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TECHNICAL REPORT

External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2022

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
ATU	Area of technical uncertainty
CDC	United States Centers for Disease Control and Prevention
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
MRSA	Meticillin-resistant *Staphylococcus aureus*
R	Resistant
S	'Susceptible, standard dosing regimen'
std	Standard deviation
VME	Very major error

Executive summary

This report describes and summarises the results of the 2022 external quality assessment (EQA) of 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. For the first time, all 30 EU/EEA countries participated in the EARS-Net EQA exercise.

The aims of the 2022 EARS-Net EQA exercise were: 1) to assess the accuracy of species identification reported by participating individual laboratories; 2) to assess the accuracy of qualitative AST results reported by participating individual laboratories; and 3) to evaluate the overall comparability of routinely collected test results, between laboratories and between European Union/European Economic Area (EU/EEA) countries.

Eligible laboratories were identified by the National EARS-Net EQA Coordinators, who had been designated by the Coordinating Competent Body in each EU/EEA country. Participating laboratories had to identify the species of six bacterial strains and submit AST results for the antibiotics included in EARS-Net surveillance, using methods routinely used in their settings. In 2022, the six EQA strains included five species that are included in EARS-Net surveillance (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*) and one species (*Pseudomonas putida*) that is not included in EARS-Net surveillance (Table 1).

On 9 June 2022, the six strains were distributed, via the National EARS-Net EQA Coordinators, to 948 laboratories in all EU/EEA countries. A webtool for submission of results, was open from 27 June 2022 until 19 August 2022. As in previous EARS-Net EQA exercises [2-4], the 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%).

Species identification was submitted by 855 laboratories, and 4 853 (95.7%) of the 5 070 reported species were correct. There was 'excellent' concordance for each of the five strains of species included in EARS-Net surveillance (97.7 to 99.3% concordance). The concordance for the *P. putida* strain, which is not included in EARS-Net surveillance, was 'satisfactory', as the correct species was only identified by 668 (80.1%) of the 834 laboratories that submitted species results for this strain.

The interpretation of AST results was evaluated for strains with a correct species identification. The evaluation was performed according to the clinical breakpoints in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints Tables v12.0[1], with the EUCAST categories 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R).

The reported interpretation of AST results was evaluated for 850 laboratories. Two laboratories did not submit any AST interpretations and two laboratories reported the wrong species for all six strains. One laboratory used Clinical and Laboratory Standards Institute (CLSI) guidelines, and so its results were not evaluated.

Among the evaluated AST results, the most frequently reported method for acquisition of the AST data was an automated system (51.4%), followed by disk or tablet diffusion (28.1%) and minimum inhibitory concentration (MIC) methods, including broth microdilution and gradient test (19.3%). 'Very good' concordance was observed for macro broth dilution (93.9%), broth microdilution (92.7%) and disk or tablet diffusion (90.7%), and 'good' concordance was observed for agar dilution (89.8%), automated system (87.8%) and gradient test (86.1%).

Overall, the submitted AST interpretations were in 'very good' concordance with the expected results, with 92.6% (n=39 925) being correct. Otherwise, major errors (MEs) and very major errors (VMEs) were observed for 4.2% and 3.2% of interpretations, respectively. At country level, one country (Bulgaria) achieved an 'excellent' level of concordance with the expected interpretation of AST results, 28 countries achieved a 'very good' level concordance, and one country (Liechtenstein) achieved a 'good' level of concordance. At laboratory level, 23.3% of the laboratories achieved an 'excellent' level of concordance, 53.1% achieved a 'very good' level of concordance, 19.9% achieved a 'good' level of concordance, 3.1% achieved a 'satisfactory' level, and 0.7% were below the 'satisfactory' level.

There were 58 species-antimicrobial agent combinations tested in the 2022 EARS-Net EQA exercise with an 'excellent' level of concordance between the submitted AST interpretations and the expected results for 46 (79.3%) of these combinations. The species-antimicrobial agent combination with the lowest level of concordance was for the levofloxacin AST result for the *P. aeruginosa* strain, with 87.4% interpretations being MEs (I → R), only 12.6% of the interpretations being correct, and results varying by AST methodology. Low concordance was also observed for AST results for the *S. pneumoniae* strain (benzylpenicillin, 70.7% concordance; azithromycin, 72.2%; ceftriaxone, 83.8%; and cefotaxime, 84.6%), the *E. coli* strain (piperacillin-tazobactam), 60.5%; amikacin, 64%; cefepime, 79.6%; and ceftazidime, 83.7%), and the *A. baumannii* strain (tobramycin, 55.8%; and gentamicin, 64.2%). All remaining species-antimicrobial agent combinations achieved at least a 'very good' concordance (>90%).

[1] EUCAST clinical breakpoints: https://www.eucast.org/clinical_breakpoints

In the 2022 EARS-Net EQA exercise, a new scoring system was implemented for the evaluation of the submitted results. The scoring took into account, for each species-antimicrobial agent combination, an assessment of the 'level of difficulty' and the 'severity of error' for the AST. Additionally, in 2022, the scoring applied a negative score if results on mandatory antimicrobial agents were not reported.

The 'level of difficulty' had two levels, 'easy' and 'difficult', reflecting the magnitude of the risk from 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 AST using breakpoints that 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 than errors for 'difficult' results.

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

Table 1. Overview of species identification results and antimicrobial susceptibility testing (AST) results reported by clinical laboratories participating in the 2022 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)	Total correct AST interpretations (N(%))	Total MEs (N (%))	Total VMEs (N (%))
2022 EARS-Net 1	*Streptococcus pneumoniae* **S:** AZM, CTX, CRO, CLR, ERY, MFX, NOR; **I:** LVX; **R:** PEN, OXA.	839	820 (97.7)	6 024	5 422 (90.0)	359 (6.0)	243 (4.0)
2022 EARS-Net 2	*Escherichia coli* **S:** FEP, COL, ETP, GEN, IPM, MEM, TZP, TGC; **I:** CAZ; **R:** AMK, AMX, AMC, AMP, CTX, CRO, CIP, LVX, MFX, OFX, TOB.	851	845 (99.3)	13 558	12 572 (92.7)	662 (4.9)	324 (2.4)
2022 EARS-Net 3	*Pseudomonas putida* **SIR:** NA	834	668 (80.1)	NA	NA	NA	NA
2022 EARS-Net 4	*Staphylococcus aureus* **S:** DAP, LNZ, NOR, RIF, VAN; **I:** CIP, LVX; **R:** FOX, OXA.	848	840 (99.1)	6 214	6 125 (98.6)	39 (0.6)	50 (0.8)
2022 EARS-Net 5	*Pseudomonas aeruginosa* **S:** AMK, COL, MEM, TOB; **I:** IPM, LVX; **R:** FEP, CAZ, CIP, PIP, TZP.	849	841 (99.1)	8 157	7 504 (92.0)	606 (7.4)	47 (0.6)
2022 EARS-Net 6	*Acinetobacter baumannii* **S:** AMK, COL; **I:** (none); **R:** IPM, GEN, IPM, LVX, MEM, TOB.	849	839 (98.8)	5 972	5 339 (89.4)	16 (0.3)	617 (10.3)
Total	NA	**855**	**4 853 (95.7)**	**39 925**	**36 962 (92.6)**	**1 682 (4.2)**	**1 281 (3.2)**

AST: antimicrobial susceptibility testing; NA: not applicable; ME: major error; VME: very major error; S: susceptible, standard dosing regimen; I: susceptible, increased exposure; R: resistant; AMC: amoxicillin-clavulanic acid; AMK: amikacin; AMP: ampicillin; AMX: amoxicillin; AZM: azithromycin; CAZ: ceftazidime; CIP: ciprofloxacin; CLR: clarithromycin; COL: colistin; CRO: ceftriaxone; CTX: cefotaxime; DAP: daptomycin; ERY: erythromycin; ETP: ertapenem; FEP: cefepime; FOX: cefoxitin; GEN: gentamicin; IPM: imipenem; LNZ: linezolid; LVX: levofloxacin; MEM: meropenem; MFX: moxifloxacin; NOR: norfloxacin; OFX: ofloxacin; OXA: oxacillin; PEN: penicillin; PIP: piperacillin; RIF: rifampicin; TGC: tigecycline; TOB: tobramycin; TZP: piperacillin-tazobactam; VAN: vancomycin.

Strain **2022 EARS-Net 1 (*Streptococcus pneumoniae*)** was resistant to benzylpenicillin and oxacillin, and susceptible to cefotaxime, ceftriaxone, moxifloxacin, norfloxacin, azithromycin, clarithromycin, and erythromycin. Its expected MIC value for levofloxacin was in the 'susceptible, increased exposure' (I) range.

In total, 97.7% (820/839) of laboratories correctly identified the species of this strain and, overall, the AST interpretations reported for the strain were in good concordance with expected results (90.0%). MEs and VMEs were observed for 6.0% and 4.0% of the reported interpretations, respectively.

There was a 'good' level of concordance with the expected results for this strain, for all reported AST methodologies (>85% of concordance for all assessed antimicrobial agents/groups), with the exception of gradient tests, for which there was 'satisfactory' concordance (minimum concordance: 83.5%).

Prediction of resistance to benzylpenicillin was problematic. One of the main reasons for the deviations appeared to be the application of incorrect clinical breakpoints. In this EQA exercise, this strain was described as being obtained from cerebrospinal fluid from a patient with clinical manifestations suggesting meningitis, and its resistance profile should be easily identifiable when applying the clinical breakpoints for meningitis. However, the expected AST result was close to the clinical breakpoints for situations other than meningitis, and so the likelihood of misclassification increased if the wrong clinical breakpoint was used. These deviations correspond to VMEs (R → S) and may indicate that resistance to penicillins can be under-reported in the EU/EEA.

Prediction of susceptibility to azithromycin, cefotaxime and ceftriaxone was also relatively poor for this strain. While some of the deviations can be attributed to the inherent method variability and were within an acceptable variation range (+/-1 dilution), there also seemed to be associations with specific methodologies, in particular with the gradient test for azithromycin, and with automated systems for the cephalosporins. These deviations correspond to MEs (S → R) and may be an indication that, for *S. pneumoniae*, resistance to these antimicrobial agents may be overestimated in the EU/EEA.

These results suggest that, in general, laboratories should become more familiar with the different clinical breakpoints for species-antimicrobial agent combinations, dependent on the clinical manifestations, as well as other general or specific EUCAST recommendations for the performance, interpretation and evaluation of the recommended methodologies for AST of *S. pneumoniae*.

Strain **2022 EARS-Net 2** ***(Escherichia coli)*** was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, amikacin and tobramycin, and susceptible to piperacillin-tazobactam, cefepime, ertapenem, imipenem, meropenem, gentamicin, tigecycline and colistin. Its expected MIC value for ceftazidime was in the I range.

In total, 99.3% (845/851) 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 (92.7%). MEs and VMEs were observed for 4.9% and 2.4% of the reported interpretations, respectively.

There was a good level of concordance with the expected results (>85% of concordance for all assessed antimicrobial agents/groups) for this strain, for every reported AST methodology.

Prediction of amikacin resistance appeared to be relatively challenging. Most of the deviations may be attributed to the inherent method variability and are within the acceptable variation range. These deviations correspond to VMEs (R → S) and might indicate that resistance to amikacin may be under-reported in the EU/EEA.

Characterisation of susceptibility to cefepime, ceftazidime and piperacillin-tazobactam was also problematic. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, there also seemed to be association with a specific methodology, in particular disk or tablet diffusion for cephalosporins. These deviations correspond to MEs (S → R or I → R) and may be an indication that, in *E. coli*, resistance to these agents is overestimated in the EU/EEA.

Similar to the strain '2022 EARS-Net 1' (*S. pneumoniae*) these results suggest that laboratories should become more familiar with EUCAST recommendations regarding AST results within the ATU or results near the clinical breakpoints. They should also review their methodologies concerning the performance and interpretation of AST results for *E. coli* for antimicrobial agents/groups that may be associated with differentially expressed genes encoding for antimicrobial resistance (AMR).

Strain **2022 EARS-Net 3 (*Pseudomonas putida*)** was from a species which is not part of the EARS-Net surveillance, and so the participating laboratories did not need to report their interpretation of AST results. The species was correctly identified by 80.1% of the participating laboratories (668/834).

Strain **2022 EARS-Net 4 (*Staphylococcus aureus*)** was resistant to oxacillin and cefoxitin, and susceptible to norfloxacin, vancomycin, linezolid, daptomycin and rifampicin. Its expected MIC values for ciprofloxacin and levofloxacin wee in the I range. As oxacillin resistance and cefoxitin resistance among *S. aureus* isolates indicates meticillin resistance, this strain was considered to be meticillin-resistant *S. aureus* (MRSA).

In total, 99.1% (840/848) 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 (98.6%). MEs and VMEs were observed for 0.6% and 0.8% of the reported interpretations, respectively.

There was a good level of concordance with the expected results (>85% of concordance for all assessed antimicrobial agents/groups) for this strain, for every reported AST methodology.

Strain **2022 EARS-Net 5 (*Pseudomonas aeruginosa*)** was resistant to piperacillin, piperacillin-tazobactam, cefepime, ceftazidime and ciprofloxacin, and susceptible to meropenem, amikacin, tobramycin and colistin. Its expected MIC values for imipenem and levofloxacin wee in the I range.

In total, 99.1% (841/849) 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 (92.0%). MEs and VMEs were observed for 7.4% and 0.6% of the reported interpretations, respectively.

Similar to the strain '2022 EARS-Net 1' (*S. pneumoniae*), there was a 'good' level of concordance with the expected results (>85% concordance overall) for all reported AST methodologies except for gradient tests, which achieved satisfactory concordance (minimum concordance: 80.9%).

As noted above, prediction of the I profile for levofloxacin had the most errors of any species-antimicrobial agent combination included in this EQA exercise, with only 12.6% of the interpretations correct and 87.4% MEs (I → R) among the 651 laboratories that reported these data. This result varied by AST method. There were four AST methodologies reported by at least four laboratories, among the 651 laboratories that reported their AST methodology for this species-antimicrobial agent combination. The best performing methodology was broth microdilution, with 48.7% correct interpretations. It was used by 39 (6%) laboratories. By contrast, the worst performing methodology, 'disk/tablet diffusion' tests, was also the most frequently reported methodology (n=221/651; 34% laboratories). Only 6.3% results from this methodology had the correct interpretation. This might indicate that resistance to levofloxacin may be overestimated in the EU/EEA for *P. aeruginosa* strains with I resistance.

These results suggest that laboratories should review their methodologies concerning the performance and interpretation of fluoroquinolone susceptibility testing results for *P. aeruginosa*, due to inherent difficulties associated with these tests.

Strain **2022 EARS-Net 6 (*Acinetobacter baumannii*)** was resistant to imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin and tobramycin; and susceptible to amikacin and colistin. None of the included antimicrobial agents had expected MIC values in the I range.

In total, 98.8% (839/849) of laboratories correctly identified the species of this strain, and, overall, the reported interpretations were in good concordance with expected results (89.4%). MEs and VMEs were observed for 0.3% and 10.3% of the reported interpretations, respectively.

There was a 'good' level of concordance with the expected results for this strain, for most of the reported AST methodologies (>85% of concordance for all assessed antimicrobial agents/groups). The two exceptions were AST results reported from 'automated systems' which achieved 'satisfactory' concordance (83.8%), and 'other methods', which did not achieve satisfactory concordance (74.2%).

Characterisation of resistance to tobramycin and gentamicin was challenging for this strain. Most of the deviations can be attributed to the inherent method variability and are within the acceptable variation range. These deviations correspond to VMEs (R → S) and might indicate that resistance to these aminoglycosides may be under-reported in the EU/EEA.

These results suggest that laboratories should review their methodologies for the performance of aminoglycoside susceptibility testing results for *Acinetobacter* spp., and the interpretation of results, as these can vary according to medium composition.

Overall, in the 2022 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 antimicrobial resistance among the participating laboratories, but rather deviations restricted to specific species-antimicrobial agent combinations included in the EQA exercise.

Some of the AST challenges identified in the EQA exercises in 2018–2021 remained present in the 2022 EARS-Net EQA exercise, such as the AST of *S. pneumoniae* for penicillin and cephalosporins, and AST of *E. coli* for piperacillin-tazobactam and ceftazidime. The most problematic issue detected in the 2022 EARS-Net EQA exercise was AST of *P. aeruginosa* for levofloxacin. This was not detected in the 2018 or 2019 EARS-Net EQA exercises, which both included this species-antimicrobial agent combination, potentially due to the different EQA methodologies. The two previous EQA exercises both defined the expected AST results according to the consensus of the AST results reported by the participating laboratories. By contrast, in the 2022 EARS-Net EQA exercise, the expected AST results were defined before the strains were sent to participating laboratories, according to consensus results from three pre-selected reference laboratories.

As standard practice, laboratories should confirm that their laboratory protocols are in accordance with the latest EUCAST recommendations and guidelines, applying current 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 2022.

In 2022, 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 2022 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 2022 EARS-Net EQA protocol[2] specified that laboratories should perform AST according to their routine procedures, using methodologies 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 three exceptions. Firstly, reporting of results for colistin was not included for any species. Secondly, ofloxacin was not included for *S. aureus* as there is no corresponding breakpoint in the EUCAST Clinical Breakpoints v12.0. Finally, norfloxacin was not included for *E. coli* as the breakpoint is only applicable to uncomplicated urinary tract infections.

The overwhelming majority of clinical laboratories in the EU/EEA are unlikely to perform, as standard practice, AST on every species-antimicrobial agent combination that can be reported to EARS-Net. For example, many will utilise 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 2022 EQA exercise, the species of one of the six EQA strains (2022 EARS-Net 3) was *P. putida*, which is not under surveillance in EARS-Net. This implied that participating laboratories did not need to report AST results for this strain (see below). The other five EQA strains are all 'EARS-Net species' (*S. pneumoniae, S. aureus, E. coli, P. aeruginosa* and *A. baumannii*), selected from the strain collection at DTU FOOD, based on their AMR profiles.

Participating laboratories were requested to consider the sample '2022 EARS-Net 1' (*S. pneumoniae*) as being obtained from the cerebrospinal fluid of a patient with clinical manifestations suggesting meningitis, and the other strains (*E. coli*, *S. aureus*, *P. aeruginosa, A. baumannii* and *P. putida*) as being from patients with bloodstream infections.

The EUCAST Clinical Breakpoints Tables v12.0[3] 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 two other reference laboratories. These were the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory, Växjö, Sweden, and by the Centre for Disease Control and Prevention (CDC), Georgia, United States of America. Subsequently, the consensus phenotypic AST profile was compared with whole-genome sequencing (WGS) data on acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs), obtained at DTU FOOD using the bioinformatics tools ResFinder v4.1 and CARD RGI (Tables 1–5). 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.

[2] 2022 EARS-Net EQA protocol: https://antimicrobialresistance.dk/ears-net-EQA.aspx

[3] EUCAST clinical breakpoints: https://www.eucast.org/clinical_breakpoints

Table 2. EUCAST clinical breakpoints for *Streptococcus pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2022 EARS-Net 1' (*S. pneumoniae*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected result**	Expected interpretation	ARGs and PMs***
	MIC (mg/L)		Zone diameter (mm)					
	S ≤	R >	S ≥	R <				
Azithromycin	0.25	0.5	Note	Note	Easy	0.125 mg/L	S	ND
Benzylpenicillin	0.06	0.06	Note	Note	Easy	2 mg/L	R	ND
Cefotaxime	0.5	0.5	Note	Note	Difficult	0.5 mg/L	S	ND
Ceftriaxone	0.5	0.5	Note	Note	Difficult	0.5 mg/L	S	ND
Clarithromycin	0.25	0.5	Note	Note	Easy	0.06 mg/L	S	ND
Erythromycin	0.25	0.5	22	19	Easy	0.06 mg/L	S	ND
Levofloxacin	0.001	2	50	16	Easy	1 mg/L	I	ND
Moxifloxacin	0.5	0.5	22	22	Easy	0.25 mg/L	S	ND
Norfloxacin	NA	NA	10	10	Easy	18 mm	S	ND
Oxacillin	NA	NA	20	Note	Easy	6 mm	R	ND

ND: Not detected. Note: Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**The level of difficulty indicates the magnitude of the 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 the breakpoint was new or recently changed.*
*** If the EUCAST clinical breakpoint tables recommends a zone diameter test, the result is shown as 'mm'. Otherwise, the EUCAST tables recommend a MIC, and the test results is therefore displayed as 'mg/L'.*
****Antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) detected in the Streptococcus pneumoniae strain through analysis with ResFinder 4.1 or CARD RGI. Additional ARGs or chromosomal PMs: None. MALDI-TOF by DTU: S. pneumoniae (score 2.24), and MLST: ST558.*

Table 3. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2022 EARS-Net 2' (*E. coli*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		zone diameter (mm)					
	S ≤	R >	S ≥	R <				
Amikacin	8	8	18	18	Difficult	>8 mg/L	R	*aac(6')-Ib-cr*
Amoxicillin	8	8	Note	Note	Easy	>32 mg/L	R	bla_{OXA-1} and $bla_{CTX-M-15}$
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>32/2 mg/L	R	bla_{OXA-1}
Ampicillin	8	8	14	14	Easy	>32 mg/L	R	bla_{OXA-1} and $bla_{CTX-M-15}$
Cefepime	1	4	27	24	Difficult	1 mg/L	S	bla_{OXA-1} and $bla_{CTX-M-15}$
Cefotaxime	1	2	20	17	Easy	16 mg/L	R	$bla_{CTX-M-15}$
Ceftazidime	1	4	22	19	Difficult	2 mg/L	I	$bla_{CTX-M-15}$
Ceftriaxone	1	2	25	22	Easy	>8 mg/L	R	$bla_{CTX-M-15}$
Ciprofloxacin	0.25	0.5	25	22	Easy	>8 mg/L	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Colistin****	2	2	Note	Note	Easy	0.5 mg/L	S	ND
Ertapenem	0.5	0.5	25	25	Easy	≤0.015 mg/L	S	ND
Gentamicin	2	2	17	17	Easy	1 mg/L	S	ND
Imipenem	2	4	22	19	Easy	≤0.125 mg/L	S	ND
Levofloxacin	0.5	1	23	19	Easy	>8 mg/L	R	*aac(6')-Ib-cr, gyrA* S83L, *gyrA* D87N, parC S80I, *parC* E84V, *parE* I529L
Meropenem	2	8	22	16	Easy	≤0.03 mg/L	S	ND
Moxifloxacin	0.25	0.25	22	22	Easy	>4 mg/L	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Ofloxacin	0.25	0.5	24	22	Easy	>2 mg/L	R	*aac(6')-Ib-cr*, *gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* E84V, *parE* I529L
Piperacillin-tazobactam***	8	8	20	20	Difficult	8/4 mg/L	S	bla_{OXA-1}
Tigecycline	0.5	0.5	18	18	Easy	0.125 mg/L	S	ND
Tobramycin	2	2	16	16	Easy	>16 mg/L	R	*aac(6')-Ib-cr*

ND: Not detected. Note: Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**The level of difficulty indicates the magnitude of the 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 the breakpoint was new or recently changed.*
***Antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) detected in the Escherichia coli strain through analysis with ResFinder 4.1 or CARD RGI. Additional ARGs or chromosomal PMs: dfrA17, sul1, catB3, aadA5, GlpT E448K, PtsI V25I, UhpT E350Q, EF-Tu R234F, AcrAB-TolC Y137H, AcrAB-TolC G103S. MALDI-TOF by DTU: E. coli (score 2.33), and MLST: ST131 (E. coli #1) / ST43 (E. coli #2).*
**** Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4mg/L tazobactam.*
***** Reporting results for colistin was not mandatory.*

Table 4. EUCAST clinical breakpoints for *Staphylococcus aureus* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2022 EARS-Net 4' (*S. aureus*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected result**	Expected interpretation	ARGs and PMs***
	MIC (mg/L)		zone diameter (mm)					
	S ≤	R >	S ≥	R <				
Cefoxitin	Note	Note	22	22	Easy	15 mm	R	*mecC*
Ciprofloxacin	0.001	1	50	21	Easy	0.25 mg/L	I	ND
Daptomycin	1	1	Note	Note	Difficult	1 mg/L	S	ND
Levofloxacin	0.001	1	50	22	Easy	0.25 mg/L	I	ND
Linezolid	4	4	21	21	Easy	2 mg/L	S	ND
Norfloxacin	NA	NA	17	17	Easy	21 mm	S	ND
Oxacillin	Note	Note	Note	Note	Difficult	4 mg/L	R	*mecC*
Rifampicin	0.06	0.06	26	26	Easy	≤0.008 mg/L	S	ND
Vancomycin	2	2	Note	Note	Easy	1 mg/L	S	ND

ND: Not detected. Note: Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**The level of difficulty indicates the magnitude of the 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 the breakpoint was new or recently changed.*
***If the EUCAST clinical breakpoint tables recommends a zone diameter test, the result is shown as 'mm'. Otherwise, the EUCAST tables recommend a MIC, and the test results is therefore displayed as 'mg/L'.*
****Antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) detected in the Staphylococcus aureus strain through analysis with ResFinder 4.1 or CARD RGI. Additional ARGs or chromosomal PMs:GlpT A100V, murA E291D, murA T396N. MALDI-TOF by DTU: S. aureus (score 2.33), and MLST: ST130.*

Table 5. EUCAST clinical breakpoints for *Pseudomonas aeruginosa* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2022 EARS-Net 5' (*P. aeruginosa*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		MIC (mg/L)					
	S ≤	R >	S ≥	R <				
Amikacin	16	16	15	15	Easy	4 mg/L	S	ND
Cefepime	0.001	8	50	21	Easy	32 mg/L	R	$bla_{OXA-485/488}$
Ceftazidime	0.001	8	50	17	Easy	>32 mg/L	R	$bla_{OXA-485/488}$
Ciprofloxacin	0.001	0.5	50	26	Difficult	1 mg/L	R	ND
Colistin***	4	4	Note	Note	Easy	1 mg/L	S	ND
Imipenem	0.001	4	50	20	Easy	1 mg/L	I	ND
Levofloxacin	0.001	2	50	18	Difficult	2 mg/L	I	ND
Meropenem	2	8	24	14	Easy	0.5 mg/L	S	ND
Piperacillin	0.001	16	50	18	Easy	>128 mg/L	R	$bla_{OXA-485/488}$
Piperacillin-tazobactam****	0.001	16	50	18	Easy	>128/4 mg/L	R	$bla_{OXA-485/488}$
Tobramycin	2	2	18	18	Easy	0.5 mg/L	S	ND

ND: Not detected. Note: Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**The level of difficulty indicates the magnitude of the 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 the breakpoint was new or recently changed.*
***Antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) detected in the Pseudomonas aeruginosa strain through analysis with ResFinder 4.1 or CARD RGI. Additional ARGs or chromosomal PMs: fosA, catB7, aph(3')-IIb, bla_{PAO}, nalC S209R, nalC G71E. MALDI-TOF by DTU: P. aeruginosa (score 2.33), and MLST: ST1633.*
**** Reporting results for colistin was not mandatory.*
***** Reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4mg/L tazobactam.*

Table 6. EUCAST clinical breakpoints for and the expected MIC value, level of difficulty in interpretation and interpretation for strain '2022 EARS-Net 6' (*Acinetobacter baumannii*), by antimicrobial agent

Antimicrobial agent	EUCAST clinical breakpoints				Level of difficulty*	Expected MIC result	Expected interpretation	ARGs and PMs**
	MIC (mg/L)		MIC (mg/L)					
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Easy	2 mg/L	S	ND
Ciprofloxacin	0.001	1	50	21	Easy	>8 mg/L	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin***	2	2	Note	Note	Easy	0.5 mg/L	S	ND
Gentamicin	4	4	17	17	Easy	16 mg/L	R	*ant(2'')-Ia*
Imipenem	2	4	24	21	Easy	16 mg/L	R	bla_{OXA-23}
Levofloxacin	0.5	1	23	20	Easy	4 mg/L	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	32 mg/L	R	bla_{OXA-23}
Tobramycin	4	4	17	17	Difficult	8 mg/L	R	*ant(2'')-Ia*

ND: Not detected. Note: Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**The level of difficulty indicates the magnitude of the 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 the breakpoint was new or recently changed.*
***Antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) detected in the Acinetobacter baumannii strain through analysis with ResFinder 4.1 or CARD RGI. Additional ARGs or chromosomal PMs: floR, sul2, tet(B), tet(G), aadA2b, aph(3'')-Ib, aph(6)-Id, bla_{CARB-2}, bla_{ADC-25}, $bla_{OXA-429}$. MALDI-TOF by DTU: A. baumannii (score 2.4), and MLST: ST1780 (A. baumannii #1) / ST764 (A. baumannii #2). ***Reporting results for colistin was not mandatory.*

Procedure for participating laboratories

The 2022 EARS-Net EQA protocol[4] specified that participating laboratories should identify the species of six bacterial strains, and then perform AST, following EUCAST recommendations[5], 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 2022 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 when performing AST can participate in the EARS-Net EQA exercise.

Distribution of EQA strains to laboratories

On 9 June 2022, an overpack was shipped to the National EARS-Net EQA Coordinator according to International Air Transport Association regulations (UN3373, biological substances category B), contained individual packages for distribution nationally. Each package was labelled with the address of a laboratory that had enrolled to participate. Every package (double pack containers (class UN 6.2)) contained six swabs (Copan Transystem™ or Stuarts transport media) 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.

[4] 2022 EARS-Net EQA protocol: https://antimicrobialresistance.dk/ears-net-EQA.aspx
[5] EUCAST recommendations: https://www.eucast.org/ast_of_bacteria

Reporting EQA results

The 2022 EARS-Net EQA protocol, test forms and a guideline on how to access the password protected webpage were available on the EARS-Net EQA website (antimicrobialresistance.dk/ears_net_EQA.aspx).

DTU FOOD also developed and hosted a dedicated password-protected EARS-Net EQA webpage for participating laboratories to submit EQA results for evaluation, using a personal login and password.

The EQA protocol specified that participants 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 (v12.0). They were also asked to provide information about the standard guideline they used, the methodology used to undertake 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 15 August 2022, however the submission period was extended until 19 August 2022. After submission of results, an email was automatically forwarded to all contacts from the respective laboratory with an attached 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 reported AST results were not evaluated for that strain.

The 2022 EARS-Net EQA protocol specified a new scoring system for the evaluation of submitted results (Table 7). It assigned scores for each species-antimicrobial agent combination based on the 'level of difficulty', the 'severity of error' of the AST, and whether or not the result was reported.

The level of difficulty indicated the magnitude of the 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: VME, 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 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 score than if the expected susceptibility category had been reported (Table 7).

If a laboratory did not report an AST result for a species-antimicrobial agent combination (see the section 'Antimicrobial susceptibility testing and selected antimicrobial agents'), this generated a negative score. This report presents the total scores, for all participating laboratories, by EQA strain, with and without application of the negative system.

By contrast, the laboratory-level feedback reports did not include a calculation of the total score for that laboratory. This is because that might give the incorrect impression that every clinical laboratory should perform AST, as standard practice, on every combination that can be reported to EARS-Net.

Moreover, total scores cannot always be compared between laboratories. For example, a laboratory that performed excellently, reporting correct AST interpretations for a subset of species-antimicrobial agent combinations could achieve the same score as laboratory that tested more combinations, and reported some incorrect interpretations. The EQA protocol recommended that laboratories analyse scores for each species-antimicrobial agent combination individually, and National EARS-Net EQA Coordinators received the raw data with the scores, to enable national analyses that incorporate appropriate knowledge of the (sub-)national setting.

It is important to note that the 2022 EARS-Net EQA methodology does not aim to provide information on the appropriateness of laboratory practices, because a survey is not the ideal methodology to acquire robust

supporting data. The survey would need to collect laboratory-level data in each laboratory, for comparison to available national guidelines. Such data would benefit from national validation; but it was not possible to include this as a secondary objective in the EARS-Net EQA exercise, and would be better suited for a separate targeted activity. Similarly, a methodological challenge for EARS-Net EQA exercises historically, has been the definition of an appropriate minimum set of species–antimicrobial agent combinations, that is appropriate for all (sub-)national settings in all 30 EU/EEA countries. In 2022, the EARS-Net DNCC discussed potentially equivalent AST tests, that might reduce the list of included species-antimicrobial agent combinations. No such reductions were identified, largely in recognition of the variety of valid laboratory practices across the EU/EEA. 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, and all results that were not reported received a negative score.

Table 7. 2022 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 (included antimicrobial agents)	-4	-4	-4	-1	-1	-1
Not reported (other antimicrobial agents)*	0	0	0	0	0	0

R: resistant; I: susceptible, increased exposure; S: susceptible, standard dosing regimen;
VME: very major error; ME: major error.
** Colistin was not included for any pathogen, ofloxacin was not included for S. aureus (no corresponding breakpoint in the EUCAST Clinical Breakpoints v12.0), norfloxacin was not included for E. coli (breakpoint only applicable to uncomplicated urinary tract infections).*

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 2022 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. Contacts only had access to the evaluation reports from their own laboratory.

The individual laboratory evaluation reports from each country were also shared with the National EARS-Net EQA Coordinators together with a detailed, country-specific national summary 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 when relevant. Participating laboratories were identified by codes known by the corresponding laboratory, the National EARS-Net EQA Coordinator and the EQA provider. A national database with all the reported results and a list connecting the anonymised laboratory identification numbers with the corresponding laboratory was also shared with the National EARS-Net EQA Coordinators. ECDC received the anonymised national summary reports as well as a database containing all submitted results.

Laboratories acquired a 'certificate for participation' if they had reported AST results for the five EQA strains that would be reportable to EARS-Net surveillance. 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.

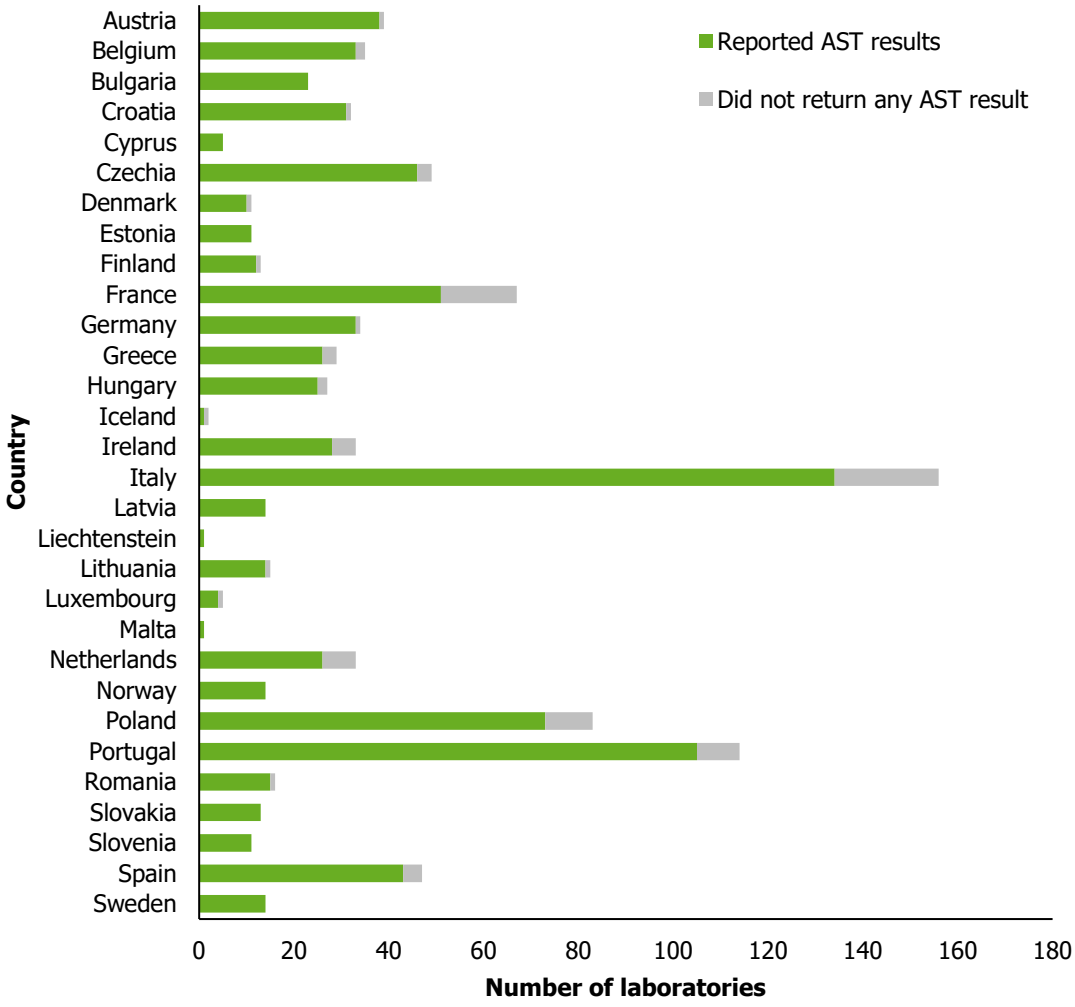
3. Results

Participation

In 2022, all 30 EU/EEA countries participated in the EARS-Net EQA exercise. National EARS-Net EQA Coordinators sent invitations to the 949 laboratories that they had identified, of which 948 (99.9%) laboratories enrolled. Subsequently, National EARS-Net EQA Coordinators received packages from DTU FOOD for each of these 948 laboratories, containing the six EQA strains for analysis.

All 948 laboratories that had enrolled received two reminder emails in the weeks approaching the submission deadline. When the deadline passed, the EQA website database contained analysable data submitted by 855 laboratories (90.3%) from 30 countries (Figure 1). The reasons to exclude laboratories from analysis included use of CLSI guidelines for AST (n=1 laboratory), or entry of data without AST interpretations. One laboratory reported using the 'NordicAST guideline', which is based on EUCAST guidelines, and so this laboratory was included. Also, eight (0.8%) laboratories in six countries (Czechia (n=2), Finland, France (n=2), Latvia, Luxembourg, and Poland) entered results onto the EQA website, for all five EQA strains that are species included in EARS-Net surveillance, but did not finalise submission of the results on the website, and so their data could not be validated. Overall, results were evaluated for 854 laboratories, corresponding to 90.1% of all laboratories that received the EQA strains. The majority of the laboratories that received EQA materials submitted EQA results for the five species under surveillance in EARS-Net (n=834; 88.0%), which was the minimal criteria to receive a certificate of participation. Most of these laboratories submitted results for all six EQA strains (n=817; 86.2%).

Figure 1. Number of participating laboratories returning external quality assessment results based on EUCAST guidelines, by country, 2022 EARS-Net EQA exercise



AST: antimicrobial susceptibility testing

Species identification results

Species identification results were submitted for 5 070 strains by 854 laboratories and 95.7% were correct (4 853 strains). Therefore, there was an overall 'excellent' concordance between the submitted and the expected results.

An overview of the species identification for the six strains and the number of laboratories reporting the correct identification is provided in Table 8. There was excellent concordance (≥95%) between the submitted species identification and the expected results for all five EQA strains belonging to species included in EARS-Net surveillance. The lowest level of concordance was reported for strain '2022 EARS-Net 3' *P. putida* (80.1%), which is the only strain belonging to a species not included in EARS-Net surveillance.

Table 8. Number and percentage of laboratories reporting the correct species in the 2022 EARS-Net EQA exercise

Strain ID	Expected species	No. of reporting laboratories	No. of laboratories reporting correct species identification	% of laboratories reporting correct species identification
2022 EARS-Net 1	*Streptococcus pneumoniae*	839	820	97.7
2022 EARS-Net 2	*Escherichia coli*	851	845	99.3
2022 EARS-Net 3	*Pseudomonas putida*	834	668	80.1
2022 EARS-Net 4	*Staphylococcus aureus*	848	840	99.1
2022 EARS-Net 5	*Pseudomonas aeruginosa*	849	841	99.1
2022 EARS-Net 6	*Acinetobacter baumannii*	849	839	98.8

Antimicrobial susceptibility testing AST results

AST results were evaluated for strains with correct species identification, for species included in EARS-Net surveillance. Therefore, strain '2022 EARS-Net 3' (*P. putida*) was not evaluated.

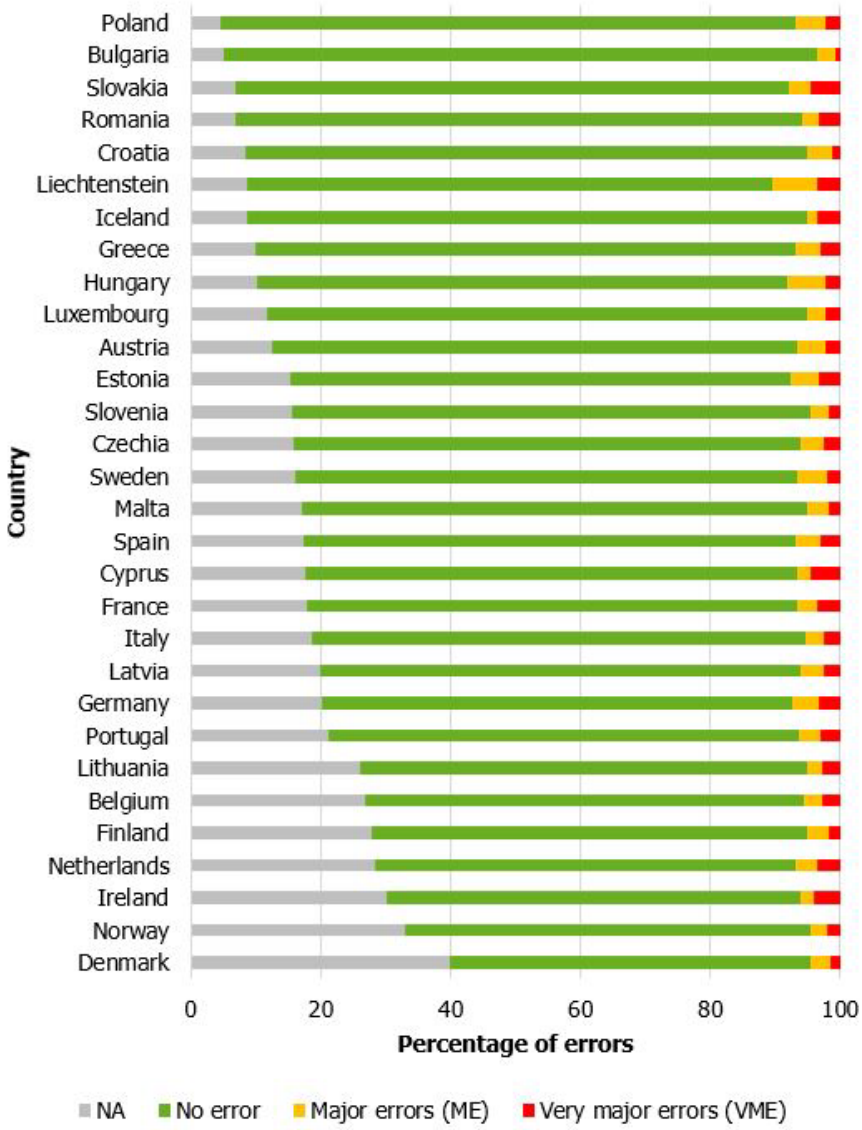
Out of the 854 laboratories that submitted EQA results, 850 laboratories submitted AST result interpretations and were analysed, two laboratories were excluded because all species identification results were wrong, and two did not submit any AST result interpretations.

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

In 2022, if every participating laboratory had reported data for every species-antimicrobial agent combination, including for colistin, there would be 48 225 results. The participating laboratories reported 39 925 AST result interpretations (Figure 2), which equates to 82.8% of that theoretical maximum.

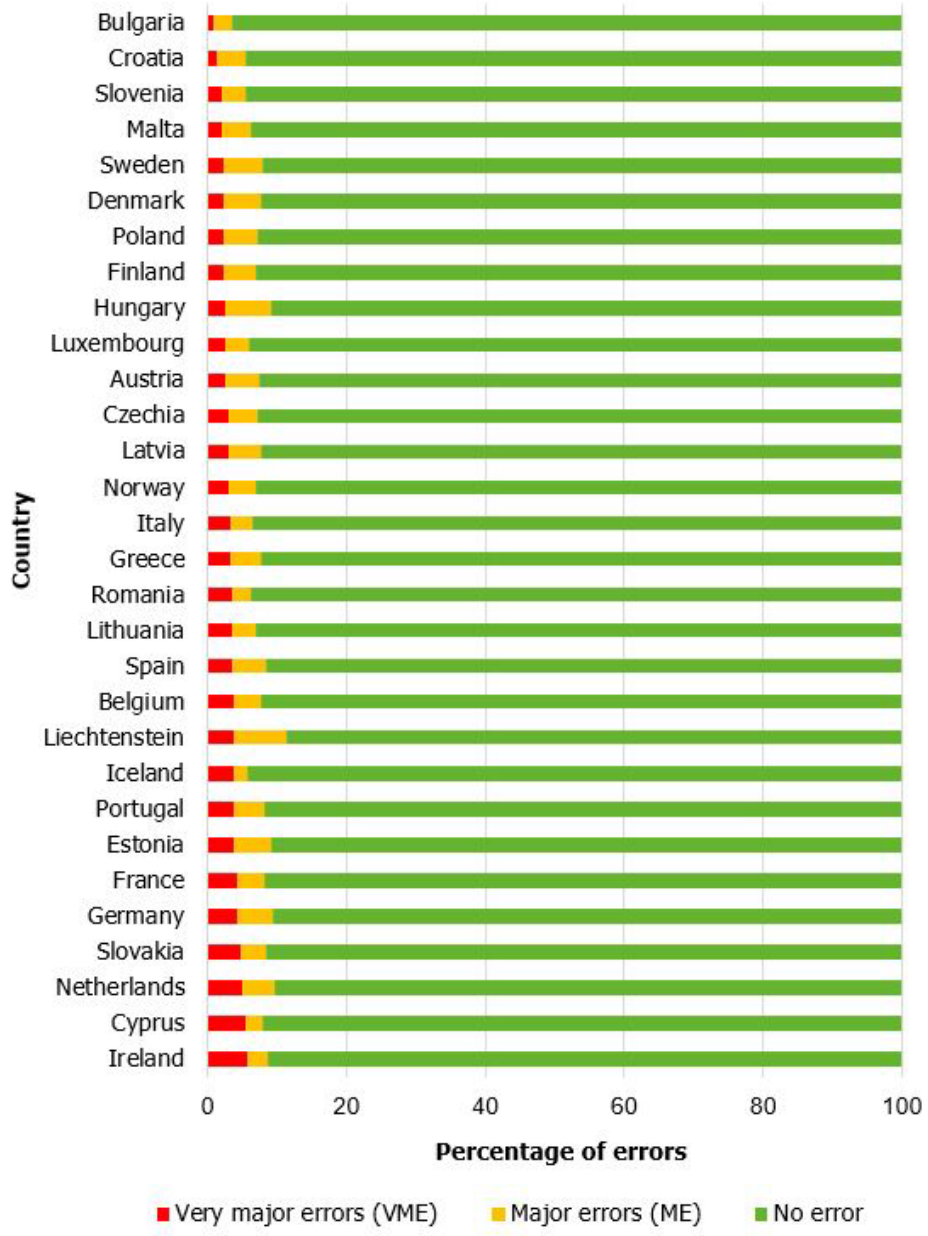
Overall, the interpretations were in 'very good concordance' with 92.6% (n=36 962) of the 39 925 reported interpretations (including colistin) being correct (Figure 3). Concordance varied by country from 88.7% ('good') to 96.4% ('excellent'). MEs were observed for 4.2% (n=1 676) of the reported interpretations (country range: 1.9% to 7.5%), and VMEs were observed for 3.2% (n=1 281; country range: 0.8% to 5.7%) for the 30 countries.

Figure 2. Reported interpretation of AST results, by country, 2022 EARS-Net EQA exercise, sorted by country according to the proportion of species-antimicrobial agent combinations with no reported results



AST: antimicrobial susceptibility testing; NA: not applicable (e.g. no data)

Figure 3. Reported interpretation of AST results excluding non-responses, by country, 2022 EARS-Net EQA exercise, sorted by country according to the proportion of AST results that were very major errors

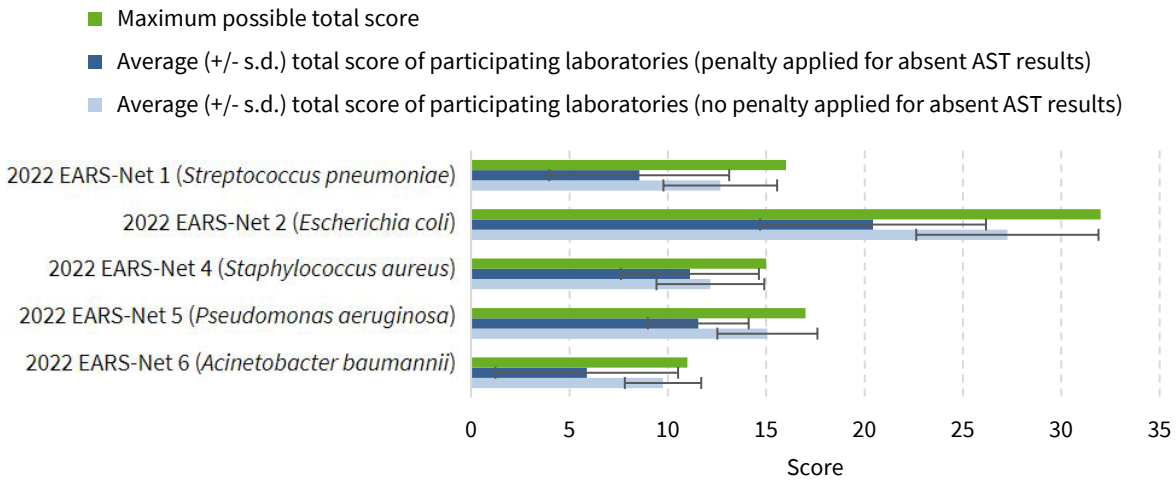


AST: antimicrobial susceptibility testing

The maximum possible score that participants could obtain for the five strains, if AST results for all species-antimicrobial agent combinations were submitted and correct, was 91. For the 850 laboratories submitting results for analysis, the average score was 23.2±36.4 when including the penalty given if results on mandatory antimicrobial agents were omitted. The average scores for submitted results without the penalty was 56.6±14.1.

Figure 4 presents three scores for each of the five strains. The first score is the maximum score that the participating laboratories could have attained, if they had reported their interpretation for every species-antimicrobial agent combination in the EQA protocol, and if all of their interpretations had been correct. The other two scores are the average of the laboratory-level scores, with or without application of a negative score for 'missing' (non-reported) species-antimicrobial agent combinations (Table 7).

Figure 4. Maximum possible score, and average total scores, for the AST results reported by participating laboratories, by EQA strain, 2022 EARS-Net EQA exercise



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

Tables 9 to present the distribution of the methods used per stains and the percentage of correct interpretations for each method. The most commonly used method was an automated system (51.4%), followed by disk or tablet diffusion (28.1%) and MIC methods including broth microdilution and gradient test (19.3%) (Table 11). Very good concordance was observed for macro broth dilution (93.9%), broth microdilution (92.7%) and disk or tablet diffusion (90.7%), and good concordance was observed for agar dilution (89.8%), automated system (87.8%) and gradient test (86.1%).

Table 9. Overview of methods used for determination of the AST results for strains '2022 EARS-Net 1' and '2022 EARS-Net 2'

Method	2022 EARS-Net 1 *Streptococcus pneumoniae*			2022 EARS-Net 2 *Escherichia coli*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	21	0.3	100.0	37	0.3	81.1
Automated system	2 238	37.2	78.1	7 268	53.6	87.5
Broth microdilution	272	4.5	84.6	1 325	9.8	90.6
Disk/Tablet diffusion	1 771	29.4	95.0	3 867	28.5	85.4
Gradient test	1 619	26.9	80.8	943	7.0	88.4
Macro broth dilution (tubes)	-	-	-	8	0.1	100.0
Other	103	1.7	93.2	110	0.8	88.2
Total	**6 024**	**100.0**	**84.4**	**13 558**	**100.0**	**87.3**

Percentage may not total 100% due to rounding.

Table 10. Overview of methods used for determination of the AST results for strains '2022 EARS-Net 4' and '2022 EARS-Net 5'

Method	2022 EARS-Net 4 *Staphylococcus aureus*			2022 EARS-Net 5 *Pseudomonas aeruginosa*		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	26	0.4	80.8	21	0.3	90.5
Automated system	3 287	52.9	93.9	4 601	56.4	91.4
Broth microdilution	345	5.6	95.7	1 004	12.3	95.3
Disk/Tablet diffusion	1 893	30.5	95.8	2 120	26.0	87.0
Gradient test	612	9.8	98.2	350	4.3	80.0
Macro broth dilution (tubes)	8	0.1	75.0	8	0.1	100.0
Other	43	0.7	81.4	53	0.6	86.8
Total	**6 214**	**100.0**	**94.8**	**8 157**	**100.0**	**90.2**

Percentage may not total 100% due to rounding.

Table 11. Overview of methods used for determination of the AST results for strains '2022 EARS-Net 6' and total results for all EQA strains

Method	2022 EARS-Net 6 *Acinetobacter baumannii*			Total		
	No. of tests performed	% of total tests performed	% correct interpretations	No. of tests performed	% of total tests performed	% correct interpretations
Agar dilution	24	0.4	100.0	**129**	**0.3**	**89.1**
Automated system	3 118	52.2	83.9	**20 512**	**51.4**	**87.8**
Broth microdilution	867	14.5	94.3	**3 813**	**9.6**	**92.7**
Disk/Tablet diffusion	1 586	26.6	97.6	**11 237**	**28.1**	**90.7**
Gradient test	337	5.6	89.6	**3 861**	**9.7**	**86.1**
Macro broth dilution (tubes)	9	0.2	100.0	**33**	**0.1**	**93.9**
Other	31	0.5	74.2	**340**	**0.9**	**87.4**
Total	**5 972**	**100.0**	**89.4**	**39 925**	**100.0**	**88.9**

Percentages might not total 100% due to rounding.

Strain '2022 EARS-Net 1' (*Streptococcus pneumoniae*)

The *S. pneumoniae* EQA strain ('2022 EARS-Net 1') was described as being obtained from cerebrospinal fluid from a patient with clinical manifestations suggesting meningitis. Therefore, AST results were interpreted according to clinical breakpoints referring to meningitis, when applicable. This strain was resistant to benzylpenicillin and oxacillin (Table 2). The strain was susceptible to cefotaxime, ceftriaxone, moxifloxacin, norfloxacin, azithromycin, clarithromycin and erythromycin, and the expected MIC value for levofloxacin was in the I range (Table 2). The level of difficulty was considered 'difficult' for cefotaxime and ceftriaxone 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'.

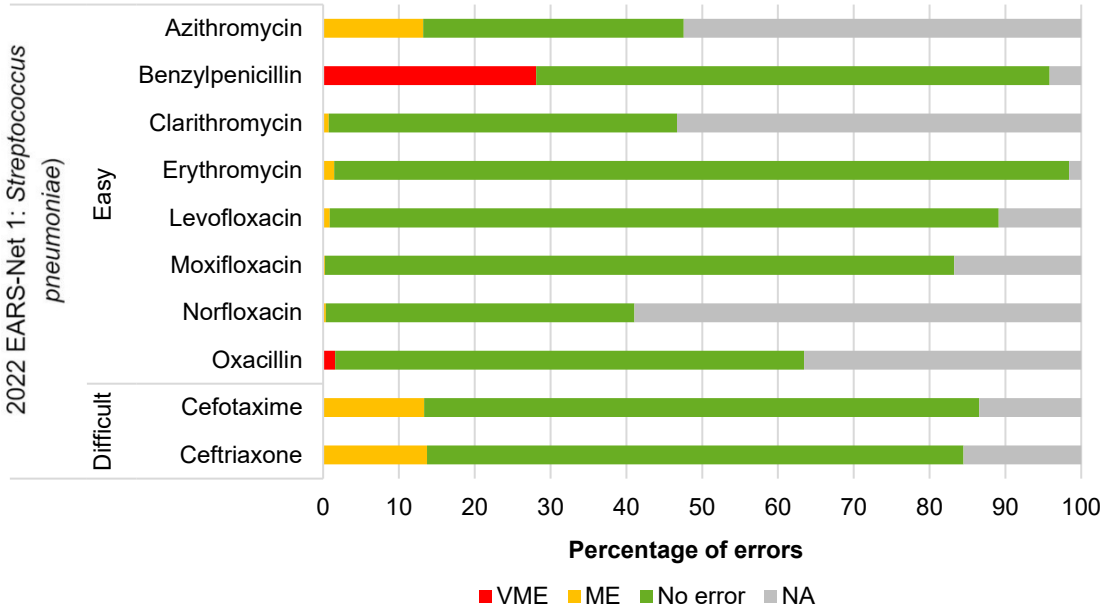
Interpretation of AST results for the *S. pneumoniae* strain were analysed for the 820 laboratories with correct species identification (Table 8). In total, 54.4% of the laboratories (n=446) would have sent the strain to a reference or other laboratory for further testing. In total, 6 024 tests were performed, and 5 422 reported interpretations were correct. Thus, the reported interpretations were in good concordance with expected results (90.0%) (Table 12). MEs were observed for 6.0% (n=359) of the reported interpretations and VMEs were observed for 4.0% (n=243) of the reported interpretations.

The following methodologies were applied: automated systems (37.2%), disk or tablet diffusion (29.4%), gradient test (26.9%), broth microdilution (4.5%), agar dilution (0.3%) and 'other methods' (1.7%) (Table 9). Overall, most methodologies achieved, as a minimum, a good level of concordance with the expected results (>85% of concordance). The exception was gradient test, which achieved a satisfactory concordance (83.5%).

VMEs were observed for benzylpenicillin and oxacillin (Figure 5). For benzylpenicillin, VMEs represented 29.3% of all submitted interpretations for this antimicrobial agent and were reported for almost all methods, except agar dilution (Table 12). For oxacillin, VMEs represented 2.5% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system, disk or tablet diffusion and 'other methods' (Table 12).

A high proportion of MEs was observed for azithromycin (27.8% of submitted results) and were reported for all methods (Figure 5, Table 12). Lower proportions of MEs were observed for ceftriaxone (16.2%) and cefotaxime (15.4%). These were reported for almost all methods, except agar dilution for ceftriaxone and 'other methods' for cefotaxime. For the remaining antimicrobial agents, there were none or very low proportions of VMEs or MEs (Figure 5, Table 12).

Figure 5. Reported interpretation of AST results for strain '2022 EARS-Net 1' (*Streptococcus pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



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

Table 12. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2022 EARS-Net 1' (*Streptococcus pneumoniae*), by antimicrobial agent and AST methodology

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	%
Azithromycin	-	-	83	96.4	16	93.8	75	90.7	178	46.1*	-	-	37	97.3	**389**	**72.2***
Benzylpenicillin	3	100.0	287	74.2*	60	75.0	21	66.7*	408	67.9*	-	-	5	40.0*	**784**	**70.7***
Cefotaxime	-	-	330	76.1*	45	84.4	20	90.0	307	93.2	-	-	6	100.0	**708**	**84.6**
Ceftriaxone	2	100.0	280	70.4*	43	79.1	26	88.5	336	95.2	-	-	4	75.0*	**691**	**83.8**
Clarithromycin	1	100.0	85	98.8	19	100.0	63	92.1	179	100.0	-	-	35	100.0	**382**	**98.4**
Erythromycin	4	100.0	384	97.9	36	100.0	326	98.8	54	100.0	-	-	1	100.0	**805**	**98.5**
Levofloxacin	3	100.0	377	98.9	27	100.0	228	99.6	90	97.8	-	-	4	100.0	**729**	**99.0**
Moxifloxacin	1	100.0	376	99.7	26	100.0	216	100.0	54	98.1	-	-	8	100.0	**681**	**99.7**
Norfloxacin	2	100.0	10	100.0	-	-	321	99.1	2	100.0	-	-	1	100.0	**336**	**99.1**
Oxacillin	5	100.0	26	96.2	-	-	475	97.7	11	100.0	-	-	2	50.0*	**519**	**97.5**
Total	**21**	**100.0**	**2 238**	**88.7**	**272**	**88.2**	**1 771**	**97.6**	**1 619**	**83.5**	**-**	**-**	**103**	**94.2**	**6 024**	**90.0**

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

Strain '2022 EARS-Net 2' (*Escherichia coli*)

The *E. coli* EQA strain ('2022 EARS-Net 2') was resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin, amikacin and tobramycin (Table 3). The strain was susceptible to piperacillin-tazobactam, cefepime, ertapenem, imipenem, meropenem, gentamicin, tigecycline and colistin, and the expected MIC value for ceftazidime was in the I range (Table 3). 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'.

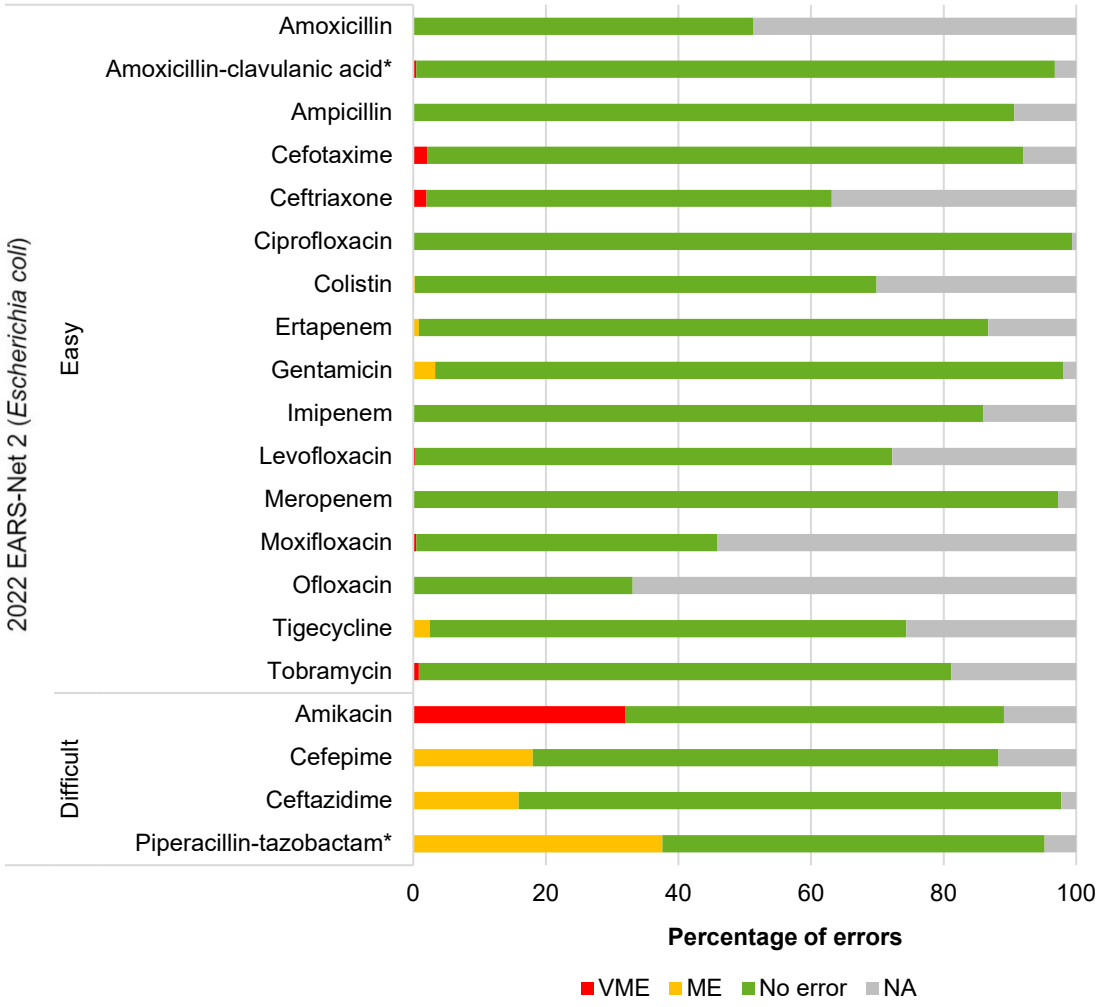
Interpretation of AST results for the *E. coli* strain were analysed for the 845 laboratories with correct species identification (Table 8). In total, 17.0% of the laboratories (n=144) would have sent the strain to a reference or other laboratory for further testing. In total, 13 558 tests were performed, and 12 572 reported interpretations were correct. Thus, the reported interpretations were in very good concordance with expected results (92.7%) (Table 13). MEs were observed for 4.9% (n=662) of the reported interpretations and VMEs were observed for 2.4% (n=324) of the reported interpretations.

The following methodologies were applied: automated systems (53.6%), disk or tablet diffusion (28.5%), broth microdilution (9.8%), gradient test (7.0%), agar dilution (0.3%), macro broth dilution (0.1%), and 'other methods' (0.8%) (Table 9). Overall, all methodologies achieved, as a minimum, a good level of concordance with the expected results (>85% of concordance).

VMEs were observed for amikacin, ceftriaxone, cefotaxime, tobramycin, moxifloxacin, amoxicillin-clavulanic acid, levofloxacin, ciprofloxacin and ampicillin (Figure 6). For amikacin, VMEs represented 36.0% of all submitted interpretations for this antimicrobial agent and were reported for all methods (Table 13). For ceftriaxone, VMEs represented 3.2% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system, disk or tablet diffusion and gradient test. For cefotaxime, VMEs represented 2.3% of all submitted interpretations for this antimicrobial agent and were reported for almost all methods, except agar dilution. For tobramycin, VMEs represented 1.0% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system, broth microdilution, disk or tablet diffusion and gradient test. For moxifloxacin, VMEs represented 1.0% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system and disk or tablet diffusion. For amoxicillin-clavulanic acid, VMEs represented 0.5% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system and broth microdilution. For levofloxacin, VMEs represented 0.3% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system. For ciprofloxacin, VMEs represented 0.1% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system. For ampicillin, VMEs represented 0.1% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system (Table 13).

A high proportion of MEs were observed for piperacillin-tazobactam (39.5% of submitted results) and for cefepime (20.4% of submitted results) and were reported for all methods (Figure 6, Table 13). Lower proportions of MEs were observed for ceftazidime (16.3%) and were reported for all methods. For the remaining antimicrobial agents, there were very low proportions of or no VMEs or MEs (Figure 6, Table 13).

Figure 6. Reported interpretation of AST results for strain '2022 EARS-Net 2' (*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 test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.*

Table 13. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2022 EARS-Net 2' (*Escherichia coli*), by antimicrobial agent and AST methodology

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	50.0*	432	69.0	69	40.6*	202	63.9*	43	53.5*	-	-	3	66.7*	**751**	**64.0***
Amoxicillin	1	100.0	150	100.0	19	100.0	85	100.0	153	100.0	-	-	24	100.0	**432**	**100.0**
Amoxicillin-clavulanic acid **	2	100.0	493	99.4	47	97.9	240	100.0	30	100.0	-	-	4	100.0	**816**	**99.5**
Ampicillin	2	100.0	448	99.8	39	100.0	238	100.0	32	100.0	-	-	5	100.0	**764**	**99.9**
Cefepime	2	50.0*	449	84.2	56	91.1	189	63.5*	43	95.3	-	-	5	20.0*	**744**	**79.6***
Cefotaxime	2	100.0	468	99.4	57	94.7	205	95.6	38	94.7	-	-	6	83.3	**776**	**97.7**
Ceftazidime	2	50.0*	493	85.0	72	87.5	216	79.2*	37	91.9	-	-	4	50.0*	**824**	**83.7**
Ceftriaxone	2	100.0	157	98.1	27	100.0	218	97.7	121	92.6	-	-	7	100.0	**532**	**96.8**
Ciprofloxacin	2	100.0	524	99.8	66	100.0	229	100.0	13	100.0	-	-	4	100.0	**838**	**99.9**
Colistin	-	-	197	99.0	362	100.0	4	100.0	15	100.0	7	100.0	4	100.0	**589**	**99.7**
Ertapenem	1	100.0	431	99.3	54	100.0	205	98.0	39	100.0	-	-	1	100.0	**731**	**99.0**
Gentamicin	2	100.0	519	97.7	63	98.4	218	93.6	21	100.0	-	-	4	75.0*	**827**	**96.6**
Imipenem	3	100.0	435	100.0	43	100.0	210	100.0	30	100.0	-	-	4	100.0	**725**	**100.0**
Levofloxacin	2	100.0	262	99.2	40	100.0	228	100.0	69	100.0	-	-	8	100.0	**609**	**99.7**
Meropenem	3	100.0	493	99.8	69	100.0	220	100.0	30	100.0	-	-	5	100.0	**820**	**99.9**
Moxifloxacin	1	100.0	82	96.3	16	100.0	224	99.6	60	100.0	-	-	4	100.0	**387**	**99.0**
Ofloxacin	2	100.0	63	100.0	9	100.0	177	100.0	21	100.0	-	-	7	100.0	**279**	**100.0**
Piperacillin-tazobactam ***	2	0.0*	475	74.5*	79	78.5*	205	29.8*	37	18.9*	-	-	5	40.0*	**803**	**60.5***
Tigecycline	2	100.0	314	94.3	89	97.8	131	100.0	89	98.9	-	-	2	100.0	**627**	**96.7**
Tobramycin	2	100.0	383	99.2	49	98.0	223	99.1	22	95.5	1	100.0	4	100.0	**684**	**99.0**
Total	**37**	**86.5**	**7 268**	**93.7**	**1 325**	**94.0**	**3 867**	**90.5**	**943**	**92.8**	**8**	**100.0**	**110**	**89.1**	**13 558**	**92.7**

*n: number of reporting laboratories; -: no data; shaded cells indicate that n<5 laboratories reported concordant results; *: below the threshold of satisfactory concordance (80%); **: 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 '2022 EARS-Net 3' (*Pseudomonas putida*)

Pseudomonas putida is not under surveillance in EARS-Net, and so no AST results needed to be submitted for this strain.

Strain '2022 EARS-Net 4' (*Staphylococcus aureus*)

The *S. aureus* EQA strain ('2022 EARS-Net 4') was resistant to oxacillin and cefoxitin (Table 4). As oxacillin resistance and cefoxitin resistance among *S. aureus* strains are indicators for meticillin resistance, this strain is considered to be meticillin-resistant *S. aureus* (MRSA). The strain was susceptible to norfloxacin, vancomycin, linezolid, daptomycin and rifampicin, and the expected MIC values for ciprofloxacin and levofloxacin were in the I range (Table 4). The level of difficulty was considered 'difficult' for oxacillin and daptomycin 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'.

Interpretation of AST results for the *S. aureus* strain were analysed for the 840 laboratories with correct species identification (Table 8). In total, 37.0% of the laboratories (n=247) would have sent the strain to a reference or other laboratory for further testing. In total, 6 214 tests were performed, and 6 125 reported interpretations were correct. Thus, the reported interpretations were in excellent concordance with expected results (98.6%) (Table 14). MEs were observed for 0.6% (n=39) of the reported interpretations and VMEs were observed for 0.8% (n=50) of the reported interpretations.

The following methodologies were applied: automated systems (52.9%), disk or tablet diffusion (30.5%), gradient test (9.8%), broth microdilution (5.6%), agar dilution (0.4%), macro broth dilution (0.1%), and 'other methods' (0.7%) (Table 10). Overall, all methodologies achieved, as a minimum, a good level of concordance with the expected results (>85% of concordance).

VMEs were observed for oxacillin and cefoxitin (Figure 7). For oxacillin, VMEs represented 6.1% of all submitted interpretations for this antimicrobial agent and were reported for most methods, except agar dilution and macro broth dilution (Table 14). For cefoxitin, VMEs represented 1.1% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system as well as disk or tablet diffusion (Table 14).

For the remaining antimicrobial agents, there were very low proportions of or no VMEs or MEs (Figure 7, Table 14).

Figure 7. Reported interpretation of AST results for strain '2022 EARS-Net 4' (*Staphylococcus aureus*) by antimicrobial agent and anticipated difficulty of identification

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

Table 14. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2022 EARS-Net 4' (*Staphylococcus aureus*), by antimicrobial agent and AST methodology

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	%
Cefoxitin	6	100.0	185	98.4	24	100.0	498	99.0	14	100.0	1	100.0	3	100.0	**731**	**98.9**
Ciprofloxacin	3	100.0	322	99.7	33	100.0	257	97.7	67	100.0	1	100.0	6	100.0	**689**	**99.0**
Daptomycin	-	-	451	99.1	45	100.0	9	100.0	124	100.0	1	100.0	2	100.0	**632**	**99.4**
Levofloxacin	3	100.0	420	99.3	27	100.0	218	99.1	49	100.0	1	100.0	5	100.0	**723**	**99.3**
Linezolid	3	66.7*	530	99.8	45	97.8	220	100.0	21	100.0	1	100.0	3	100.0	**823**	**99.6**
Norfloxacin	2	100.0	15	93.3	-	-	305	98.4	1	100.0	-	-	1	0.0*	**324**	**97.8**
Oxacillin	3	100.0	444	93.2	35	88.6	106	98.1	90	94.4	1	100.0	15	93.3	**694**	**93.9**
Rifampicin	3	66.7*	427	99.3	34	94.1	271	98.9	33	97.0	1	100.0	4	100.0	**773**	**98.7**
Vancomycin	3	66.7*	493	99.8	102	99.0	9	100.0	213	100.0	1	100.0	4	100.0	**825**	**99.6**
Total	**26**	**88.5**	**3 287**	**98.6**	**345**	**97.7**	**1 893**	**98.8**	**612**	**99.0**	**8**	**100.0**	**43**	**95.3**	**6 214**	**98.6**

*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 '2022 EARS-Net 5' (*Pseudomonas aeruginosa*)

The *P. aeruginosa* EQA strain ('2022 EARS-Net 5') was resistant to piperacillin, piperacillin-tazobactam, cefepime, ceftazidime and ciprofloxacin (Table 5). The strain was susceptible to meropenem, amikacin, tobramycin and colistin, and the expected MIC values for imipenem and levofloxacin were in the I range (Table 5). The level of difficulty was considered 'difficult' for ciprofloxacin and levofloxacin 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'.

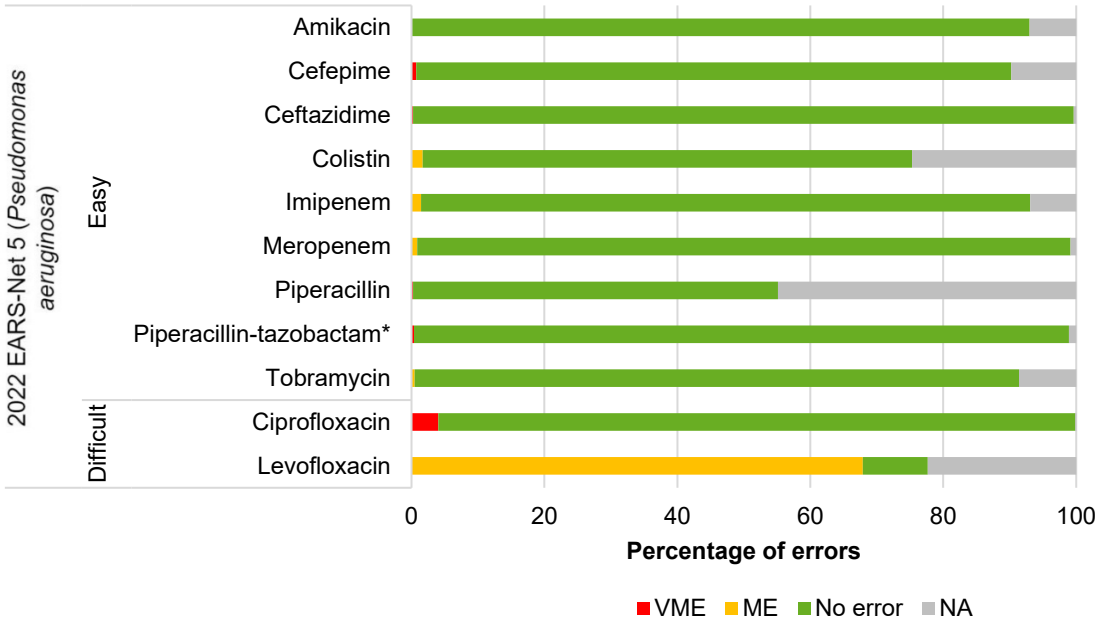
Interpretation of AST results for the *P. aeruginosa* strain were analysed for the 841 laboratories with correct species identification (Table 8). In total, 17.7% of the laboratories (n=149) would have sent the strain to a reference or other laboratory for further testing. In total, 8 157 tests were performed, and 7 504 reported interpretations were correct. Thus, the reported interpretations were in very good concordance with expected results (92.0%) (Table 15). MEs were observed for 7.4% (n=606) of the reported interpretations and VMEs were observed for 0.6% (n=47) of the reported interpretations.

The following methodologies were applied: automated systems (56.4%), disk or tablet diffusion (26.0%), broth microdilution (12.3%), gradient test (4.3%), agar dilution (0.3%), macro broth dilution (0.1%), and 'other methods' (0.6%) (Table 10). Overall, most methodologies achieved, as a minimum, a good level of concordance with the expected results (>85% of concordance). The exception was gradient test, which achieved a satisfactory concordance (80.9%).

VMEs were observed for ciprofloxacin, cefepime, piperacillin, piperacillin-tazobactam and ceftazidime (Figure 8). For ciprofloxacin, VMEs represented 4.1% of all submitted interpretations for this antimicrobial agent and were reported for most methods, except agar dilution and gradient test (Table 15). For cefepime, VMEs represented 0.8% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system, broth microdilution and disk or tablet diffusion. For piperacillin, VMEs represented 0.4% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system. For piperacillin-tazobactam, VMEs represented 0.4% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system, disk or tablet diffusion and gradient test. For ceftazidime, VMEs represented 0.2% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system and broth microdilution (Table 15).

A high proportion of MEs was observed for levofloxacin (87.4% of submitted results) and was reported for all methods (Figure 8, Table 15). For the remaining antimicrobial agents, there were very low proportions of or no VMEs or MEs (Figure 8, Table 15).

Figure 8. Reported interpretation of AST results for strain '2022 EARS-Net 5' (*Pseudomonas aeruginosa*) by antimicrobial agent and anticipated difficulty of identification



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

Table 15. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2022 EARS-Net 5' (*Pseudomonas aeruginosa*), by antimicrobial agent and AST methodology

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	470	100.0	73	100.0	208	100.0	23	100.0	-	-	4	100.0	**779**	**100.0**
Cefepime	2	100.0	475	99.6	52	96.2	202	99.0	21	100.0	-	-	4	100.0	**756**	**99.2**
Ceftazidime	2	100.0	501	99.8	74	98.6	231	100.0	22	100.0	1	100.0	4	100.0	**835**	**99.8**
Ciprofloxacin	2	100.0	503	97.4	74	95.9	224	92.4	30	100.0	-	-	4	75.0*	**837**	**95.9**
Colistin	-	-	204	99.0	387	97.4	7	100.0	20	90.0	7	100.0	6	100.0	**631**	**97.8**
Imipenem	3	100.0	462	98.5	51	94.1	209	99.5	51	98.0	-	-	4	100.0	**780**	**98.5**
Levofloxacin	2	0.0*	310	11.6*	39	48.7*	221	6.3*	76	17.1*	-	-	3	0.0*	**651**	**12.6***
Meropenem	3	100.0	487	99.4	79	98.7	204	98.5	54	100.0	-	-	4	100.0	**831**	**99.2**
Piperacillin	2	100.0	246	99.2	48	100.0	135	100.0	20	100.0	-	-	11	100.0	**462**	**99.6**
Piperacillin-tazobactam**	2	100.0	484	99.8	76	100.0	241	99.6	22	95.5	-	-	4	100.0	**829**	**99.6**
Tobramycin	2	100.0	459	99.8	51	100.0	238	98.7	11	100.0	-	-	5	100.0	**766**	**99.5**
Total	**21**	**90.5**	**4 601**	**93.3**	**1 004**	**96.0**	**2 120**	**89.0**	**350**	**80.9**	**8**	**100.0**	**53**	**92.5**	**8 157**	**92.0**

*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 piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam. Percentages might not total 100% due to rounding.*

Strain '2022 EARS-Net 6' (*Acinetobacter baumannii*)

The *A. baumannii* EQA strain ('2022 EARS-Net 6') was resistant to imipenem, meropenem, ciprofloxacin, levofloxacin, gentamicin and tobramycin (Table 6). The strain was susceptible to amikacin and colistin (Table 6). The level of difficulty was considered 'difficult' for tobramycin since the expected MIC value was less than two dilutions away from the clinical breakpoints. For the remaining antimicrobial agents, the level of difficulty was considered 'easy'.

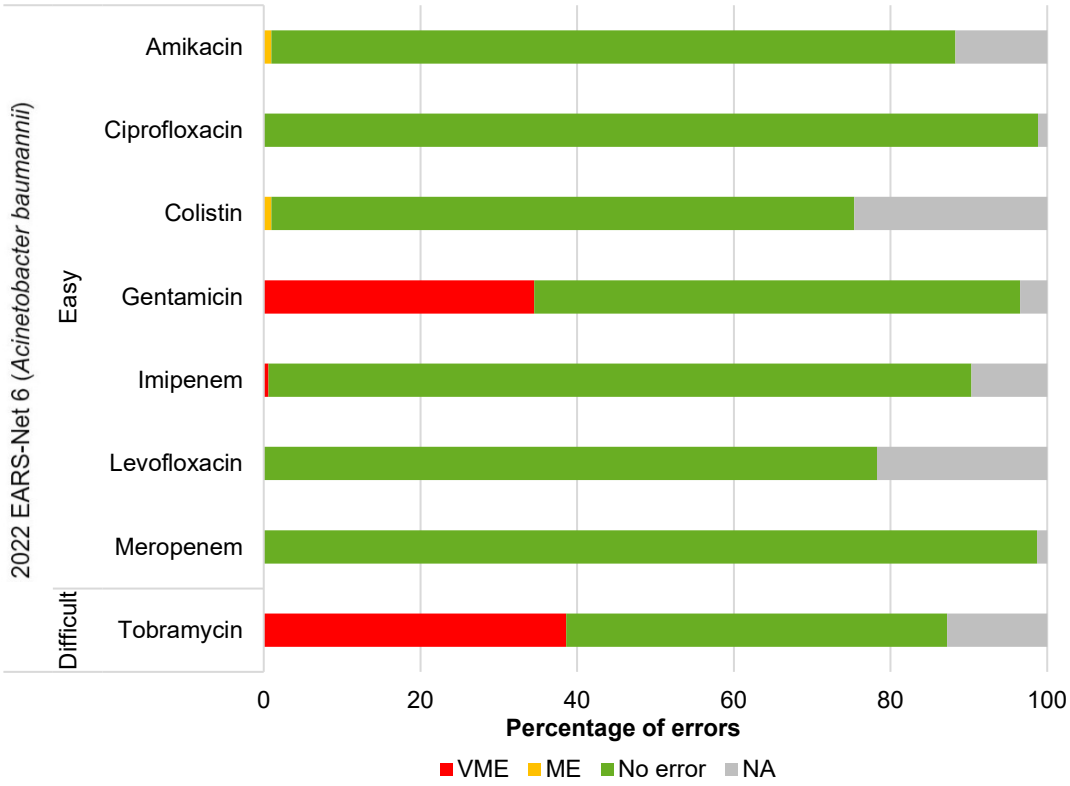
Interpretation of AST results for the *A. baumannii* strain were analysed for the 839 laboratories with correct species identification (Table 8). In total, 39.4% of the laboratories (n=331) would have sent the strain to a reference or other laboratory for further testing. In total, 5 972 tests were performed, and 5 339 reported interpretations were correct. Thus, the reported interpretations were in good concordance with expected results (89.4%) (Table 16). MEs were observed for 0.3% (n=16) of the reported interpretations and VMEs were observed for 10.3% (n=617) of the reported interpretations.

The following methodologies were applied: automated systems (52.2%), disk or tablet diffusion (26.6%), broth microdilution (14.5%), gradient test (5.6%), agar dilution (0.4%), macro broth dilution (0.2%), and 'other methods' (0.5%) (Table 9). Overall, most methodologies achieved, as a minimum, a good level of concordance with the expected results (>85% of concordance). The exceptions were automated systems, which achieved a satisfactory concordance (83.9%), and 'other methods', which did not achieve satisfactory concordance (74.2%).

VMEs were observed for tobramycin, gentamicin and imipenem (Figure 9). For tobramycin, VMEs represented 44.2% of all submitted interpretations for this antimicrobial agent and were reported for most methods, except agar dilution (Table 16). For gentamicin, VMEs represented 35.8% of all submitted interpretations for this antimicrobial agent and were reported for almost all methods, except agar dilution. For imipenem, VMEs represented 0.7% of all submitted interpretations for this antimicrobial agent and were reported when using an automated system (Table 16).

For the remaining antimicrobial agents, there were very low proportions of or no VMEs or MEs (Figure 9, Table 16).

Figure 9. Reported interpretation of AST results for strain '2022 EARS-Net 6' (*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 16. Number of antimicrobial susceptibility tests performed and the percentage of correct AST interpretations for strain '2022 EARS-Net 6' (*Acinetobacter baumannii*), by antimicrobial agent and AST methodology

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	3	100.0	360	99.7	73	100.0	206	97.6	37	100.0	1	100.0	4	100.0	**684**	**98.9**
Ciprofloxacin	3	100.0	454	100.0	69	100.0	221	100.0	19	100.0	-	-	4	100.0	**770**	**100.0**
Colistin	-	-	172	97.1	372	99.5	8	100.0	17	100.0	7	100.0	4	100.0	**580**	**98.7**
Gentamicin	3	100.0	432	49.8*	69	73.9*	215	97.2	31	45.2*	-	-	4	0.0*	**754**	**64.2***
Imipenem	5	100.0	395	98.7	51	100.0	197	100.0	48	100.0	-	-	4	100.0	**700**	**99.3**
Levofloxacin	2	100.0	290	100.0	37	100.0	207	100.0	70	100.0	-	-	3	100.0	**609**	**100.0**
Meropenem	5	100.0	430	100.0	75	100.0	204	100.0	51	100.0	-	-	4	100.0	**769**	**100.0**
Tobramycin	3	100.0	380	37.6*	42	52.4*	223	90.6	24	37.5*	-	-	4	0.0*	**676**	**55.8***
Total	**24**	**100.0**	**2 913**	**84.0**	**788**	**94.9**	**1 481**	**97.8**	**297**	**89.2**	**8**	**100.0**	**31**	**74.2**	**5 542**	**89.4**

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.*

Results from the feedback survey of participating laboratories

A link to the feedback survey was shared with all contacts for the participating laboratories via email on 17 November 2022 (three weeks after receiving information about the release of the evaluation reports), with deadline of 1 December 2022. The survey questions can be found in Annex 2. In total, 129 laboratories provided feedback (15.1% of the 855 laboratories submitting results).

Corrective actions had been taken by 61 out of the 129 laboratories providing feedback (47.3%), and three laboratories were still evaluating the results. The main actions taken were re-testing of isolate(s), verification of reagents, evaluation of the procedures, review of Standard Operating Procedures, updating/validation of methods, and training of laboratory personnel. For 35 laboratories out of the 129 laboratories (27.1%), all EQA analytical test results conformed to expected results and no further action was taken.

Sixty-three laboratories (48.8%) replied that they would use the results as documentation for accreditation and/or licensing purposes.

Overall, 82.3% of the laboratories (n=106) were satisfied with the individual evaluation report. Twenty-three laboratories provided additional comments, and the majority of the comments were regarding the new scoring system; they did not support that a penalty was given if results on mandatory antimicrobial agents were omitted, they would like more information about the scoring system, or they would like to have a total score.

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. A comparison between national laboratories is available in the national summary report shared with the National EARS-Net EQA Coordinators at the same time as the evaluation reports are released.

Some participants commented they would like to have the possibility to modify their data until the deadline, and to have access to the submitted data after release of the individual evaluation reports. The submitted data are included in the evaluation reports that can be downloaded from the webtool, however data cannot be updated at this stage.

Some laboratories found it difficult to navigate in the webtool and/or to get access to the website. Adequate adjustments will be applied to the guidelines and information emails for the 2023 EARS-Net EQA exercise.

One laboratory commented that information about the type of sample was not provided, however this information is described in the protocol.

4. Discussion

All 30 EU/EEA countries participated in the 2022 EARS-Net EQA exercise, with Liechtenstein participating for the first time. A total of 948 laboratories were invited to participate and 855 (90.3%) submitted results for validation. In previous EARS-Net EQA exercises in 2018, 2019 and 2021, 90.3–93.7% laboratories submitted results [2,3,4].

In both 2018 and 2019, 952 laboratories in the EU/EEA signed up for the annual EARS-Net EQA exercise, and between 860 and 892 laboratories submitted results [2,3,4]. ECDC did not initiate an EARS-Net EQA exercise in 2020, due to its response to the SARS-CoV-2 virus pandemic (COVID-19). In 2021, only 642 laboratories signed up for participation and 592 submitted results. This number, lower than what was observed in previous years, was likely due to the ongoing pandemic which required the allocation of laboratorial resources. When comparing the overall results between years, it is important to remember that the species and antimicrobial agents were not the same.

In 2022, species identification was a component of the EQA exercise, and it was decided to include species relevant for EARS-Net surveillance in 2022 and not only species that are part of the surveillance. The submitted species identification results were in excellent concordance with the expected results for the five EQA strains that are species included in EARS-Net surveillance (97.7% to 99.3%), but lower ('satisfactory'; 80.1%) for the *P. putida* strain ('2022 EARS-Net 3'), i.e. a species that is not included in EARS-Net surveillance.

The distribution of AST methods used in the 2022 EARS-Net EQA exercise is similar to those observed in previous years as 51.4% of submitted results were obtained using automated systems (50.3% to 54.7% in previous years), 28.1% of the submitted results were obtained using disk or tablet diffusion (28.0% to 39.8% in previous years) and 19.3% of the submitted results were obtained using MIC methods including broth microdilution and gradient test (8.3% to 16.8% in previous years) [2,3,4]. Very good concordance was observed for macro broth dilution (93.9%), broth microdilution (92.7%) and disk or tablet diffusion (90.7%), and good concordance was observed for agar dilution (89.8%), automated system (87.8%) and gradient test (86.1%).

The concordance of AST results at national level for the 2022 EARS-Net EQA exercise was similar to the results from the 2021 EQA exercise [2]. In 2022, almost all (N=28/30) countries achieved a very good level of concordance, one country (Bulgaria) achieved an excellent level of concordance; and Liechtenstein, during its first ever participation in an EARS-Net EQA exercise, achieved a 'satisfactory' level of concordance. At laboratory level, the vast majority of laboratories achieved a level of concordance that was 'good' or better (96.3% laboratories). Otherwise, 3.1% laboratories achieved a satisfactory level of concordance, and 0.7% were below the satisfactory level.

At the level of the submitted AST interpretations, the vast majority of the 58 included species-antimicrobial agent combinations had 'very good' concordance with the expected results (92.6% interpretations), and an 'excellent' level of concordance was achieved for 46 (79.3%) combinations. This is similar to the percentage of 'excellent' results in EARS-Net EQA exercises from 2021 (80.2%), 2019 (75.6%) and 2018 (80.0%) [2,3,4].

The lowest level of concordance was observed for the *P. aeruginosa* strain, for which only 12.6% of the interpretation of levofloxacin AST results were correct. Low concordance was also observed for *S. pneumoniae* results for benzylpenicillin (70.7%), azithromycin (72.2%), ceftriaxone (83.8%), and cefotaxime (84.6%), for *E. coli* results for piperacillin-tazobactam (60.5%), amikacin (64.0%), cefepime (79.6%), and ceftazidime (83.7%), and for *A. baumannii* results for tobramycin (55.8%) and gentamicin (64.2%). All remaining species-antimicrobial agent combinations achieved at least a very good concordance (>90%).

Strain **2022 EARS-Net 1 (*Streptococcus pneumoniae*)** was resistant to benzylpenicillin, but prediction of this profile was problematic and concordance did not reach a satisfactory level (70.7%). One of the main reasons for the low concordance achieved for this antimicrobial agent in this species was the application of incorrect clinical breakpoints. The strain was described as being obtained from cerebrospinal fluid from a patient with clinical manifestations suggesting meningitis, and presented an expected benzylpenicillin MIC = 2 mg/L. Thus, this profile should be easily identifiable as resistant when applying the clinical breakpoints for meningitis (S ≤ 0.06 mg/L and R > 0.06 mg/L), even when accounting for the permitted inherent method variability of plus or minus one dilution. However, if applying the clinical breakpoints dedicated to situations other than meningitis (S ≤ 0.06 mg/L and R > 2 mg/L), the likelihood of misclassification increases due to the closeness of the expect result to the breakpoint. These deviations correspond to VMEs (R → S) and might indicate that resistance of *S. pneumoniae* isolates to penicillins can be under-reported in the EU/EEA. The under-reporting might not be necessarily due to limitations of certain methodologies, but instead due to incorrect application of the most recent clinical breakpoints.

Prediction of susceptibility to azithromycin was also poor and did not reach a satisfactory level (72.2% of concordance). Most deviations were observed in association with the methodology of gradient test: for this method only 46.1% of concordance was achieved, while for the other applied methods the concordance between the expected and submitted results was very good or excellent (>90%). These deviations are major errors (S → R) and may be an indication that, in *S. pneumoniae*, azithromycin resistance is overestimated in the EU/EEA, especially considering that a high proportion (45.8%) of participating laboratories using the method with poor performance for AST of this antimicrobial agent.

The strain was susceptible to cephalosporins (cefotaxime and ceftriaxone) and the concordance of results for both antimicrobial agents reached at least a satisfactory level (83.8% - 84.6%). The deviations were observed for most methods, although concordance was worse with automated systems (70.4% - 76.1%). The expected MIC results (MIC = 0.5 mg/L, for both antimicrobial agents) were very close to the clinical breakpoints (S ≤ 0.5 mg/L and R > 0.5 mg/L, for both). Thus, the prediction of these AST profiles was considered difficult and the deviations observed in these cases might be attributed to the inherent method variability, since the expected MIC values correspond to a borderline concentration, increasing the probability of misclassification. These deviations are major errors (S → R) and may be an indication that, for *S. pneumoniae*, resistance is overestimated in the EU/EEA, especially considering that high proportions (40.5% - 46.6%) of participating laboratories using the method with poor performance for AST of these antimicrobial agents. Similarly to benzylpenicillin, participants were expected to apply clinical breakpoints for meningitis when analysing results for these antimicrobial agents. However, the breakpoints for situations other than meningitis (S ≤ 0.5 mg/L and R > 2 mg/L) should not yield a misclassification of the strain as resistant, due to the large enough interval between the expected MIC result and the breakpoint leading to a R classification. Therefore, it's expected that for these cephalosporins, the impact of choosing an incorrect breakpoint is less accentuated than what was observed for benzylpenicillin.

Concordance of results for the remaining antimicrobial agents was excellent (≥95%) for strain 2022 EARS-Net 1.

Strain **2022 EARS-Net 2 (*Escherichia coli*)** was resistant to amikacin, but concordance of results for this antimicrobial agent was poor and did not reach a satisfactory level (64.0%). The deviations were observed for all methods. 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 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 probability of misclassification. These deviations correspond to VMEs (R → S) and might indicate that resistance of *E. coli* isolates to amikacin can be under-reported in the EU/EEA.

A similar justification can be applied to the problematic results observed for cefepime (which did not reach a satisfactory level, with 79.6% of concordance) and ceftazidime (with results at a satisfactory level, with 83.7% concordance). The deviations were observed for most methods, with the most concerning situation corresponding to the disk or tablet diffusion methodology (63.5% to 79.2% concordance), due to its frequent use by the laboratories (25.4% - 26.2%). 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' AST predictions. 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 harboured by the strain.

The same situation was observed for piperacillin-tazobactam, for which results did not reach a satisfactory level (60.5%). The deviations were observed for all methods. The determination of the expected MIC value (MIC = 8/4 mg/L) was considered '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 antimicrobial susceptibility/resistance of this strain to piperacillin-tazobactam. The differential expression of the $bla_{\text{OXA-1}}$ gene harboured by the strain could furthermore exacerbate the deviations.

The deviations previously described, observed in cefepime, ceftazidime and piperacillin-tazobactam, correspond to major errors (S → R or I → R) and may be an indication that, for *E. coli,* resistance to these agents is overestimated in the EU/EEA.

Concordance of results for the remaining antimicrobial agents was excellent (≥95%) for strain 202 EARS-Net 2.

Strain **2022 EARS-Net 3 (*Pseudomonas putida*)** was from a species which is not under surveillance within EARS-Net. Therefore, according to the EARS-Net surveillance protocol, and the EARS-Net EQA protocol, pariticipants did not need to report its AST results. Species identification of this strain had the poorest concordance (80.1%), which was classified as satisfactory, as opposed to the excellent concordance (≥95%) for the other five EQA strains. It is possible that participants were not aware that species not under EARS-Net surveillance could be included in this EQA exercise, despite this information being described in the respective protocols. It is also possible that the deviations are due to incompleteness of diagnostic databases (such as lack of specific MALDI-TOF MS spectrum). However, the EQA protocol did not collect data regarding the speciation methodology, and so this theory is merely speculative.

Strain **2022 EARS-Net 4 (*Staphylococcus aureus*)**, i.e. the MRSA strain, was the only strain that had very few results reported incorrectly. The concordance with expected results was excellent (≥95%) for almost all antimicrobial agents with the exception of oxacillin, which nevertheless had a very good concordance (93.9%).

Overall, the concordance of results for all AST methodologies was excellent (≥95%) for strain 2022 EARS-Net 4, with the exception of agar dilution where only a good level of concordance was achieved (88.5%), but the low number of tests performed with this method must be taken into account (n=26).

Strain **2022 EARS-Net 5 (*Pseudomonas aeruginosa*)** had a MIC value for levofloxacin in the 'susceptible, increased exposure' (I) range, and prediction of the susceptibility profile to this antimicrobial agent did not reach a satisfactory level of concordance (12.6%). The deviations were observed for all methods, and the best concordance was seen for broth microdilution (48.7%). The expected MIC result (MIC = 2 mg/L) was very close to the clinical breakpoints (S ≤ 0.001 mg/L and R > 2 mg/L). Thus, the prediction of this AST profiles was considered 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 probability of misclassification. These deviations correspond to MEs (I → R) and might indicate that resistance of *P. aeruginosa* to levofloxacin is overestimated in the EU/EEA. It is also possible that the strain harbours a currently unknown genetic mechanism of resistance to fluoroquinolones, which might be inducible or differentially expressed, since it presents phenotypic resistance to ciprofloxacin.

Concordance of results for the remaining antimicrobial agents was excellent (≥95%) for strain 2022 EARS-Net 5.

Strain **2022 EARS-Net 6 (*Acinetobacter baumannii*)** was resistant to tobramycin, for which results did not reach a satisfactory level (55.8%). The deviations were observed for most methods. The expected MIC result (MIC = 8 mg/L) was very close to the clinical breakpoints (S ≤ 4 mg/L and R > 4 mg/L). Thus, the prediction of this AST profile was considered 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 probability of misclassification.

The strain was also resistant to gentamicin and prediction of the resistance profile to this antimicrobial agent did not reach a satisfactory level of concordance (64.2%). The deviations were observed for most methods. Contrary to what was described for tobramycin, the expected MIC result of this strain for gentamicin (MIC = 16 mg/L) was not close to the clinical breakpoints, thus it is unlikely that deviations are attributable to inherent variation of the methodologies.

Both types of deviations correspond to VMEs (R → S) and might indicate that resistance of *A. baumannii* isolates to these aminoglycosides can be under-reported in the EU/EEA.

Concordance of results for the remaining antimicrobial agents was excellent (≥95%) for strain 2022 EARS-Net 6.

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

Common issues identified in this EQA exercise

In previous EARS-Net EQA exercises, in 2018 [2], 2019 [3] and 2021 [4]), AST with identified issues included:

- *S. pneumoniae* with intermediate [past terminology] results for penicillin;
- *S. pneumoniae* with I results for cephalosporins;
- *E. coli* with intermediate [previous terminology] or R results for piperacillin-tazobactam;
- *E. coli* with R results for colistin;
- *E. coli* with S or R results for amoxicillin-clavulanic acid;
- *E. coli* with I results for ceftazidime;
- *E. coli* with I or R results for fluoroquinolones;
- *E. coli* with R results for tigecycline;
- *E. coli* with S results for gentamicin;
- *S. aureus* with intermediate [previous terminology] results for vancomycin;
- *P. aeruginosa* with S results for ceftazidime;
- *P. aeruginosa* with S results for piperacillin-tazobactam.

The laboratories participating in the 2022 EARS-Net EQA exercise generally did not report the same issues, with the exception of *S. pneumoniae* for penicillin and cephalosporins and in *E. coli* for piperacillin-tazobactam and ceftazidime. However, there were additional issues in the 2022 EQA exercise not noted in the three previous EQA exercises. These included:

- *S. pneumoniae* with S results for azithromycin;
- *E. coli* with R results for amikacin;
- *E. coli* with S results for cefepime;
- *P. aeruginosa* with I results for levofloxacin;
- *A. baumannii* with R results for tobramycin;
- *A. baumannii* with R results for gentamicin.

Overall, results of the 2022 EARS-Net EQA exercise did not show a systematic overestimation or underestimation of resistance in the EU/EEA, with deviations being distributed through both types of errors (MEs and VMEs). Furthermore, results did not highlight any systematic underperformance of a certain methodology when compared to the remaining reported methodologies, except for the specific species-antimicrobial agent combinations previously described above.

5. Conclusions

The number of participating laboratories is approaching the number observed before the SARS-CoV-2 virus pandemic, which is a welcome trend.

The 2022 EARS-Net EQA exercise once again included species identification, which was absent from the 2021 EQA exercise. The submitted species identification results were highly accurate (≥97.7%) for all species that are reportable to EARS-Net, which implying that that the EARS-Net surveillance data on species is accurate, overall.

The submitted AST interpretations also imply that EARS-Net surveillance data are mostly accurate, although MEs were observed for 4.2% of the reported interpretations, and VMEs were observed for 3.2% of the reported interpretations. Both MEs and VMEs imply the possibility for sub-optimal treatment outcomes. 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 may suggest that some participants do not always strictly adhere to the most current guidelines. Furthermore, certain antimicrobial groups, for specific species, presented higher percentages of deviations, namely benzylpenicillin, cephalosporins and azithromycin in *S. pneumoniae*, piperacillin-tazobactam, certain cephalosporins and amikacin in *E. coli*, levofloxacin in *P. aeruginosa*, and aminoglycosides in *A. baumannii*.

The findings indicate that AMR is heterogeneously reported in the EU/EEA. The VMEs (R → S or R → I) showed a tendency of under-reporting reduced susceptibility in *S. pneumoniae* to benzylpenicillin, in *E. coli* to amikacin, and in *A. baumannii* to tobramycin and gentamicin. At the same time, the major errors (S → R or I → R) indicate a trend of over-reporting resistance of *S. pneumoniae* to azithromycin, cefotaxime and ceftriaxone, *E. coli* to piperacillin-tazobactam, cefepime and ceftazidime, and *P. aeruginosa* to levofloxacin. One frequent justification for these deviations was the inherent method variability of plus or minus one dilution in MIC methodologies, especially when the expected MIC values corresponded to borderline concentrations very close to the clinical breakpoints, which increased the probability of misclassification. Furthermore, it was observed that clinical metadata was overlooked, leading to the choice of incorrect breakpoints. Finally, it should be noted that some of the strains harboured known genetic mechanisms associated with resistance to certain antimicrobial groups, and although genotypic characterisation of the strains was outside of the scope of this exercise, it is possible for the laboratories to screen for AMR determinants. Therefore, when considering both phenotypic and genotypic data, the final reporting of results could present lower proportions of deviations. Specifically, detection of genes mediating resistance to aminoglycosides in the *A. baumannii* strain would probably lead to re-testing or re-evaluation of AST results, and to the potential correct classification of those R profiles. Detection of genes encoding extended-spectrum beta-lactamases in the *E. coli* strain would also be likely to promote increased attention in interpretation of cephalosporin and other β-lactams AST results, or even confirmatory testing using other methods. However, one possible consequence of detecting antimicrobial resistance genes is the tendency to further over-report decreased susceptibility profiles.

The analysis of the overall performance of the different AST methods showed few differences between methodologies, except for a slightly poorer performance of the gradient test. Specific shortcomings were observed in AST of *S. pneumoniae* for amikacin when using the gradient test and for cephalosporins when using automated systems. Simultaneously, the disk or tablet diffusion method for AST of cephalosporins in *E. coli* also yielded concerning results. In conclusion, there is no exclusive pattern of over- or under-reporting decreased susceptibility profiles in the EU/EEA.

6. Recommendations

The 2022 EARS-Net EQA exercise concluded that only 23.0% of the laboratories participating in the 2022 EARS-Net EQA exercise achieved at least 95% of concordance with the expected AST results, and specific areas of difficulty have been identified. 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 can also suggest that some participants do not always strictly adhere to the most current guidelines. In such cases, laboratories should review their reporting practices and confirm that the 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 in Europe may occur. Although genotypic analysis of AMR genes or chromosomal point mutations could potentially solve some of the deviations reported by the laboratories, the focus of this EQA exercise was phenotypic testing, and the observed under- and overestimation should be kept 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. A finding worthy of further investigation is the low performance of AST for *P. aeruginosa* with I for levofloxacin, especially among results generated with disk/tablet diffusion methodologies, which was the most common methodology.

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 several laboratories to review their methodologies as follows:

- Ensuring that they are familiar with the existence of different clinical breakpoints for the same species-antimicrobial agent combination, dedicated to different clinical manifestations;
- Performing and reading results for aminoglycosides susceptibility testing, particularly in non-fermenting gram-negative bacilli, since results can vary due to differences in medium composition;
- Performing and reading results for fluoroquinolone susceptibility testing, due to inherent difficulties associated with the reading of these AST results. Reading and interpreting inhibition zone diameters when performing disk diffusion or tablet diffusion is notoriously difficult, thus special attention should be given to this issue and, if necessary, appropriate training established;
- 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;
- Becoming familiar with recommendations regarding AST results within the ATU or results near the clinical breakpoints;
- Becoming familiar with other general or specific recommendations regarding the performance, interpretation and evaluation of AST for certain species-antimicrobial agent combinations;
- Opting to use the recommended AST methods for each species-antimicrobial agent combination being tested.

Furthermore, participants with poor performance, as described in their individual evaluation reports, should ensure that adequate internal quality control strains are being applied and monitored to ensure reliability of results, and that relevant quality management systems and control measures are in place.

Continued regular participation in the annual EQA exercise by the laboratories reporting to EARS-Net is required to evaluate and review their performance. 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 1A. Number of laboratories receiving material and submitting results for the 2022 EARS-Net EQA exercise

EU/EEA country	Number of laboratories receiving material for the EQA exercise	Number of laboratories submitting data		Number of laboratories included in the analysis of AST results	
	N	N	%	N	%
Austria	39	38	97.4	38	100.0
Belgium	35	33	94.3	33	100.0
Bulgaria	23	23	100.0	23	100.0
Croatia	32	31	96.9	31	100.0
Cyprus	5	5	100.0	5	100.0
Czechia	50	46	92.0	46	100.0
Denmark	11	10	90.9	10	100.0
Estonia	11	11	100.0	11	100.0
Finland	13	12	92.3	12	100.0
France***	67	51	76.1	50	98.0
Germany	34	33	97.1	33	100.0
Greece	29	26	89.7	26	100.0
Hungary	27	25	92.6	25	100.0
Iceland	2	1	50.0	1	100.0
Ireland	33	28	84.8	28	100.0
Italy**, ***	156	134	85.9	132	98.5
Latvia*	14	14	100.0	13	92.9
Liechtenstein	1	1	100.0	1	100.0
Lithuania	15	14	93.3	14	100.0
Luxembourg	5	4	80.0	4	100.0
Malta	1	1	100.0	1	100.0
Netherlands	33	26	78.8	26	100.0
Norway	14	14	100.0	14	100.0
Poland	83	73	88.0	73	100.0
Portugal**	114	105	92.1	104	99.0
Romania	16	15	93.8	15	100.0
Slovakia	13	13	100.0	13	100.0
Slovenia	11	11	100.0	11	100.0
Spain	47	43	91.5	43	100.0
Sweden	14	14	100.0	14	100.0
Total	**948**	**855**	**90.2**	**850**	**99.4**

** One laboratory reported results using the Clinical and Laboratory Standards Institute (CLSI) guidelines and data from this laboratory was not included in the evaluation.*
*** One laboratory was 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 2022 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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