EURL-PH-AMR
EU Reference Laboratory for public health in the field of AMR

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External Quality Assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2025

National summary report for Italy

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
DTU Food	Technical University of Denmark, National Food Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
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
EURL-PH-AMR	EU Reference Laboratory for Public Health in the Field of AMR
I	Susceptible, increased exposure
MIC	Minimum inhibitory concentration
ME	Major error
R	Resistant
S	Susceptible, standard dosing regimen
s.d.	Standard deviation
VME	Very major error

Figures

Tables

1. Introduction

This report presents and summarises the national results from the external quality assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2025. Participating laboratories are identified by unique codes, which are known to the respective laboratory, the national EQA coordinator, and the EQA provider.

The objectives of the 2025 EARS-Net EQA exercise were to:

- Assess the quality of species identification performed by participating laboratories.
- Evaluate the accuracy of qualitative antimicrobial susceptibility testing (AST) results reported.
- Determine the overall comparability of routinely collected AST results across laboratories and EU/EEA countries.

This report provides a summary of the results, including a brief conclusion regarding the capacity of participating laboratories and, where relevant, recommendations for improvement.

The 2025 EQA focused on species identification and AST interpretation for six distributed strains: (*Acinetobacter baumannii* (n=2), *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*).

1.1 Participation

In the 2025 EARS-Net EQA, it was decided to include species relevant for the EARS-Net surveillance for species identification. In total, six strains were included and all six were covered by EARS-Net surveillance.

In total, 977 laboratories from 29 EU/EEA countries registered for the 2025 EARS-Net EQA, and 895 laboratories (91.6%) submitted data. This participation rate is comparable to the 2024 EQA, in which 957 laboratories from 30 EU/EEA countries registered and 871 submitted data (91.0%).

In Italy, 217 laboratories registered for the 2025 EARS-Net EQA and received the six strains for analysis. Results were submitted by 199 laboratories comprising 4 national, 25 regional, 165 local, and 5 categorised as 'other' laboratories. No results were submitted by 18 laboratories (IT251, IT256, IT261, IT143, IT155, IT184, IT151, IT507, IT045, IT209, IT518, IT529, IT253, IT215, IT225, IT218, IT144, IT265).

Adherence to the EUCAST guideline is mandatory for participation in the EARS-Net EQA. The guideline is updated annually in the beginning of the year, and laboratories are expected to follow the latest version (v15.0) when analysing results. Overall, 167 laboratories followed EUCAST v 15.0, 23 laboratories followed EUCAST v 14.0, 2 laboratories followed EUCAST v 13.0, and 7 laboratories followed another EUCAST version.

Strain ‘2025 EARS-Net 6’ (*Streptococcus pneumoniae*) showed reduced viability compared to the other EQA strains. Participants were therefore strongly encouraged to process this strain immediately upon receipt. However, several laboratories were unable to revive the sample. As a result, the minimum requirement for receiving a certificate of participation (submitting interpretation of AST results) was revised to include only strains ‘2025 EARS-Net 1’ to ‘2025 EARS-Net 5’, instead of all six strains. Overall, 195 laboratories out of 199 laboratories submitted interpretation for strains '2025 EARS-Net 1' to '2025 EARS-Net 5' following the EUCAST guideline and received the Certificate of Participation in 2025 EARS-Net EQA.

A total of 177 laboratories (88.9%) submitted AST results for all six strains.

2. Materials and Methods

2.1 Strains and antimicrobial susceptibility testing

For this EQA, strains of *Acinetobacter baumannii* (n=2), *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Streptococcus pneumoniae* were selected from the strain collection at the Technical University of Denmark, National Food Institute (DTU Food). Selection was based on antimicrobial resistance profiles and the recommendations from the European Centre for Disease Prevention and Control (ECDC).

The expected results were established by evaluating the consensus AST results generated at DTU Food using broth microdilution and/or disk diffusion. The results were further evaluated through confirmatory testing provided by two reference laboratories: the EUCAST Development Laboratory (Växjö, Sweden) and the Microbiological Diagnostic Unit Public Health Laboratory (The Doherty Institute, Australia).

The consensus phenotypic AST profiles were compared with whole-genome sequencing (WGS) data to identify acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs). The WGS analysis was performed at DTU Food using the bioinformatics tools ResFinder v4.6.0, AMRFinderPlus v4.0.19, and CARD RGI v6.0.4 (Annex 1).

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

The antimicrobial agents included in this EQA corresponded to the panel of pathogen-antimicrobial agent combinations under surveillance by EARS-Net, as outlined in the AMR reporting protocol 2025[1] (Annex 2).

Participating laboratories were instructed to perform AST according to their routine procedures, i.e., automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient diffusion, or other methods following EUCAST recommendations (https://www.eucast.org/ast_of_bacteria/).

Interpretation of AST results was based on EUCAST clinical breakpoints tables v15.0 (https://www.eucast.org/clinical_breakpoints/) as detailed in Annex 1. This allowed categorisation of the AST results as “resistant” (R), “susceptible, increased exposure” (I), or “susceptible, standard dosing regimen” (S).

2.2 Procedure

The protocol, test forms, and user guide for the reporting webtool are available on the 2025 EARS-Net EQA website: https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net.

All participating laboratories were invited to submit their results to the EARS-Net EQA webtool using secure, individual login credentials. The deadline for data submission was 10 August 2025. Results were assessed using a scoring algorithm that accounted for both the difficulty of the AST determination and the severity of errors.

Participants were encouraged to complete an electronic feedback survey, assessed via a link sent to the designated contact persons for each laboratory. The purpose of this evaluation was to collect feedback to inform and improve future EQA exercises. The questions were provided by ECDC.

2.3 Scoring antimicrobial susceptibility results

In the 2025 EARS-Net EQA, the scoring system for the interpretation of AST results incorporated both the “level of difficulty” and the “severity of error” for each organism–antimicrobial combination.

Level of Difficulty

The level of difficulty reflected the likelihood of misclassification and was categorize as either ‘easy’ or ‘difficult’.

‘Difficult’ situations included cases where:

- An AST result with a one-fold dilution difference from the expected MIC value would result in a different S/I/R interpretation; and/or
- The expected MIC value falls within the area of technical uncertainty (ATU); and/or
- The relevant EUCAST clinical breakpoint had been recently changed or newly introduced in the latest EUCAST breakpoint table.

‘Easy’ situations included cases where:

- An AST result with a one-fold dilution difference from the expected MIC value would lead to the same S/I/R interpretation; and/or
- The EUCAST clinical breakpoint had not been recently changed or added

Severity of Error

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

VME was defined as reporting false susceptibility, i.e. the expected result was resistant (R), but the reported result was susceptible (S or I).

ME was defined as reporting false resistance, i.e. the expected result was susceptible (S or I), but the reported result was resistant (R).

The scoring of each result reflected both the level of difficulty and the severity of error (Table 1). This scoring system was also applied in the 2023 and 2024 EARS-Net EQA exercises.

Scoring concordance

The concordance of submitted species identification and AST interpretations with the expected results was categorised as ‘excellent’ (≥95% of interpretations in concordance with expected results), ‘very good’ (>90% to <95%) or ‘good’ (>85 to ≤90%). There was also the category ‘satisfactory’ (>80 to ≤85%) for results that could be improved.

Table 1. Scoring system for reported AST results in the 2025 EARS-Net EQA

		Difficulty of result and expected interpretation					
		Easy			Difficult		
		R	I	S	R	I	S
Obtained interpretation	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

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

3. Results

3.2 Species identification results

For each strain, the species should be identified. In total, 1142 out of 1149 (99.4%) strains submitted with interpretation of AST results had the correct species identification. An overview of the species identification for the six strains and the number of laboratories reporting the correct identification is given in Table 2. All laboratories reported the correct species identification for all submitted strains except IT183, which reported an incorrect species for 2 out of 6 submitted strains, IT522, which reported an incorrect species for 1 out of 4 submitted strains, IT263, which reported an incorrect species for 1 out of 6 submitted strains, IT138, which reported an incorrect species for 2 out of 6 submitted strains, and IT230, which reported an incorrect species for 1 out of 6 submitted strains.

Table 2. Number and percentage of laboratories reporting the correct species in the 2025 EARS-Net EQA

Italy Strain ID	Expected species	No. of labs submitting data with interpretation of AST results	No. of labs reporting correct species identification	% of labs reporting correct species identification
2025 EARS-Net 1	*Klebsiella pneumoniae*	197	196	99.5
2025 EARS-Net 2	*Acinetobacter baumannii*	199	197	99.0
2025 EARS-Net 3	*Staphylococcus aureus*	199	199	100.0
2025 EARS-Net 4	*Acinetobacter baumannii*	198	198	100.0
2025 EARS-Net 5	*Escherichia coli*	198	197	99.5
2025 EARS-Net 6	*Streptococcus pneumoniae*	158	155	98.1

3.3 Antimicrobial susceptibility testing (AST) results

AST results were evaluated for strains with correct species identification.

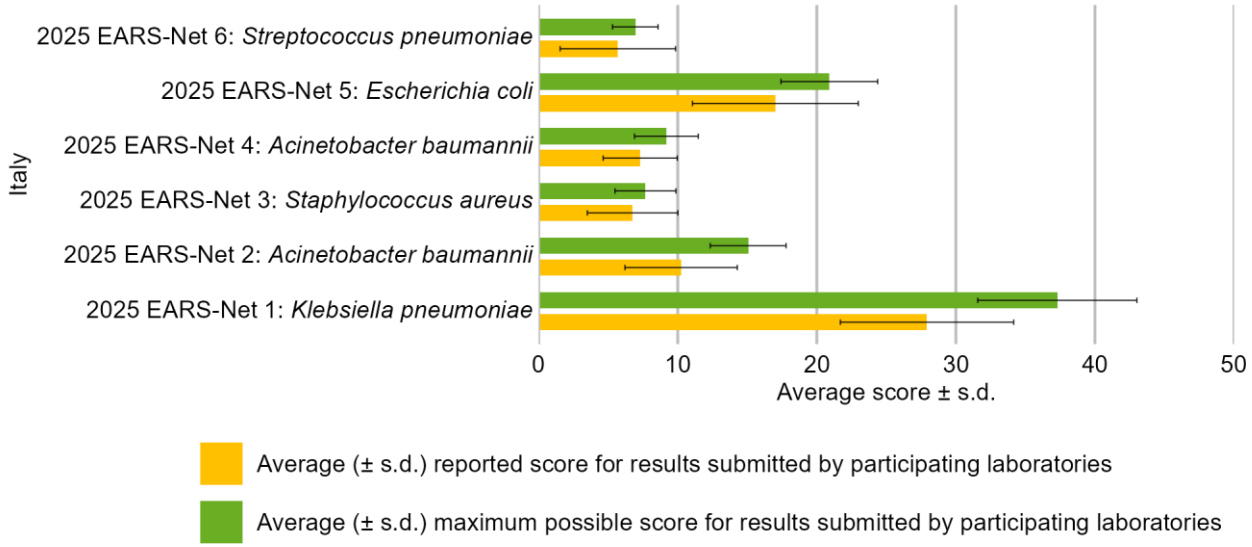
Participants were asked to report AST results, i.e., MIC or zone diameter values and their categorisation as “resistant” (R), “susceptible, increased exposure” (I), and “susceptible, standard dosing regimen” (S) for the species covered by EARS-Net surveillance. Only the categorisation was evaluated, whereas the quantitative values were used as supplementary information.

For the 2025 EARS-Net EQA, each laboratory could report interpretation for 86 different strain-antimicrobial combinations with a total maximum score of 122.

For the 199 laboratories submitting results with correct species identification, interpretation of AST results were reported for 13426 out of the 16526 possible strain-antimicrobial combinations, and 12516 (93.2%) were reported with the correct interpretation with an average score for submitted results of 72.9 ± 18.4. The maximum possible score for the reported results was 94.6 ± 17.2.

Figure 1 presents the average maximum possible score for reported results ± s.d., and the average score for reported results ± s.d. for the laboratories reporting results for each of the six strains.

Figure 1. Average maximum possible score of reported results ± s.d., and average score of the reported results ± s.d. for each strain, in 2025 EARS-Net EQA

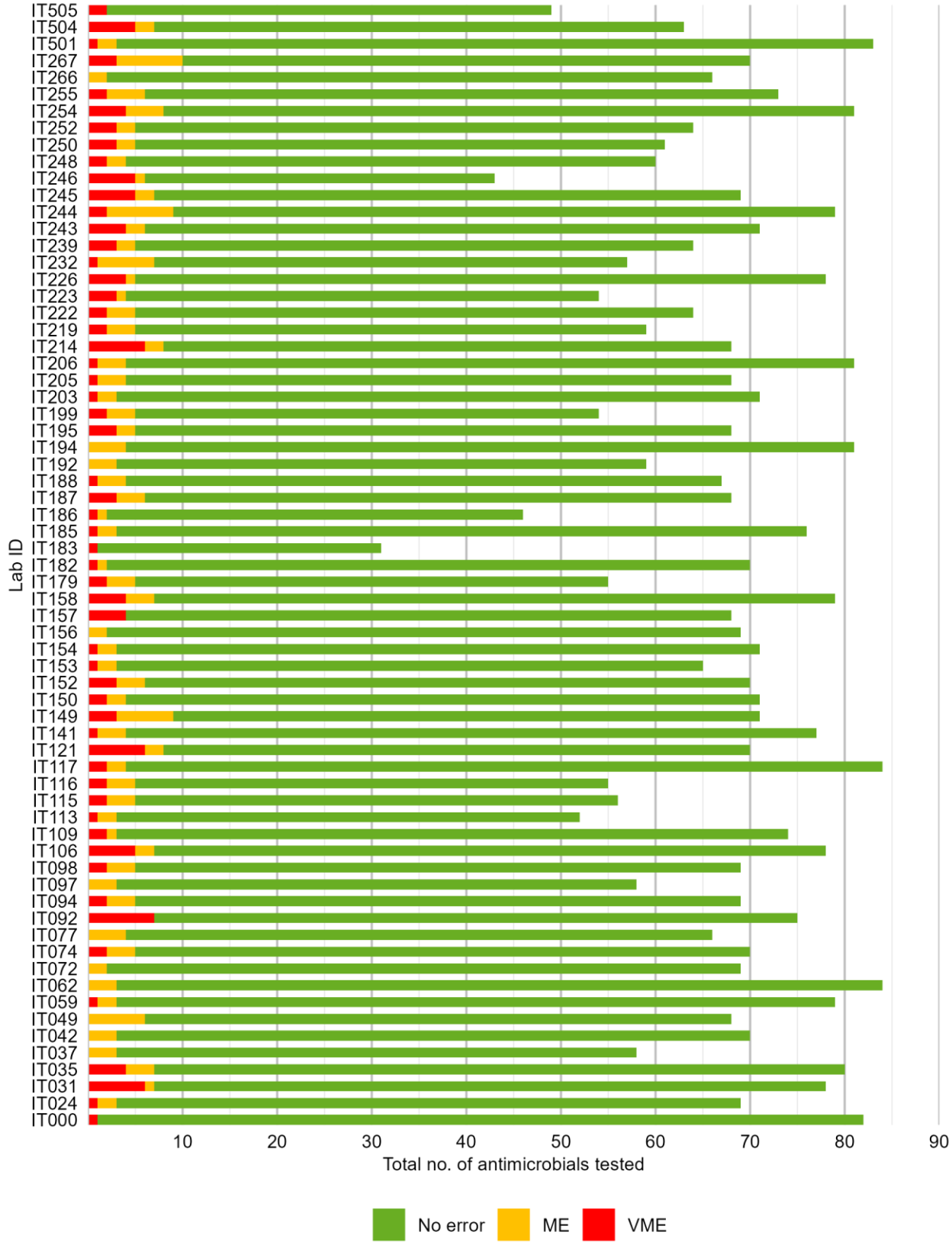


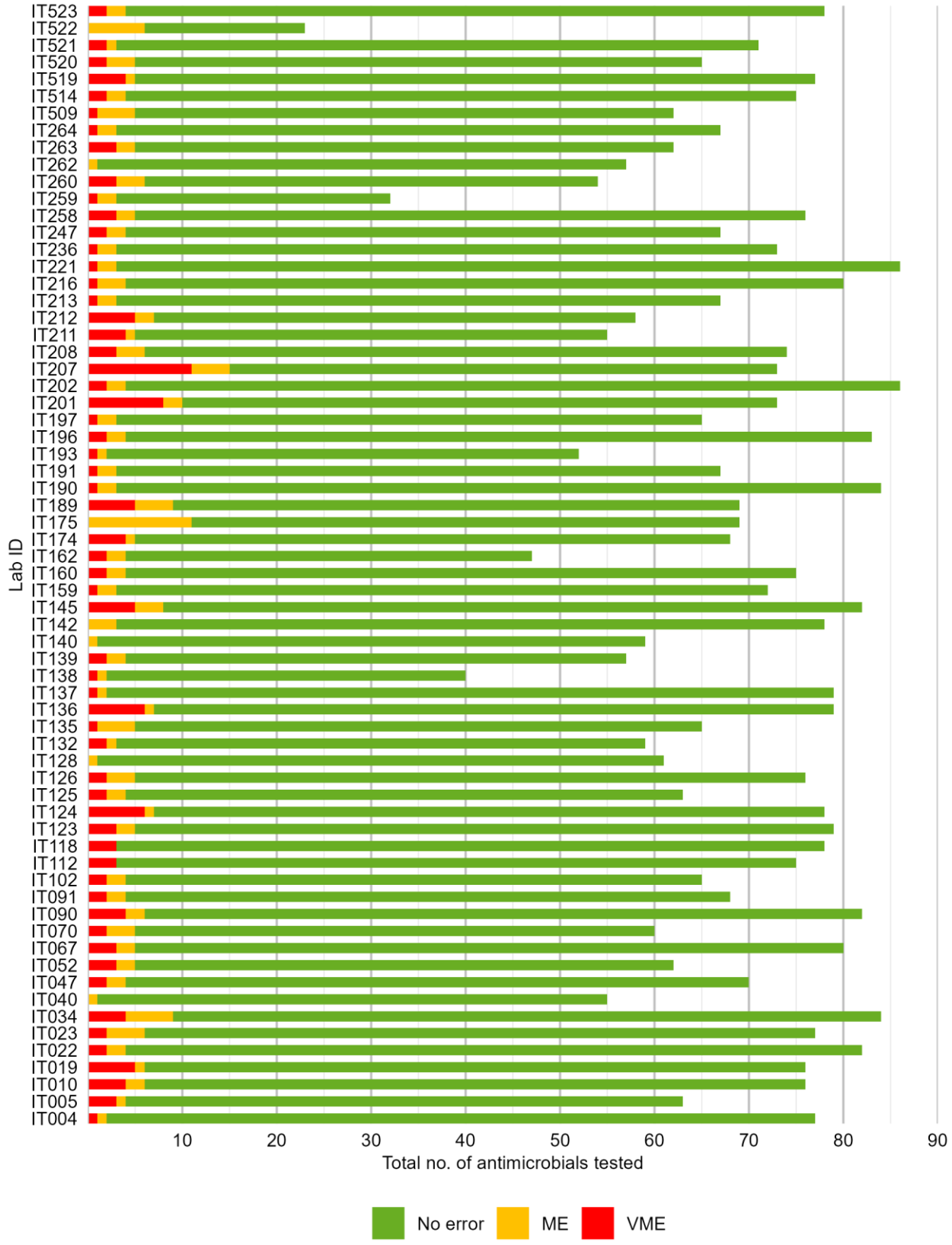
Key: s.d. – standard deviation.

Of the 13426 AST interpretations reported by the 199 laboratories, 12516 (93.2%) were correct, 468 (3.5%) were ME and 442 (3.3%) were VME. The distribution of results per laboratory is presented in Figure 2.

An overview of the methods used to determine the antimicrobial resistance in the six strains, along with the percentage of correct interpretations, is presented in Tables 3-5. The most frequently used method was automated system accounting for 79.7% of all tests (Table 6). The lowest concordance with expected interpretations was observed with the agar dilution (83.3%).

Figure 2. Reported interpretation of all AST results for the 2025 EARS-Net EQA by laboratory




Lab ID
IT523
IT522
IT521
IT520
IT519
IT514
IT509
IT264
IT263
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IT221
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30
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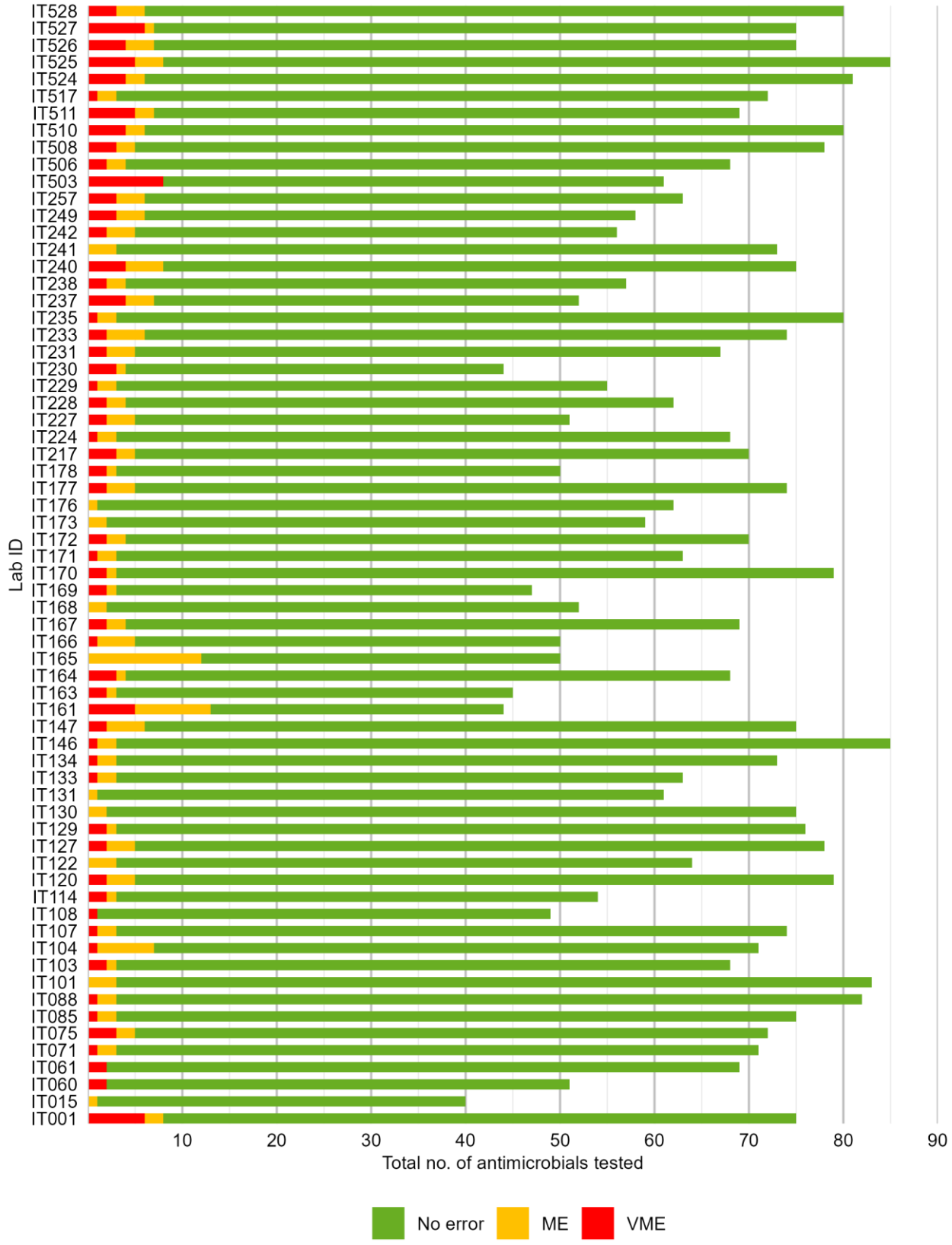
50
60
70
80
90
Total no. of antimicrobials tested
No error
ME
VME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Table 3. Overview of methods used for determination of the AST results for strains '2025 EARS-Net 1' and '2025 EARS-Net 2'

Italy	2025 EARS-Net 1 *Klebsiella pneumoniae*			2025 EARS-Net 2 *Acinetobacter baumannii*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	3	0.08	66.7	2	0.1	100.0
Automated system	2 975	80.6	88.8	1 199	76.7	86.5
Broth microdilution	475	12.9	86.3	272	17.4	89.0
Disk/Tablet diffusion	82	2.2	91.5	60	3.8	91.7
Gradient test	149	4.0	85.9	27	1.7	88.9
Macro broth dilution	1	0.03	100.0	0	-	-
Other	6	0.2	83.3	4	0.3	75.0
Total	**3 691**	**100.0**	**88.4**	**1 564**	**100.0**	**87.1**

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 4. Overview of methods used for determination of the AST results for strains '2025 EARS-Net 3' and '2025 EARS-Net 4'

Italy	2025 EARS-Net 3 *Staphylococcus aureus*			2025 EARS-Net 4 *Acinetobacter baumannii*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	0	-	-	1	0.06	100.0
Automated system	1 136	80.3	97.9	1 208	77.3	98.3
Broth microdilution	103	7.3	98.1	265	17.0	92.8
Disk/Tablet diffusion	109	7.7	97.2	58	3.7	75.9
Gradient test	65	4.6	98.5	27	1.7	92.6
Macro broth dilution	0	-	-	0	-	-
Other	1	0.07	100.0	4	0.3	75.0
Total	**1 414**	**100.0**	**97.9**	**1 563**	**100.0**	**96.4**

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 5. Overview of methods used for determination of the AST results for strains '2025 EARS-Net 5' and '2025 EARS-Net 6'

Italy	2025 EARS-Net 5 *Escherichia coli*			2025 EARS-Net 6 *Streptococcus pneumoniae*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	0	-	-	0	-	-
Automated system	3 363	81.7	97.1	821	76.2	97.0
Broth microdilution	475	11.5	91.8	73	6.8	93.2
Disk/Tablet diffusion	90	2.2	93.3	87	8.1	98.9
Gradient test	185	4.5	93.5	92	8.5	90.2
Macro broth dilution	1	0.02	100.0	0	-	-
Other	3	0.07	66.7	4	0.4	100.0
Total	**4 117**	**100.0**	**96.2**	**1 077**	**100.0**	**96.3**

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Table 6. Overview of methods used for determination of the AST results for all six strains

Italy	Total		
Method	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	6	0.04	83.3
Automated system	10 702	79.7	93.8
Broth microdilution	1 663	12.4	90.4
Disk/Tablet diffusion	486	3.6	92.6
Gradient test	545	4.1	91.2
Macro broth dilution	2	0.01	100.0
Other	22	0.2	81.8
Total	**13 426**	**100.0**	**93.2**

Percentage may not total 100% due to rounding. Key: AST – antimicrobial susceptibility testing

Reported intention of participating laboratories to send a strain to a reference laboratory

When submitting AST results, participating laboratories were given the option to indicate whether they would send the strain to a reference laboratory for further microbiological analysis.

For strain '2025 EARS-Net 1' (*K. pneumoniae*), 36 of 196 laboratories (18.4%) would send the strain. This includes 13 out of 82 laboratories with VME.

For strain '2025 EARS-Net 2' (*A. baumannii*), 31 of 197 laboratories (15.7%) would send the strain for further analysis. This includes 18 out of 125 laboratories with VME.

For strain '2025 EARS-Net 3' (*S. aureus*), 29 of 199 laboratories (14.6%) would send the strain for further analysis. The 2 laboratories with VME would not send the strain for further analysis.

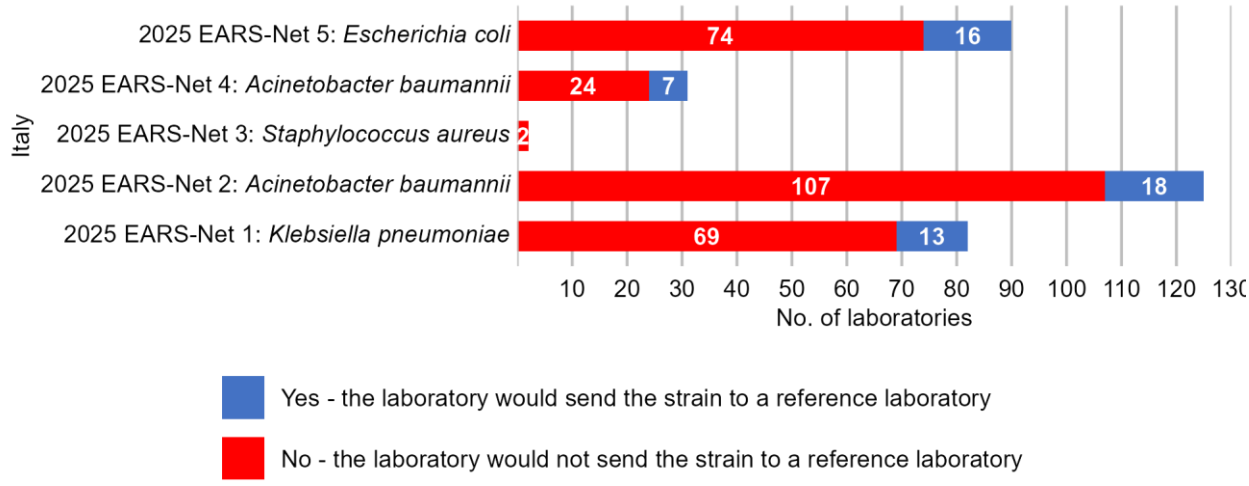
For strain '2025 EARS-Net 4' (*A. baumannii*), 32 of 198 laboratories (16.2%) would send the strain for further analysis. This includes 7 out of 31 laboratories with VME.

For strain '2025 EARS-Net 5' (*E. coli*), 34 of 197 laboratories (17.3%) would send the strain for further analysis. This includes 16 out of 90 laboratories with VME.

For strain '2025 EARS-Net 6' (*S. pneumoniae*), 35 of 155 laboratories (22.6%) would send the strain for further analysis. None of the laboratories reported any VME.

Figure 3 provides an overview of the laboratories reporting very major (VME) that indicated they would send the strains for further analysis.

Figure 3. Laboratories with very major errors (VME) intention to send a strain to a reference laboratory for further testing in 2025 EARS-Net EQA, by strain



Number in the columns: Number of laboratories

Antimicrobial agents tested for each EQA strain

The EQA protocol[2] states that participating laboratories should perform AST on the species-antimicrobial agent combination that can be reported to EARS-Net if they perform that test within their standard practice. The overwhelming majority of clinical laboratories in the EU/EEA are unlikely to perform, as standard practice, AST on all these combinations. For example, many laboratories will utilise the services of reference laboratories.

For strain ‘2025 EARS-Net 1’ (*K. pneumoniae*), 8 out of 196 laboratories tested all 23 antimicrobials (Figure 4).

For strain ‘2025 EARS-Net 2’ (*A. baumannii*), 66 out of 197 laboratories tested all 9 antimicrobials (Figure 6).

For strain ‘2025 EARS-Net 3’ (*S. aureus*), 32 out of 199 laboratories tested all 9 antimicrobials (Figure 9).

For strain ‘2025 EARS-Net 4’ (*A. baumannii*), 63 out of 198 laboratories tested all 9 antimicrobials (Figure 11).

For strain ‘2025 EARS-Net 5’ (*E. coli*), 8 out of 197 laboratories tested all 26 antimicrobials (Figure 14).

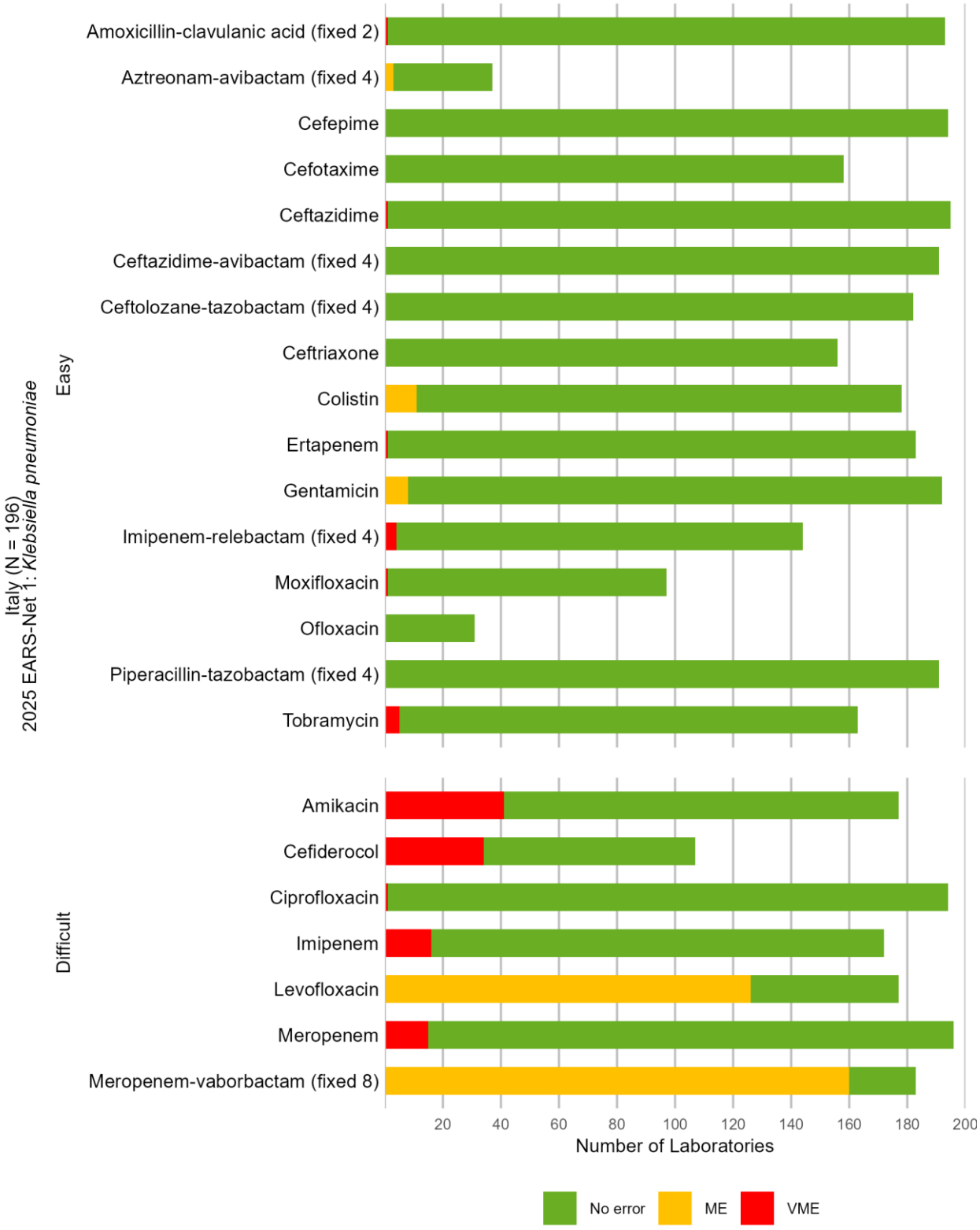
For strain ‘2025 EARS-Net 6’ (*S. pneumoniae*), 10 out of 155 laboratories tested all 10 antimicrobials (Figure 16).

Strain ‘2025 EARS-Net 1’ (*K. pneumoniae*)

Overall, 196 of the 197 laboratories that submitted interpretations of results correctly identified the species for the strain ’2025 EARS-Net 1’. Each laboratory could submit results from 23 antimicrobials (maximum 4508 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

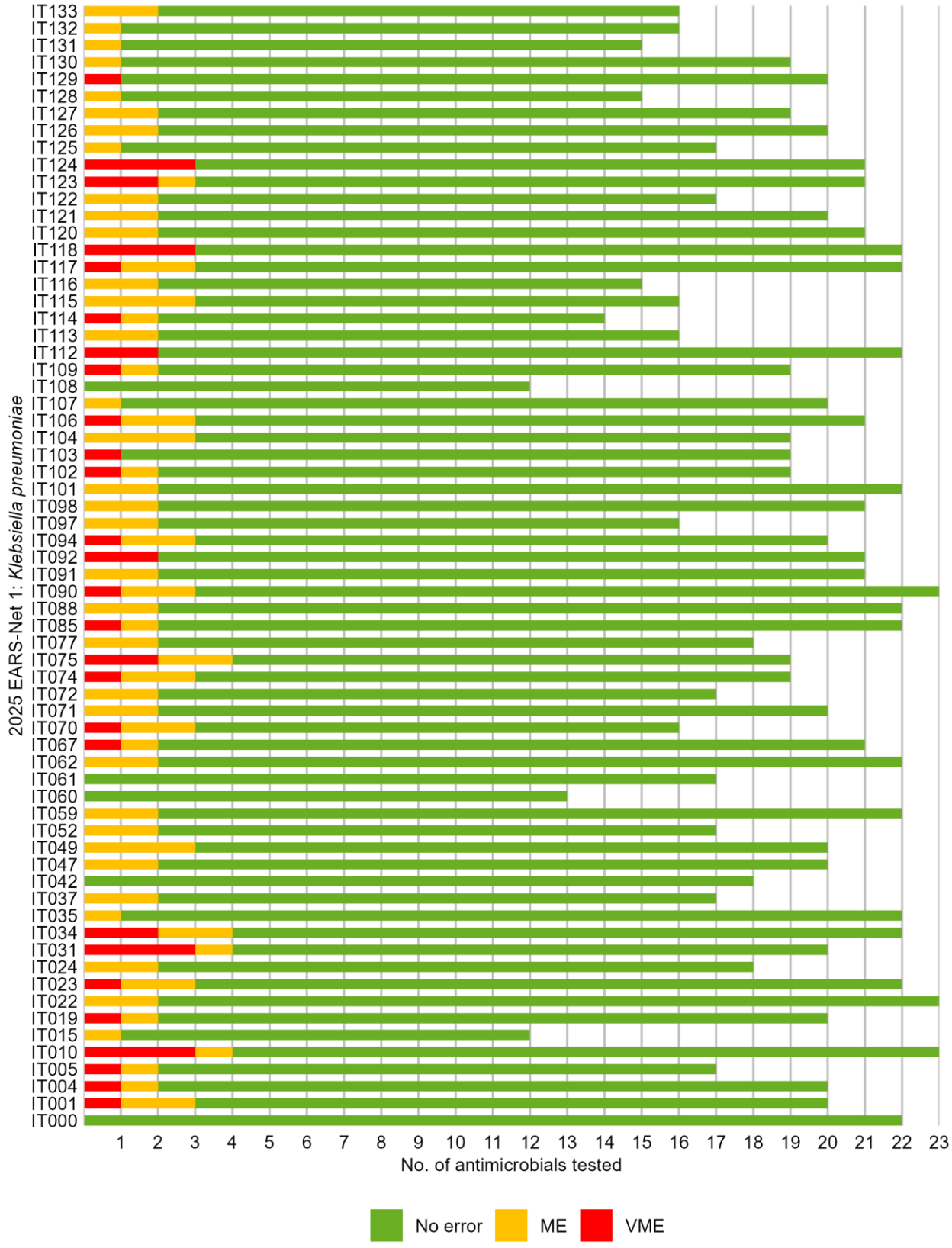
Overall, 3691 AST results were submitted and the interpretations were correct for 3263 (88.4%) of the results, 308 (8.3%) of the interpretations were ME and 120 (3.3%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2025 EARS-Net 1' were reported for amikacin, amoxicillin-clavulanic acid (fixed 2), cefiderocol, ceftazidime, ciprofloxacin, ertapenem, imipenem, imipenem-relebactam (fixed 4), meropenem, moxifloxacin, tobramycin (Figure 4). An overview of the reported results for all laboratories is presented in Figure 5.

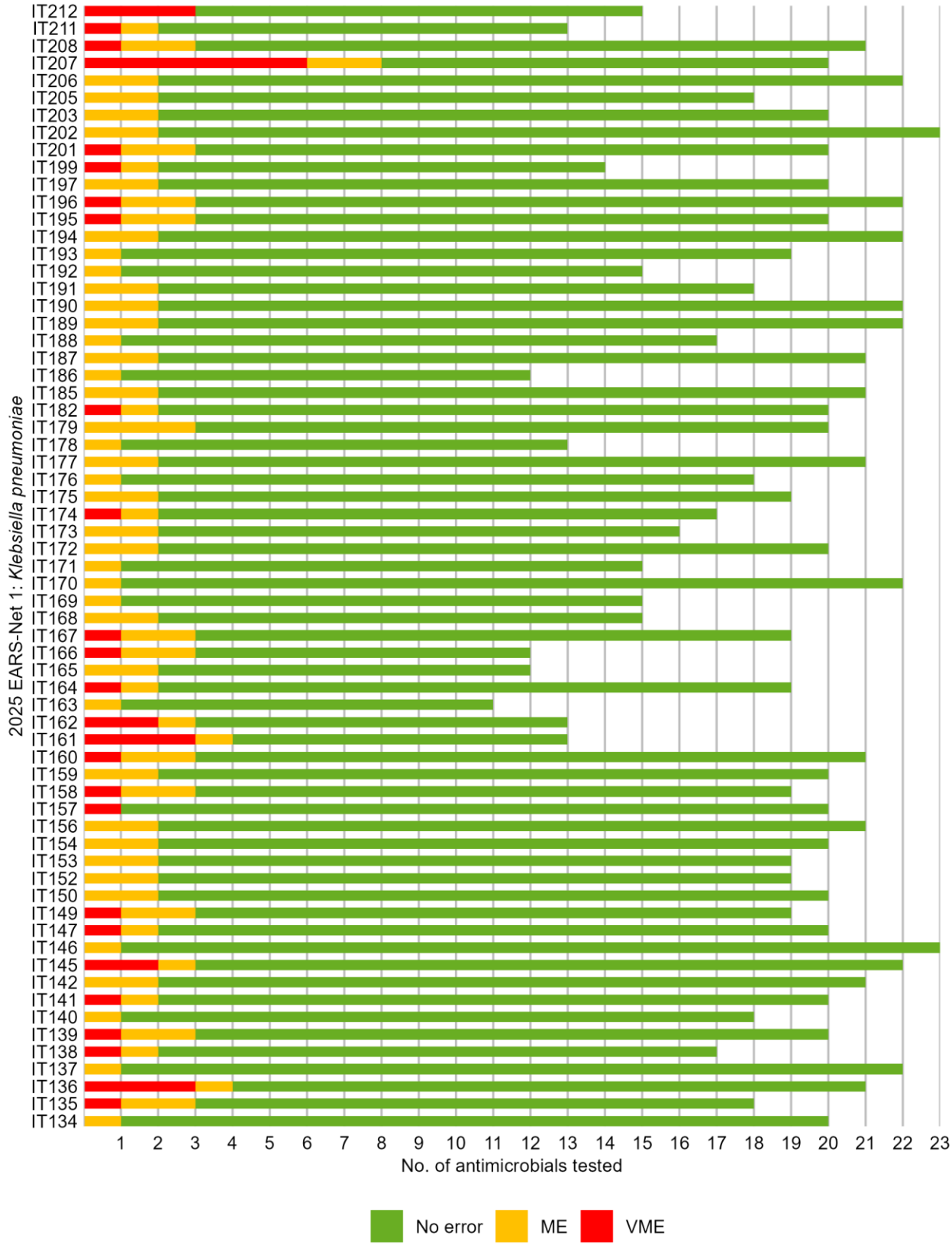
Figure 4. Reported interpretation of AST results for strain '2025 EARS-Net 1' (*Klebsiella pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 5. Reported interpretation of AST results for strain '2025 EARS-Net 1' (*Klebsiella pneumoniae*) by laboratory




2025 EARS-Net 1: Klebsiella pneumoniae
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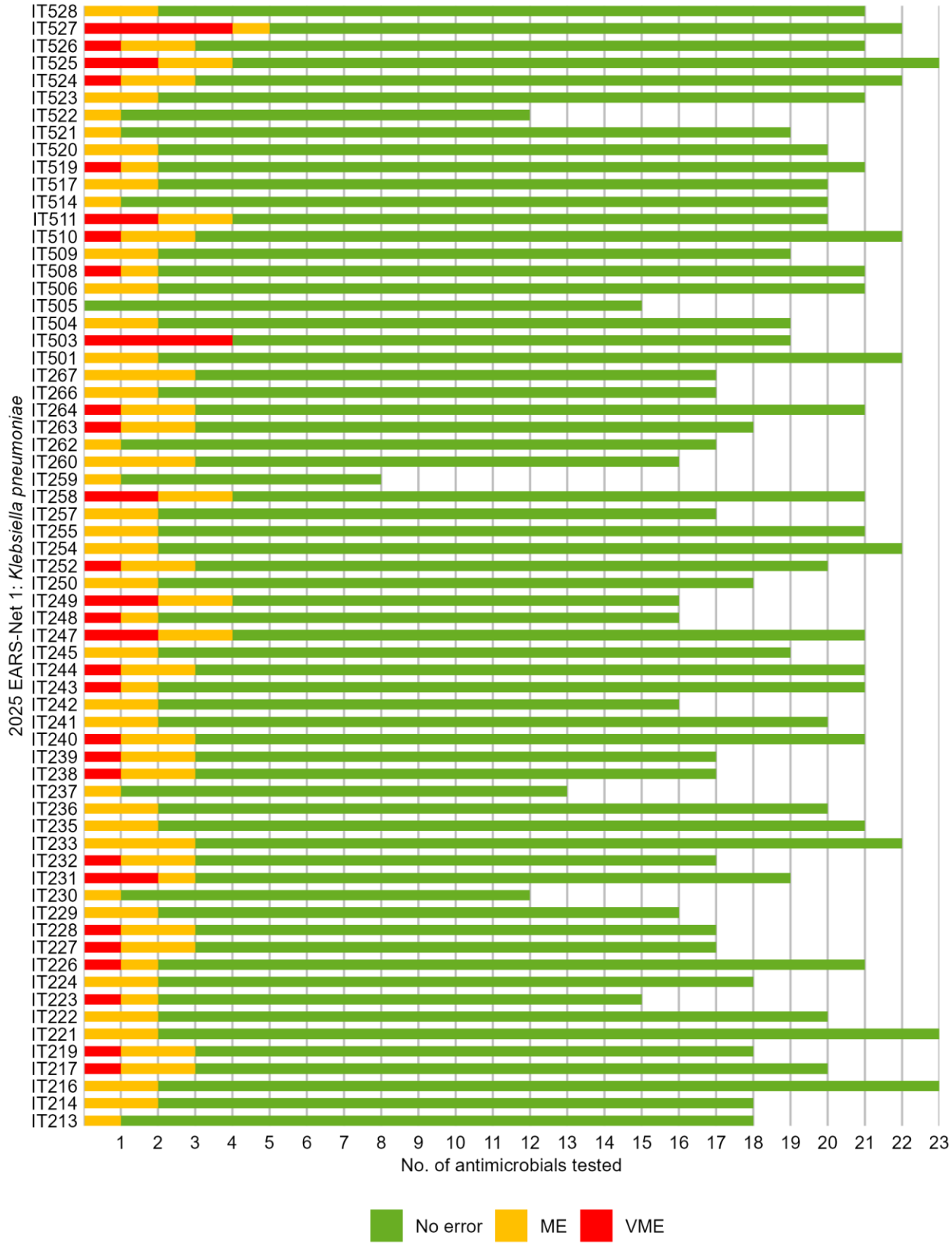
IT138
IT137
IT136
IT135
IT134
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
No. of antimicrobials tested
No error
ME
VME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the strain ‘2025 EARS-Net 1’, 6 laboratories were in full concordance with the expected interpretations, 9 laboratories had an 'excellent' concordance with the expected interpretation (≥ 21

95%), 78 laboratories had a 'very good' concordance (< 95% and ≥ 90%), 66 laboratories had a 'good' concordance (< 90% and ≥ 85%), 29 laboratories had a 'satisfactory' concordance (< 85% and ≥ 80%), and 8 laboratories had < 80% concordance.

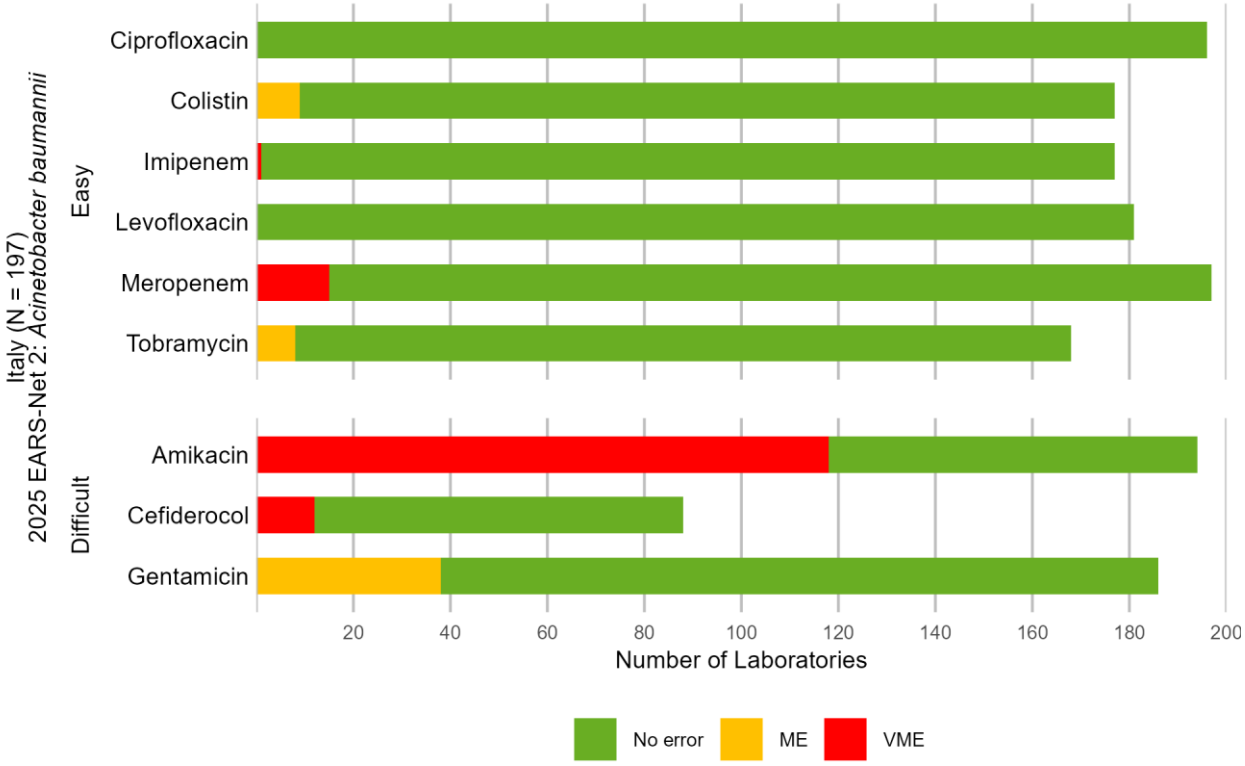
In Italy, for the strain '2025 EARS-Net 1', VMEs were observed for amikacin, imipenem, meropenem and cefiderocol. Deviations in amikacin corresponded to 23.2% of all submitted interpretations for that antimicrobial and were reported when using automated system and broth microdilution. Deviations in imipenem corresponded to 9.3% of all submitted interpretations for that antimicrobial and were reported through all methods. Deviations in meropenem corresponded to 7.7% of all submitted interpretations for that antimicrobial and were reported through all methods. Deviations in cefiderocol corresponded to 31.8% of all submitted interpretations for that antimicrobial and were reported through all methods. For all four antimicrobials the expected AST result was less than two dilutions or less than four millimeters away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures. proportions of VME (≤5%) were observed for ceftazidime, ciprofloxacin, ertapenem, moxifloxacin, tobramycin, amoxicillin-clavulanic acid, imipenem-relebactam. A high proportion of MEs was observed for levofloxacin (71.2% of submitted results, reported through all methods) and meropenem-vaborbactam (87.4% of submitted results, reported through all methods). For both antimicrobials, the expected AST results were less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures.

Strain ‘2025 EARS-Net 2’ (*Acinetobacter baumannii*)

Overall, 197 of the 199 laboratories that submitted interpretations of results correctly identified the species for the strain ’2025 EARS-Net 2’. Each laboratory could submit results from 9 antimicrobials (maximum 1773 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

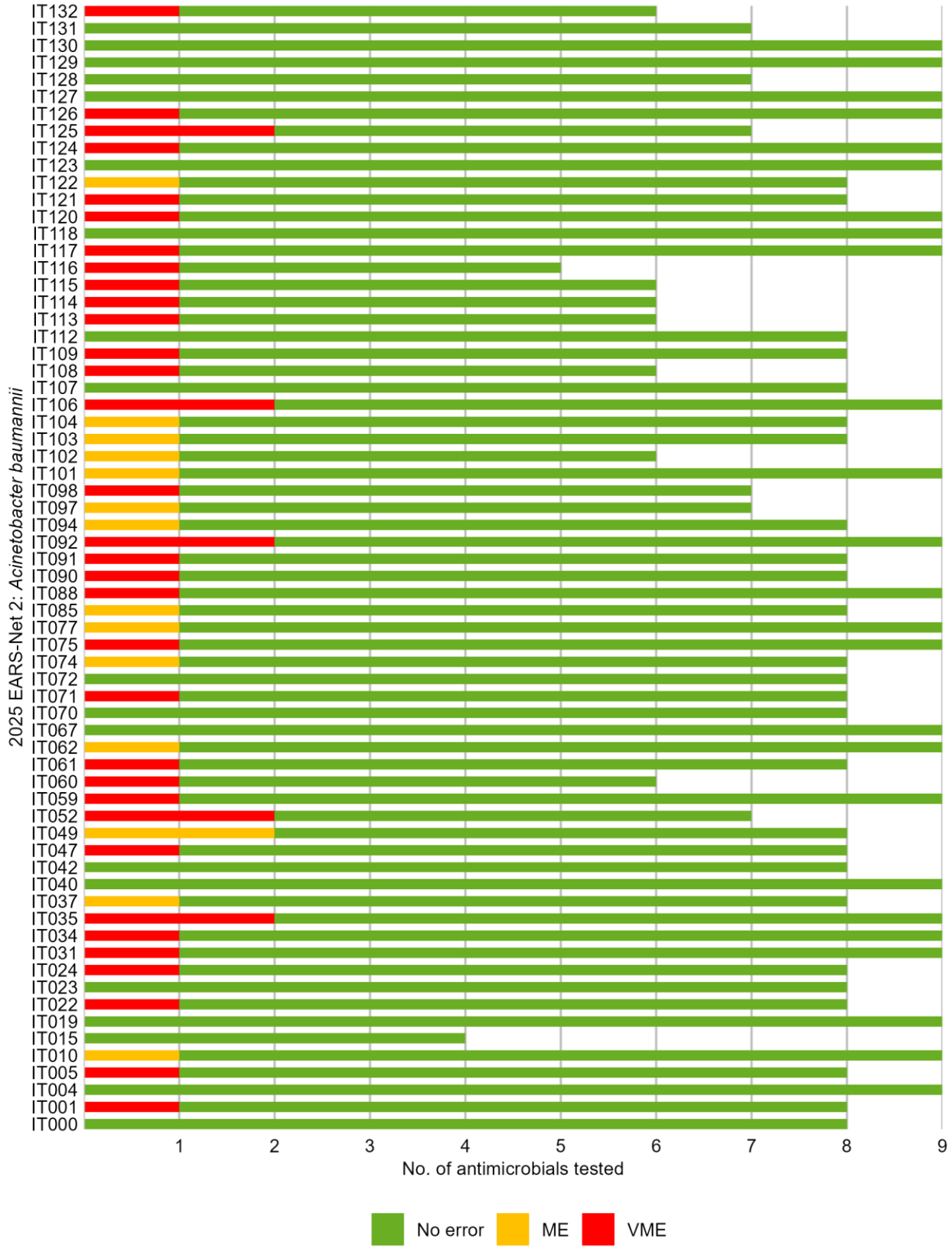
Overall, 1564 AST results were submitted and the interpretations were correct for 1363 (87.1%) of the results, 55 (3.5%) of the interpretations were ME and 146 (9.3%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2025 EARS-Net 2' were reported for amikacin, cefiderocol, imipenem, meropenem (Figure 6). An overview of the reported results for all laboratories is presented in Figure 7.

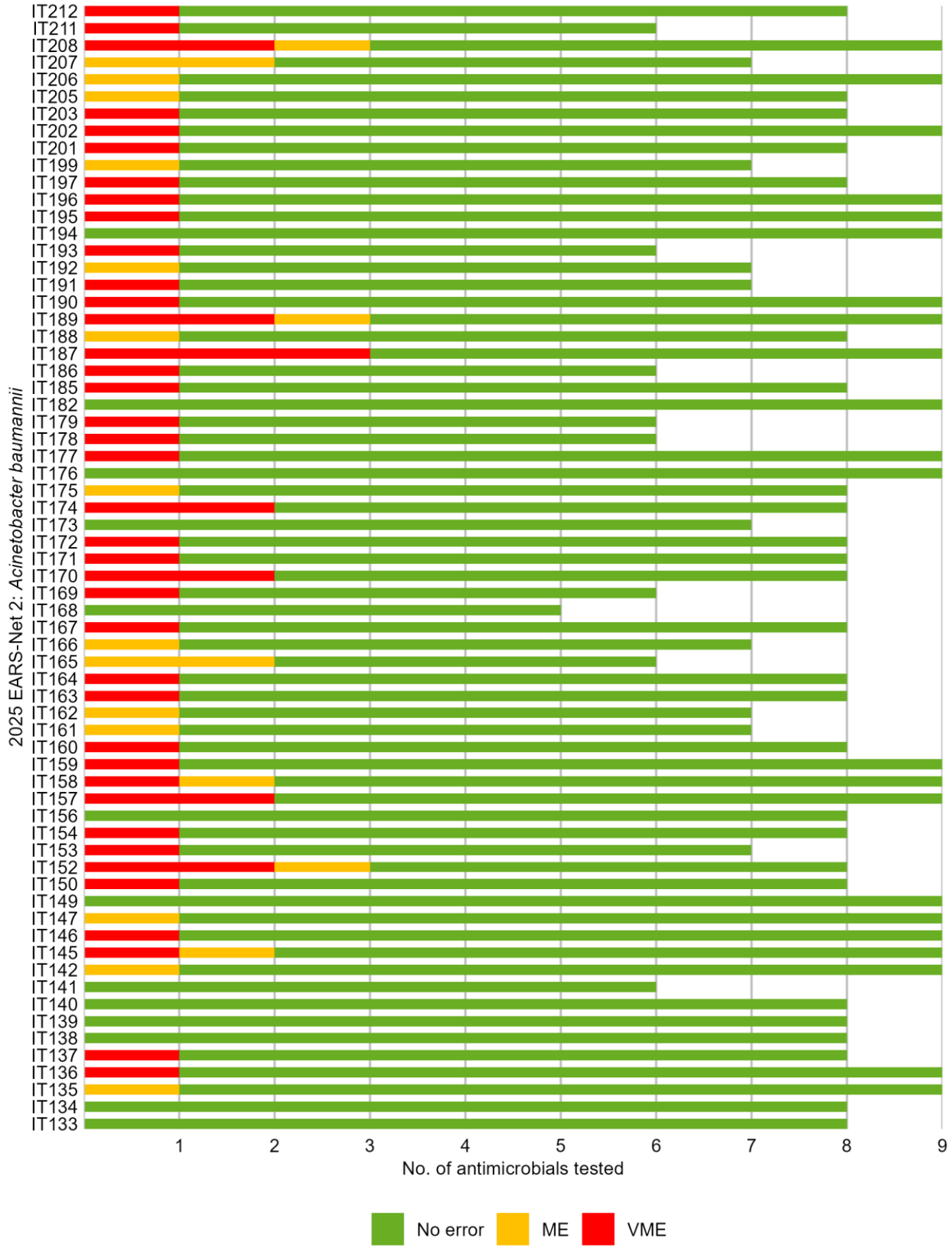
Figure 6. Reported interpretation of AST results for strain ‘2025 EARS-Net 2’ (*Acinetobacter baumannii*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 7. Reported interpretation of AST results for strain '2025 EARS-Net 2' (*Acinetobacter baumannii*) by laboratory




2025 EARS-Net 2: Acinetobacter baumannii
IT212
IT211
IT208
IT207
IT206
IT205
IT203
IT202
IT201
IT199
IT197
IT196
IT195
IT194
IT193
IT192
IT191
IT190
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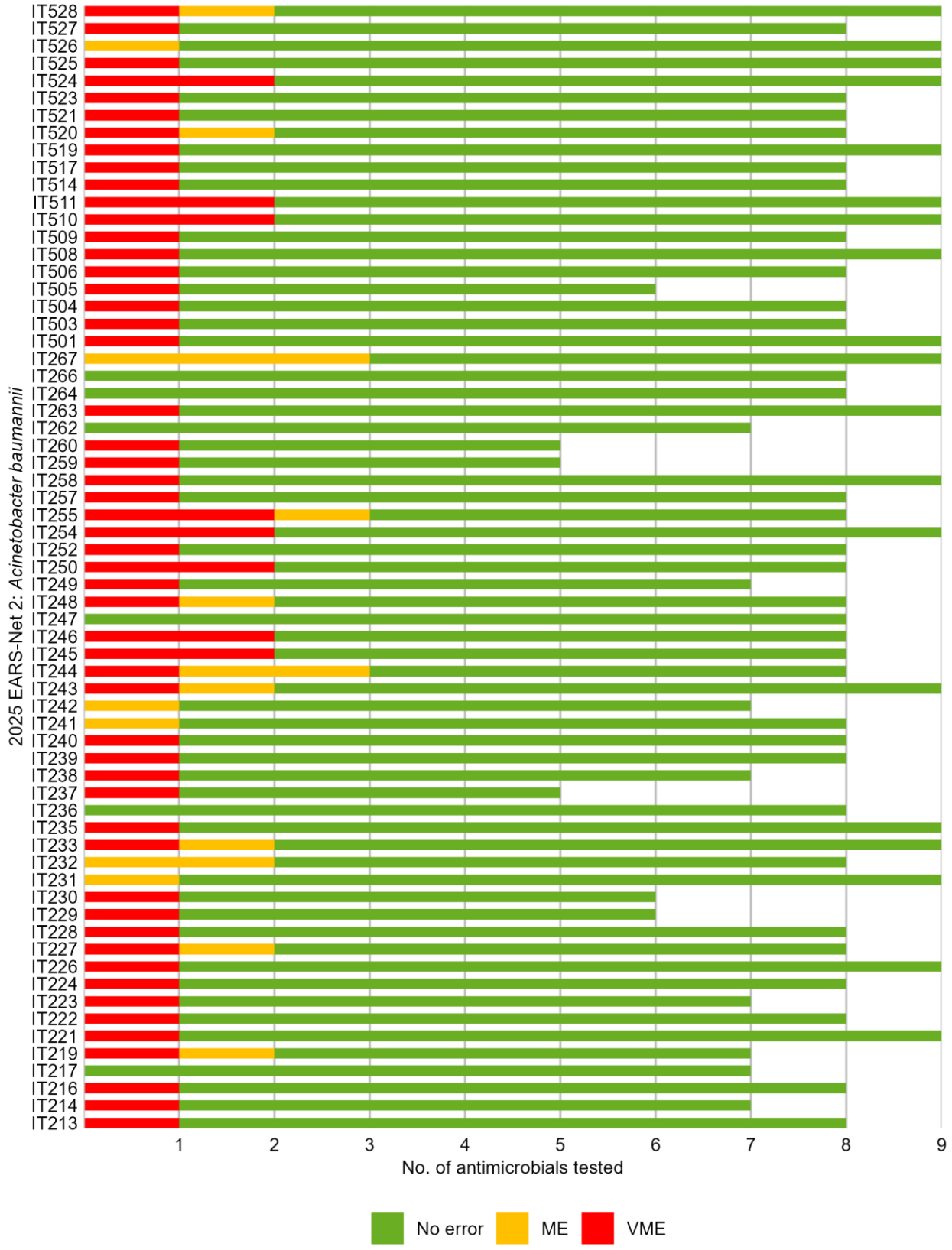
IT153
IT152
IT150
IT149
IT147
IT146
IT145
IT142
IT141
IT140
IT139
IT138
IT137
IT136
IT135
IT134
IT133
1
2
3
4
5
6
7
8
9
No. of antimicrobials tested
No error
ME
VME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the strain ‘2025 EARS-Net 2’, 38 laboratories were in full concordance with the expected interpretations, 104 laboratories had a 'good' concordance (< 90% and ≥ 85%), 20 laboratories

had a 'satisfactory' concordance (< 85% and ≥ 80%), and 35 laboratories had < 80% concordance.

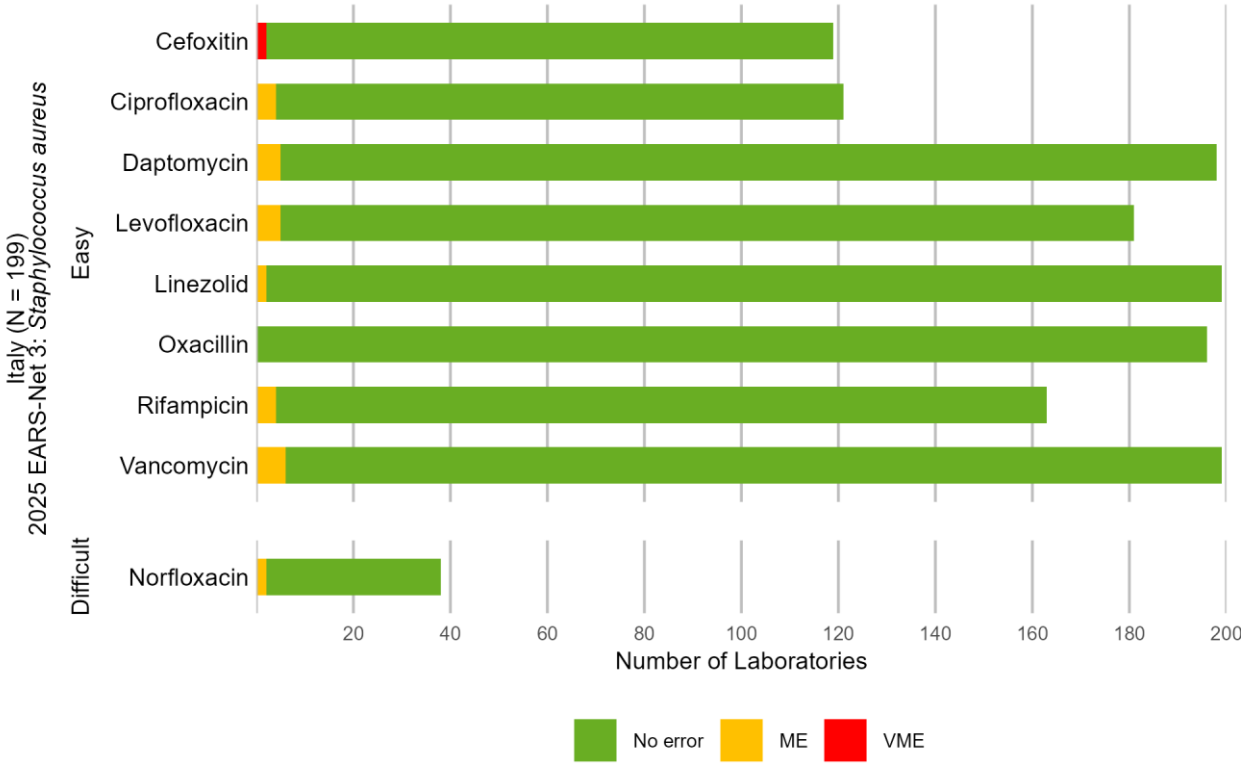
In Italy, for the strain '2025 EARS-Net 2', VMEs were observed for amikacin, meropenem and cefiderocol. Deviations in amikacin corresponded to 60.8% of all submitted interpretations for that antimicrobial and were reported when using automated systems, broth microdilution and gradient test. Deviations in meropenem corresponded to 7.6% of all submitted interpretations for that antimicrobial and were reported when using automated system. Deviations in cefiderocol corresponded to 13.6% of all submitted interpretations for that antimicrobial and were reported through most methods. For amikacin and cefiderocol the expected AST result was less than two dilutions or less than four millimeters away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures. For meropenem the expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures. Low proportions of VME (≤5%) were observed for imipenem. A high proportion of MEs was observed for gentamicin (20.4% of submitted results, reported when using automated system, broth microdilution and disk/tablet diffusion). The expected AST result was less than two dilutions away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures.

Strain ‘2025 EARS-Net 3’ (*Staphylococcus aureus*)

For the 2025 EARS-Net EQA, The 199 laboratories that submitted interpretations of results correctly identified the species for the strain ’2025 EARS-Net 3’. Each laboratory could submit results from 9 antimicrobials (maximum 1791 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

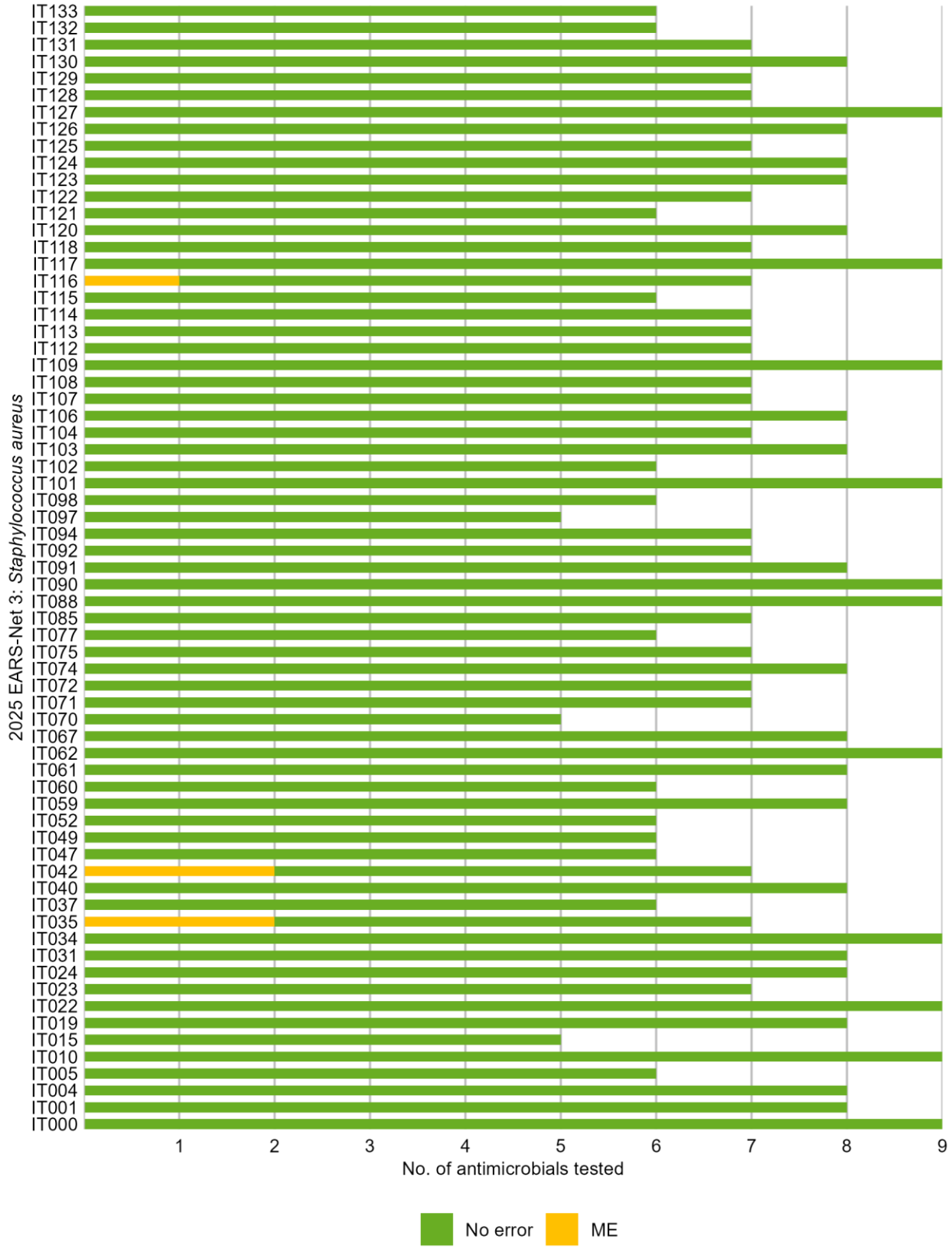
Overall, 1414 AST results were submitted and the interpretations were correct for 1384 (97.9%) of the results, 28 (2%) of the interpretations were ME and 2 (0.1%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2025 EARS-Net 3' were reported for cefoxitin (Figure 8). An overview of the reported results for all laboratories is presented in Figure 9.

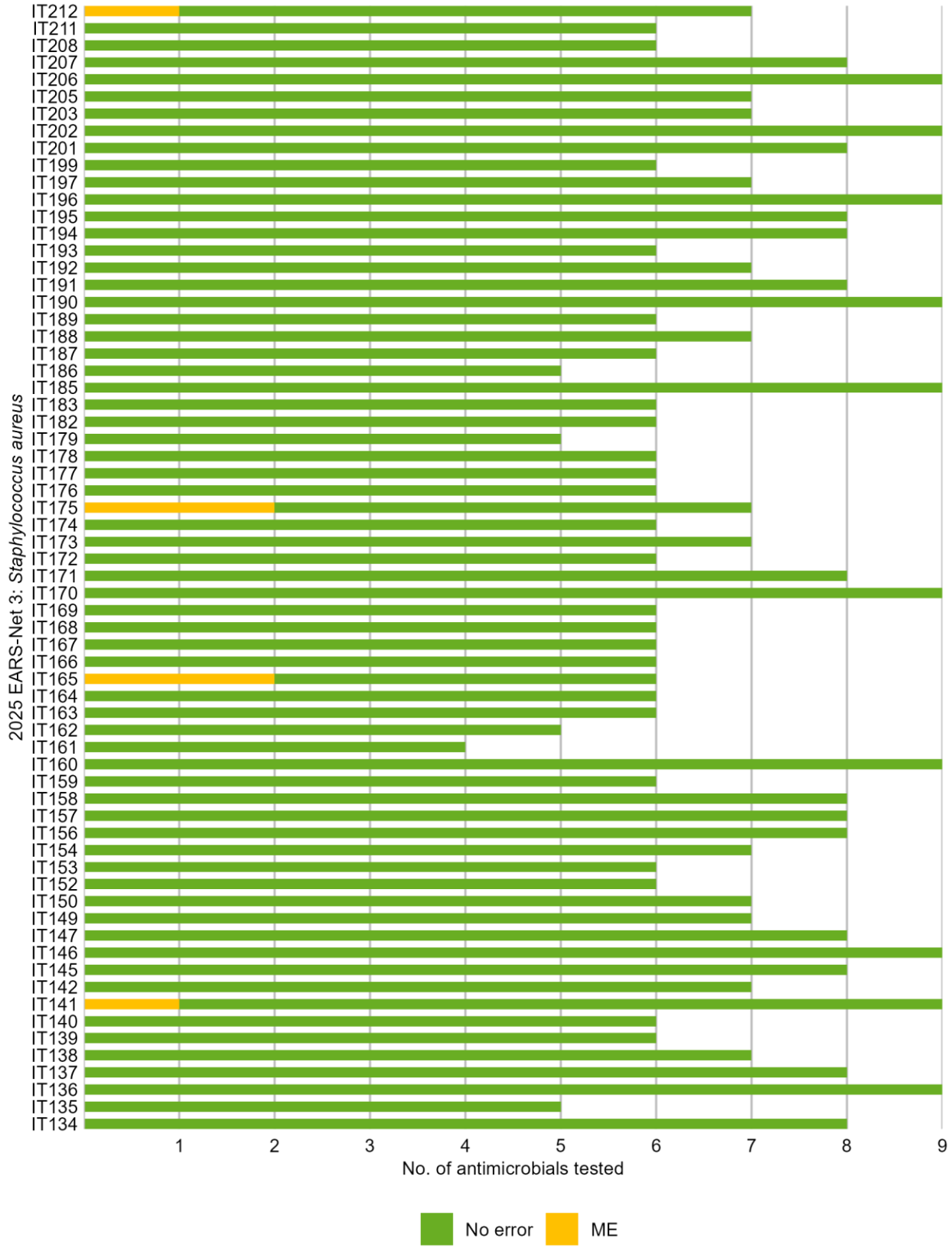
Figure 8. Reported interpretation of AST results for strain ‘2025 EARS-Net 3’ (*Staphylococcus aureus*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 9. Reported interpretation of AST results for strain '2025 EARS-Net 3' (*Staphylococcus aureus*) by laboratory




2025 EARS-Net 3: Staphylococcus aureus
IT212
IT211
IT208
IT207
IT206
IT205
IT203
IT202
IT201
IT199
IT197
IT196
IT195
IT194
IT193
IT192
IT191
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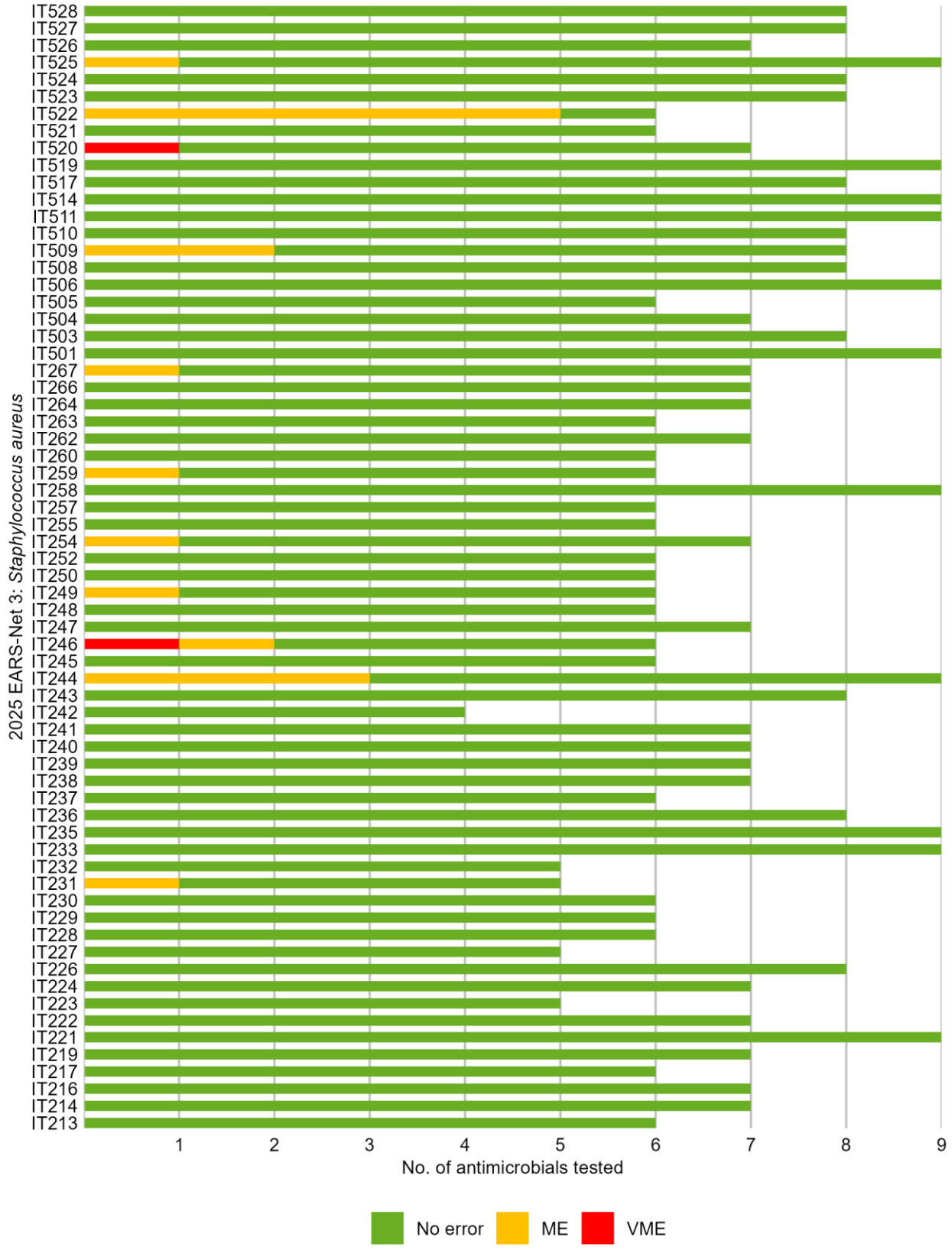
IT152
IT150
IT149
IT147
IT146
IT145
IT142
IT141
IT140
IT139
IT138
IT137
IT136
IT135
IT134
1
2
3
4
5
6
7
8
9
No. of antimicrobials tested
No error
ME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the strain ‘2025 EARS-Net 3’, 181 laboratories were in full concordance with the expected interpretations, 7 laboratories had a 'good' concordance (< 90% and ≥ 85%), 3 laboratories had a

'satisfactory' concordance (< 85% and ≥ 80%), and 8 laboratories had < 80% concordance.

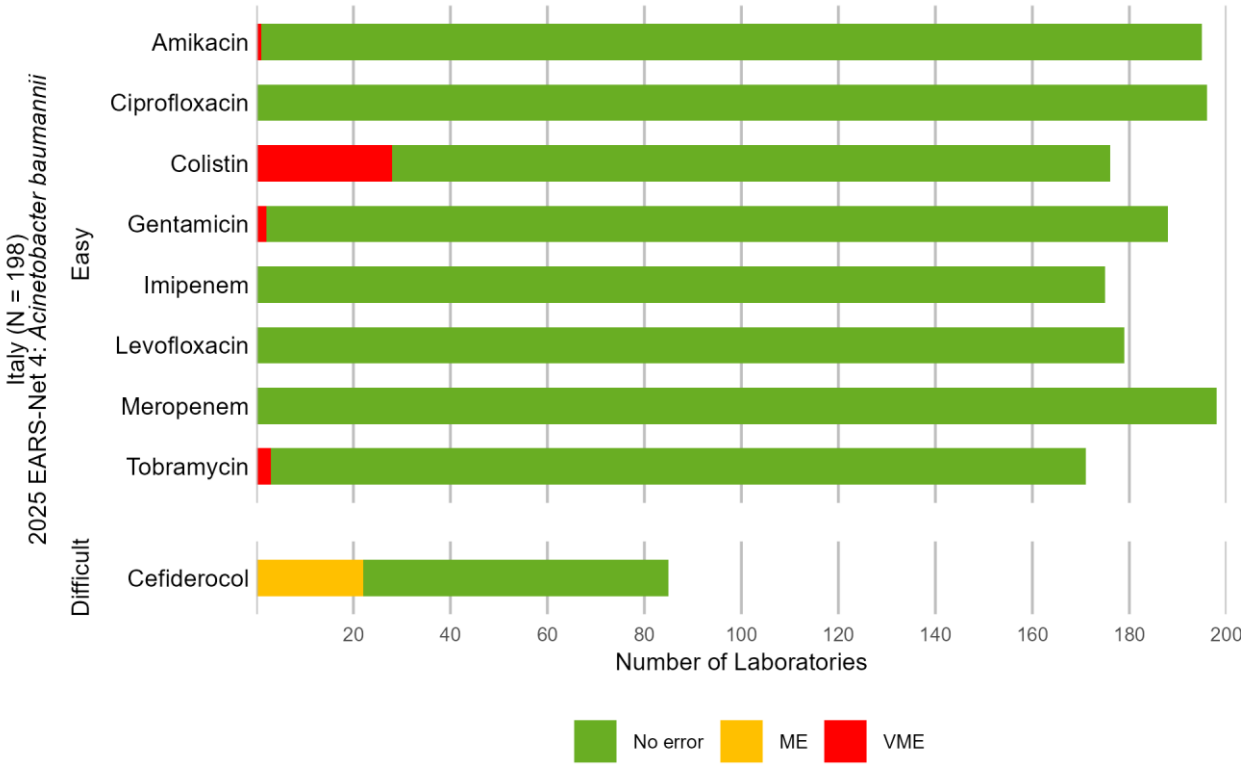
In Italy, for the strain '2025 EARS-Net 3', low proportions of VMEs (≤5%) were observed for cefoxitin. No high proportions of MEs were observed.

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

The 198 laboratories that submitted interpretations of results correctly identified the species for the strain '2025 EARS-Net 4'. Each laboratory could submit results from 9 antimicrobials (maximum 1782 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

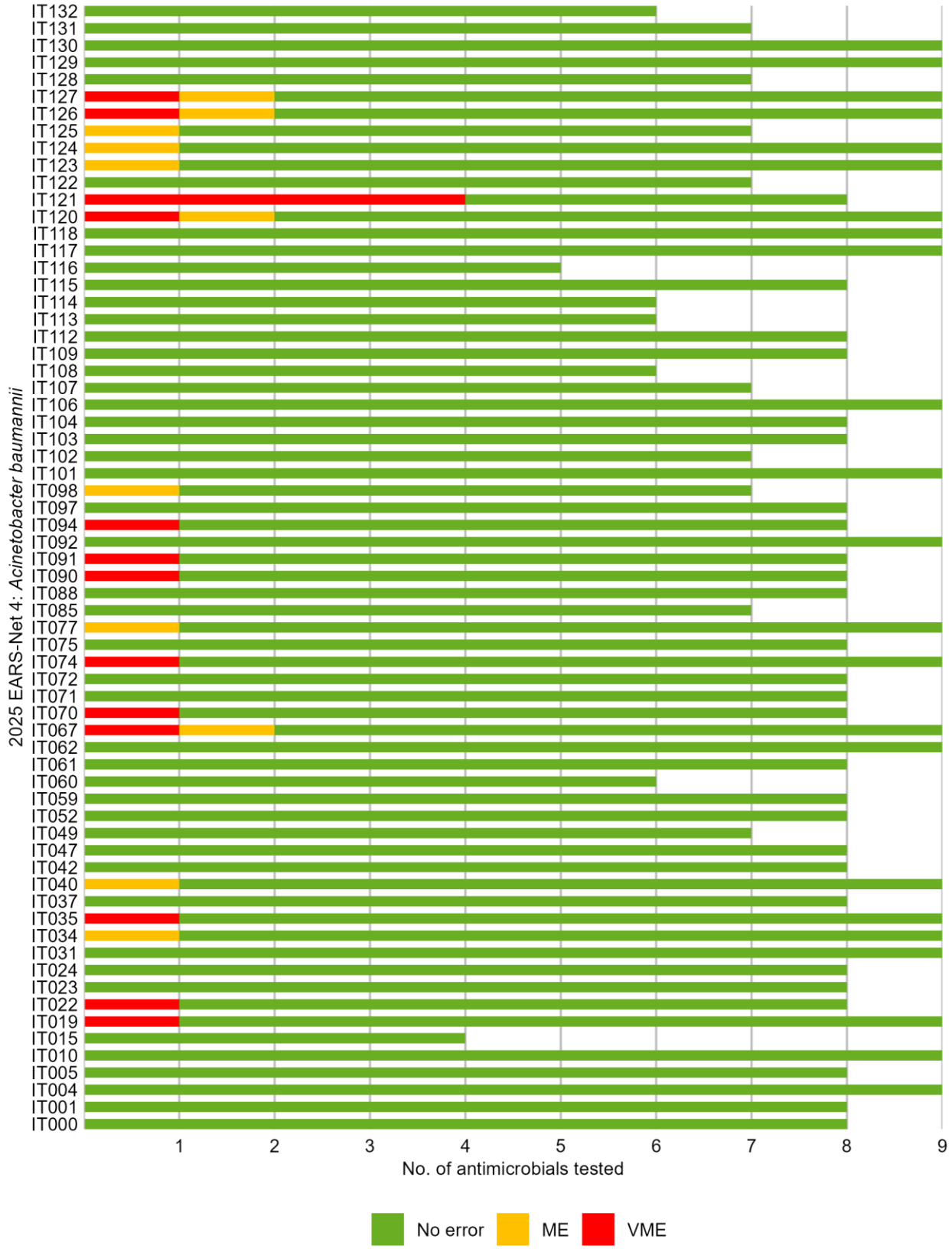
Overall, 1563 AST results were submitted and the interpretations were correct for 1507 (96.4%) of the results, 22 (1.4%) of the interpretations were ME and 34 (2.2%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2025 EARS-Net 4' were reported for amikacin, colistin, gentamicin, tobramycin (Figure 10). An overview of the reported results for all laboratories is presented in Figure 11.

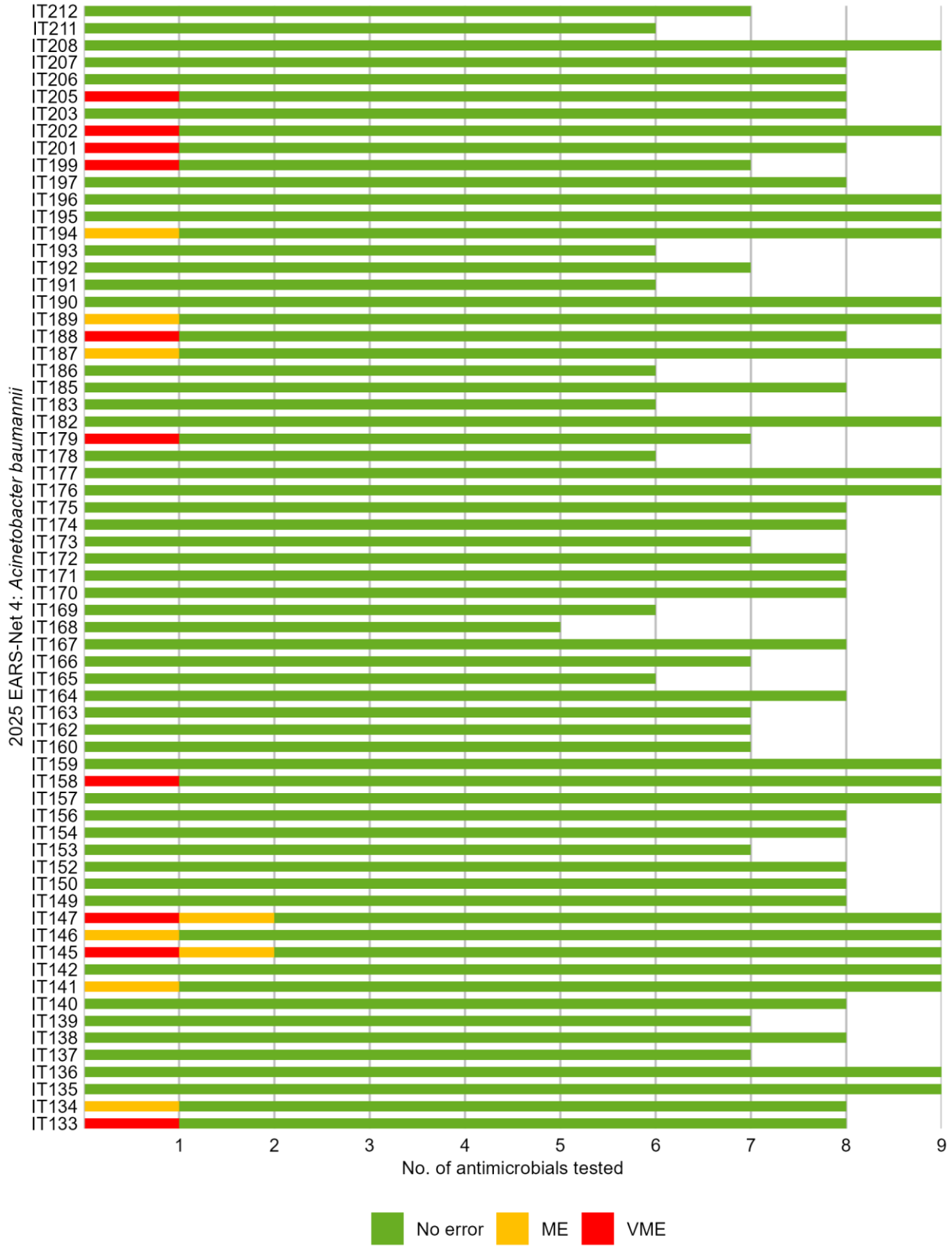
Figure 10. Reported interpretation of AST results for strain '2025 EARS-Net 4' (*Acinetobacter baumannii*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 11. Reported interpretation of AST results for strain '2025 EARS-Net 4' (*Acinetobacter baumannii*) by laboratory




2025 EARS-Net 4: Acinetobacter baumannii
IT212
IT211
IT208
IT207
IT206
IT205
IT203
IT202
IT201
IT199
IT197
IT196
IT195
IT194
IT193
IT192
IT191
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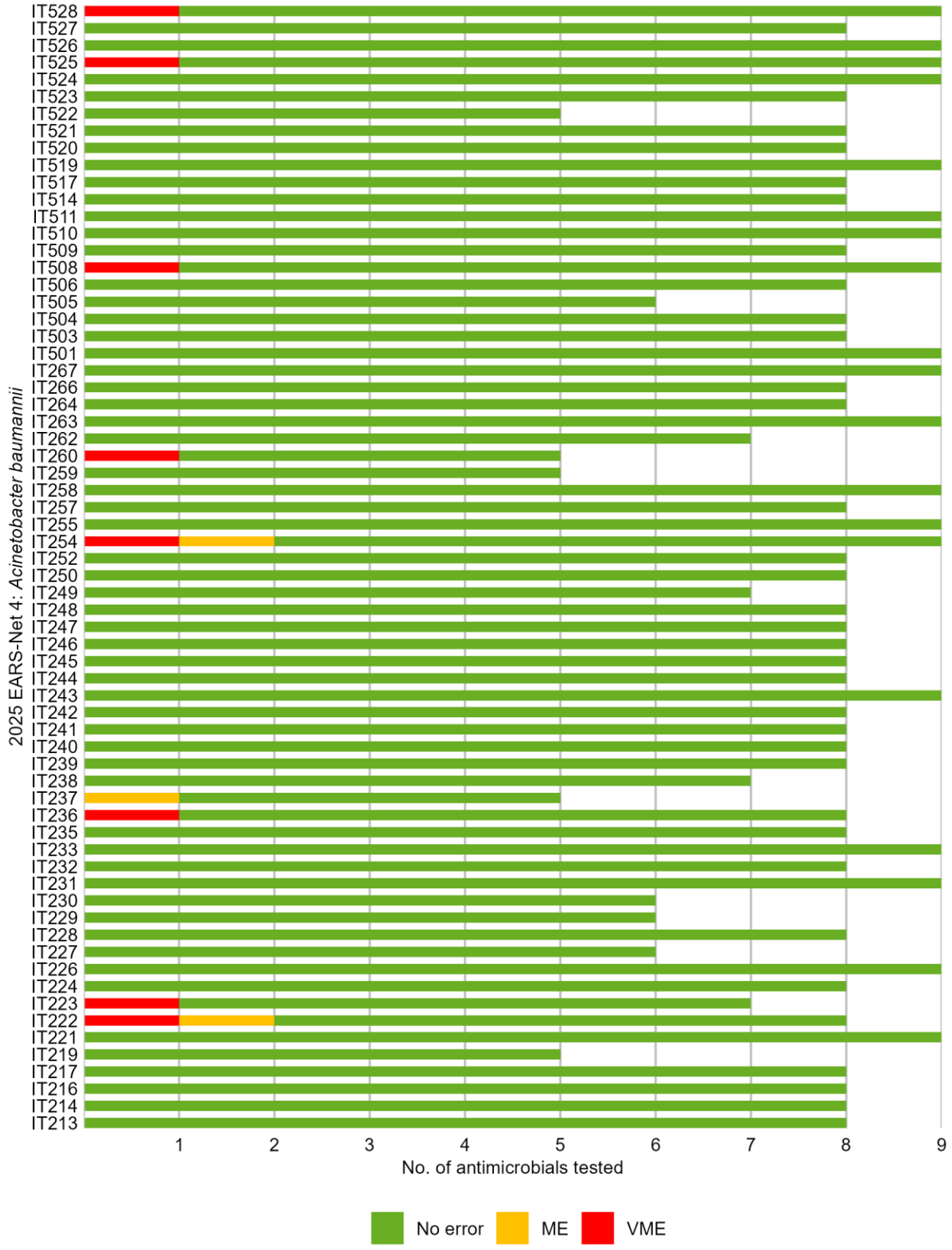
IT146
IT145
IT142
IT141
IT140
IT139
IT138
IT137
IT136
IT135
IT134
IT133
1
2
3
4
5
6
7
8
9
No. of antimicrobials tested
No error
ME
VME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the ‘2025 EARS-Net 4’, 153 laboratories were in full concordance with the expected interpretations, 34 laboratories had a 'good' concordance (< 90% and ≥ 85%), 2 laboratories had

a 'satisfactory' concordance (< 85% and ≥ 80%), and 9 laboratories had < 80% concordance.

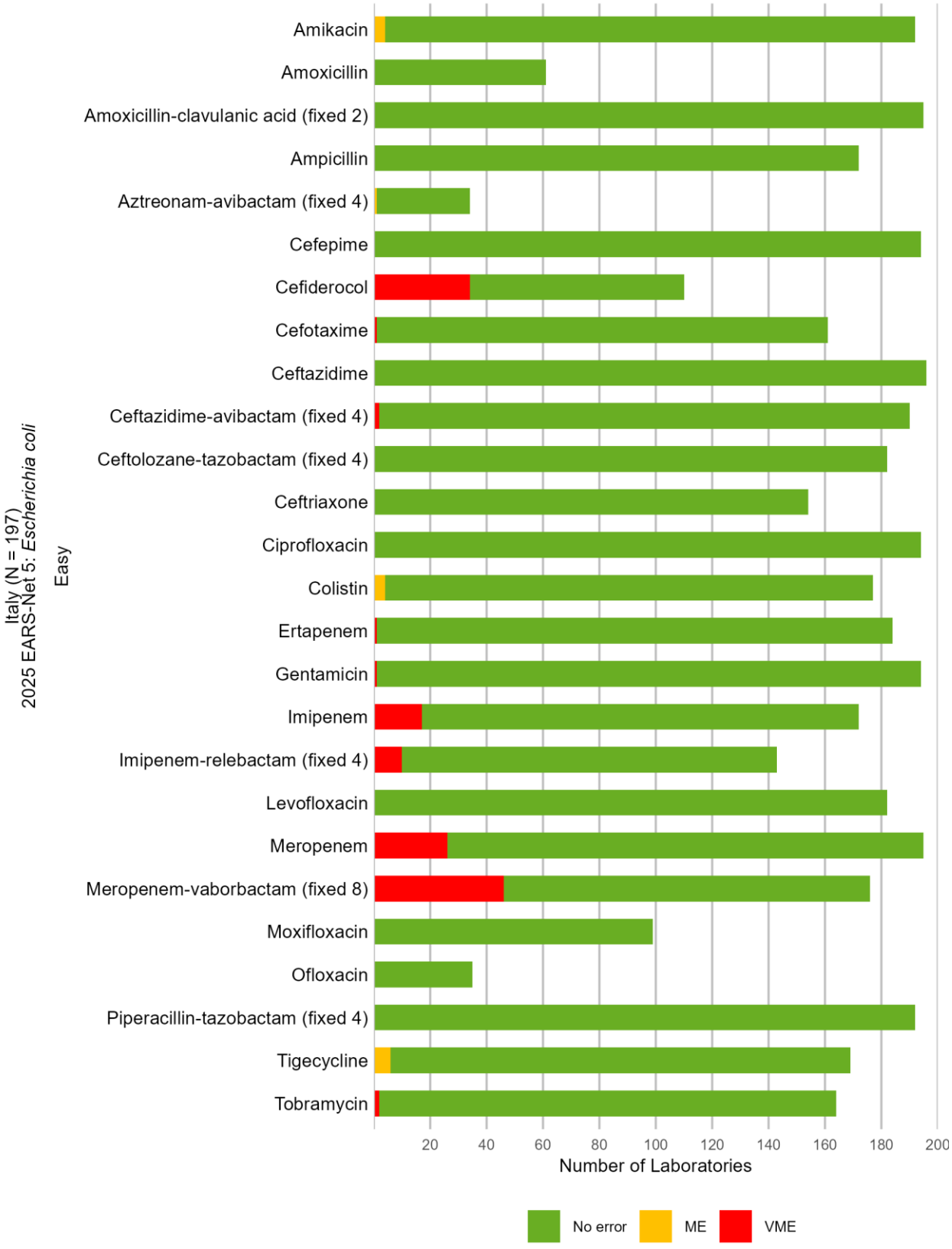
In Italy, for the strain '2025 EARS-Net 4', VMEs were observed for colistin. These deviations corresponded to 15.9% of all submitted interpretations for that antimicrobial and were reported when using automated system, broth microdilution and gradient test. The expected AST result was at least two dilutions away from the clinical breakpoint, thus these deviations should not be due to inherent method variation. They might be attributable to systematic or random errors in the laboratories' procedures. Low proportions of VMEs (≤5%) were observed for amikacin, gentamicin, tobramycin. A high proportion of MEs was observed for cefiderocol (25.9% of submitted results, reported through most methods). The expected AST result was less than four millimeters away from the clinical breakpoint, thus these deviations can be due to inherent method variation. However, they might also be attributable to systematic or random errors in the laboratories' procedures.

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

In the 2025 EARS-Net EQA, Overall, 197 of the 198 laboratories that submitted interpretations of results correctly identified the species for the strain '2025 EARS-Net 5'. Each laboratory could submit results from 23 antimicrobials (maximum 5122 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

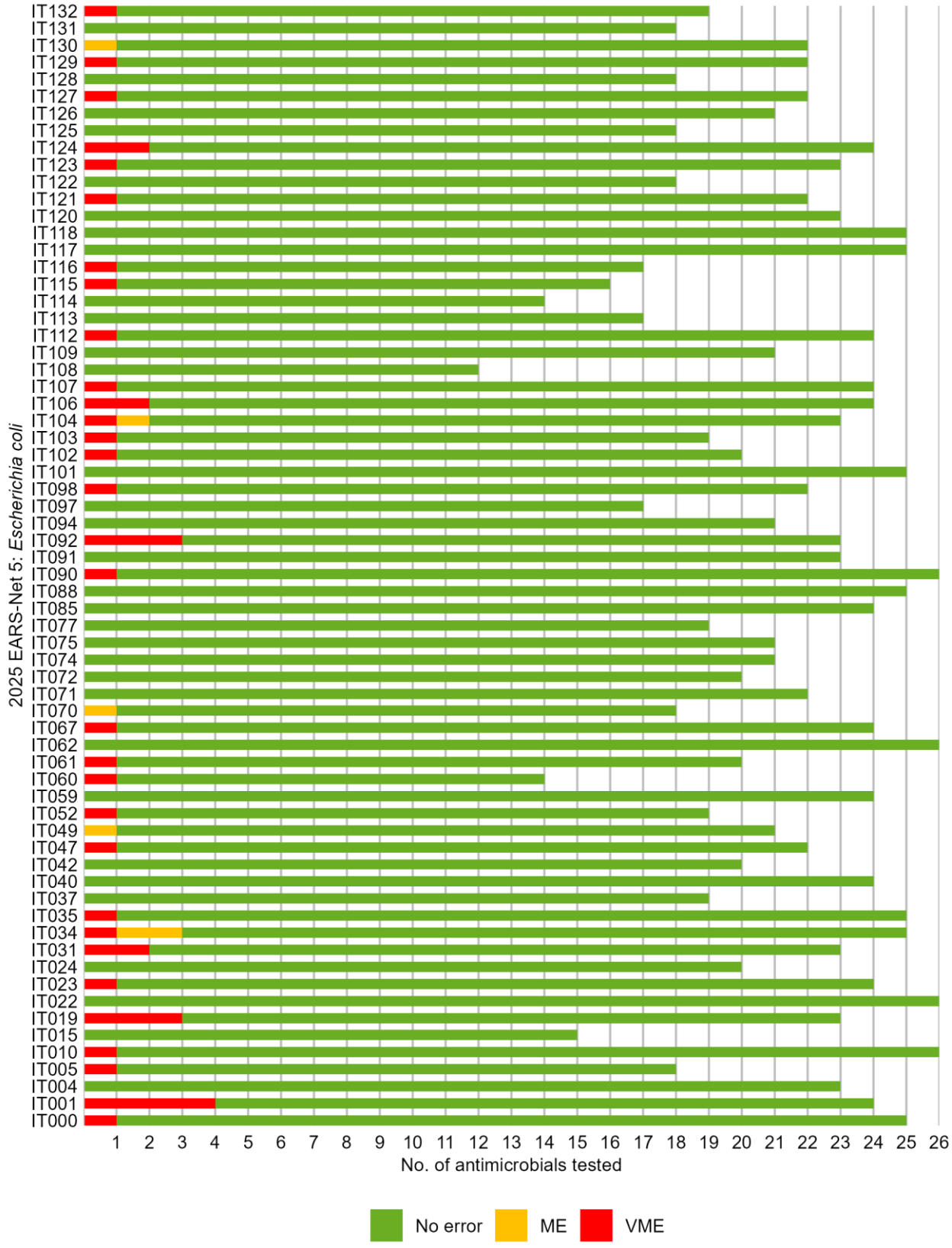
Overall, 4117 AST results were submitted and the interpretations were correct for 3962 (96.2%) of the results, 15 (0.4%) of the interpretations were ME and 140 (3.4%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2025 EARS-Net 5' were reported for cefiderocol, cefotaxime, ceftazidime-avibactam (fixed 4), ertapenem, gentamicin, imipenem, imipenem-relebactam (fixed 4), meropenem, meropenem-vaborbactam (fixed 8), tobramycin (Figure 12). An overview of the reported results for all laboratories is presented in Figure 13.

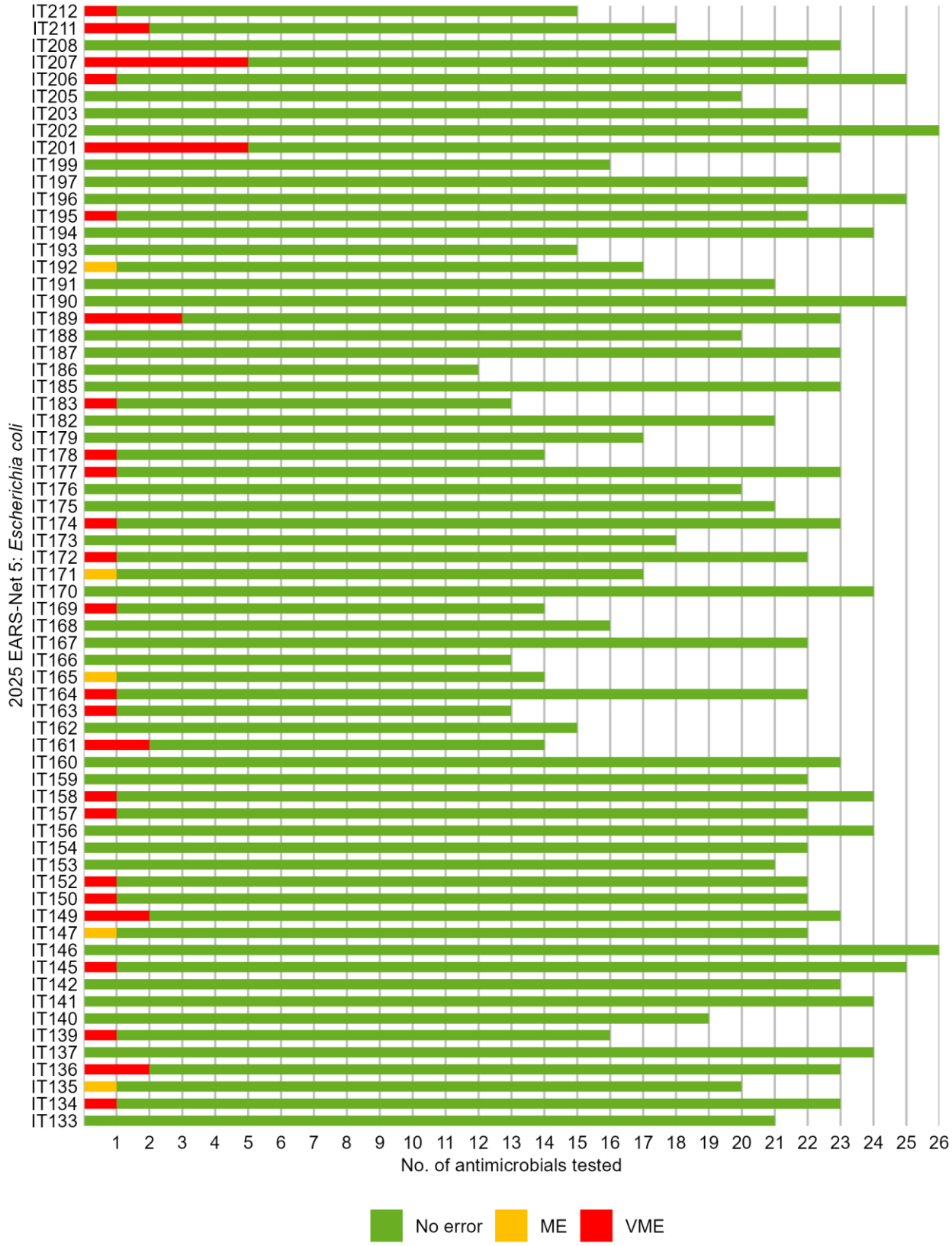
Figure 12. Reported interpretation of AST results for strain '2025 EARS-Net 5' (*Escherichia coli*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 13. Reported interpretation of AST results for strain '2025 EARS-Net 5' (*Escherichia coli*) by laboratory




2025 EARS-Net 5: Escherichia coli
IT212
IT211
IT208
IT207
IT206
IT205
IT203
IT202
IT201
IT199
IT197
IT196
IT195
IT194
IT193
IT192
IT191
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IT152
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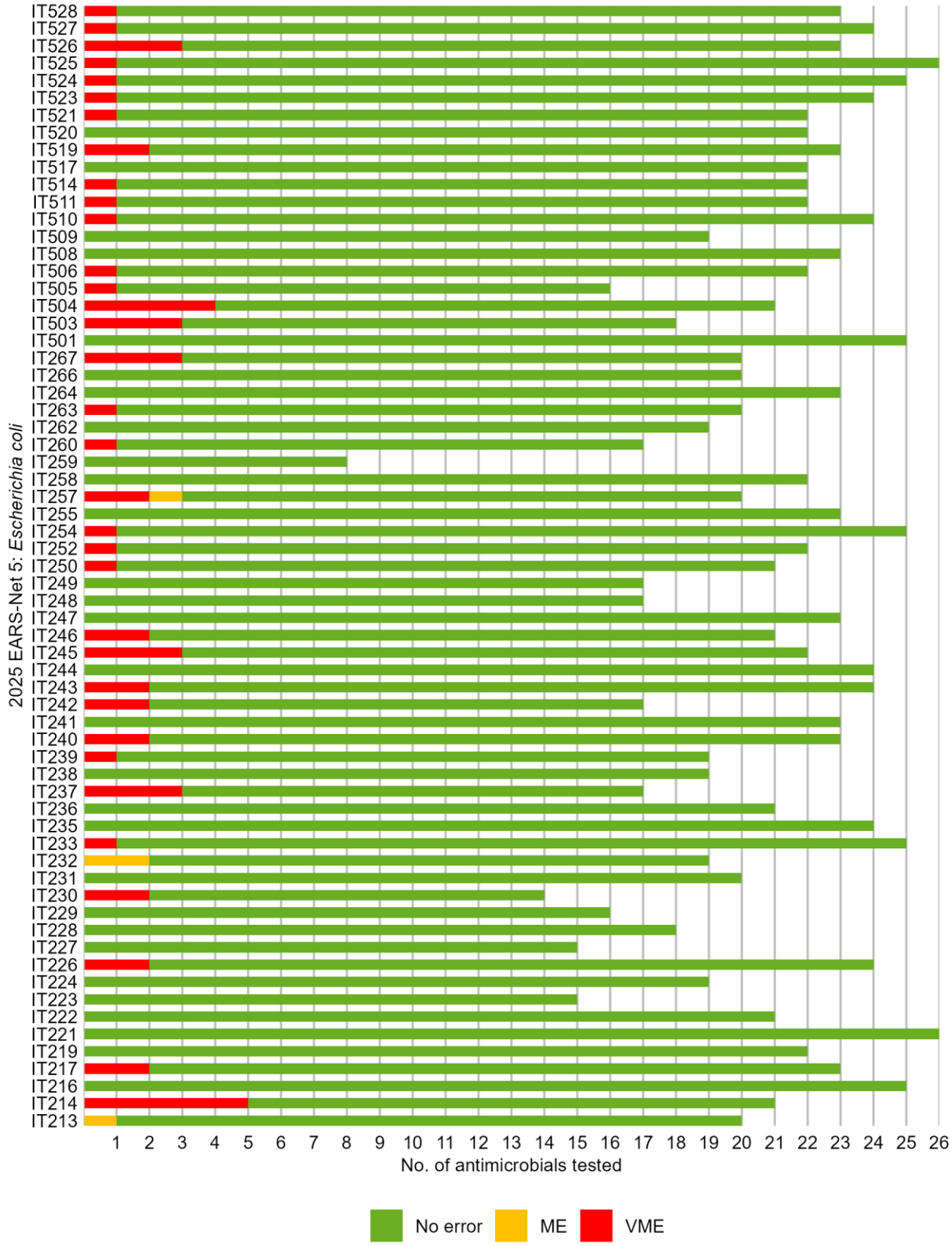
IT149
IT147
IT146
IT145
IT142
IT141
IT140
IT139
IT137
IT136
IT135
IT134
IT133
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
No. of antimicrobials tested
No error
ME
VME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the ‘2025 EARS-Net 5’, 97 laboratories were in full concordance with the expected interpretations, 48 laboratories had an 'excellent' concordance with the expected interpretation (≥ 40

95%), 32 laboratories had a 'very good' concordance (< 95% and ≥ 90%), 13 laboratories had a 'good' concordance (< 90% and ≥ 85%), 4 laboratories had a 'satisfactory' concordance (< 85% and ≥ 80%), and 3 laboratories had < 80% concordance.

In Italy, for the strain '2025 EARS-Net 5', VMEs were observed for imipenem (9.9% of submitted results, reported when using automated system and broth microdilution), meropenem (13.3% of submitted results, reported when using automated system and broth microdilution), cefiderocol (30.9% of submitted results, reported through all methods), imipenem-relebactam (7% of submitted results, reported when using automated system, broth microdilution and gradient test) and meropenem-vaborbactam (26.1% of submitted results, reported through all methods). Low proportions of VMEs (≤5%) were observed for cefotaxime, ertapenem, gentamicin, tobramycin, ceftazidime-avibactam. No high proportions of MEs were observed. For all antimicrobials included in this EQA for strain '2025 EARS-Net 5', the expected AST results were at least two dilutions or four millimeters away from the clinical breakpoint. Therefore, any deviations observed for this strain should not be due to inherent method variation, and might be attributable to systematic or random errors in the laboratories' procedures.

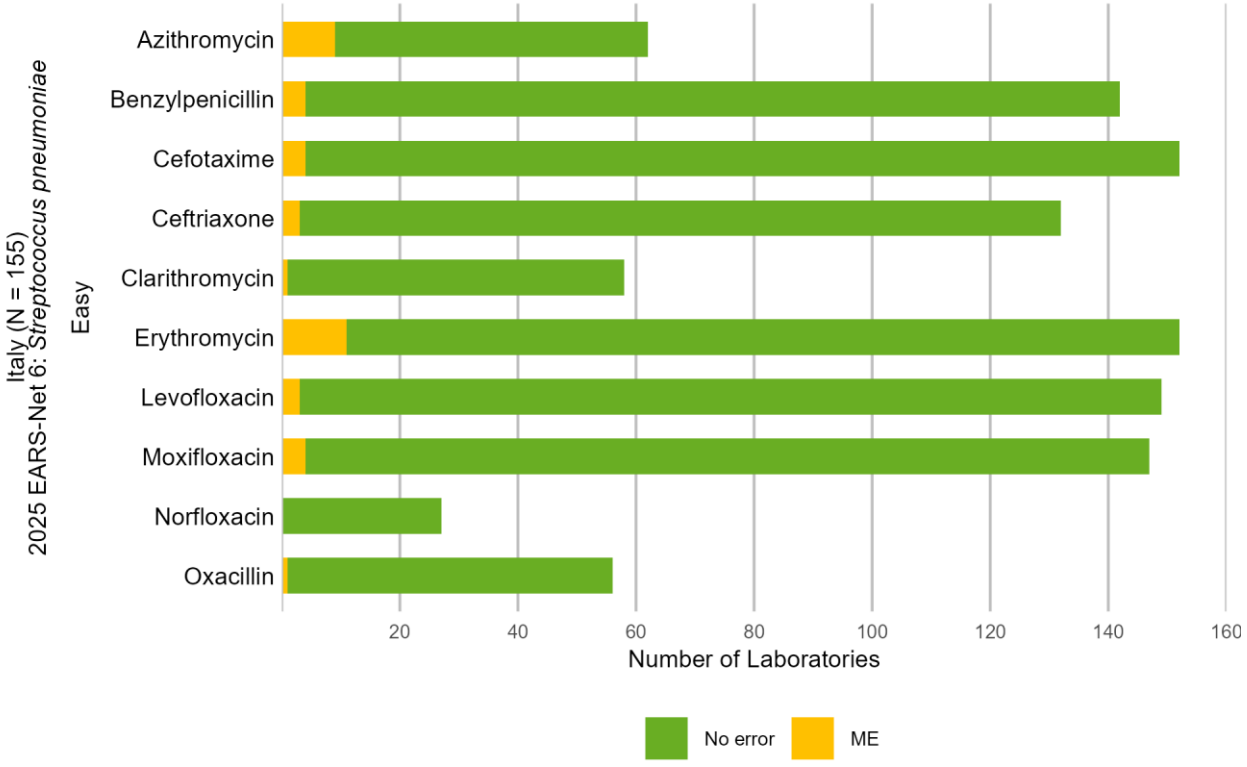
Strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*)

Strain '2025 EARS-Net 6' (Streptococcus pneumoniae) showed reduced viability compared to the other EQA strains. Participants were therefore strongly encouraged to process this strain immediately upon receipt. However, several laboratories were unable to revive the sample.

Overall, 155 of the 158 laboratories that submitted interpretations of results correctly identified the species for the strain '2025 EARS-Net 6'. Each laboratory could submit results from 10 antimicrobials (maximum 1550 submissions). Annex 2 provides an overview of the antimicrobials included in the 2025 EARS-Net EQA.

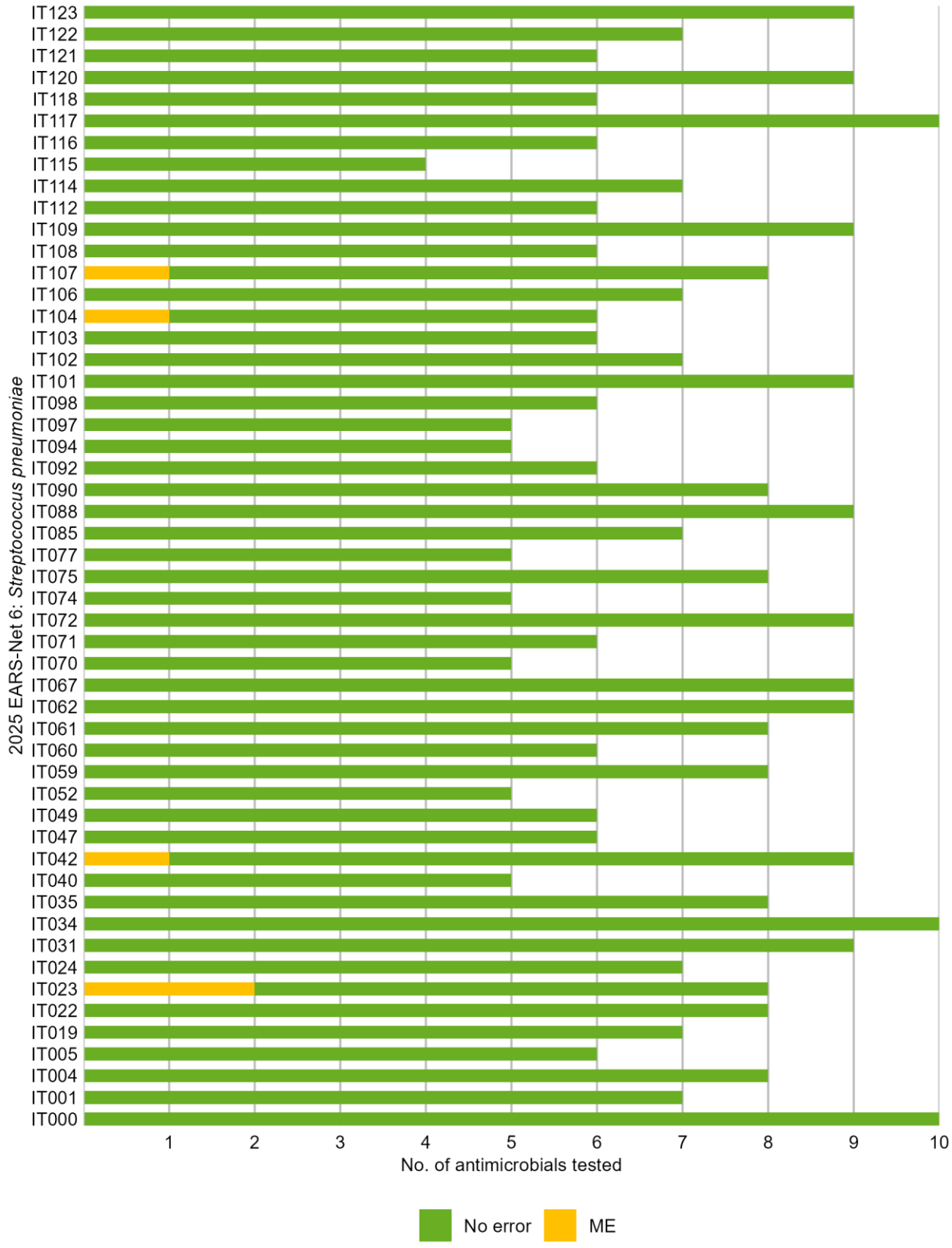
Overall, 1077 AST results was submitted and the interpretation were correct for 1037 (96.3%) of the results, 40 (3.7%) of the interpretations were ME and none of the interpretations were VME (Figure 14). An overview of the reported results for all laboratories is presented in Figure 15.

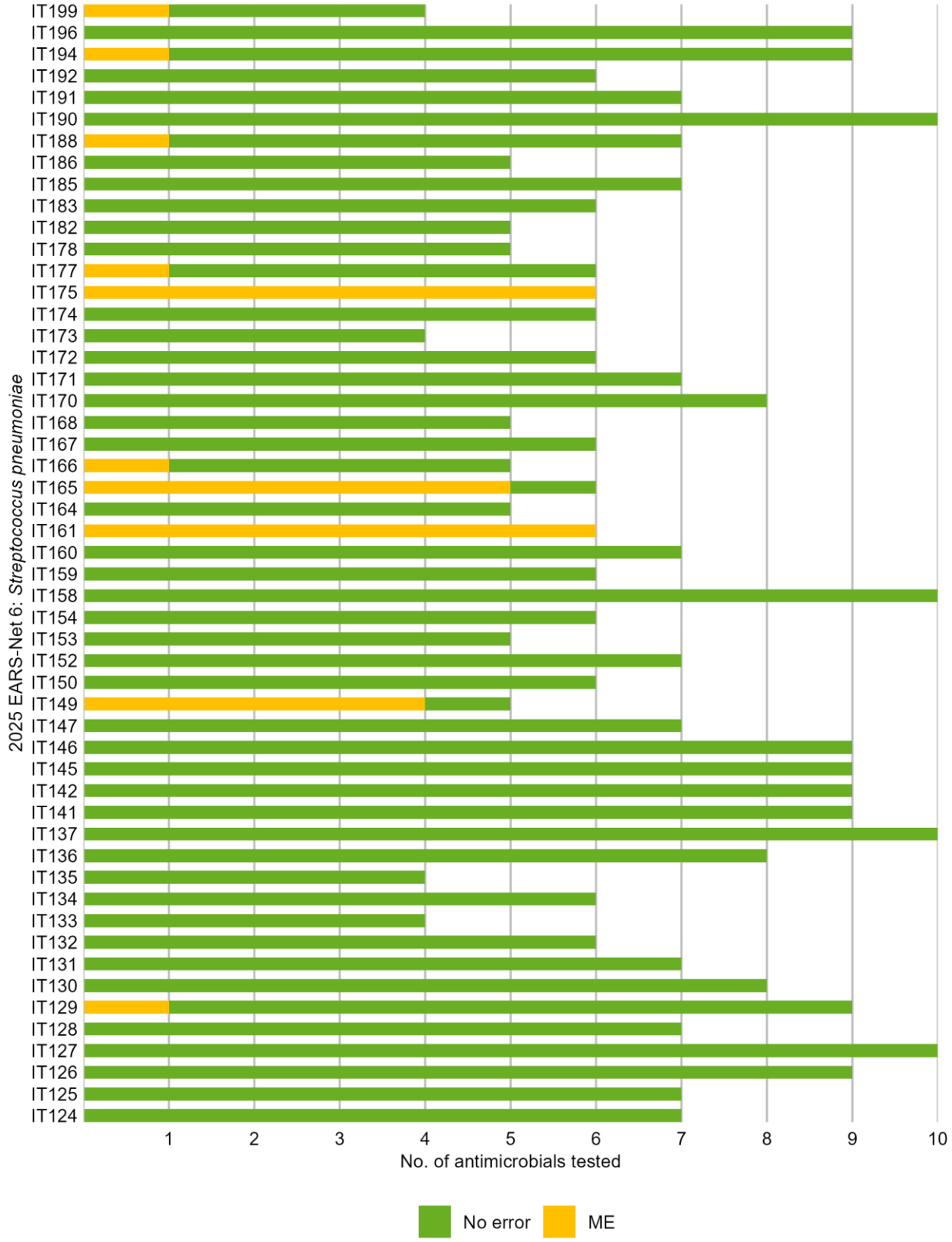
Figure 14. Reported interpretation of AST results for strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*) by antimicrobial agent and anticipated difficulty of identification



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Figure 15. Reported interpretation of AST results for strain '2025 EARS-Net 6' (*Streptococcus pneumoniae*) by laboratory




2025 EARS-Net 6: Streptococcus pneumoniae
IT199
IT196
IT194
IT192
IT191
IT190
IT188
IT186
IT185
IT183
IT182
IT178
IT177
IT175
IT174
IT173
IT172
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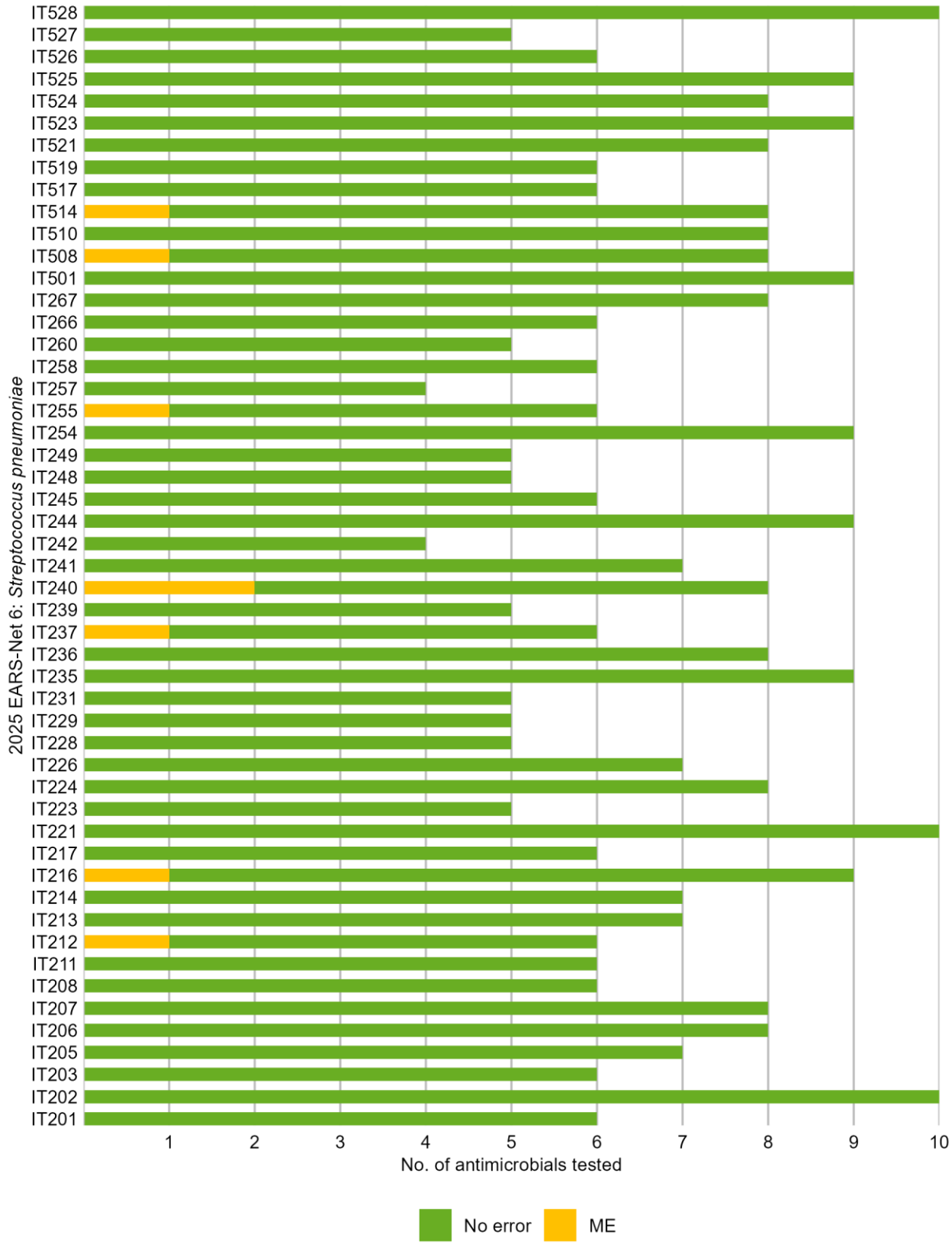
IT146
IT145
IT142
IT141
IT137
IT136
IT135
IT134
IT133
IT132
IT131
IT130
IT129
IT128
IT127
IT126
IT125
IT124
1
2
3
4
5
6
7
8
9
10
No. of antimicrobials tested
No error
ME



Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

For the ‘2025 EARS-Net 6’, 134 laboratories were in full concordance with the expected interpretations, 8 laboratories had a 'good' concordance (< 90% and ≥ 85%), 6 laboratories had a

'satisfactory' concordance (< 85% and ≥ 80%), and 7 laboratories had < 80% concordance.

In Italy, for the strain '2025 EARS-Net 6', no VMEs could be reported as the strain was susceptible to all antimicrobials included in this EQA. High proportions of MEs were observed for azithromycin (14.5% of submitted results, reported when using automated system and gradient test). For all antimicrobials included in this EQA for strain '2025 EARS-Net 6', the expected AST results were at least two dilutions or four millimeters away from the clinical breakpoint. Therefore, any deviations observed for this strain should not be due to inherent method variation, and might be attributable to systematic or random errors in the laboratories’ procedures.

4. Conclusions and recommendation for improvement

For the 2025 EARS-Net EQA, correct species identification was submitted for 1142 strains (100.0%) out of 1172 strains, submitted by the 199 laboratories in Italy.

Interpretation of AST results was reported for 13426 out of the 16526 possible strain-antimicrobial combinations. Overall, there was 'very good' concordance with the expected interpretations as 12516 (93.2%) were correct out of the 13426 tests performed with 71 (35.7%) laboratories meeting the 'excellent' level of 95% concordance for the reported interpretations.

The following methodologies were applied by the laboratories when performing the tests: agar dilution (0.04%), automated system (79.7%), broth microdilution (12.4%), disk/tablet diffusion (3.6%), gradient test (4.1%), and other (0.2%)

4.1 Recommendations

We recommend the following actions to identify root causes to address the observed deviations:

- Confirm the protocols in use are in accordance with the latest EUCAST recommendations and guidelines;
- Ensure the adequate control strains are being applied and monitored to guarantee reliability of results;
- Ensure that relevant quality management systems and control measures are in place;
- Be aware of method variability when applying the different AST methods, especially the automated system, gradient test and broth microdilution methods;
- Ensure compliance with the most recent EUCAST recommendations and warnings regarding AST of cefiderocol, including applying the disk diffusion method and being aware of possible variation in results depending on the media and disks used for AST;
- Ensure compliance with the most recent EUCAST recommendations and warnings regarding AST of colistin, including applying the broth microdilution method;
- Be aware of random or systematic deviations during AST of aminoglycosides, due to variations in media composition or other factors;
- Be aware and potentially seek consultancy regarding the testing and reading of results for macrolides, for carbapenems and for the more recent beta-lactam/beta-lactamase inhibitor combinations;
- Consider seeking consultancy for the issues detected in this EQA and additional training of technical staff, to enhance capabilities and performance.

5. References

1) Antimicrobial resistance (AMR) reporting protocol 2025. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2025. Available from: https://www.ecdc.europa.eu/en/publications-data/reporting-protocol-antimicrobial-resistance-amr
2) 2025 EARS-Net EQA protocol. DTU Food, 2025. Available from: https://www.food.dtu.dk/english/topics/antimicrobial-resistance/ears-net

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

Table 7. EUCAST clinical breakpoints for *Klebsiella pneumoniae* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 1' (*K. pneumoniae*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*			EUCAST zone diameter breakpoints (mm)*			Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	ATU	S ≥	R <	ATU				
Amikacin	8	8		18	18		Difficult	16	R	aac(6')-Ib, aph(3')-VI
Amoxicillin-clavulanic acid iv (fixed 2 mg/L)	8	8		19	19	19-20	Easy	>64	R	blaNDM-1, blaDHA-1
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	0.06	S	(Absent from databases)
Cefepime	1	4		27	24		Easy	>16	R	blaNDM-1, blaCTX-M-15
Cefiderocol	2	2		23	23	21-23	Difficult	18 mm	R	(Absent from databases)
Cefotaxime	1	2		20	17		Easy	>8	R	blaNDM-1, blaDHA-1, blaCTX-M-15
Ceftazidime	1	4		22	19		Easy	>16	R	blaNDM-1, blaDHA-1, blaCTX-M-15
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	>16	R	blaNDM-1
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Easy	>8	R	(Absent from databases)
Ceftriaxone	1	2		27	24		Easy	>4	R	blaCTX-M-15
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Difficult	1	R	qnrS1, qnrB4

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*			EUCAST zone diameter breakpoints (mm)*			Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	ATU	S ≥	R <	ATU				
Colistin	2	2		Note	Note		Easy	0.5	S	None
Ertapenem	0.5	0.5		23	23		Easy	>4	R	blaNDM-1
Gentamicin	2	2		17	17		Easy	0.5	S	None
Imipenem	2	4		22	19		Difficult	8	R	blaNDM-1
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	8	R	(Absent from databases)
Levofloxacin	0.5	1		23	19		Difficult	1	I	qnrS1, qnrB4
Meropenem	2	8		22	16		Difficult	16	R	blaNDM-1
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Difficult	8	S	(Absent from databases)
Moxifloxacin	0.25	0.25		22	22		Easy	2	R	qnrS1, qnrB4
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	qnrS1, qnrB4
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64	R	blaNDM-1, blaDHA-1
Tobramycin	2	2		16	16		Easy	>8	R	aac(6')-Ib

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

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

Table 8. EUCAST clinical breakpoints for *Acinetobacter baumannii* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 2' (*A. baumannii),* by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*		EUCAST zone diameter breakpoints (mm)*		Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Difficult	16	R	aph(3')-VI
Cefiderocol	Note	Note	Note	Note	Difficult	14 mm	R	(Absent from databases)
Ciprofloxacin	0.001	1	50	21	Easy	>4	R	gyrA S81L, parC S84L, parC V104I, parC D105E
Colistin	2	2	Note	Note	Easy	<=0,5	S	None
Gentamicin	4	4	17	17	Difficult	4	S	None
Imipenem	2	4	24	21	Easy	>8	R	blaNDM-1
Levofloxacin	0.5	1	23	20	Easy	>4	R	gyrA S81L, parC S84L, parC V104I, parC D105E
Meropenem	2	8	21	15	Easy	>16	R	blaNDM-1
Tobramycin	4	4	17	17	Easy	1	S	None

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. Most relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening. For cefiderocol, wild-type isolates were registered as 'S', non-wild type isolates which may be associated with impaired clinical response were reported as 'I', and likely resistant isolates were registered as 'R'.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

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

Table 9. EUCAST clinical breakpoints for *Staphylococcus aureus* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 3' (*S. aureus),* by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*		EUCAST zone diameter breakpoints (mm)*		Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	S ≥	R <				
Oxacillin	Note	Note	Note	Note	Easy	>8	R	mecA
Cefoxitin	Note	Note	22	22	Easy	11 mm	R	mecA
Ciprofloxacin	0.001	2	50	17	Easy	<=0,5	I	None
Levofloxacin	0.001	1	50	22	Easy	<=0,25	I	None
Norfloxacin	-	-	17	17	Difficult	20 mm	S	None
Vancomycin	2	2	Note	Note	Easy	<=1	S	None
Linezolid	4	4	21	21	Easy	<=2	S	None
Daptomycin	1	1	Note	Note	Easy	<=0,25	S	None
Rifampicin	0.06	0.06	26	26	Easy	<=0,08	S	None

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

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

Table 10. EUCAST clinical breakpoints, expected AST results for *Acinetobacter baumannii* and the level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 4' (*A. baumannii*), by antimicrobial agent

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

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. Most relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening. For cefiderocol, wild-type isolates were registered as 'S', non-wild type isolates which may be associated with impaired clinical response were reported as 'I', and likely resistant isolates were registered as 'R'.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

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

Table 11. EUCAST clinical breakpoints for *Escherichia coli* and the expected AST results, level of difficulty in interpretation and expected interpretations for strain '2025 EARS-Net 5' (*E. coli*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*			EUCAST zone diameter breakpoints (mm)*			Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	ATU	R <	S ≤	ATU				
Amikacin	8	8		18	18		Easy	4	S	aac(6')-Ib-cr
Amoxicillin	8	8		Note	Note		Easy	>32	R	blaNDM-5, blaOXA-1, blaOXA-181, blaCMY-2
Amoxicillin-clavulanic acid (fixed 2 mg/L)	8	8		19	19	19-20	Easy	>32	R	blaNDM-5, blaOXA-1, blaOXA-181, blaCMY-2
Ampicillin	8	8		14	14		Easy	>32	R	blaNDM-5, blaOXA-1, blaOXA-181, blaCMY-2
Aztreonam-avibactam (fixed 4 mg/L)	4	4		25	25	22-24	Easy	2	S	(Absent from databases)
Cefepime	1	4		27	24		Easy	>16	R	blaNDM-5, blaCTX-M-15, blaOXA-1, blaOXA-181
Cefiderocol	2	2		23	23	21-23	Easy	16 mm	R	(Absent from databases)
Cefotaxime	1	2		20	17		Easy	>8	R	blaNDM-5, blaCTX-M-15, blaCMY-2
Ceftazidime	1	4		22	19		Easy	>16	R	blaNDM-5, blaCTX-M-15, blaCMY-2
Ceftazidime-avibactam (fixed 4 mg/L)	8	8		13	13		Easy	>16	R	blaNDM-5
Ceftolozane-tazobactam (fixed 4 mg/L)	2	2		22	22	19-21	Easy	>8	R	–(Absent from databases)
Ceftriaxone	1	2		27	24		Easy	>4	R	blaCTX-M-15
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	>4	R	qnrS1, aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parE S458A
Colistin	2	2		Note	Note		Easy	<=0,5	S	None
Ertapenem	0.5	0.5		23	23		Easy	>4	R	blaNDM-5, blaOXA-181
Gentamicin	2	2		17	17		Easy	>16	R	aac(3)-Iid
Imipenem	2	4		22	19		Easy	>8	R	blaNDM-5, blaOXA-181
Imipenem-relebactam (fixed 4 mg/L)	2	2		22	22	20-22	Easy	>4	R	–(Absent from databases)
Levofloxacin	0.5	1		23	19		Easy	>4	R	qnrS1, aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parE S458A

Meropenem	2	8		22	16		Easy	>16	R	blaNDM-5, blaOXA-181
Meropenem-vaborbactam (fixed 8 mg/L)	8	8		20	20	15-19	Easy	>16	R	–(Absent from databases)
Moxifloxacin	0.25	0.25		22	22		Easy	>8	R	qnrS1, aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parE S458A
Ofloxacin	0.25	0.5		24	22		Easy	>2	R	qnrS1, aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parE S458A
Piperacillin-tazobactam (fixed 4 mg/L)	8	8	16	20	20	19	Easy	>64	R	blaNDM-5, blaOXA-1, blaOXA-181, blaCMY-2
Tigecycline	0.5	0.5		18	18		Easy	<=0,25	S	None
Tobramycin	2	2		16	16		Easy	>8	R	aac(6')-Ib-cr

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

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

Table 12. EUCAST clinical breakpoints for *Streptococcus pneumoniae* and the expected MIC value, level of difficulty in interpretation and interpretation for strain '2025 EARS-Net 6' (*S. pneumoniae*), by antimicrobial agent

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)*		EUCAST zone diameter breakpoints (mm)*		Level of difficulty**	Expected result***	Expected interpretation	Genetic determinants of AMR****
	S ≤	R >	S ≥	R <				
Oxacillin	-	-	20	20	Easy	27 mm	S	None
Azithromycin	0.25	0.25	Note	Note	Easy	<=0,06	S	None
Benzylpenicillin	0.06	1	Note	Note	Easy	0.015	S	None
Cefotaxime	0.5	2	Note	Note	Easy	<=0,03	S	None
Ceftriaxone	0.5	2	Note	Note	Easy	<=0,03	S	None
Clarithromycin	0.25	0.25	Note	Note	Easy	<=0,03	S	None
Erythromycin	0.25	0.25	22	22	Easy	0.03	S	None
Levofloxacin	0.001	2	50	16	Easy	1	I	None
Moxifloxacin	0.5	0.5	22	22	Easy	0.125	S	None
Norfloxacin	-	-	10	10	Easy	20 mm	S	None

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

* EUCAST Clinical Breakpoint Tables v15.0, valid from 01-01-2025. Note: Please refer to notes in the breakpoint tables. All relevant breakpoints were used as they appear, regardless of whether they were bracketed (indicating that monotherapy is unlikely) or recommended for screening.

** The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

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

**** Additional determinants of AMR: None.

7. Annex 2. Antimicrobials included in the 2025 EARS-Net EQA

Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2025: Microorganism and antimicrobial agent combinations under surveillance by EARS-Net (isolates from blood and/or cerebrospinal fluid). Available at: https://www.ecdc.europa.eu/sites/default/files/documents/EARS-Net-reporting-protocol.pdf.

As indicated in the text preceding the table, “When, according to the EUCAST guidelines, a specific type of test is to be used, the method is indicated next to the antimicrobial.”

Table 13. Antimicrobials included in the 2025 EARS-Net EQA

Microorganism	Antimicrobial agent
***Acinetobacter* species (ACISPP)**	Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
***Enterococcus faecalis* (ENCFAE) and *Enterococcus faecium* (ENCFAI)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
***Escherichia coli* (ESCCOL)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (CZA) Ceftriaxone (CRO) Cefepime (FEP) Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Ertapenem (ETP) Tigecycline (TGC) Aztreonam-avibactam (AZA) Colistin (COL) - Broth microdilution
***Klebsiella pneumoniae* (KLEPNE)**	Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP)

Microorganism	Antimicrobial agent
	Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (CZA) Ceftriaxone (CRO) Cefepime (FEP) Cefiderocol (FDC) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Ertapenem (ETP) Aztreonam-avibactam (AZA) Colistin (COL) - Broth microdilution
Pseudomonas aeruginosa **(PSEAER)**	Piperacillin/Tazobactam (TZP) Piperacillin (PIP) Ceftazidime (CAZ) Ceftazidime-avibactam (CZA) Cefepime (FEP) Cefiderocol (FDC) Ceftolozane-tazobactam (CZT) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Imipenem-relebactam (IMR) Meropenem (MEM) Meropenem-vaborbactam (MEV) Colistin (COL) - Broth microdilution
Staphylococcus aureus **(STAAUR)**	Cefoxitin (FOX) – Disk diffusion Oxacillin (OXA)* – MIC test Levofloxacin (LVX) Ciprofloxacin (CIP) Norfloxacin (NOR) – Disk diffusion Vancomycin (VAN) – MIC test Rifampin (RIF) Linezolid (LNZ) Daptomycin (DAP) – MIC test
Streptococcus pneumoniae **(STRPNE)**	Oxacillin (OXA) – Disk diffusion Penicillin (PEN) – MIC test Clarithromycin (CLR) – MIC test Erythromycin (ERY) Azithromycin (AZM) – MIC test Levofloxacin (LVX) Moxifloxacin (MFX) Norfloxacin (NOR) – Disk diffusion Cefotaxime (CTX) – MIC test Ceftriaxone (CRO) – MIC test