherichia coli*** S: AMK, COL, ETP, FEP, GEN, IPM, MEM, TGC I: CAZ R: AMC, AMP, AMX, CIP, CRO, CTX, LVX, MFX, OFX, TOB, TZP	867	861 (99.3%)	13 931	12 566 (90.2%)	832 (6.0%)	533 (3.8%)
2023 EARS-Net2	*Klebsiella pneumoniae* S: AMK, CIP, COL, IPM, LVX, MEM, MFX, OFX I: FEP R: AMC, CAZ, CRO, CTX, ETP, GEN, TOB, TZP	867	859 (99.1%)	12 081	11 220 (92.9%)	652 (5.4%)	209 (1.7%)
2023 EARS-Net 3	*Enterococcus faecalis* S: AMP, AMX, GEN, TEC, VAN R: LNZ	864	857 (99.2%)	4 450	4 336 (97.4%)	89 (2.0%)	25 (0.6%)
2023 EARS-Net 4	*Klebsiella pneumoniae**** R: AMC, AMK, CAZ, CIP, COL, CRO, CTX, ETP, FEP, GEN, IPM, LVX, MEM, MFX, OFX, TOB, TZP	866	858 (99.1%)	12 171	12 053 (99.0%)	0 (-)	118 (1.0%)
2023 EARS-Net 5	*Acinetobacter baumannii* S: COL, IPM, MEM R: AMK, CIP, GEN, LVX, TOB	866	856 (98.8%)	6 185	5 922 (95.7%)	23 (0.4%)	240 (3.9%)
2023 EARS-Net 6	*Enterococcus faecium* S: GEN, LNZ, TEC R: AMP, AMX, VAN	867	859 (99.1%)	4 454	4 344 (97.5%)	87 (2.0%)	23 (0.5%)
Total		**869**	**5 150 (99.0)**	**53 272**	**50 441 (94.7%)**	**1 683 (3.2%)**	**1 148 (2.2%)**

** All samples were considered to be obtained from patients with bloodstream infections.*
*** The 2023 EARS-Net 1 strain was identical to the 2022 EARS-Net 2 strain. See explanation on page 21.*
**** The 2023 EARS-Net 4 strain was identical to the 2021 KPN 21.1 strain. See explanation on page 27.*
AST: antimicrobial susceptibility testing; NA: not applicable; S: susceptible, standard dosing regimen; I: susceptible, increased exposure; R: resistant; AMC: amoxicillin-clavulanic acid, AMK: amikacin, AMP: ampicillin, AMX: amoxicillin, CAZ: ceftazidime, CIP: ciprofloxacin, COL: colistin, CRO: ceftriaxone, CTX: cefotaxime, ETP: ertapenem, FEP: cefepime, GEN: gentamicin, IPM: imipenem, LNZ: linezolid, LVX: levofloxacin, MEM: meropenem, MFX: moxifloxacin, OFX: ofloxacin, TEC: teicoplanin, TGC: tigecycline, TOB: tobramycin, TZP: piperacillin-tazobactam, VAN: vancomycin.

Strain '**2023 EARS-Net 1' (*Escherichia coli*)** was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin and tobramycin. The strain was susceptible to cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, tigecycline and colistin, and the expected MIC value for ceftazidime was in the I range (Table 1, Table 2). The strain harboured two beta-lactamase genes that contributed to the complex beta-lactam resistance profile: *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{CTX-M-15}}$ (Table 2).

In total, 99.3% (861/867) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for the strain were in 'very good' concordance with expected results (90.2%). MEs and VMEs were observed for 6.0% and 3.8% of the reported interpretations, respectively.

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

Prediction of susceptibility to amikacin was problematic as 70.8% (554 out of 783) of the submitted results were not in concordance with the expected result. Prediction of resistance to piperacillin-tazobactam was also poor since 57.9% (485 out of 838) of the submitted results were not concordant. Characterisation of susceptibility to cefepime (16.6% errors, 124 out of 749) and ceftazidime (12.5% errors, 109 out of 847) was also challenging. These deviations can be attributed to the inherent method variability, since results within the acceptable variation range (+/-1 dilution) would lead to an incorrect AST interpretation. In addition, the reported AST results for cephalosporins for laboratories using 'disk or tablet diffusion' had a lower concordance with the expected method than those from other methods. The deviations in amikacin and cephalosporins correspond to MEs (S → R or I → R) and may indicate that, for *E. coli*, resistance to these antimicrobials may be overestimated in the EU/EEA. The deviations in piperacillin-tazobactam correspond to VMEs (R → S) and may indicate that resistance to piperacillin-tazobactam can be under-reported in the EU/EEA. These issues may be exacerbated when AST results are close to the current breakpoints, which increases the difficulty of AST. Consideration should also be given to the level of difficulty of the AST determinations for the four antimicrobials ('difficult'). The results observed in this EQA may therefore reflect the fact that the strain was particularly challenging, rather than evidencing general trends in estimations of AMR within Europe.

This strain was also part of the 2022 EARS-Net EQA exercise (strain '2022 EARS-Net 2' (*E. coli*)) and was the most challenging for participating laboratories in 2022 [2]. Therefore, it was decided to include the exact same *E. coli* strain in the panel for the 2023 EARS-Net EQA exercise. When taking into account the results submitted for this strain by all participating laboratories, for all antimicrobial agents excluding amikacin and piperacillin-tazobactam, the highest variation was the decrease in ME for cefepime, from 20.4% in 2022 to 16.6% in 2023. The difference in results for piperacillin-tazobactam and amikacin was more complex because the consensus results obtained for these antimicrobial agents were not the same between years. In 2022, 60.5% of participating laboratories reported a correct AST interpretation for piperacillin-tazobactam (expected interpretation of S). In 2023, with a new expected interpretation of R, this percentage decreased to 40.6%. In 2022, 64.0% of participating laboratories reported a correct AST interpretation for amikacin (expected interpretation of R). In 2023, with a new expected interpretation of S, this proportion decreased to 29.2%.

These results indicate that laboratories should become familiar with recommendations on interpretation of AST results near the clinical breakpoints, as well as other general or specific EUCAST recommendations for the performance, interpretation and evaluation of the various AST methods. They should also review their methods for the performance and interpretation of results for species/antimicrobial combinations that may be associated with differential expression of AMR genes.

Strain '**2023 EARS-Net 2' *(Klebsiella pneumoniae)*** was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin and tobramycin. The strain was susceptible to imipenem, meropenem, ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin, had conditional susceptibility '(S)' to amikacin and colistin, and the expected MIC value for cefepime was in the I range (Table 1, Table 3). The strain harboured three beta-lactamase genes that contributed to the complex beta-lactam resistance profile, specifically *bla*$_{\text{VEB-1}}$, *bla*$_{\text{SHV-11}}$ (or a similar *bla*$_{\text{SHV}}$ variant) and *bla*$_{\text{OXA-10}}$.

In total, 99.1% (859/867) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for this strain were in 'very good' concordance with expected results (92.9%). MEs and VMEs were observed for 5.4% and 1.7% of the reported interpretations, respectively.

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

Prediction of susceptibility to amikacin (33.3% errors, 261 out of 784), cefepime (21.1% errors, 160 out of 758) and imipenem (17.5% errors, 131 out of 748) appeared to be challenging. Some of the deviations (specifically those observed for cefepime and imipenem) may be attributed to the inherent method variability and are within the acceptable variation range. However, the deviations observed for amikacin should not be justifiable by the natural methodological variability and might be due to variations in the methods and/or material used for AST [7-9]. All these deviations represent MEs (S → R) and may indicate that resistance to these antimicrobial agents is overestimated in the EU/EEA.

Strain '**2023 EARS-Net 3' (*Enterococcus faecalis*)** was resistant to linezolid and susceptible to amoxicillin, ampicillin, teicoplanin and vancomycin. The strain did not present high-level aminoglycoside resistance to gentamicin (Table 1, Table 4). The strain harboured the *optrA* gene which confers resistance to linezolid.

In total, 99.2% (857/864) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for this strain were in 'excellent' concordance with expected results (97.4%). MEs and VMEs were observed for 2.0% and 0.6% of the reported interpretations, respectively.

There was an 'excellent' level of concordance with the expected results (>95% of concordance) for every reported AST method.

The results observed for gentamicin were perhaps suboptimal, with 9.2% (62 out of 673) of the submitted results not in concordance with the expected result, with unexpected reports of positive high-level aminoglycoside resistance. These deviations may have derived from misinterpretation of the EQA protocol, with participants reporting the natural aminoglycoside resistance, including gentamicin resistance, of the test strain. Concordance of

AST results for the remaining antimicrobial agents was 'excellent', supporting a hypothesis that laboratories generally correctly apply EUCAST guidelines and breakpoints for *E. faecalis.* Furthermore, this supports the idea that misreporting of gentamicin results in this EQA exercise was likely due to misinterpretation. Therefore, these results do not strongly suggest that there is anomalous reporting of aminoglycoside resistance in *E. faecalis* in the EU/EEA, and neither do they indicate systemic problems with the methods applied by the participating laboratories.

Strain **'2023 EARS-Net 4' (*Klebsiella pneumoniae*)** was resistant to all antimicrobials included in EARS-Net(Table 1, Table 5). The strain harboured five beta-lactamase genes that contributed to the extensive beta-lactam resistance profile, specifically *bla*$_{\text{NDM-5}}$, *bla*$_{\text{SHV-1}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{OXA-181}}$. The strain also harboured the 16S rRNA methylase gene *rmtB*, responsible for resistance to aminoglycosides, and it presented a point mutation in the *mgrB* gene which was responsible for colistin resistance. Finally, the strain harboured various mutations and genes contributing to fluoroquinolone resistance, including point mutations in the *gyrA* and *parC* genes and the acquired AMR gene *qnrS1*.

In total, 99.1% (858/866) of laboratories correctly identified the species of this test strain and, overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (99.0%). As the strain was resistant to all the antimicrobial agents included, there could not be MEs. VMEs were observed for 1.0% of the reported interpretations.

There was an 'excellent' level of concordance with the expected results (>95% of concordance) for almost all AST methods. The exception was 'other methods', which only achieved a 'good' concordance (90.0%).

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

This strain had previously been included in the 2021 EARS-Net EQA exercise ('EARS-Net KPN 21.1') [3]. In 2021, it was the *K. pneumoniae* strain with the highest concordance of AST results. Therefore, it was included in the 2023 EQA exercise to facilitate comparison of the performance of AST methods with the challenging '2023 EARS-Net 2' strain. When comparing results between the 2021 and 2023 EARS-Net EQA exercises, there was little variability of results for this strain, with less than 1.5% of variation in the proportion of VMEs observed for each antimicrobial agent.

Strain **'2023 EARS-Net 5' (*Acinetobacter baumannii*)** was resistant to amikacin, gentamicin, tobramycin, ciprofloxacin and levofloxacin. The strain was susceptible to imipenem, meropenem and colistin (Table 1, Table 6). The strain harboured the *ant(2'')-Ia* gene which is responsible for resistance to gentamicin and tobramycin, as well as point mutations in the *gyrA* and *parC* genes, responsible for resistance to fluoroquinolones.

In total, 98.8% (856/866) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for the strain were in 'excellent' concordance with expected results (95.7%). MEs and VMEs were observed for 0.4% and 3.9% of the reported interpretations, respectively.

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

Characterisation of resistance to amikacin was challenging, as 30.0% (235 out of 783) of the submitted results were not in concordance with the expected result. Most of the deviations could be attributed to the inherent method variability and are within the acceptable variation range. These deviations corresponded to VMEs (R → S) and may indicate that, for this species, resistance to amikacin is under-reported in the EU/EEA.

The results indicate that laboratories should review their methods for the performance and interpretation of results of aminoglycoside susceptibility testing for *Acinetobacter* spp., as these can vary according to method and/or material used for testing [7-9].

Strain **'2023 EARS-Net 6' (*Enterococcus faecium*)** was resistant to amoxicillin, ampicillin and vancomycin, and susceptible to linezolid and teicoplanin. The strain was naturally resistant to gentamicin, but did not present high-level aminoglycoside resistance to this antimicrobial (Table 1, Table 7). The strain presented point mutations in the *pbp5* gene, which are responsible for resistance to the penicillins.

In total, 99.1% (859/867) of laboratories correctly identified the species of this test strain and, overall, the reported interpretations were in 'excellent' concordance with expected results (97.5%). MEs and VMEs were observed for 2.0% and 0.5% of the reported interpretations, respectively.

There was an 'excellent' level of concordance with the expected results (>95% of concordance) for every reported AST method.

As also observed for strain '2023 EARS-Net 3' (*E. faecalis*), the results observed for gentamicin were somewhat problematic, with unexpected reports of high-level aminoglycoside resistance (8.8% errors, 59 out of 672). These deviations are likely to be due to misinterpretation of the EQA protocol, and do not seem to indicate anomalous reporting of aminoglycoside resistance in *E. faecium* in the EU/EEA.

Overall, in the 2023 EARS-Net EQA exercise, the AST interpretations by the participating laboratories, located in all EU/EEA countries, were in 'very good' concordance with the expected results. There was no overall pattern of over- or under-reporting of AMR among the participating laboratories, but rather deviations limited to specific species-antimicrobial agent combinations included in the EQA exercise.

Some of the AST challenges identified in previous EQA exercises in 2019–2022 were still evident in the 2023 EARS-Net EQA exercise, such as testing for beta-lactam susceptibility in Enterobacterales isolates, and testing for aminoglycoside susceptibility in several species. The most problematic issue detected in the 2023 EARS-Net EQA exercise was amikacin susceptibility testing of *E. coli* isolates, which was assessed as being difficult for this particular strain, both during the current and previous EARS-Net EQA exercises.

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

1. Introduction

From 2000 to 2009, an annual external quality assessment (EQA) exercise for antimicrobial susceptibility testing (AST) was delivered to clinical laboratories participating in the European Antimicrobial Resistance Surveillance System (EARSS). In 2010, this activity was transferred to the European Centre for Disease Prevention and Control (ECDC) as the European Antimicrobial Resistance System Network (EARS-Net). This report describes and summarises the results of the EQA performance by laboratories participating in EARS-Net in 2023.

In 2023, the EARS-Net EQA exercise was carried out in collaboration with the Technical University of Denmark, National Food Institute (DTU Food). Since 2000, DTU Food has provided capacity-building for diagnostics and AST as well as EQA services globally in its capacity as a World Health Organization Collaborating Centre for antimicrobial resistance (AMR) and Genomics, European Union Reference Laboratory for AMR, and the Food and Agriculture Organization of the United Nations Reference Laboratory for AMR.

The 2023 EARS-Net EQA exercise aimed to 1) assess the quality of species identification by participating laboratories; 2) assess the accuracy of the qualitative AST results reported by participating laboratories; and 3) evaluate the overall comparability of routinely collected AST results between laboratories and European Union/European Economic Area (EU/EEA) countries.

2. Study design and methods

Antimicrobial susceptibility testing, and selected antimicrobial agents

The 2023 EARS-Net EQA protocol [5] specified that 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 antimicrobial agents selected for this EQA exercise correspond to the panel of species–antimicrobial agent combinations under surveillance by EARS-Net [1], with one exception. Testing for norfloxacin for *E. coli* and *K. pneumoniae* was not included as the breakpoint is only applicable to uncomplicated urinary tract infections.

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

Selection and characteristics of the EQA strains

In the 2023 EQA exercise, participating laboratories were asked to consider all six samples as if they had been obtained from patients with bloodstream infections.

The EUCAST Clinical Breakpoints Tables v13.0 [6] were used for the interpretation of AST results. This permitted categorisation of the expected AST results into three categories: susceptible, standard dosing regimen (S), susceptible, increased exposure (I), and resistant (R). The expected results were determined by examining the consensus AST results obtained by DTU Food through broth microdilution and/or disk diffusion, and results from confirmatory testing provided by three other reference laboratories. These were the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory, Växjö, Sweden; the Microbiological Diagnostic Unit Public Health Laboratory, The Doherty Institute, Australia; and the Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Japan. The consensus phenotypic AST profile was then compared with whole-genome sequencing (WGS) data on acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs), obtained at DTU Food using the bioinformatics tools ResFinder v4.1, AMRFinderPlus and CARD RGI (Tables 2–7). Finally, after the preparation of the agar swab cultures/charcoal swabs for shipment to participants, MIC determinations were performed at DTU Food to confirm that the vials contained the correct strains with the expected AST results.

Table 2. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2023 EARS-Net 1' (*E. coli*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result (mg/L)	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		Zone diameter (mm)					
	S <	R >	S >	R <				
Amikacin	8	8	18	18	Difficult	8	S	*aac(6')-Ib-cr*
Amoxicillin	8	8	Note***	Note	Easy	>64	R	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Amoxicillin-clavulanic acid****	8	8	19	19	Easy	>64/2	R	*bla*$_{OXA-1}$
Ampicillin	8	8	14	14	Easy	>32	R	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefepime	1	4	27	24	Difficult	1	S	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefotaxime	1	2	20	17	Easy	16	R	*bla*$_{CTX-M-15}$
Ceftazidime	1	4	22	19	Difficult	2	I	*bla*$_{CTX-M-15}$
Ceftriaxone	1	2	25	22	Easy	32	R	*bla*$_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	25	22	Easy	>4	R	*aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L*
Colistin	2	2	Note	Note	Easy	0,5	S	ND
Ertapenem	0.5	0.5	25	25	Easy	<=0.015	S	ND
Gentamicin	2	2	17	17	Easy	1	S	ND
Imipenem	2	4	22	19	Easy	<=0.12	S	ND
Levofloxacin	0.5	1	23	19	Easy	>8	R	*aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L*
Meropenem	2	8	22	16	Easy	<=0.03	S	ND
Moxifloxacin	0.25	0.25	22	22	Easy	>8	R	*aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L*
Ofloxacin	0.25	0.5	24	22	Easy	>2	R	*aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L*
Piperacillin-tazobactam****	8	8	20	20	Difficult	16/4	R	*bla*$_{OXA-1}$
Tigecycline	0.5	0.5	18	18	Easy	<=0.25	S	ND
Tobramycin	2	2	16	16	Easy	>16	R	*aac(6')-Ib-cr*

ND: Not detected.
** The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
*** Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI. Additional antimicrobial resistance genes or chromosomal point mutations: mph(A), catB3, aadA5, sul1, dfrA17. MALDI-TOF by DTU:* E. coli *(score 2,24), and MLST: ST-131 (scheme* E. coli *#1).*
**** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
***** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
ARG - antimicrobial resistance gene; PM - point mutation.

Table 3. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2023 EARS-Net 2' (*K. pneumoniae*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result (mg/L)	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		Zone diameter (mm)					
	S ≤	R >	S ≥	R <				
Amikacin	8	8	18	18	Easy	4	S	*aac(6')-Ia*
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	*bla*$_{SHV-11}$, *bla*$_{SHV-11}$
Cefepime	1	4	27	24	Difficult	4	I	*bla*$_{SHV-11}$, *bla*$_{SHV-11}$
Cefotaxime	1	2	20	17	Easy	8	R	*bla*$_{SHV-11}$, *bla*$_{SHV-11}$
Ceftazidime	1	4	22	19	Easy	>32	R	*bla*$_{SHV-11}$, *bla*$_{SHV-11}$
Ceftriaxone	1	2	25	22	Easy	16	R	*bla*$_{SHV-11}$
Ciprofloxacin	0.25	0.5	25	22	Easy	0.03	S	ND
Colistin	2	2	Note****	Note	Easy	0.5	S	ND
Ertapenem	0.5	0.5	25	25	Easy	2	R	ND
Gentamicin	2	2	17	17	Difficult	4	R	*ant(2'')-Ia*
Imipenem	2	4	22	19	Difficult	2	S	ND
Levofloxacin	0.5	1	23	19	Easy	0.06	S	ND
Meropenem	2	8	22	16	Easy	1	S	ND
Moxifloxacin	0.25	0.25	22	22	Easy	0.06	S	ND
Ofloxacin	0.25	0.5	24	22	Easy	0.125	S	ND
Piperacillin-tazobactam***	8	8	20	20	Easy	>128/4	R	*bla*$_{SHV-11}$, *bla*$_{SHV-11}$, *bla*$_{OXA-10}$
Tobramycin	2	2	16	16	Easy	8	R	*aac(6')-Ia, ant(2'')-Ia*

ND: Not detected.
** The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
*** Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI. bla$_{SHV-11}$ was an imperfect match (other identified variants: bla$_{SHV-40}$, bla$_{SHV-56}$, bla$_{SHV-79}$, bla$_{SHV-85}$, bla$_{SHV-89}$). Additional antimicrobial resistance genes or chromosomal point mutations: blaOXA-436, ARR-2, aadA1, cml, cmlA1, sul1, OqxA (intrinsic), OqxB (intrinsic), fosA (intrinsic), fosA7 (intrinsic), ompK36 N49S, ompK36 L59V, ompK36 G189T, ompK36 F198Y, ompK36 F207Y, ompK36 A217S, ompK36 T222L, ompK36 D223G, ompK36 Q227_None679del, ompK36 I228_None229insK, ompK36 E232R, ompK36 N304E, ompK37 I70M, ompK37 I128M, acrR P161R, acrR G164A, acrR F172S, acrR R173G, acrR L195V, acrR F197I, acrR K201M (ompK36 A217S, ompK37 I70M and ompK37 I128M potentially associated with carbapenem resistance). MALDI-TOF by DTU:* K. pneumoniae *(score 2,57), and MLST: ST-37.*
**** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
***** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
ARG - antimicrobial resistance gene; PM - point mutation.

Table 4. EUCAST clinical breakpoints for *Enterococcus faecalis* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2023 EARS-Net 3' (*E. faecalis*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result (mg/L)	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		Zone diameter (mm)					
	S ≤	R >	S ≥	R <				
Amoxicillin	4	8	Note***	Note	Easy	1	S	ND
Ampicillin	4	8	10	8	Easy	1	S	ND
Gentamicin (test for HLAR)	128	128	8	8	Easy	16	S	ND
Linezolid	4	4	20	20	Easy	>8	R	*optrA*
Teicoplanin	2	2	16	16	Easy	≤0.5	S	ND
Vancomycin	4	4	12	12	Easy	2	S	ND

HLAR: High-level aminoglycoside resistance
ND: Not detected.
** The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
*** Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI.*
Additional antimicrobial resistance genes or chromosomal point mutations: erm(B), tet(L), tet(M), fexA, str, Isa(A) (intrinsic).
MALDI-TOF by DTU: E. faecalis *(score 2,35), and MLST: ST-22.*
**** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
ARG - antimicrobial resistance gene; PM - point mutation.

Table 5. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretation for strain '2023 EARS-Net 4' (*K. pneumoniae*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result (mg/L)	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		MIC (mg/L)					
	S ≤	R >	S ≥	R <				
Amikacin	8	8	18	18	Easy	>32	R	*rmtB*
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	bla_{NDM-5}, bla_{OXA-1}, $bla_{OXA-181}$, bla_{SHV-1}
Cefepime	1	4	27	24	Easy	32	R	bla_{NDM-5}, bla_{OXA-1}, $bla_{OXA-181}$, bla_{SHV-1}, $bla_{CTX-M-15}$
Cefotaxime	1	2	20	17	Easy	>64	R	bla_{NDM-5}, bla_{SHV-1}, $bla_{CTX-M-15}$
Ceftazidime	1	4	22	19	Easy	>128	R	bla_{NDM-5}, bla_{SHV-1}, $bla_{CTX-M-15}$
Ceftriaxone	1	2	25	22	Easy	>64	R	bla_{SHV-1}, $bla_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	25	22	Easy	>4	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Colistin	2	2	Note****	Note	Easy	32	R	*mgrB* W20R
Ertapenem	0.5	0.5	25	25	Easy	>16	R	bla_{NDM-5}, $bla_{OXA-181}$
Gentamicin	2	2	17	17	Easy	>16	R	*rmtB*
Imipenem	2	4	22	19	Easy	16	R	bla_{NDM-5}, $bla_{OXA-181}$
Levofloxacin	0.5	1	23	19	Easy	>8	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Meropenem	2	8	22	16	Easy	>16	R	bla_{NDM-5}, $bla_{OXA-181}$
Moxifloxacin	0.25	0.25	22	22	Easy	>8	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Ofloxacin	0.25	0.5	24	22	Easy	>2	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Piperacillin-tazobactam***	8	8	20	20	Easy	>128/4	R	bla_{NDM-5}, bla_{OXA-1}, $bla_{OXA-181}$, bla_{SHV-1}, $bla_{CTX-M-15}$
Tobramycin	2	2	16	16	Easy	>16	R	*rmtB*

ND: Not detected.
** The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
*** Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI.*
bla_{SHV-1} was an imperfect match (other identified variants: bla_{SHV-26}, bla_{SHV-78}, bla_{SHV-98}, $bla_{SHV-145}$, $bla_{SHV-179}$, $bla_{SHV-194}$, $bla_{SHV-199}$). Additional antimicrobial resistance genes or chromosomal point mutations: bla_{TEM-1B}, mph(A), catB3, erm(B), tet(A), aph(3')-Ia, aadA2, sul1, dfrA12, fosA5 (intrinsic), OqxA (intrinsic), OqxB (intrinsic), ompK36 A217S, ompK36 N218H, ompK36 F207W, ompK36 L191S, ompK36 T254S, ompK36 Q227_None679del, ompK36 L228V, ompK36 n304_None305insE, ompK36 N49S, ompK36 E232R, ompK36 D224E, ompK36 L59V, ompK36 A190_None568del, ompK37 I70M, ompK37 I128M, acrR G164A, acrR F172S, acrR P161R, acrR R173G, acrR L195V, acrR K201M, acrR F197I (ompK36 A217S, ompK36 N218H, ompK37 I70M and ompK37 I128M potentially associated with carbapenem resistance). MALDI-TOF by DTU: K. pneumoniae *(score 2,48), and MLST: ST-16.*
**** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
***** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
ARG - antimicrobial resistance gene; PM - point mutation.

Table 6. EUCAST clinical breakpoints for *Acinetobacter baumannii* and the expected AST results, level of difficulty in interpretation and expected interpretation for strain '2023 EARS-Net 5' (*A. baumannii*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result (mg/L)	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		MIC (mg/L)					
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Difficult	16	R	ND
Ciprofloxacin	0.001	1	50	21	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin	2	2	Note***	Note	Easy	0,5	S	ND
Gentamicin	4	4	17	17	Easy	>16	R	*ant(2'')-Ia*
Imipenem	2	4	24	21	Easy	0.25	S	ND
Levofloxacin	0.5	1	23	20	Easy	8	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	1	S	ND
Tobramycin	4	4	17	17	Easy	>16	R	*ant(2'')-Ia*

ND: Not detected.
** The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
*** Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI.*
Additional antimicrobial resistance genes or chromosomal point mutations: bla_{CARB-2}, tet(39), tet(B), tet(G), aph(3')-Ib, aph(3'')-Ib, aph(6)-Id, aadA2, sul2, bla_{OXA-51} (intrinsic), bla_{ADC-25} (likely intrinsic). The strain appears to harbour multiple copies of genes associated with aminoglycoside resistance. Certain copies of those genes might in fact correspond to other variants able to confer amikacin resistance (e.g. other aph(3') variants). MALDI-TOF by DTU: A. baumannii *(score 2,42), and MLST: ST-1552 (scheme A. baumannii #1).*
**** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
ARG - antimicrobial resistance gene; PM - point mutation.

Table 7. EUCAST clinical breakpoints for *Enterococcus faecium* and the expected AST results, level of difficulty in interpretation and expected interpretation for strain '2023 EARS-Net 6' (*E. faecium*), by antimicrobial agent

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

HLAR: High-level aminoglycoside resistance.
ND: Not detected.
**The level of difficulty indicates the magnitude of risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or if the breakpoint was new or recently changed.*
***Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1, AMRFinderPlus or CARD RGI.*
PBP5-R: pbp5 M485A, pbp5 D204G, pbp5 S27G, pbp5 R34Q, pbp5 E525D, pbp5 N496K, pbp5 V24A, pbp5 T324A, pbp5 A499T, pbp5 E100Q, pbp5 L177I, pbp5 E629V, pbp5 A216S, pbp5 A68T, pbp5 P667S, pbp5 E85D, pbp5 G66E, pbp5 K144Q, pbp5 T172A, pbp5 V586L. Additional antimicrobial resistance genes or chromosomal point mutations: tet(M), msr(C), aac(6')-Ii (intrinsic), gyrA S83Y, parC S80I. MALDI-TOF by DTU: E. faecium *(score 2,47), and MLST: ST-17.*
**** Please refer to notes in the EUCAST clinical breakpoints tables v. 13.0.*
ARG - antimicrobial resistance gene; PM - point mutation.

Procedure for participating laboratories

The 2023 EARS-Net EQA protocol [5] specified that participating laboratories should identify the species of six bacterial strains, and then perform AST, following EUCAST recommendations [6] on species that are included in EARS-Net surveillance. If the species identification was incorrect, the reported AST results were not evaluated.

Identification of eligible laboratories

Each participating country designated a 'National EARS-Net EQA Coordinator' for the 2023 EARS-Net EQA exercise. The National EARS-Net EQA Coordinators were asked to provide a list of laboratories that were eligible to participate, and those laboratories received an information letter. Since 2019, only laboratories using EUCAST guidelines to perform AST can participate in the EARS-Net EQA exercise.

Distribution of EQA strains to laboratories

On 12 June 2023, a shipment was sent to each National EARS-Net EQA Coordinator according to International Air Transport Association regulations (UN3373, biological substances category B), containing individual packages for further national distribution. Each individual package (double pack containers (class UN 6.2)) was labelled with the address of a laboratory that had enrolled to participate. Every individual package contained six swabs (Copan Transystem™), each containing a pure culture of one of the six EQA strains. Each package also contained a cover letter with safety instructions, and information on how to process the swabs on arrival at a laboratory.

Reporting EQA results

The 2023 EARS-Net EQA protocol, test forms and a guide on how to access the password-protected webpage for submission of results were available on the EARS-Net EQA website (EARS-Net EQA (antimicrobialresistance.dk). The dedicated password-protected EARS-Net EQA webpage for participating laboratories to submit EQA results for evaluation, using a personal login and password, was developed and hosted by DTU Food.

The EQA protocol specified that participants should report AST results, specifically minimum inhibitory concentration (MIC) or zone diameter values, and their respective categorisation as S, I, or R, based on the most recent clinical breakpoints in EUCAST guidelines (v13.0). Participants were also asked to provide information on the standard guideline they used, the method for undertaking AST (agar dilution, automated system, broth microdilution, disk or tablet diffusion, gradient test, macro broth dilution, or other), and whether they would send the strain to a reference laboratory for further testing.

The deadline for submission of results was 4 August 2023; however the submission period was extended until 11 August 2023. After submission of results, an email was automatically forwarded to all contacts from the respective laboratory with a report containing their submitted results.

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

Evaluation of reported EQA results

Scoring concordance

Similar to previous EARS-Net EQA exercises, the concordance of submitted species identification and AST interpretations with the expected results was categorised as 'excellent' (≥95% of interpretations in concordance with expected results), 'very good' (>90% to <95%), 'good' (>85 to ≤90%) or 'satisfactory' (>80 to ≤85%) [2,3,4].

Scoring antimicrobial susceptibility results

If a laboratory reported the incorrect species for an EQA strain, the interpretations of AST results were not evaluated for that strain.

The 2023 EARS-Net EQA protocol specified the scoring system for the evaluation of submitted results (Table 8). It assigned scores for each species-antimicrobial agent combination based on the 'level of difficulty' and the 'severity of error' for the submitted AST interpretation.

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

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

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

For EARS-Net EQA exercises, the definition of an appropriate minimum set of species–antimicrobial agent combinations that is relevant for all (sub-)national settings in all 30 EU/EEA countries, has always been a methodological challenge. The EARS-Net EQA methodology is designed to provide information to support assessment of EARS-Net surveillance data quality. Therefore, every species-antimicrobial agent combination that can be reported to EARS-Net is included in the EQA exercise, but laboratories were not penalised for missing results in the 2023 EARS-Net EQA exercise.

Table 8. 2023 EARS-Net EQA exercise scoring system for reported AST results

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

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

Reporting EQA results

Only laboratories using EUCAST guidelines received a laboratory evaluation report and were included in the analysis for the national summary reports and this 2023 EARS-Net EQA Annual Report.

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

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

Laboratories acquired a 'certificate for participation' if they had reported interpretation of AST results for the six strains included in the 2023 EARS-Net EQA. Laboratories only had access to the certificate from their own laboratory, via the password protected webpage. National EARS-Net EQA Coordinators received copies of all issued certificates, for their country only.

Feedback survey of the National EARS-Net EQA Coordinators

In 2023, ECDC invited all National EARS-Net EQA Coordinators to participate in a feedback survey investigating the usefulness of the 2022 EARS-Net EQA. Every year the National EARS-Net EQA Coordinators receive the national summary report, the national laboratory evaluation reports, and the raw national data. The feedback survey included questions about the level of EQA activities (see below), distribution of the national summary report, usage and follow up on results.

1. In 2022, in your country, do you consider the EARS-Net EQA to be the main EQA activity for antimicrobial susceptibility testing (AST) for local clinical laboratories, or a supplementary EQA activity?
2. To date, which institutions/teams have received the national-level report for the 2022 EARS-Net EQA that was sent to the National EQA coordinator in December 2022?
3. To date, how have the results from the 2022 EARS-Net EQA been used in your country?
4. To date, was there follow-up with laboratories that had unexpected results during the 2022 EARS-Net EQA?

3. Results

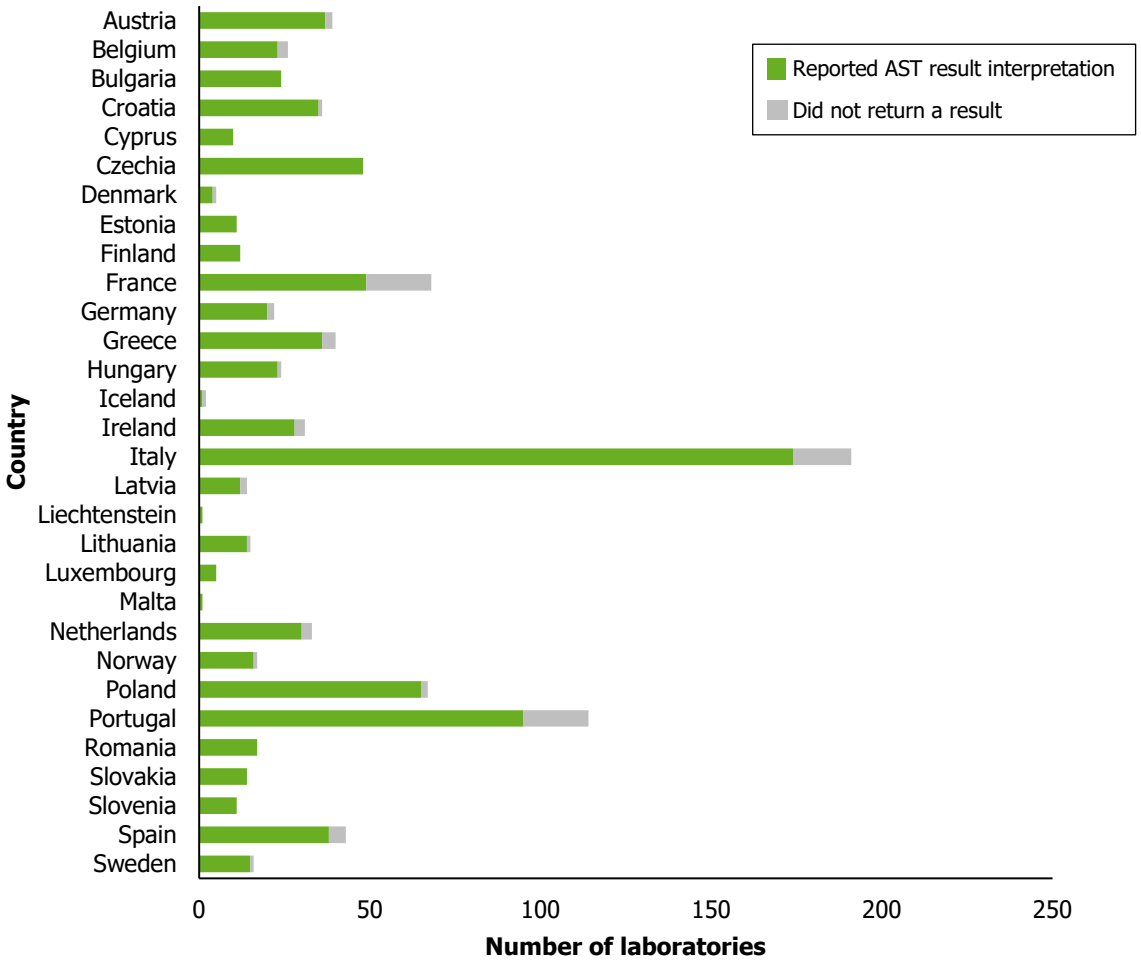
Participation

In 2023, all 30 EU/EEA countries participated in the EARS-Net EQA exercise. DTU Food sent information letters to the 957 laboratories identified by the National EARS-Net EQA Coordinators, and 951 (99.3%) laboratories enrolled. National EARS-Net EQA Coordinators then received packages from DTU Food for each of the 951 laboratories, containing the six EQA strains, for further national distribution.

One week before the submission deadline, a reminder email was sent to the laboratories that had enrolled but had not yet submitted results, with a one-week extension of the submission deadline. After the expiry of the extended deadline (11 August 2023), 871 (91.6%) laboratories from 30 countries had submitted results (Figure 1). Two laboratories were excluded from analysis because they had entered data without AST interpretations. One laboratory reported using the 'NordicAST guideline', which is based on EUCAST guidelines, and this laboratory was included in the analysis. Overall, results were evaluated for 869 laboratories, corresponding to 91.4% of all laboratories that received the EQA strains. The majority of the laboratories that received EQA materials submitted AST result interpretations for all six isolates (n=863; 99.1%), which was the minimum criterion for receiving a certificate of participation.

Twelve (1.3%) laboratories in nine countries (Austria, Belgium, Germany, Greece, Ireland, Italy (n=2), Latvia, Poland and Portugal (n=3)) entered results on the EQA webpage but did not finalise submission of the results, so their data could not be validated.

Figure 1. Number of participating laboratories returning interpretation of AST results, based on EUCAST guidelines, by country, 2023 EARS-Net EQA exercise



AST= antimicrobial susceptibility testing.

Species identification results

Species identification results were submitted for 5 197 strains by 869 laboratories and 99.1% (5 150 strains) were correct. Therefore, the overall concordance between the submitted and expected results was 'excellent'.

An overview of the species identification for the six strains and the number of laboratories reporting the correct identification is provided in Table 9. There was excellent concordance (≥95%) between the submitted species identification and the expected results for all six EQA strains. The lowest concordance was reported for strain '2023 EARS-Net 5' *Acinetobacter baumannii* (98.8%) and the highest concordance was reported for strain '2023 EARS-Net 1' *Escherichia coli* (99.3%).

Table 9. Number and percentage of laboratories reporting the correct species in the 2023 EARS-Net EQA exercise

Strain ID	Expected species	No. of reporting laboratories	No. of laboratories reporting correct species identification	Percentage of laboratories reporting correct species identification
2023 EARS-Net 1	*Escherichia coli*	867	861	99.3
2023 EARS-Net 2	*Klebsiella pneumoniae*	867	859	99.1
2023 EARS-Net 3	*Enterococcus faecalis*	864	857	99.2
2023 EARS-Net 4	*Klebsiella pneumoniae*	866	858	99.1
2023 EARS-Net 5	*Acinetobacter baumannii*	866	856	98.8
2023 EARS-Net 6	*Enterococcus faecium*	867	859	99.1

Antimicrobial susceptibility testing results

AST results were evaluated for strains with correct species identification.

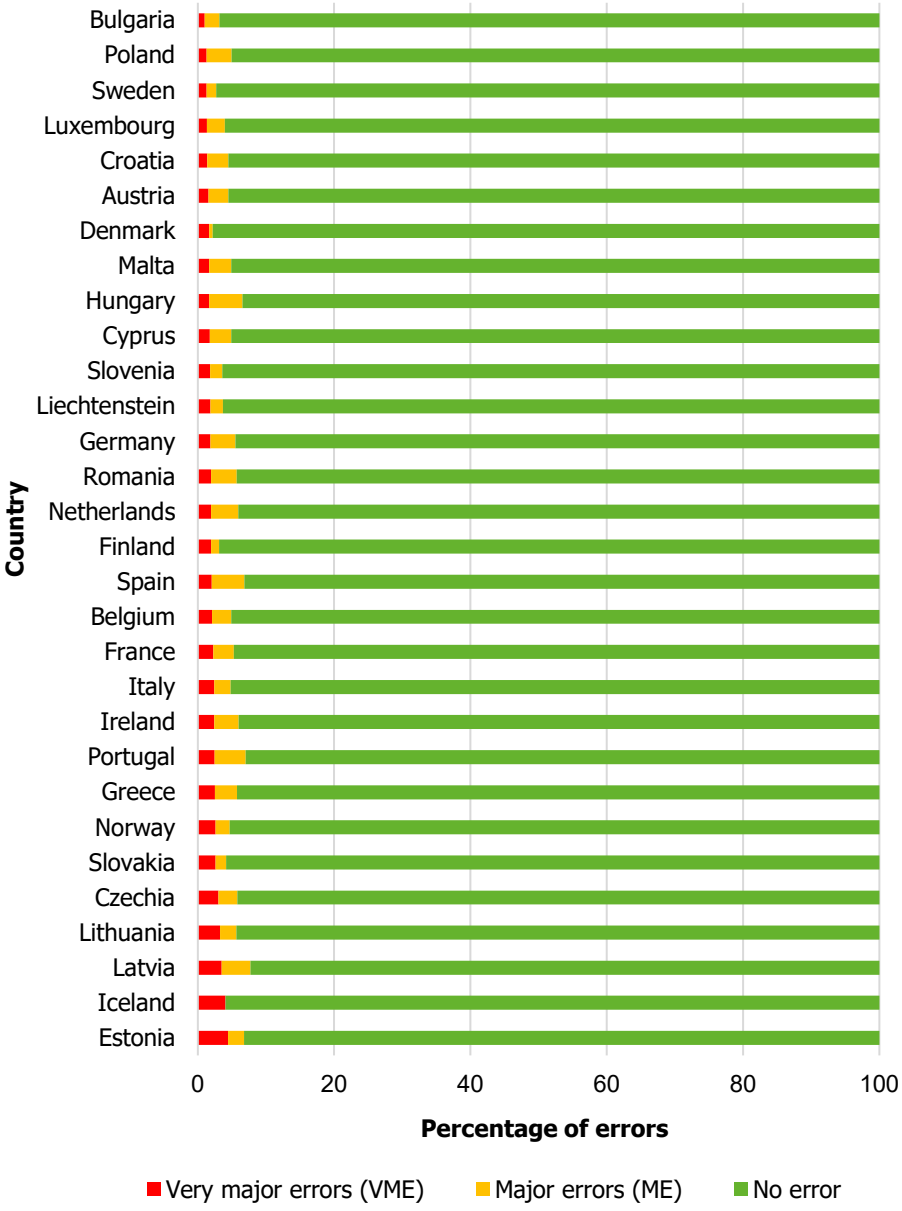
EQA results were submitted to the EARS-Net EQA webpage by 871 laboratories, and AST results were analysed for 865 of these laboratories. Data were not analysed for six of the 871 laboratories, as they had either not submitted any AST results (N=2 laboratories) or they had reported an incorrect species for every EQA strain (n=4 laboratories).

The participants were asked to report AST results (i.e. MIC or zone diameter values and their categorisation) as S, I or R. Only the interpretations of AST results were evaluated. The quantitative values were used as supplementary information.

In 2023, if every participating laboratory had reported data for every species-antimicrobial agent combination for the strains for which they submitted results, there would have been 64 084 results. The participating laboratories reported 53 272 AST result interpretations, which equates to 83.1% of the theoretical maximum.

Overall, the interpretations were in 'very good concordance' with 94.7% (n=50 441) of the 53 272 reported interpretations being correct (Figure 2). Concordance varied by country from 92.3% ('very good') to 97.8% ('excellent'). MEs were observed for 3.2% (n=1 683) of the reported interpretations (country range: 0.0% to 4.9%), and VMEs were observed for 2.2% (n=1 148; country range: 1.0% to 4.4%) for the 30 countries.

Figure 2. Reported interpretation of AST results, by country, 2023 EARS-Net EQA exercise, sorted by country according to the proportion of AST results representing very major errors

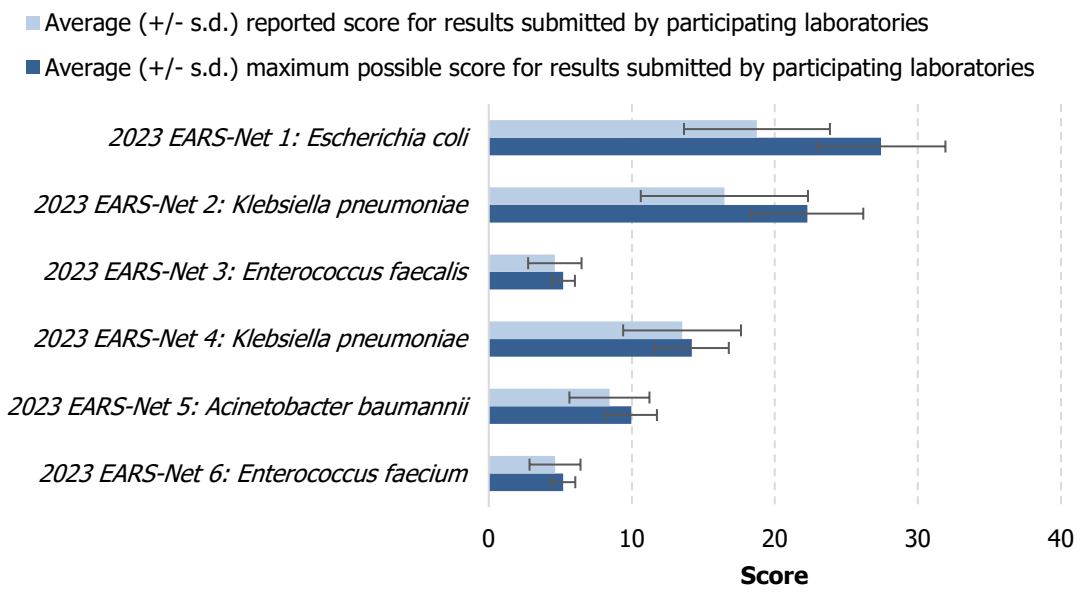


AST = antimicrobial susceptibility testing

The maximum possible score that participants could obtain for the six strains, if AST results for all species-antimicrobial agent combinations were submitted and correct, was 98. The average maximum possible score for the reported results was 83.5±14.4 (i.e. the average score that would be achieved by the laboratories if all submitted results had been correct). For the 865 laboratories with correct species identification and submitting AST result interpretations, the average score was 65.8±15.4.

Figure 3 presents the average maximum possible score and the average score of reported results for each strain.

Figure 3. Average maximum possible score, and average total scores, for the AST results reported by participating laboratories, by EQA strain, 2023 EARS-Net EQA exercise



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

For each strain, Tables 10 to 12 present the distribution of the methods used per strain and the percentage of correct AST interpretations for each method. The most commonly used method was an automated system (53.9%), followed by disk or tablet diffusion (27.3%), and MIC methods including broth microdilution and gradient test (18.0%) (Table 13). Excellent concordance was observed for agar dilution (99.0%), macro broth dilution (98.6%), broth microdilution (95.6%) and disk or tablet diffusion (95.2%), and very good concordance was observed for gradient tests (94.6%) and automated systems (94.2%) (Table 13).

Table 10. Overview of methods used for determination of AST results for strains '2023 EARS-Net 1' and '2023 EARS-Net 2'

Method	2023 EARS-Net 1 *Escherichia coli*			2023 EARS-Net 2 *Klebsiella pneumoniae*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	25	0.2	100.0	21	0.2	95.2
Automated system	7 492	53.8	88.5	6 426	53.2	92.9
Broth microdilution	1 440	10.3	92.4	1 352	11.2	95.6
Disk/tablet diffusion	3 963	28.4	91.5	3 374	27.9	92.7
Gradient test	920	6.6	94.2	847	7.0	88.9
Macro broth dilution (tubes)	25	0.2	96.0	17	0.1	100.0
Other	66	0.5	93.9	44	0.4	95.5
Total	**13 931**	**100.0**	**90.2**	**12 081**	**100.0**	**92.9**

Percentage may not total 100% due to rounding.

Table 11. Overview of methods used for determination of AST results for strains '2023 EARS-Net 3' and '2023 EARS-Net 4'

Method	2023 EARS-Net 3 *Enterococcus faecalis*			2023 EARS-Net 4 *Klebsiella pneumoniae*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	11	0.2	100.0	28	0.2	100.0
Automated system	2 470	55.5	97.2	6 588	54.1	99.5
Broth microdilution	311	7.0	97.4	1 401	11.5	97.9
Disk/tablet diffusion	1 062	23.9	97.3	3 413	28.0	99.4
Gradient test	564	12.7	98.8	673	5.5	96.0
Macro broth dilution (tubes)	1	0.0	100.0	18	0.1	100.0
Other	31	0.7	96.8	50	0.4	90.0
Total	**4 450**	**100.0**	**97.4**	**12 171**	**100.0**	**99.0**

Percentage may not total 100% due to rounding.

Table 12. Overview of methods used for determination of AST results for strains '2023 EARS-Net 5' and '2023 EARS-Net 6'

Method	2023 EARS-Net 5 *Acinetobacter baumannii*			2023 EARS-Net 6 *Enterococcus faecium*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	8	0.1	100.0	11	0.2	100.0
Automated system	3 306	53.5	95.0	2 434	54.6	97.3
Broth microdilution	903	14.6	95.7	279	6.3	97.1
Disk/tablet diffusion	1 631	26.4	97.7	1 105	24.8	97.4
Gradient test	306	4.9	93.1	602	13.5	98.7
Macro broth dilution (tubes)	7	0.1	100.0	3	0.1	100.0
Other	24	0.4	91.7	20	0.4	100.0
Total	**6 185**	**100.0**	**95.7**	**4 454**	**100.0**	**97.5**

Percentages might not total 100% due to rounding.

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

Method	Total		
	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	104	0.2	99.0
Automated system	28 716	53.9	94.2
Broth microdilution	5 686	10.7	95.6
Disk/tablet diffusion	14 548	27.3	95.2
Gradient test	3 912	7.3	94.6
Macro broth dilution (tubes)	71	0.1	98.6
Other	235	0.4	94.0
Total	**53 272**	**100.0**	**94.7**

Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 1' (*Escherichia coli*)

The *E. coli* EQA strain ('2023 EARS-Net 1') was described as being obtained from a patient with bloodstream infection. This strain was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin and tobramycin (Table 2). The strain was susceptible to cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, tigecycline and colistin, and the expected MIC value for ceftazidime was in the I range (Table 2). The level of difficulty was considered 'difficult' for piperacillin-tazobactam, cefepime, ceftazidime and amikacin since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'. The strain harboured two beta-lactamase genes that contributed to the complex beta-lactam resistance profile, as defined in the expected results, specifically *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{CTX-M-15}}$.

In the 2022 EARS-Net EQA exercise, strain '2022 EARS-Net 2' (*E. coli*) was the most challenging for participating laboratories [2]. It was therefore decided to include the exact same *E. coli* strain as strain '2023 EARS-Net 1' in the panel for the 2023 EARS-Net EQA exercise. To ensure harmonisation between expected results included in the 2023 EARS-Net EQA exercise, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA exercise. The expected results were essentially in agreement with the results obtained and described in the 2022 EARS-Net EQA exercise. However, the consensus obtained for piperacillin-tazobactam for the 2023 EARS-Net EQA exercise was MIC=16/4 mg/L, and this was therefore interpreted as 'Resistant', whereas for the 2022 EARS-Net EQA exercise, the expected result was MIC=8/4 mg/L with an interpretation of 'Susceptible, standard dosing regimen'. Furthermore, the consensus obtained for amikacin for the 2023 EARS-Net EQA exercise was MIC=8 mg/L, and this was therefore interpreted as 'Susceptible, standard dosing regimen', whereas for the 2022 EARS-Net EQA exercise, the expected result was MIC>8 mg/L with an interpretation of 'Resistant'. The variation observed between the 2022 and 2023 expected results is within the acceptable method variation (+/- 1 dilution) and is likely to be due to the complex genetic resistance mechanisms harboured by the strain, as well as cumulative small variations in the material used for testing.

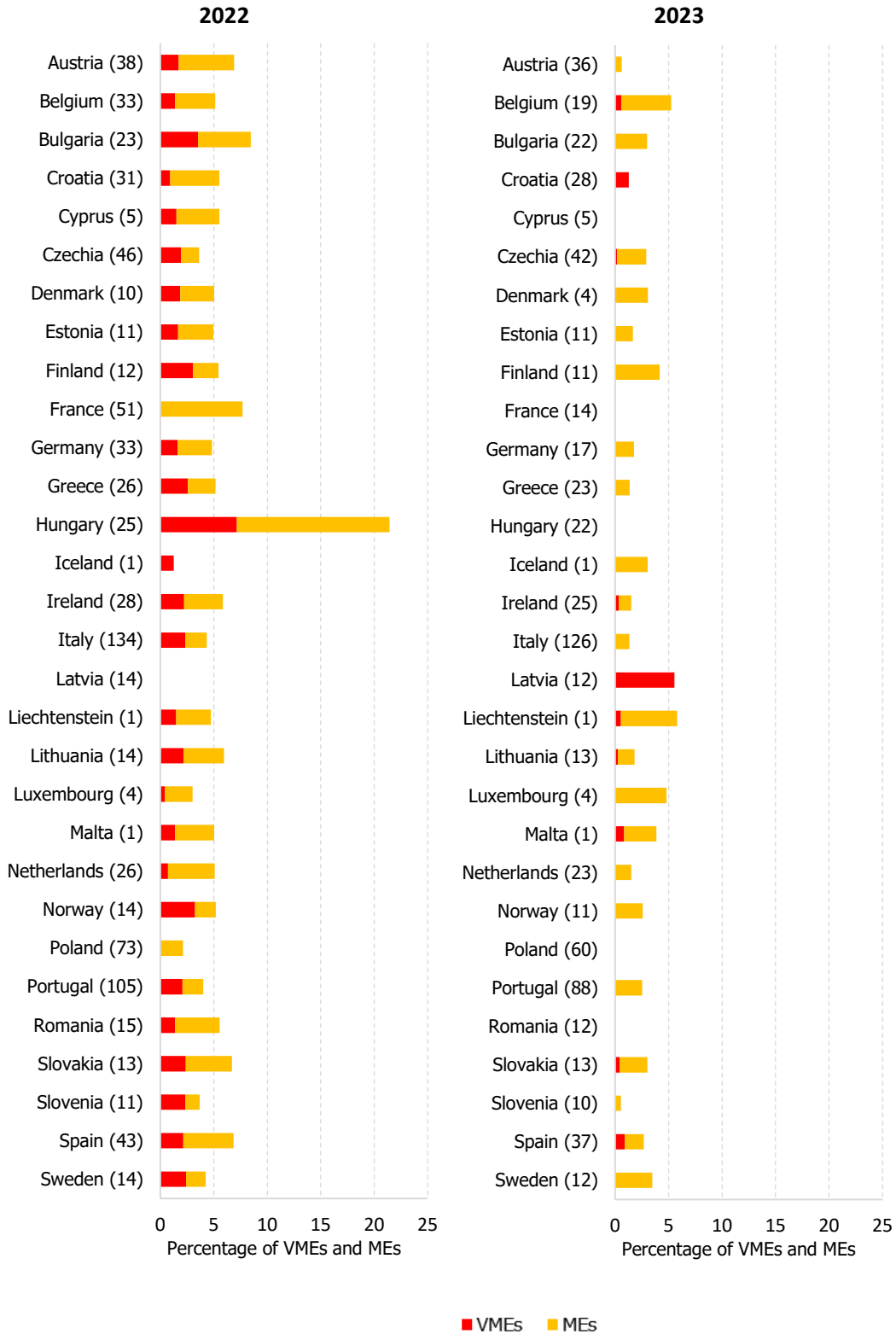
When all the results submitted by all participating laboratories were taken into account, for all antimicrobial agents excluding amikacin and piperacillin-tazobactam, the highest variation was the decrease in MEs for cefepime, from 20.4% in 2022 to 16.6% in 2023.

In 2022, 60.5% of participating laboratories reported a correct AST interpretation for piperacillin-tazobactam (expected interpretation as S). In 2023, with a new expected interpretation of R, this proportion decreased to 40.6%.

In 2022, 64.0% of participating laboratories reported a correct AST interpretation for amikacin (expected interpretation as R). In 2023, with a new expected interpretation as S, this proportion decreased to 29.2%.

At the EU/EEA level, 703 laboratories submitted interpretation of AST results for both years, and when comparing results between the 2022 and 2023 EARS-Net EQA exercises, there was little variability of results for this strain (excluding the results obtained for amikacin and piperacillin-tazobactam) (Figure 4).

Figure 4. Reported errors (%) of interpretation for AST results (not including piperacillin-tazobactam and amikacin) for the same strain (i.e. '2022 EARS-Net 2' and '2023 EARS-Net 1') for those laboratories providing results in both 2022 and 2023 (n=703), by country



The number in brackets is the number of laboratories providing AST results for both 2022 and 2023.
VME - very major error; ME - major error.

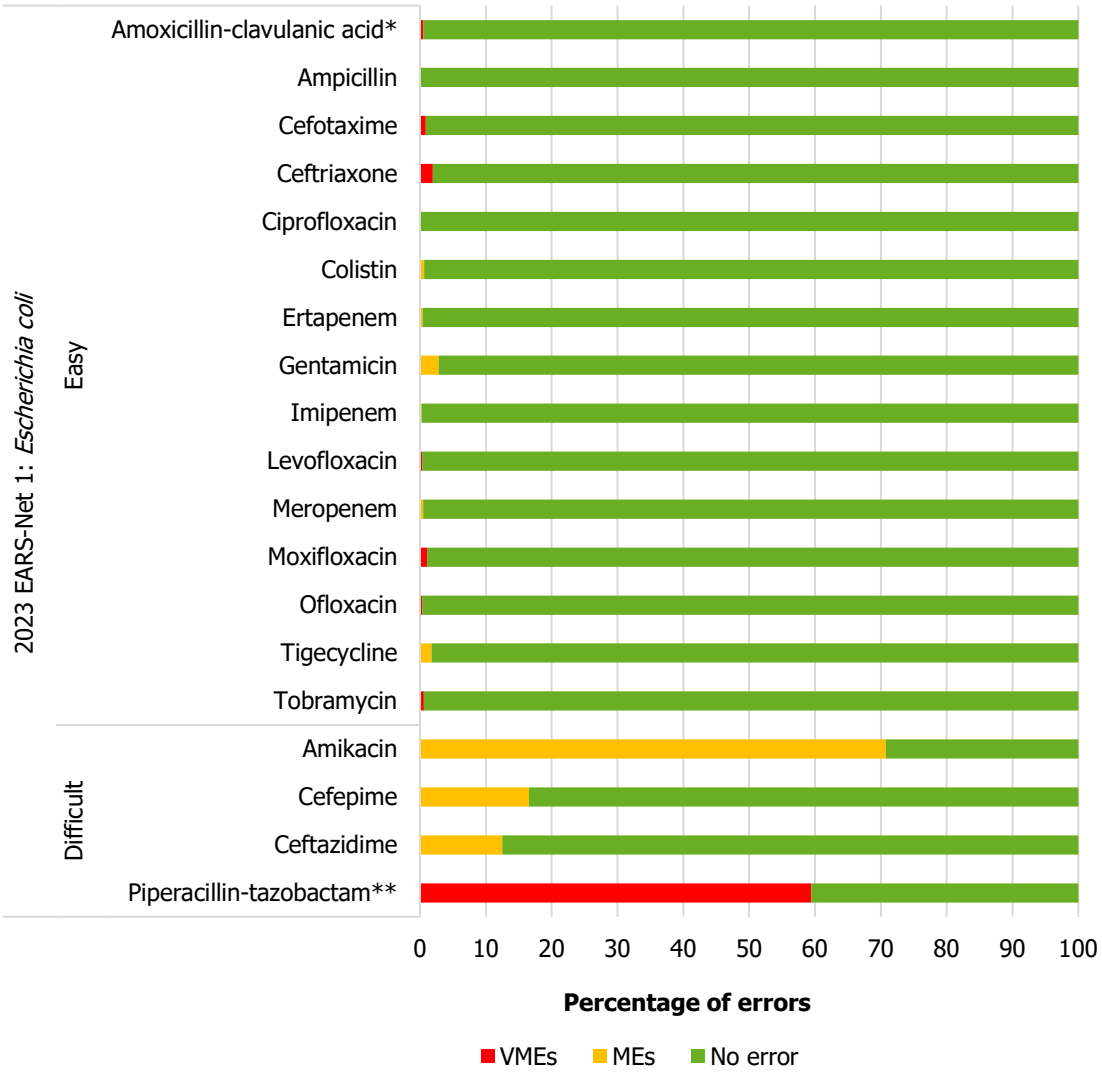
Interpretation of AST results for the *E. coli* strain were analysed for the 861 laboratories with correct species identification (Table 9). In total, 18.5% of the laboratories (n=159) would have sent the strain to a reference or other laboratory for further testing. In total, 13 931 tests were performed, and 12 566 reported interpretations were correct. Thus, the reported interpretations were in 'very good' concordance with expected results (90.2%) (Table 14). MEs were observed for 6.0% (n=832) and VMEs for 3.8% (n=533) of the reported interpretations. In 2022, for the same strain, the reported interpretations were in very good concordance with expected results (92.7%).

The following methods were applied: automated systems (53.8%), disk or tablet diffusion (28.4%), broth microdilution (10.3%), gradient test (6.6%), agar dilution (0.2%) and 'other methods' (0.5%) (Table 10). Overall, most methods achieved, as a minimum, a 'very good' level of concordance with the expected results (>90% of concordance). The exception was automated systems, which achieved a 'good' concordance (88.5%) (Table 10).

VMEs were observed for amoxicillin-clavulanic acid, ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin (Figure 5). VMEs for piperacillin-tazobactam corresponded to 59.4% of all submitted interpretations for that antimicrobial agent and were reported for almost all methods, except agar dilution (Table 14). For the other antimicrobials, VMEs represented <2% of all submitted interpretations for those antimicrobial agents (Table 14).

A high proportion of MEs was observed for amikacin (70.8% of submitted results) and these were reported for all methods except agar dilution and macro broth dilution (Figure 5). Lower proportions of MEs were observed for cefepime (16.6%), ceftazidime (12.5%), and gentamicin (2,5%). For the remaining antimicrobial agents, there were no, or very low proportions of MEs (Figure 5, Table 14).

Figure 5. Reported interpretation of AST results for strain '2023 EARS-Net 1' (*Escherichia coli*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data)
** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
*** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 14. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2023 EARS-Net 1' (*Escherichia coli*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	1	100.0	452	23.0*	84	57.1*	208	29.3*	33	33.3*	1	100.0	4	75.0*	**783**	**29.2***
Amoxicillin	1	100.0	148	100.0	20	100.0	66	100.0	188	100.0	-	-	15	100.0	**438**	**100.0**
Amoxicillin-clavulanic acid**	1	100.0	520	99.6	40	100.0	251	99.6	25	96.0	-	-	2	100.0	**839**	**99.5**
Ampicillin	2	100.0	449	100.0	45	100.0	247	100.0	29	96.6	1	100.0	2	100.0	**775**	**99.9**
Cefepime	2	100.0	446	89.0	63	95.2	195	65.1*	39	89.7	1	100.0	3	100.0	**749**	**83.4**
Cefotaxime	1	100.0	483	99.4	58	98.3	216	99.1	26	96.2	1	100.0	3	100.0	**788**	**99.1**
Ceftazidime	1	100.0	500	90.2	71	93.0	223	79.8*	48	87.5	1	100.0	3	66.7*	**847**	**87.5**
Ceftriaxone	2	100.0	201	98.0	24	100.0	212	98.1	124	97.6	-	-	3	100.0	**566**	**98.1**
Ciprofloxacin	1	100.0	533	99.8	70	100.0	238	100.0	10	100.0	1	100.0	3	100.0	**856**	**99.9**
Colistin	1	100.0	214	98.6	402	99.8	4	100.0	6	100.0	9	100.0	2	100.0	**638**	**99.4**
Ertapenem	3	100.0	442	99.5	61	100.0	220	99.5	28	100.0	1	100.0	1	100.0	**756**	**99.6**
Gentamicin	1	100.0	526	97.9	65	95.4	228	96.1	20	95.0	1	100.0	3	100.0	**844**	**97.2**
Imipenem	1	100.0	439	99.8	53	100.0	217	99.5	32	100.0	1	100.0	3	100.0	**746**	**99.7**
Levofloxacin	2	100.0	279	99.3	45	100.0	228	100.0	68	100.0	1	100.0	2	100.0	**625**	**99.7**
Meropenem	1	100.0	501	99.6	70	100.0	240	99.2	25	100.0	1	100.0	3	100.0	**841**	**99.5**
Moxifloxacin	1	100.0	64	96.9	16	100.0	214	99.5	71	98.6	-	-	3	100.0	**369**	**98.9**
Ofloxacin	2	100.0	80	100.0	10	100.0	170	99.4	26	100.0	-	-	4	100.0	**292**	**99.7**
Piperacillin-tazobactam***	1	100.0	494	24.1*	83	31.3*	219	76.3*	38	71.1*	1	0.0*	2	0.0*	**838**	**40.6***
Tigecycline	-	-	324	98.1	99	97.0	140	100.0	66	97.0	3	100.0	2	100.0	**634**	**98.3**
Tobramycin	-	-	397	99.2	61	100.0	227	99.6	18	100.0	1	100.0	3	100.0	**707**	**99.4**
Total	**25**	**100.0**	**7 492**	**88.5**	**1 440**	**92.4**	**3 963**	**91.5**	**920**	**94.2**	**25**	**96.0**	**66**	**93.9**	**13 931**	**90.2**

n: number of reporting laboratories; '-': no data; shaded cells indicate that n<5 laboratories reported concordant results.
** Below the threshold of satisfactory concordance (80%).*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
**** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 2' (*Klebsiella pneumoniae*)

The *K. pneumoniae* EQA strain ('2023 EARS-Net 2') was described as being obtained from a patient with bloodstream infection. This strain was resistant to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin and tobramycin (Table 3). The strain was susceptible to imipenem, meropenem, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, amikacin and colistin, and the expected MIC value for cefepime was in the I range (Table 3). The level of difficulty was considered to be 'difficult' for cefepime, imipenem and gentamicin since the expected MIC values were less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered to be 'easy'. The strain harboured three beta-lactamase genes that contributed to the complex beta-lactam resistance profile, as defined in the expected results, specifically *bla*$_{VEB-1}$, *bla*$_{SHV-11}$ (or a similar *bla*$_{SHV}$ variant) and *bla*$_{OXA-10}$.

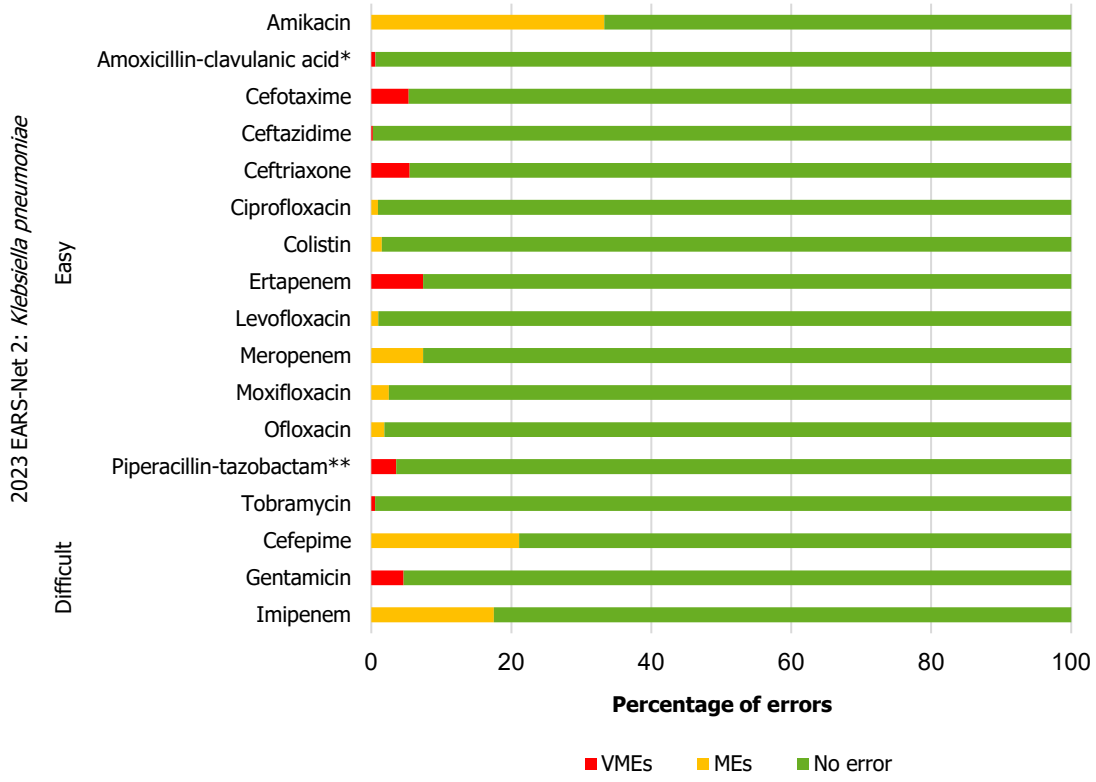
Interpretation of AST results for the *K. pneumoniae* strain were analysed for the 859 laboratories with correct species identification (Table 9). In total, 51.3% of the laboratories (n=441) would have sent the strain to a reference or other laboratory for further testing. In total, 12 081 tests were performed, and 11 220 reported interpretations were correct. Thus, the reported interpretations were in 'very good' concordance with expected results (92.9%) (Table 15). MEs were observed for 5.4% (n=652) and VMEs for 1.7% (n=209) of the reported interpretations.

The following methods were applied: automated systems (53.2%), disk or tablet diffusion (27.9%), broth microdilution (11.2%), gradient test (7.0%), agar dilution (0.2%), macro broth dilution (0.1%), and 'other methods' (0.4%) (Table 10). Overall, all methods achieved, as a minimum, a 'very good' level of concordance with the expected results (>90% of concordance). The exception was gradient test, which achieved a 'good' concordance (88.9%) (Table 10).

VMEs were observed for amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, ertapenem, gentamicin, piperacillin-tazobactam and tobramycin (Figure 6). VMEs in ertapenem (7.4% of all submitted interpretations for that antimicrobial), ceftriaxone (5.5%), cefotaxime (5.4%), gentamicin (4.6%) and piperacillin-tazobactam (3.5%) were mainly reported when using automated systems, broth microdilution, disk or tablet diffusion, and gradient test (Table 15). For the other antimicrobials, VMEs represented <1% of all submitted interpretations (Table 15).

A high proportion of MEs was observed for amikacin (33.3% of submitted results), cefepime (21.1%) and imipenem (17.5%) and they were reported for all methods except agar dilution and macro broth dilution (Figure 6, Table 15). Lower proportions of MEs were observed for meropenem (7.4%) and moxifloxacin (2.5%). For the remaining antimicrobial agents, there were no, or very low proportions of MEs (Figure 6, Table 15).

Figure 6. Reported interpretation of AST results for strain '2023 EARS-Net 2' (*Klebsiella pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).
** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
*** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 15. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2023 EARS-Net 2' (*Klebsiella pneumoniae*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	1	100.0	446	61.0*	87	90.8	210	69.0*	36	61.1*	1	100.0	3	100.0	**784**	**66.7***
Amoxicillin-clavulanic acid**	1	100.0	522	99.0	44	100.0	252	100.0	20	100.0	-	-	2	100.0	**841**	**99.4**
Cefepime	2	100.0	449	82.9	70	92.9	196	61.2*	37	94.6	1	100.0	3	100.0	**758**	**78.9***
Cefotaxime	1	100.0	475	97.9	61	85.2	211	91.5	31	83.9	1	100.0	3	100.0	**783**	**94.6**
Ceftazidime	1	100.0	523	99.8	72	100.0	238	99.6	13	100.0	1	100.0	3	100.0	**851**	**99.8**
Ceftriaxone	2	100.0	207	98.1	24	100.0	205	92.2	129	91.5	-	-	1	100.0	**568**	**94.5**
Ciprofloxacin	1	100.0	530	99.4	67	97.0	238	98.7	14	100.0	1	100.0	3	100.0	**854**	**99.1**
Colistin	1	100.0	215	98.1	425	98.6	3	100.0	8	100.0	6	100.0	2	100.0	**660**	**98.5**
Ertapenem	3	100.0	413	94.2	65	95.4	200	94.5	71	74.6*	1	100.0	2	100.0	**755**	**92.6**
Gentamicin	1	0.0*	524	99.0	74	74.3*	211	93.8	28	100.0	1	100.0	3	66.7*	**842**	**95.4**
Imipenem	1	100.0	389	75.8*	66	95.5	166	90.4	121	85.1	1	100.0	4	100.0	**748**	**82.5**
Levofloxacin	2	100.0	265	98.9	40	97.5	230	99.6	74	100.0	1	100.0	2	50.0*	**614**	**99.0**
Meropenem	1	100.0	426	93.7	93	97.8	176	92.6	135	85.2	1	100.0	4	100.0	**836**	**92.6**
Moxifloxacin	1	100.0	60	100.0	13	92.3	209	98.6	75	93.3	-	-	1	100.0	**359**	**97.5**
Ofloxacin	1	100.0	63	95.2	8	100.0	169	98.8	25	100.0	-	-	3	100.0	**269**	**98.1**
Piperacillin-tazobactam***	1	100.0	520	95.6	74	100.0	241	97.5	10	90.0	-	-	2	100.0	**848**	**96.5**
Tobramycin	-	-	399	99.5	69	100.0	219	99.1	20	100.0	1	100.0	3	100.0	**711**	**99.4**
Total	**21**	**95.2**	**6 426**	**92.9**	**1 352**	**95.6**	**3 374**	**92.7**	**847**	**88.9**	**17**	**100.0**	**44**	**95.5**	**12 081**	**92.9**

n: number of reporting laboratories; '-': no data; shaded cells indicate that n<5 laboratories reported concordant results.
** Below the threshold of satisfactory concordance (80%).*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
**** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 3' (*Enterococcus faecalis*)

The *E. faecalis* EQA strain ('2023 EARS-Net 3') was described as being obtained from a patient with bloodstream infection. This strain was resistant to linezolid and susceptible to amoxicillin, ampicillin, teicoplanin and vancomycin. The strain did not present high-level aminoglycoside resistance to gentamicin (Table 3). For all antimicrobial agents, the level of difficulty was considered to be 'easy'. The strain harboured the *optrA* gene which confers resistance to linezolid, as defined in the expected results. The *optrA* gene is also responsible for decreased susceptibility to antimicrobial agents not included in this EQA exercise, specifically other oxazolidinones such as tedizolid, and phenicols, such as chloramphenicol and florfenicol.

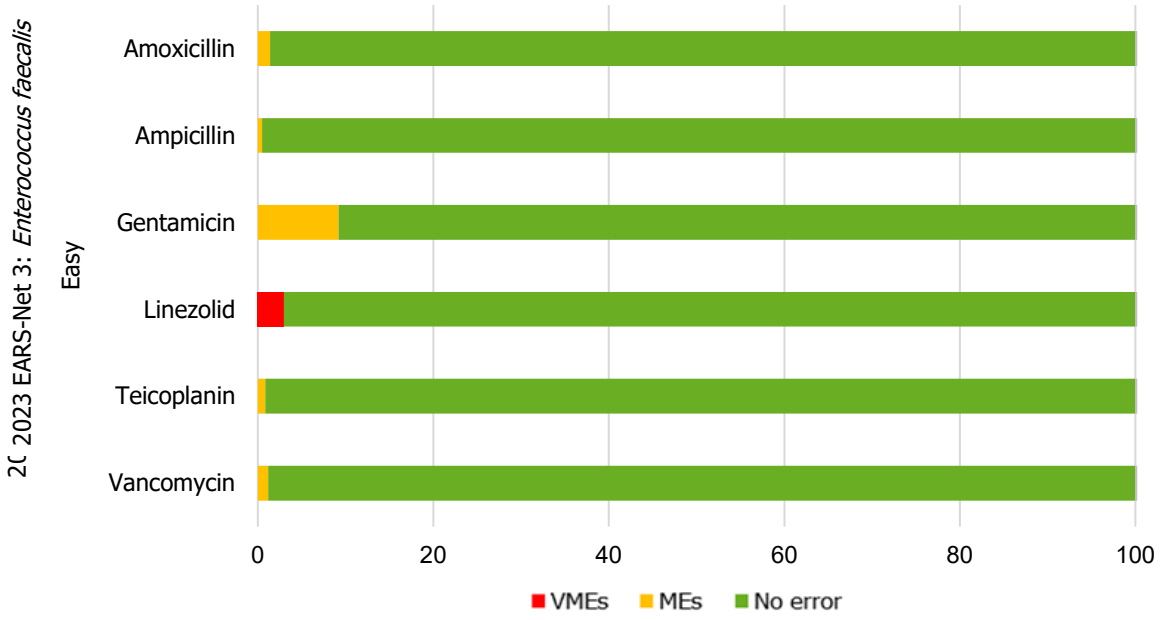
Interpretation of AST results for the *E. faecalis* strain were analysed for the 857 laboratories with correct species identification (Table 9). In total, 39.5% of the laboratories (n=338) would have sent the strain to a reference or other laboratory for further testing. In total, 4 450 tests were performed, and 4 336 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (97.4%) (Table 16). MEs were observed for 2.0% (n=89) and VMEs for 0.6% (n=25) of the reported interpretations.

The following methods were applied: automated systems (54.1%), disk or tablet diffusion (23.9%), broth microdilution (7.0%), gradient test (12.7%), agar dilution (0.2%), macro broth dilution (0.02%), and 'other methods' (0.7%) (Table 10). All methods achieved an 'excellent' level of concordance with the expected results (>95% of concordance) (Table 11).

VMEs were observed for linezolid and were mainly reported when using broth microdilution, disk or tablet diffusion, and gradient test (Figure 7, Table 15).

A high proportion of MEs was observed for gentamicin (9.2% of submitted results) and were reported for all methods except agar dilution (Figure 6, Table 15). For the remaining antimicrobial agents, there were no, or very low proportions of MEs (Figure 6, Table 15).

Figure 7. Reported interpretation of AST results for strain '2023 EARS-Net 3' (*Enterococcus faecalis*) by antimicrobial agent and anticipated difficulty of identification



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

Table 16. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2023 EARS-Net 3' (*Enterococcus faecalis*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amoxicillin	-	-	134	98.5	15	93.3	57	96.5	214	99.5	-	-	12	100.0	**432**	**98.6**
Ampicillin	1	100.0	526	99.4	48	100.0	243	99.6	21	100.0	-	-	4	100.0	**843**	**99.5**
Gentamicin	4	100.0	314	85.0	39	92.3	254	96.1	59	96.6	-	-	3	100.0	**673**	**90.8**
Linezolid	1	100.0	455	98.0	60	93.3	197	95.9	126	96.8	-	-	3	100.0	**842**	**97.0**
Teicoplanin	2	100.0	524	99.4	73	100.0	136	97.1	67	100.0	-	-	3	100.0	**805**	**99.1**
Vancomycin	3	100.0	517	99.0	76	100.0	175	97.7	77	100.0	1	100.0	6	83.3	**855**	**98.8**
Total	**11**	**100.0**	**2 470**	**97.2**	**311**	**97.4**	**1 062**	**97.3**	**564**	**98.8**	**1**	**100.0**	**31**	**96.8**	**4 450**	**97.4**

n: number of reporting laboratories; '-': no data.
Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 4' (*Klebsiella pneumoniae*)

The *K. pneumoniae* EQA strain ('2023 EARS-Net 4') was described as being obtained from a patient with bloodstream infection. This strain was resistant to all antimicrobials included in the EQA exercise (Table 4). For all antimicrobial agents the level of difficulty was considered to be 'easy'. The strain harboured five beta-lactamase genes that contributed to the extensive beta-lactam resistance profile, as defined in the expected results, specifically bla_{NDM-5}, bla_{SHV-1}, $bla_{CTX-M-15}$, bla_{OXA-1} and $bla_{OXA-181}$. Furthermore, the strain harboured the 16S rRNA methylase gene *rmtB*, responsible for resistance to aminoglycosides, and it presented a point mutation in the *mgrB* gene which is part of the intrinsic genetic mechanisms regulating lipopolysaccharide modifications and therefore responsible for colistin resistance. Finally, the strain had various mutations and genes contributing to fluoroquinolone resistance, including point mutations in the *gyrA* and *parC* genes and the acquired AMR gene *qnrS1*.

The strain '2023 EARS-Net 4' had previously been included in the 2021 EARS-Net EQA exercise ('EARS-Net KPN 21.1') [3]. In 2021, it was the *K. pneumoniae* strain with the highest concordance of AST results. Therefore, it was included in the 2023 EQA exercise to facilitate comparison of the performance of AST methods with the challenging '2023 EARS-Net 2' strain. To ensure harmonisation between expected results included in the 2023 EQA exercise, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA exercise. The results obtained were in full agreement with the results obtained and described in the 2021 EARS-Net EQA exercise.

When comparing results between the 2021 and 2023 EARS-Net EQA exercises, there was little variability for this strain. When the results submitted by all participating laboratories were taken into account, there was less than 1.5% of variation in the percentage of VMEs observed for each antimicrobial agent.

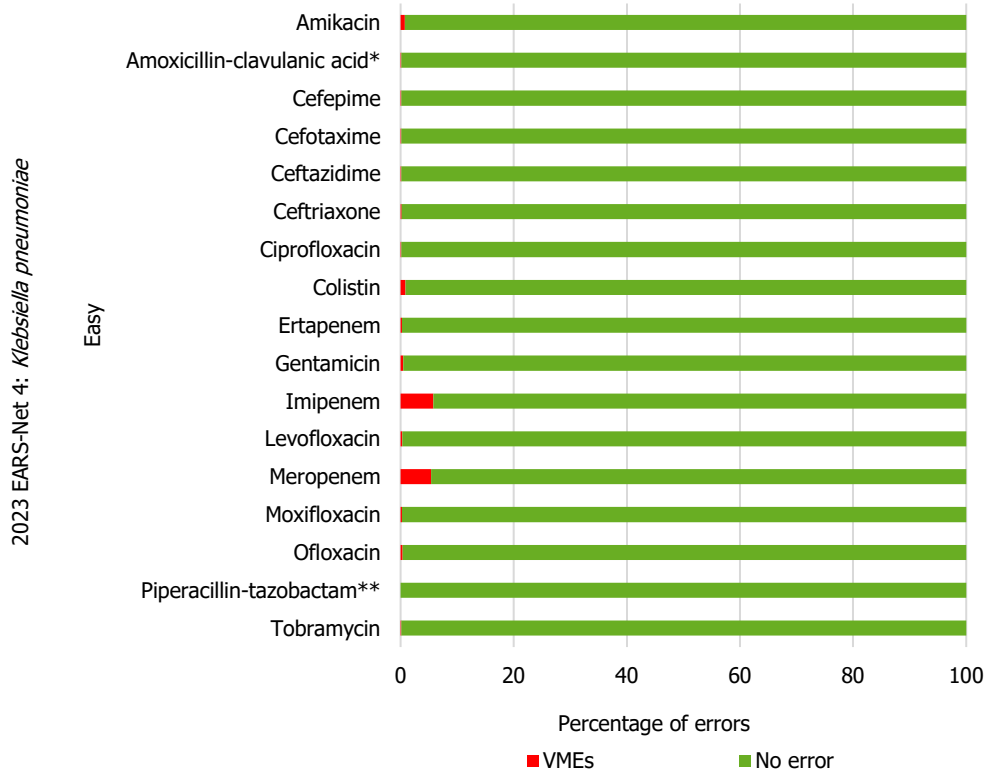
Interpretation of AST results for the *K. pneumoniae* strain were analysed for the 858 laboratories with correct species identification (Table 9). In total, 62.3% of the laboratories (n=534) would have sent the strain to a reference or other laboratory for further testing. In total, 12 171 tests were performed, and 12 053 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (99.0%) (Table 17). MEs were not observed (n=0) and VMEs were observed for 1.0% of the reported interpretations (n=118). In 2021, for the same strain, the reported interpretations were also in 'excellent' concordance with expected results (98.8%).

The following methods were applied: automated systems (54.1%), disk or tablet diffusion (28.0%), gradient test (5.5%), broth microdilution (11.5%), agar dilution (0.2%), macro broth dilution (0.1%), and 'other methods' (0.4%) (Table 11). Overall, most methods achieved an 'excellent' level of concordance with the expected results (>95% of concordance). The exception was 'other methods' (90.0%), which achieved a 'good' concordance (Table 11).

VMEs were observed for all antimicrobial agents except piperacillin-tazobactam (Figure 8). VMEs for imipenem (5.8% of all submitted interpretations for that antimicrobial agent) and meropenem (5.4%) were reported for most methods, except agar dilution and macro broth dilution (Table 17). For the other antimicrobial agents, VMEs represented <1% of all submitted interpretations (Table 17).

As the strain was resistant to all the antimicrobials included, there could not be MEs.

Figure 8. Reported interpretation of AST results for strain '2023 EARS-Net 4' (*Klebsiella pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



AST: antimicrobial susceptibility testing; VME: very major error; ME: major error; NA: not appliable (e.g. no data).
** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
*** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 17. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2023 EARS-Net 4' (*Klebsiella pneumoniae*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	2	100.0	457	99.3	88	97.7	219	99.5	18	100.0	1	100.0	3	100.0	**788**	**99.2**
Amoxicillin-clavulanic acid**	2	100.0	520	99.8	46	100.0	248	100.0	19	100.0	-	-	3	100.0	**838**	**99.9**
Cefepime	1	100.0	468	100.0	71	100.0	207	99.5	15	100.0	1	100.0	2	100.0	**765**	**99.9**
Cefotaxime	2	100.0	476	100.0	64	100.0	216	99.5	23	100.0	1	100.0	3	100.0	**785**	**99.9**
Ceftazidime	1	100.0	523	99.8	75	100.0	234	100.0	11	100.0	1	100.0	3	100.0	**848**	**99.9**
Ceftriaxone	3	100.0	211	99.5	27	100.0	213	100.0	118	100.0	-	-	2	100.0	**574**	**99.8**
Ciprofloxacin	1	100.0	530	99.8	74	100.0	229	100.0	15	100.0	1	100.0	3	100.0	**853**	**99.9**
Colistin	-	-	221	99.5	441	99.1	2	100.0	12	91.7	6	100.0	3	100.0	**685**	**99.1**
Ertapenem	3	100.0	428	99.5	67	100.0	205	100.0	53	100.0	1	100.0	2	100.0	**759**	**99.7**
Gentamicin	1	100.0	533	99.6	71	98.6	220	99.5	14	100.0	1	100.0	3	100.0	**843**	**99.5**
Imipenem	1	100.0	417	97.8	69	87.0	175	94.3	92	85.9	1	100.0	4	25.0*	**759**	**94.2**
Levofloxacin	3	100.0	277	100.0	46	100.0	224	99.1	70	100.0	1	100.0	2	100.0	**623**	**99.7**
Meropenem	2	100.0	448	97.1	92	85.9	195	97.9	96	86.5	1	100.0	4	50.0*	**838**	**94.6**
Moxifloxacin	2	100.0	74	100.0	14	100.0	209	99.5	70	100.0	-	-	3	100.0	**372**	**99.7**
Ofloxacin	2	100.0	79	100.0	8	100.0	167	99.4	24	100.0	-	-	5	100.0	**285**	**99.6**
Piperacillin-tazobactam***	1	100.0	524	100.0	79	100.0	235	100.0	8	100.0	1	100.0	2	100.0	**850**	**100.0**
Tobramycin	1	100.0	402	99.8	69	100.0	215	100.0	15	100.0	1	100.0	3	100.0	**706**	**99.9**
Total	**28**	**100.0**	**6 588**	**99.5**	**1 401**	**97.9**	**3 413**	**99.4**	**673**	**96.0**	**18**	**100.0**	**50**	**90.0**	**12 171**	**99.0**

n: number of reporting laboratories; '-: no data; shaded cells indicate that n<5 laboratories reported concordant results;
** Below the threshold of satisfactory concordance (80%).*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid.*
**** Reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 5' (*Acinetobacter baumannii*)

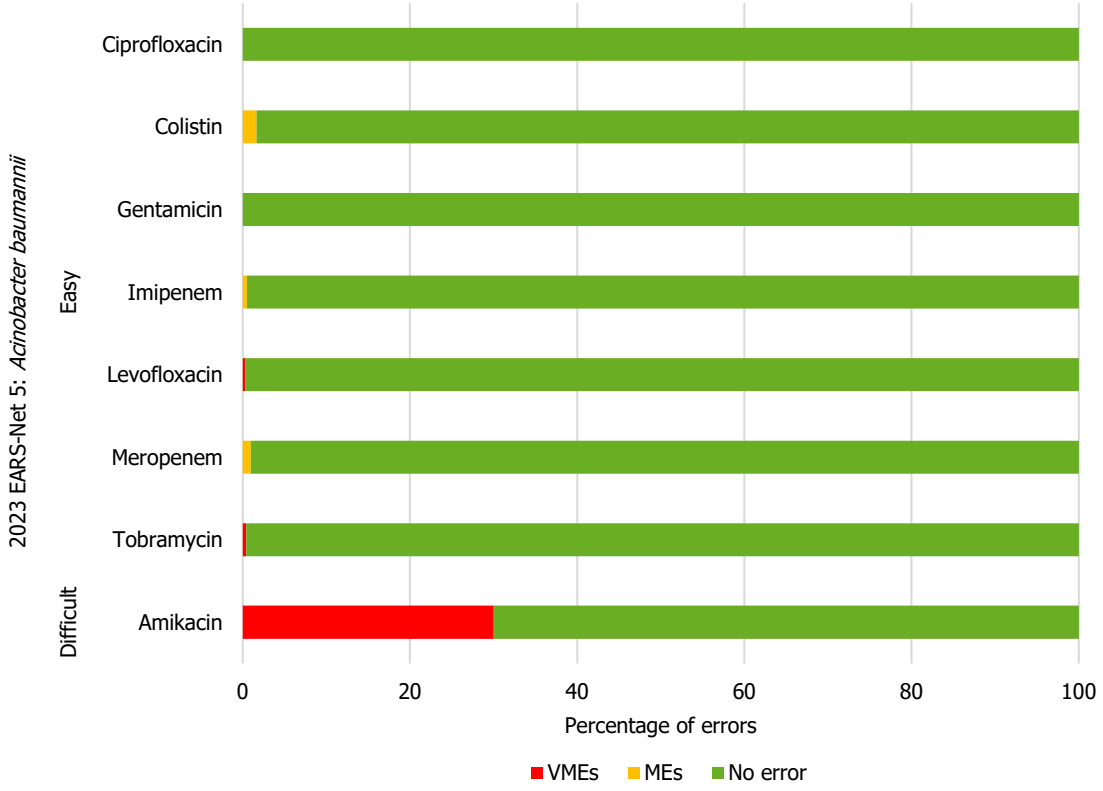
The *A. baumannii* EQA strain ('2023 EARS-Net 5') was described as being obtained from a patient with bloodstream infection. This strain was resistant to amikacin, gentamicin, tobramycin, ciprofloxacin and levofloxacin (Table 5). The strain was susceptible to imipenem, meropenem and colistin (Table 5). The level of difficulty was considered to be 'difficult' for amikacin since the expected MIC value was less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered to be 'easy'. The strain harboured the *ant(2'')-Ia* gene which encodes an enzyme responsible for inactivating aminoglycoside antimicrobials, thereby conferring resistance to gentamicin and tobramycin, as defined in the expected results. It also presented point mutations in the *gyrA* and *parC* genes, responsible for resistance to fluoroquinolones.

Interpretation of AST results for the *A. baumannii* strain were analysed for the 856 laboratories with correct species identification (Table 9). In total, 18.8% of the laboratories (n=161) would have sent the strain to a reference or other laboratory for further testing. In total, 6 185 tests were performed, and 5 922 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (95.7%) (Table 18). MEs were observed for 0.4% (n=23) and VMEs for 3.9% (n=240) of the reported interpretations.

The following methods were applied: automated systems (53.5%), disk or tablet diffusion (26.4%), broth microdilution (14.6%), gradient test (4.9%), agar dilution (0.1%), macro broth dilution (0.1%), and 'other methods' (0.4%) (Table 11). Overall, most methods achieved an 'excellent' level of concordance with the expected results (>95% of concordance). The exceptions were gradient test (93.1%) and 'other methods' (91.7%), which achieved a 'very good' concordance (Table 12).

VMEs were observed for amikacin, tobramycin and levofloxacin (Figure 9). VMEs for amikacin corresponded to 30.0% of all submitted interpretations for this antimicrobial agent and they were reported for all methods except agar dilution (Table 18). For the other antimicrobial agents, VMEs represented <1% of all submitted interpretations (Table 18). For the remaining antimicrobial agents, there were very low percentages of MEs (Figure 9, Table 18).

Figure 9. Reported interpretation of AST results for strain '2023 EARS-Net 5' (*Acinetobacter baumannii*) by antimicrobial agent and anticipated difficulty of identification



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

Table 18. Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2023 EARS-Net 5' (*Acinetobacter baumannii*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/table diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	2	100.0	403	61.5*	87	66.7*	242	87.2	46	60.9*	-	-	3	33.3*	783	70.0*
Ciprofloxacin	1	100.0	505	100.0	71	100.0	247	100.0	22	100.0	-	-	3	100.0	849	100
Colistin	-	-	222	99.1	421	97.9	4	100.0	11	100.0	7	100.0	2	100.0	667	98.4
Gentamicin	1	100.0	505	100.0	71	100.0	232	100.0	24	100.0	-	-	3	100.0	836	100.0
Imipenem	1	100.0	442	99.3	63	100.0	219	100.0	51	98.0	-	-	4	100.0	780	99.5
Levofloxacin	1	100.0	321	100.0	43	100.0	237	99.6	69	98.6	-	-	2	100.0	673	99.7
Meropenem	1	100.0	486	99.4	85	100.0	209	98.1	66	98.5	-	-	4	100.0	851	99.1
Tobramycin	1	100.0	422	99.8	62	98.4	241	99.6	17	100.0	-	-	3	100.0	746	99.6
Total	**8**	**100.0**	**3 306**	**95.0**	**903**	**95.7**	**1 631**	**97.7**	**306**	**93.1**	**7**	**100.0**	**24**	**91.7**	**6 185**	**95.7**

n: number of reporting laboratories; '-': no data; shaded cells indicate that n<5 laboratories reported concordant results.
** Below the threshold of satisfactory concordance (80%).*
Percentages might not total 100% due to rounding.

Strain '2023 EARS-Net 6' (*Enterococcus faecium*)

The *E. faecium* EQA strain ('2023 EARS-Net 6') was described as being obtained from a patient with bloodstream infection. This strain was resistant to amoxicillin, ampicillin and vancomycin, and susceptible to linezolid and teicoplanin (Table 2). The strain did not have high-level aminoglycoside resistance to gentamicin (Table 2). For all antimicrobial agents, the level of difficulty was considered to be 'easy'. The strain presented 20 point mutations in the *pbp5* gene, which are responsible for resistance to penicillins, as defined in the expected results. These mutations lead to lower affinity between the expressed penicillin-binding protein PBP5 and beta-lactam antimicrobials.

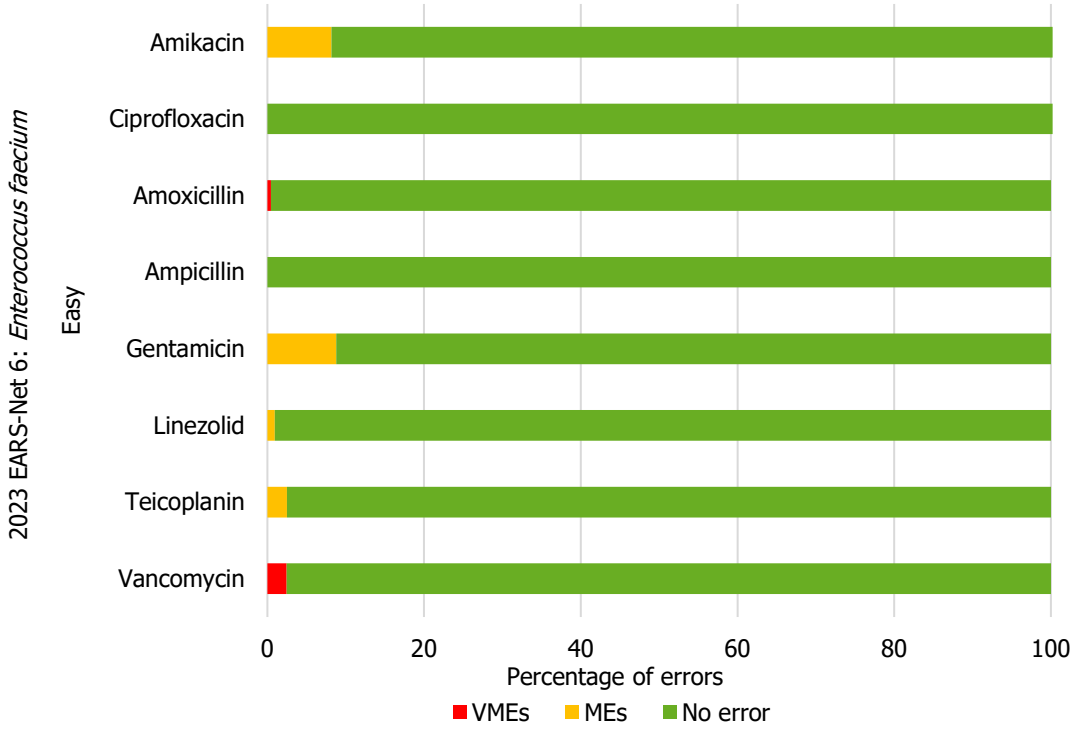
Interpretation of AST results for the *E. faecium* strain were analysed for the 859 laboratories with correct species identification (Table 9). In total, 38.6% of the laboratories (n=331) would have sent the strain to a reference or other laboratory for further testing. In total, 4 454 tests were performed, and 4 344 reported interpretations were correct. Thus, the reported interpretations were in 'excellent' concordance with expected results (97.5%) (Table 19). MEs were observed for 2.0% (n=87) and VMEs for 0.5% (n=23) of the reported interpretations.

The following methods were applied: automated systems (54.6%), disk or tablet diffusion (24.8%), broth microdilution (6.3%), gradient test (13.5%), agar dilution (0.2%), macro broth dilution (0.1%), and 'other methods' (0.4%) (Table 10). All methods achieved an 'excellent' level of concordance with the expected results (>95% of concordance).

VMEs were observed for amoxicillin and vancomycin (Figure 10). VMEs for vancomycin represented 2.4% of all submitted interpretations for that antimicrobial agent and were mainly reported when using disk or tablet diffusion (Figure 10, Table 15). For amoxicillin, VMEs represented 0.5% of all submitted interpretations (Table 14).

A high proportion of MEs was observed for gentamicin (8.8% of submitted results) and they were reported for all methods except agar dilution (Figure 6, Table 15). For the remaining antimicrobial agents, there were no, or very low proportions of MEs (Figure 6, Table 15).

Figure 10. Reported interpretation of AST results for strain '2023 EARS-Net 6' (*Enterococcus faecium*) by antimicrobial agent and anticipated difficulty of identification



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

Table 19. Number of antimicrobial susceptibility tests performed and percentage of correct AST interpretations for strain '2023 EARS-Net 6' (*Enterococcus faecium*), by antimicrobial agent and AST method

Antimicrobial agent	Agar dilution		Automated system		Broth microdilution		Disk/tablet diffusion		Gradient test		Macro broth dilution (tubes)		Other		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amoxicillin	1	100.0	131	100.0	16	100.0	67	100.0	200	99.0	-	-	12	100.0	427	99.5
Ampicillin	2	100.0	524	100.0	38	100.0	256	100.0	23	100.0	-	-	1	100.0	844	100.0
Gentamicin	4	100.0	315	86.7	30	86.7	265	96.2	57	94.7	-	-	1	100.0	672	91.2
Linezolid	1	100.0	528	98.9	47	97.9	235	99.6	36	100.0	-	-	1	100.0	848	99.1
Teicoplanin	1	100.0	487	97.7	72	97.2	130	95.4	112	99.1	1	100.0	2	100.0	805	97.5
Vancomycin	2	100.0	449	98.7	76	98.7	152	92.1	174	98.9	2	100.0	3	100.0	858	97.6
Total	**11**	**100.0**	**2 434**	**97.3**	**279**	**97.1**	**1 105**	**97.4**	**602**	**98.7**	**3**	**100.0**	**20**	**100.0**	**4 454**	**97.5**

n: number of reporting laboratories; '-': no data.
Percentages might not total 100% due to rounding.

Feedback survey of participating laboratories

A link to the feedback survey was shared with all contacts in the participating laboratories via email on 10 October 2023 (three weeks after receiving information on the release of the evaluation reports), with a deadline to reply by 1 November 2023. The survey questions can be found in Annex 2. In total, 187 laboratories provided feedback (21.5% of the 871 laboratories submitting results). The response was higher in 2023 than in 2022 (15.1%).

Corrective action had been taken by 82 (43.7%) of the 187 laboratories providing feedback. The main actions taken were re-testing of isolate(s), verification of reagents, evaluation of the procedures, review of standard operating procedures and updating/validating methods. For 56 (30.0%) of the 187 laboratories, all EQA analytical test results conformed to expected results and no further action was taken.

Ninety (48.1%) laboratories replied that they would use the results as documentation for accreditation and/or licensing purposes. This is similar to 2022 (48.8%).

Overall, 173 (92.5%) laboratories were satisfied with the individual evaluation report. This is an increase from 2022 (82.3%). Fifteen laboratories provided additional comments, and the majority of the comments were related to their own results. Twenty-two laboratories provided suggestions for improvement of the next EQA. A few laboratories asked for additional information, such as the AMR genes detected in EQA strains. Information on AMR genes had been uploaded to the website when the evaluation reports were released, and all participants were informed via email.

Some laboratories expressed a desire to receive information on the results obtained by other laboratories to be able to compare results, or to have trend data included in the evaluation. The description of results for all national laboratories is available in the respective national summary report shared with the National EARS-Net EQA Coordinators.

Feedback survey of the National EARS-Net EQA Coordinators

In 2023, ECDC invited all National EARS-Net EQA Coordinators to participate in a feedback survey investigating the usefulness of the 2022 EARS-Net EQA exercise. Every year, the National EARS-Net EQA Coordinators receive the national summary report, the national laboratory evaluation reports, and the raw national data. The feedback survey included questions about the level of EQA activities, distribution of the national summary report, usage and follow-up on results.

In total, 20 of the 30 EU/EEA countries participating in the 2022 EARS-Net EQA exercise responded to the feedback survey (Belgium, Croatia, Cyprus, Czechia, Denmark, Estonia, Finland, Germany, Iceland, Ireland, Italy, Latvia, Lichtenstein, Lithuania, Malta, Netherlands, Poland, Slovakia, Slovenia and Spain).

Question 1 was 'In 2022, in your country, do you consider the EARS-Net EQA to be the main EQA activity for antimicrobial susceptibility testing (AST) for local clinical laboratories, or a supplementary EQA activity?' In 12 countries, the 2022 EARS-Net EQA exercise was a supplement to other EQA-like activities. In three countries, fewer laboratories participated in the EARS-Net EQA exercise than in other EQA-like activities. For five countries, the EARS-Net EQA exercise was the main EQA activity for AST (Table 20).

Question 2 was 'To date, which institutions/teams have received the national-level report for the 2022 EARS-Net EQA that was sent to the National EQA coordinator in December 2022?' In 19 countries, the report was shared with participating laboratories, and in three of these countries, the report was also shared with laboratories that were eligible to participate, but did not participate in 2022. Five countries also shared the reports with other teams within their institution and with national institutions in three countries (Table 20).

Question 3 was 'To date, how have the results from the 2022 EARS-Net EQA been used in your country?' Eight countries included the results in presentations and national reports. Six countries had produced or updated national

training materials, and six countries had assessed the suitability of the national guidance/guidelines/recommendations for AST based on the results and updated, where necessary. In three countries, the results and possible actions were discussed with the laboratories. One country reported that each laboratory had prepared corrective measures based on its results, as necessary (Table 20).

Question 4 asked 'To date, was there follow-up with laboratories that had unexpected results during the 2022 EARS-Net EQA?' In seven countries, there were no need for follow-up; in seven other countries, there had been no follow-up; and in the remaining six countries, the laboratories with unexpected results had been contacted by national-level or sub-national-level staff to discuss the results (Table 20).

Table 20. Information provided by the National EARS-Net EQA Coordinators for a feedback survey focussing on the 2022 EARS-Net EQA, distributed by ECDC

Question 1: 'In 2022, in your country, do you consider the EARS-Net EQA to be the main EQA activity for antimicrobial susceptibility testing (AST) for local clinical laboratories, or a supplementary EQA activity?'	No. of responses
In most local clinical laboratories in my country, the 2022 EARS-Net EQA supplemented other EQA-like activities for AST for local clinical laboratories.	12
In most local clinical laboratories in my country, the 2022 EARS-Net EQA was the main EQA activity for AST.	5
In my country, fewer local laboratories participated in the 2022 EARS-Net EQA than in other EQA-like activities for AST.	6
Question 2: 'To date, which institutions/teams have received the national-level report for the 2022 EARS-Net EQA that was sent to the National EQA coordinator in December 2022?'	**No. of responses**
The laboratories that participated in the EARS-Net EQA in 2022.	19
Laboratories that were eligible to participate, but did not participate, in the EARS-Net EQA in 2022.	3
Other teams within your institution.	5
Official national institutions (e.g. ministries, government agencies).	3
Question 3: 'To date, how have the results from the 2022 EARS-Net EQA been used in your country?'	**No. of responses**
The 2022 EARS-Net EQA results were included in national-level outputs, such as presentations and/or reports.	8
The 2022 EARS-Net EQA results were used to produce or update training materials for local/regional/national laboratories or hospitals.	6
The 2022 EARS-Net EQA results were used to assess the current suitability of national guidance/guidelines/recommendations for AST.	5
The results gave rise to discussions on the quality of routine laboratory procedures with national AST committee	1
Each laboratory prepared corrective measures based on its results, as necessary.	1
Results are discussed with laboratories, deviations from results are placed under closer surveillance to prevent failure in AST	1
The results were discussed with the laboratories in a common meeting.	1
The 2022 EARS-Net EQA results contributed to a change in national guidance/guidelines/recommendations for AST.	1
Question 4: 'To date, was there follow-up with laboratories that had unexpected results during the 2022 EARS-Net EQA?'	**No. of responses**
Laboratories with unexpected EQA results in the 2022 EARS-Net EQA were contacted by national-level staff to discuss those results.	4
I am aware that laboratories with unexpected EQA results in the 2022 EARS-Net EQA were contacted by sub-national-level staff to discuss those results.	2
There was no follow-up with laboratories that had unexpected results during the 2022 EARS-Net EQA.	7
The national report did not identify any participating laboratories requiring direct follow-up of their 2022 EARS-Net EQA results.	7

4. Summary and discussion

Participation

For the second year in a row, all 30 EU/EEA countries participated in the 2023 EARS-Net EQA exercise. A total of 951 laboratories were invited to participate and 871 (91.6%) submitted results for validation. This percentage is similar to previous EARS-Net EQA exercises in 2019, 2021 and 2022, in which 90.3–93.7% laboratories submitted results [2,3,4].

When comparing the overall results between years, it is important to remember that the species and antimicrobial agents were not the same. Similarly, ECDC did not initiate an EARS-Net EQA exercise in 2020, due to its response to the COVID-19 pandemic. In 2021, only 642 laboratories signed up to participate and 592 submitted results [3]. This number, lower than that observed in other years, was probably due to the ongoing COVID-19 pandemic which required the allocation of laboratorial resources to COVID-19.

Speciation and overall AST results

As in previous years, species identification was a component of the EQA exercise in 2023 and the submitted species identification results were in 'excellent' concordance (98.8 to 99.3%) with the expected results for each of the six EQA strains. There was no additional information collected from four laboratories that reported the species incorrectly for all six strains, to determine whether this was a clerical or true error. The laboratories are included in the country reports that were sent to national teams.

The distribution of AST methods used in the 2023 EARS-Net EQA exercise was similar to that observed in previous years, as 53.9% of submitted results were obtained using automated systems (50.3% to 54.7% in previous years), 27.3% using disk or tablet diffusion (28.0% to 39.8% in previous years), and 18.0% using MIC methods including broth microdilution and gradient test (8.3% to 19.3% in previous years) [2,3,4,5]. In 2023, 'excellent' concordance was observed for agar dilution (99.0%), macro broth dilution (98.6%), broth microdilution (95.6%) and disk or tablet diffusion (95.2%), and 'very good' concordance was observed for gradient tests (94.4%) and automated systems (94.2%). More generally, these results indicate that the methods applied by laboratories in Europe are robust and accurate, for the species and antimicrobial agents included in this EARS-Net EQA exercise.

Concordance of AST results at national level for the 2023 EARS-Net EQA exercise was better than for the 2022 EQA exercise [2]. In 2023, 17 countries achieved an 'excellent' level of concordance, which is better than in 2022 where only one country achieved an 'excellent' level of concordance. Thirteen countries achieved a 'very good' level of concordance in 2023, compared to 28 countries in 2022.

At laboratory level, the vast majority of the participating laboratories achieved an 'excellent' (n=439, 50.7%) or 'very good' (n=345, 39.8%) level of concordance, including 40 laboratories with no errors at all. Otherwise, 7.7% (n=67 in 20 countries) achieved a 'good' level of concordance, 1.2% (n=10 in five countries) laboratories achieved a 'satisfactory' level of concordance, and 0.6% (n=5 in four countries) were below the 'satisfactory' level. This shows that overall participating laboratories comply with the most recent EUCAST guidelines and breakpoints, and are able to produce reliable AST results from clinical samples.

Overall, concordance of all submitted AST interpretations with the expected results was 'very good', as 94.7% of all submitted interpretations were correct. For the individual species-antimicrobial agent combinations included in the EQA exercise (n=74), the vast majority showed results in 'excellent' concordance with those expected (n=58 or 78.4% of the combinations), and a 'very good' level of concordance was achieved for eight combinations (10.8%). This is similar to the percentage of 'excellent' results in EARS-Net EQA exercises from 2022 (79.3%), 2021 (80.2%) and 2019 (75.6%) [2,3,4].

The lowest level of concordance was observed for the *E. coli* strain, for which only 29.2% of the results interpreting amikacin AST were correct. Non-satisfactory levels of concordance were also observed for the same strain with piperacillin-tazobactam (40.6%), for *K. pneumoniae* (strain '2023 EARS-Net 2') results with amikacin (66.7%) and cefepime (78.9%), and for *A. baumannii* (strain '2023 EARS-Net 5') with amikacin (70.0%). All remaining species-antimicrobial agent combinations achieved at least a satisfactory concordance (>80%). Strain '2023 EARS-Net 4' (*K. pneumoniae*), which was resistant to all antimicrobial agents included in the EQA exercise, was the strain with the best overall concordance of results, with 'excellent' concordance (99.0%).

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

Strain-specific AST results

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

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

Suboptimal results were also observed for cefepime (which reached a 'satisfactory' level with 83.4% of concordance) and ceftazidime (with a 'good' level with 87.5% concordance). These deviations correspond to MEs (S → R or I → R) and were observed for most methods. The situation of most concern was the use of the disk or tablet diffusion method (only 65.1% to 79.8% concordance), due to its frequent use by the laboratories for AST for these antimicrobial agents (26.04% - 26.3%). The expected MIC values for cefepime and ceftazidime (MIC = 1 mg/L and MIC = 2 mg/L, respectively) were very close to the clinical breakpoints (S ≤ 1 mg/L and R > 4 mg/L, for both antimicrobial agents), which were also classified as 'difficult'. Furthermore, variations in results for these cephalosporins can also be derived from the differential expression of the $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$ genes that were harboured by the test strain. These results may be an indication that, for *E. coli*, resistance to these agents is overestimated in the EU/EEA. However, when compared with results from the 2022 EARS-Net EQA exercise, the results in the 2023 EARS-Net EQA exercise show an improvement for these two cephalosporins. In 2022, submitted interpretations for cefepime had not reached a satisfactory level of concordance (79.6%), but the submitted interpretations for ceftazidime were at 'satisfactory' level (83.7%) rather than 'good ' in 2023.

For strain '2023 EARS-Net 1', the concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

Strain **'2023 EARS-Net 2' (*Klebsiella pneumoniae*)** was susceptible to amikacin and cefepime, but prediction of these profiles was problematic and concordance did not reach a satisfactory level (66.7% and 78.9%, respectively). Problematic results were also observed for imipenem, which reached a 'satisfactory' level with 82.5% of concordance. These deviations corresponded to MEs (S → R) and were more prevalent when using automated systems, disk/tablet diffusion and gradient tests. The expected MIC results for cefepime (MIC = 4 mg/L) and imipenem (MIC = 2 mg/L) were very close to the clinical breakpoints (i.e. S ≤ 1 mg/L and R > 4 mg/L for cefepime, and S ≤ 2 mg/L and R > 4 mg/L for imipenem). Thus, the prediction of these AST profiles was considered difficult and the observed deviations can be attributed to the inherent method variability, since the expected MIC values correspond to borderline concentrations, thereby increasing the likelihood of misclassification. However, the expected amikacin MIC = 4 mg/L was considered to be an 'easy' determination, because the acceptable method variation (+/- 1 dilution) would yield the same classification (S). Therefore the natural methodological variability should not be a justification for these deviations. The deviations may potentially be justified by variations in the methods and/or the material used for testing [7-9]. These results may be an indication that resistance to these agents in *K. pneumoniae* isolates is overestimated in the EU/EEA.

For strain '2023 EARS-Net 2', the concordance of results for the remaining antimicrobial agents was 'very good' (>90% to <95%) or 'excellent' (≥95%).

Strain **'2023 EARS-Net 3' (*Enterococcus faecalis*)** was naturally resistant to gentamicin but did not present high-level aminoglycoside resistance. For this strain, prediction of high-level aminoglycoside resistance was the main issue, although the level of concordance was still 'very good' (90.8%). The deviations corresponded to MEs (S → R) and did not seem to be associated with a specific AST method. One of the main reasons for the lower concordance was misinterpretation of the EQA protocol, which instructed participants to report isolates not presenting high-level aminoglycoside resistance as susceptible (S) to gentamicin, but the information might have been missed by some participating laboratories, which then reported the natural aminoglycoside resistance, including gentamicin resistance, of the test strain. The expected gentamicin MIC = 16 mg/L should be easily identifiable as not being high-level aminoglycoside-resistant, therefore methodological variability should not be a justification for these deviations. The results do not seem to indicate anomalous reporting of aminoglycoside resistance in *E. faecalis* in the EU/EEA, nor do they illustrate problems with the methods applied by the laboratories.

For strain '2023 EARS-Net 3', concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

Strain **'2023 EARS-Net 4' (*Klebsiella pneumoniae*)** was resistant to all antimicrobials included in the EQA exercise and it was the strain with the best overall concordance of results: with 'excellent' concordance (99.0%). The strain had previously been included in the 2021 EARS-Net EQA exercise, where results were very similar, with 'excellent' concordance (98.8%).

Moreover, for each individual antimicrobial agent, concordance with expected results was also 'excellent' (≥95%), with the exception of imipenem and meropenem, which still had a 'very good' concordance (94.2% and 94.6%, respectively). Overall, concordance of results for all AST methods was also 'excellent' (≥95%). The exception was for 'other methods', which only achieved a 'good' concordance (90.0%).

Strain **'2023 EARS-Net 5' (*Acinetobacter baumannii*)** was resistant to amikacin, but concordance of results for this antimicrobial agent was poor (70.0%) and did not reach a satisfactory level. These deviations corresponded to VMEs (R → S) and were observed for most methods, except agar dilution, which was only applied twice. The expected MIC result (MIC = 16 mg/L) was very close to the clinical breakpoints (S ≤ 8 mg/L and R > 8 mg/L). Thus, the prediction of amikacin susceptibility/resistance was considered difficult and the observed deviations could be attributed to the inherent method variability, since the expected MIC value corresponds to a borderline concentration, increasing the likelihood of misclassification. The deviations could also represent, or have been exacerbated by, variations in the methods and/or material used for testing [7-9].

For strain '2023 EARS-Net 5', concordance of results for the remaining antimicrobial agents was excellent (≥95%).

Strain **'2023 EARS-Net 6' (*Enterococcus faecium*)** was naturally resistant to gentamicin but did not present high-level aminoglycoside resistance to the antimicrobial. As also observed for strain '2023 EARS-Net 3' (*E. faecalis*), prediction of this profile was the most problematic in this strain, although it still achieved a 'very good' level of concordance (91.2%). These deviations correspond to MEs (S → R) and did not seem to be associated with a specific AST method. The justification for the deviations is the same as previously described, probably resulting from the misinterpretation of the EQA protocol, with participants reporting the natural aminoglycoside-resistant profile of the strain. These results do seem to indicate anomalous reporting of aminoglycoside resistance in *E. faecium* in the EU/EEA, nor do they illustrate problems with the methods applied by the laboratories.

For strain '2023 EARS-Net 6', concordance of results for the remaining antimicrobial agents was 'excellent' (≥95%).

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

Common issues identified in this EQA exercise

In previous EARS-Net EQA exercises, 2019 [4], 2021 [3] and 2022 [2], the determination and interpretation of AST results had issues for the following:

- *E. coli* with I or R results for fluoroquinolones;
- *E. coli* with S or R results for gentamicin and amikacin;
- *E. coli* with R result for tigecycline;
- *E. coli* with S, I or R results for carbapenems;
- *E. coli* with I or R results for ceftazidime;
- *E. coli* with S result for cefepime;
- *E. coli* with S or R results for amoxicillin-clavulanic acid and piperacillin-tazobactam;
- *K. pneumoniae* with R result for tobramycin and gentamicin;
- *K. pneumoniae* with I and S results for imipenem and meropenem;
- *K. pneumoniae* with R result for cefepime;
- *K. pneumoniae* with R result for amoxicillin-clavulanic acid;
- *P. aeruginosa* with R result for amikacin;
- *P. aeruginosa* with S result for ceftazidime;
- *P. aeruginosa* with borderline results for colistin;
- *P. aeruginosa* with I result for levofloxacin;
- *A. baumannii* with R result for tobramycin and gentamicin;
- No problematic issues for *E. faecalis* and *E. faecium* strains, as these had not recently been included EARS-Net EQA exercises;

- Other issues related to species not included in the 2023 EARS-Net EQA (*Staphylococcus aureus* and *Streptococcus pneumoniae*).

The laboratories participating in the 2023 EARS-Net EQA exercise reported issues for several of the same species-antimicrobial combinations that were problematic in previous EQA exercises, specifically:

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

Furthermore, prediction of a negative high-level resistance to aminoglycosides from gentamicin results was problematic for both *E. faecium* and *E. faecalis* strains, but it is suggested that these results reflect a misinterpretation of the EQA protocol rather than problems with the methods applied by the laboratories.

Overall, results of the 2023 EARS-Net EQA exercise did not show a systematic overestimation or underestimation of AMR in the EU/EEA, with deviations being distributed across both types of errors (MEs and VMEs). However, they show that there are still difficulties and that there has been a lack of improvement regarding the prediction of AST profiles for beta-lactam antimicrobials in *E. coli* and *K. pneumoniae*. The results also support a continuing trend across species of difficulties in predicting either S or R results for aminoglycosides.

Finally, the results did not highlight any systematic underperformance of a certain AST method when compared to other reported methods, and the deviations were generally distributed throughout all of the methods applied. One situation where a specific method seemed to influence the percentage of correct results was the use of disk or tablet diffusion for prediction of cephalosporin resistance in *E. coli*.

5. Conclusions

In 2023, the number of participating laboratories had stabilised and reached a number similar to that before the COVID-19 pandemic.

The submitted species identification results (≥98.8% correct results for all species that are reportable to EARS-Net) imply that species data reported to EARS-Net are accurate overall.

The submitted AST interpretations also imply that AST data reported to EARS-Net are mostly accurate, although overall MEs were observed for 3.2% and VMEs 2.2% of the reported interpretations. Both MEs and VMEs suggest the possibility for sub-optimal treatment outcomes, albeit in a small percentage of bloodstream infections. The MEs and VMEs detected in this EARS-Net EQA exercise included species-antimicrobial agent combinations that were classified as 'easy' (with expected AST results far from the clinical breakpoints). This suggests that some participating laboratories did not always strictly adhere to the most recent EUCAST guidelines. Furthermore, for specific species, certain antimicrobial agents or groups presented higher percentages of deviations, namely amikacin, piperacillin-tazobactam and certain cephalosporins in *E. coli*, amikacin, cefepime and imipenem in *K. pneumoniae*, and amikacin in *A. baumannii*. These problematic species-antimicrobial combinations had been observed in previous EQA exercises and highlight an opportunity for improvement at EU/EEA level.

The findings may also indicate that AMR is heterogeneously reported in the EU/EEA. The VMEs (R → S) showed a tendency to under-report resistance to piperacillin-tazobactam in *E. coli*, and resistance to amikacin in *A. baumannii*. Inversely, the MEs (S → R or I → R) indicate a trend of over-reporting of resistance to amikacin, cefepime and ceftazidime in *E. coli*, and resistance to amikacin, imipenem and cefepime in *K. pneumoniae*. One frequent justification for these deviations was the inherent method variability of plus or minus one dilution in MIC methods, especially when the expected MIC values corresponded to borderline concentrations very close to the clinical breakpoints, which increased the likelihood of misclassification. Furthermore, some of the strains harboured known genetic mechanisms associated with resistance to certain antimicrobial groups, and although genotypic characterisation of the strains was outside the scope of this exercise, it is conceivable that laboratories could screen isolates for AMR genetic determinants during their routine procedures. Therefore, when considering both phenotypic and genotypic data, the final reporting of results could present lower proportions of deviations. For example, detection of genes encoding extended-spectrum beta-lactamases in the *E. coli* strain would be likely to promote increased attention in interpretation of AST results for cephalosporins and other beta-lactams, or even confirmatory AST using other methods. However, one possible consequence of detecting AMR genes is the tendency to further over-report decreased susceptibility profiles. The 2023 EARS-Net EQA exercise also revealed an overall tendency to incorrectly report high-level aminoglycoside resistance in *E. faecalis* and *E. faecium*, however this was probably due to misinterpretation of the EQA protocol. These results do not seem to indicate anomalous reporting of resistance to aminoglycosides in *E. faecalis* and *E. faecium* in the EU/EEA, nor do they illustrate problems with the methods applied by the laboratories.

The analysis of the overall performance of the different AST methods showed few differences between methods, except for an overall slightly poorer performance of automated systems and gradient tests. Specific shortcomings were observed in AST of *E. coli* for cephalosporins when using the disk or tablet diffusion method to test for cephalosporin susceptibility.

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

6. Recommendations

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

Furthermore, results from this EQA exercise indicate that both under- and overestimation of AMR percentages may occur in Europe. Although genotypic analysis of AMR genes or chromosomal point mutations could potentially solve some of the deviations reported by the participating laboratories, the focus of this EQA exercise was phenotypic testing, and the observed under- and overestimation should be borne in mind when interpreting EARS-Net surveillance data. Overall, surveillance or control efforts should consider the specific deviations observed for each specific antimicrobial agent or group, as well as focusing on AST results close to current breakpoints. Findings worthy of further investigation are the low performance of AST for aminoglycosides, across species, and the problems with determining AMR profiles for beta-lactam antimicrobials in *Enterobacterales*.

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

- strengthening awareness and potentially seeking advice regarding AST and reading of results for the problematic species-antimicrobial combinations detected in the EARS-Net EQA exercises;
- revising criteria for performing and reading results for aminoglycosides susceptibility testing, since the variability in the AST results for aminoglycosides may have been due to differences in medium composition;
- revising criteria for performing and reading results for species-antimicrobial agent combinations that may be associated with differential expression of AMR genes, such as for β-lactam antimicrobials, for example performing screening tests to detect the genes encoding for extended-spectrum β-lactamases, AmpC enzymes or carbapenemases;
- opting to use the recommended AST methods for each species-antimicrobial agent combination being tested and confirming that the AST protocols in use are in accordance with the latest EUCAST recommendations and guidelines (including the general or specific recommendations regarding the performance, interpretation and evaluation of AST for certain species-antimicrobial agent combinations) and ensuring that adequate control strains are being used and monitored to guarantee reliability of results;
- becoming familiar with EUCAST recommendations regarding AST results within the ATU or results near the clinical breakpoints;
- ensuring that the relevant quality management systems and control measures are in place, including but not limited to monitoring AST results over time, to allow detection random and systematic deviations;
- strengthening awareness of method variability when applying the different AST methods, especially those showing lower percentages of concordance in this EQA exercise and previous EQA exercises (i.e. automated system and gradient tests);
- seeking advice from relevant national stakeholders, such as National Focal Points for Antimicrobial Resistance, Operational Contact Points or National Reference Laboratories, to ensure compliance with national and international guidelines.

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

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Annex 1. List of participating countries

Table A1. Number of laboratories receiving material and submitting results for the 2023 EARS-Net EQA exercise

EU/EEA country	Number of laboratories receiving material for the EQA exercise	Number of laboratories submitting data		Number of laboratories included in the analysis of AST results	
	N	N	%	N	%
Austria	38	37	97.4	37	100.0
Belgium	26	23	88.5	23	100.0
Bulgaria	24	24	100.0	24	100.0
Croatia*	36	35	97.2	34	97.1
Cyprus	10	10	100.0	10	100.0
Czechia	48	48	100.0	48	100.0
Denmark	4	4	100.0	4	100.0
Estonia	11	11	100.0	11	100.0
Finland	12	12	100.0	12	100.0
France	68	49	72.1	49	100.0
Germany***	22	21	95.5	20	95.2
Greece	40	36	90.0	36	100.0
Hungary	24	23	95.8	23	100.0
Iceland	1	1	100.0	1	100.0
Ireland	31	28	90.3	28	100.0
Italy	190	174	91.6	174	100.0
Latvia	14	12	85.7	12	100.0
Liechtenstein	1	1	100.0	1	100.0
Lithuania	15	14	93.3	14	100.0
Luxembourg	5	5	100.0	5	100.0
Malta	1	1	100.0	1	100.0
Netherlands	33	30	90.9	30	100.0
Norway	17	16	94.1	16	100.0
Poland	67	65	97.0	65	100.0
Portugal**	113	95	84.1	93	97.9
Romania	17	17	100.0	17	100.0
Slovakia	14	14	100.0	14	100.0
Slovenia*	11	11	100.0	10	90.9
Spain***	43	39	90.7	38	97.4
Sweden	15	15	100.0	15	100.0
Total	951	871	91.6	865	99.3

** One laboratory was excluded from the antimicrobial susceptibility testing (AST) evaluation because all species identifications were wrong.*
*** Two laboratories were excluded from the antimicrobial susceptibility testing (AST) evaluation because all species identifications were wrong.*
**** One laboratory was excluded because interpretations for the antimicrobial susceptibility testing (AST) results were not submitted.*

Annex 2. Feedback survey questionnaire

Save a backup on your local computer (disable if you are using a public/shared computer)

EARS-Net EQA 2023 feedback survey

Fields marked with * are mandatory.

Disclaimer

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

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

Fields marked with * are mandatory.

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

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

Please specify which corrective actions were taken.

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

- ☐ Yes.
- ☐ No.
- ☐ Not applicable.

Please specify.

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

- ☐ Yes.
- ☐ No.

If no, please specify.

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

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

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

Submit

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