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

National summary report for France

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Contents

1. Introduction

This report describes and summarises the national results of the external quality assessment (EQA) of performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2022. Participating laboratories are identified by codes known by the corresponding laboratory, the national EQA coordinator and the EQA provider.

The current EARS-Net EQA aims to: 1) assess the accuracy of species identification reported by participating individual laboratories; 2) assess the accuracy of qualitative antimicrobial susceptibility test results reported by participating individual laboratories; 3) evaluate the overall comparability of routinely collected test results between laboratories and EU/EEA countries.

The report provides a summary of results including a short conclusion on the capacity of participating laboratories, and if needed, recommendations for improvement. Results from all participating laboratories are included as an Appendix.

The 2022 EQA focuses on species identification of six strains (one strain was not included in the EARS-Net surveillance), and antimicrobial susceptibility testing (AST) of the bacterial species included in the EARS-Net surveillance (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*).

In total, 67 laboratories from France were invited to participate in the 2022 EARS-Net EQA and received the six strains for analysis, and 51 laboratories submitted data for evaluation. No results were submitted by the laboratories with ID Numbers FRn0839, FRn0740, FRn0737, FRn0181, FRn0756, FRn0187, FRn0709, FRn0218, FRn0738, FRn0231, FRn0743, FRn0364, FRn0838, FRn0399, FRn0414, FRn0479 (N=16).

2. Materials and Methods

2.1 Strains and antimicrobial susceptibility testing

The *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains were selected for this EQA from the strain collection at DTU Food based on their antimicrobial resistance profiles. Further, *Pseudomonas putida* strain was selected for species identification only. Expected AST results were generated by performing minimum inhibitory concentration (MIC) determinations through broth microdilution (BMD) for all test strains, or by determining zone diameters through disk diffusion when applicable, in triplicate, at the Technical University of Denmark, National Food Institute (DTU Food). The AST profiles were validated by two reference laboratories: The Centre for Disease Control and Prevention (CDC), Georgia, US and EUCAST Development Laboratory (EUCAST), Uppsala, Sweden. Expected results for each antimicrobial and strain combination were determined by the consensus AST results obtained by DTU Food, and subsequently genotypically compared to acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) by whole-genome sequencing (WGS) and using the bioinformatics tools ResFinder v4.1 and CARD RGI (Table 1-5). Finally, a MIC determination was performed at DTU Food after preparation of the agar swab culture/charcoal swab for shipment to participants to confirm that the vials contained the correct strains with the expected AST results.

The antimicrobial agents selected for this EQA correspond to the panel of pathogen and antimicrobial agent combinations under surveillance by EARS-Net presented in the antimicrobial resistance (AMR) reporting protocol 2022[1].

Participating laboratories should perform AST according to the laboratory's applied routine procedures, i.e. automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient-diffusion, or others following EUCAST recommendations (https://www.eucast.org/ast_of_bacteria/).

The EUCAST clinical breakpoints tables v12.0 were applied for the interpretation of the obtained AST results (https://www.eucast.org/clinical_breakpoints/) (Table 1-5 and 2). This allowed for categorisation of the test results into three categories: "resistant" (R), "susceptible, increased exposure" (I), and "susceptible, standard dosing regimen" (S).

Table 1. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2022 EARS-Net 1: *Streptococcus pneumoniae*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	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

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. "Easy" are results far from the breakpoint, where the categorisation is obvious. "Difficult" are results close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added.
**Antimicrobial resistance genes and chromosomal point mutations detected in the *Streptococcus pneumoniae* strain through analysis with ResFinder 4.1 or CARD RGI. ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: None. MALDI-TOF by DTU: *Streptococcus pneumoniae* (score 2.24), and MLST: ST-558.
*** Please refer to notes in the EUCAST clinical breakpoints tables v12.0.

Table 2. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2022 EARS-Net 2: *Escherichia coli*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	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*$_{OXA-1}$

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	S ≤	R >	S ≥	R <				
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

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. “Easy” are results far from the breakpoint, where the categorisation is obvious. “Difficult” are results close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added.

**Antimicrobial resistance genes and chromosomal point mutations detected in the *Escherichia coli* strain through analysis with ResFinder 4.1 or CARD RGI. ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *dfrA17*, *sul1*, *catB3*, *aadA5*, *GlpT* E448K, *PtsI* V25I, *UhpT* E350Q, *EF-Tu* R234F, *AcrAB-TolC* Y137H, *AcrAB-TolC* G103S. MALDI-TOF by DTU: *Escherichia coli* (score 2.33), and MLST: ST-131 (*E. coli* #1) / ST-43 (*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.
**** Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
***** Reporting results for colistin was not mandatory.

Table 3. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2022 EARS-Net 4: *Staphylococcus aureus*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	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

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. “Easy” are results far from the breakpoint, where the categorisation is obvious. “Difficult” are results close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added.
**Antimicrobial resistance genes and chromosomal point mutations detected in the *Staphylococcus aureus* strain through analysis with ResFinder 4.1 or CARD RGI. ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *GlpT* A100V, *murA* E291D, *murA* T396N. MALDI-TOF by DTU: *Staphylococcus aureus* (score 2.33), and MLST: ST-130.
*** Please refer to notes in the EUCAST clinical breakpoints tables v12.0.

Table 4. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2022 EARS-Net 5: *Pseudomonas aeruginosa*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	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*$_{\text{OXA-485/488}}$
Ceftazidime	0.001	8	50	17	Easy	>32 mg/L	R	*bla*$_{\text{OXA-485/488}}$
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

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	S ≤	R >	S ≥	R <				
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

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. "Easy" are results far from the breakpoint, where the categorisation is obvious. "Difficult" are results close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added.
**Antimicrobial resistance genes and chromosomal point mutations detected in the *Pseudomonas aeruginosa* strain through analysis with ResFinder 4.1 or CARD RGI. ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *fosA*, *catB7*, *aph(3')-IIb*, *bla*$_{PAO}$, *nalC* S209R, *nalC* G71E. MALDI-TOF by DTU: *Pseudomonas aeruginosa* (score 2.33), and MLST: ST-1633.
*** Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**** 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 5. EUCAST clinical breakpoints, expected MIC value, level of difficulty in interpretation and interpretation for strain 2022 EARS-Net 6: *Acinetobacter baumannii*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L or mm)	Expected interpretation	(ARGs and PMs)**
	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*

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. "Easy" are results far from the breakpoint, where the categorisation is obvious. "Difficult" are results close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint has been recently changed or added.
**Antimicrobial resistance genes and chromosomal point mutations detected in the *Acinetobacter baumannii* strain through analysis with ResFinder 4.1 or CARD RGI. ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *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: *Acinetobacter baumannii* (score 2.4), and MLST: ST-1780 (*A. baumannii* #1) / ST-764 (*A. baumannii* #2).
*** Please refer to notes in the EUCAST clinical breakpoints tables v12.0.
**** Reporting results for colistin was not mandatory.

2.2 Procedure

Protocol, test forms, guideline and a video tutorial were available on the 2022 EARS-Net EQA website (antimicrobialresistance.dk/ears_net_EQA.aspx).

All participating laboratories were invited to enter the obtained results into the EARS-Net EQA web-based database using a secure personal login and password. The deadline for submission of data was 19 August 2022. The results were evaluated using a scoring algorithm taking into account the difficulty of classification and the severity of error.

All participants were encouraged to complete an electronic evaluation form using a link forwarded to contact persons for the participating laboratories with the aim to improve future EQA exercises. The evaluation questions were provided by ECDC.

2.3 New scoring system

In the 2022 EARS-Net EQA a new scoring system was implemented in the evaluation of interpretated results. The scoring took “level of difficulty” and “severity of error” into account for each organism/antimicrobial combination.

The level of difficulty indicates 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: very major error (VME), major error (ME) and no error. Both VME and ME were penalised. VME was reporting false susceptibility – expecting an R but obtaining an S or I. If the only categories were I and R, then reporting I instead of R was also a VME. ME was reporting false resistance – expecting an S or I but obtaining an R. The scoring of a result reflected the severity of an error.

Moreover, in 2022 a penalty was given if results on mandatory antimicrobials were omitted. Please see an overview of mandatory antimicrobials in Annex 1. These correspond to all antimicrobials under surveillance in EARS-Net, for each relevant species, with three exceptions: firstly, reporting of results for colistin was not mandatory in any species; secondly, testing of ofloxacin for *Staphylococcus aureu*s was not included in the 2022 EARS- Net EQA exercise due to the lack of a breakpoint in the EUCAST Clinical Breakpoints v12.0; finally, testing of norfloxacin for *Escherichia coli* isolates was also excluded due to the breakpoint only being applicable to uncomplicated urinary tract infections.

Table 6 shows the scoring system according to difficulty of result, category of error, and mandatory or not mandatory reporting of antimicrobials.

Table 6. Scoring system of the 2022 EARS-Net EQA exercise

		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
	Not reported (mandatory antimicrobials)	-4	-4	-4	-1	-1	-1
	Not reported (colistin)	0	0	0	0	0	0

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

3. Results

In the 2022 EARS-Net EQA, it was decided to include species relevant for the EARS-Net surveillance for species identification. In total, six strains were included in the 2022 EARS-Net EQA. Of these, five strains were covered by EARS-Net surveillance and results on the species identification and AST should be reported. The remaining strain belonged to a species not covered by EARS-Net, and thus only species identification should be reported. For each strain, the species had to be assigned before AST results could be submitted. If the species identification was incorrect, the reported AST results were not evaluated.

Overall, 51 out of 67 (76.1%) laboratories from France submitted results for one or more of the six strains.

A total of 45 (88.2%) laboratories submitted results for all six strains and 50 (98%) laboratories submitted AST results for the five strains covered by EARS-Net. Submitting AST results for the five strains covered by EARS-Net was a minimum requirement for receiving a certificate of participation. All the laboratories used the EUCAST guideline when performing the AST.

3.1 Species identification results

For each strain the species should be identified. In total, 286 out of 302 (94.7%) strains submitted had the correct species identification. An overview of the species identification for the six strains and the number of laboratories reporting the correct identification are given in Table 7. The lowest level of concordance of the identified species was reported for strain 2022 EARS-Net 3 *Pseudomonas putida* (90.2%).

Strain 2022 EARS-Net 3 was *Pseudomonas putida* which is not a species included in the EARS-Net surveillance. Only the species identification should be reported as the 2022 EARS-Net EQA exercise included identification of the species for all six strains, but AST results for the bacterial strains covered by the EARS-Net surveillance.

Only the AST data submitted for strains with correct species identification could be evaluated.

Table 7. Species identification for the six strains and number of laboratories reporting the correct species (%)

Strain ID France	Expected species	No. of labs reporting (N)	No. of labs reporting correct species identification (n)	No. of labs reporting correct species identification (%)
2022 EARS-Net 1	*Streptococcus pneumoniae*	51	51	100
2022 EARS-Net 2	*Escherichia coli*	50	48	96
2022 EARS-Net 3	*Pseudomonas putida*	51	46	90.2
2022 EARS-Net 4	*Staphylococcus aureus*	50	47	94
2022 EARS-Net 5	*Pseudomonas aeruginosa*	50	47	94
2022 EARS-Net 6	*Acinetobacter baumannii*	50	47	94

3.2 Antimicrobial susceptibility testing (AST) results

The 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. In the 2022 EARS-Net EQA, five of the strains were included in the EARS-Net surveillance and the reported AST results were evaluated if the species identification was correct. Only the categorisation was evaluated, whereas the quantitative values were used as supplementary information. All the laboratories used the EUCAST guideline when performing the AST.

Interpretation of AST results were reported for 2,276 out of the 2,604 possible strain/antimicrobial combinations including colistin, and 2,093 (92%) were reported with the correct interpretation.

The maximum expected score for the five strains was 91. For the 51 laboratories submitting results for analysis, the average score was 23.9±27.6 when including the penalty given if results on mandatory antimicrobials were omitted. When only including the scores for submitted results, the average score was 52±17.4.

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

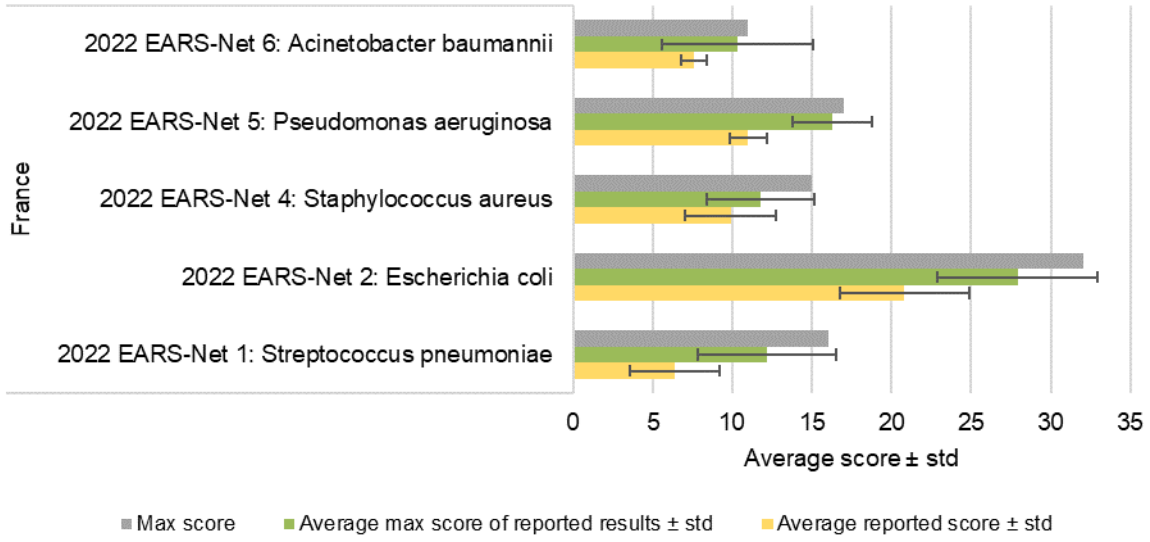

Figure 1. Maximum possible score, average max possible score of reported results ± std, and average score of the reported results ± std for each strain

An overview of the methods used for the determination of the antimicrobial resistance for the five strains included in the EARS-Net surveillance and the percentage of correct interpretations is given in Tables 8, 9 and 10. The most commonly used method was Disk/Tablet diffusion (47.9%) (Table 10). The lowest level of concordance with expected interpretations was reported when using the Gradient test (76.5%) (Table 10).

Table 8. Overview of methods used for determination of the AST results for strains 2022 EARS-Net 1 and 2

France	2022 EARS-Net 1 *Streptococcus pneumoniae*			2022 EARS-Net 2 *Escherichia coli*		
Method	Number of AST performed	% of total AST performed	% correct interpretation	Number of AST performed	% of total AST performed	% correct interpretation
Agar dilution	3	0.9	100.0	-	-	-
Automated system	34	9.9	76.5	363	46.8	88.4
Broth microdilution	3	0.9	100.0	41	5.3	90.2
Disk/Tablet diffusion	149	43.2	86.6	322	41.5	83.2
Gradient test	149	43.2	68.5	44	5.7	90.9
Other	7	2.0	71.4	5	0.6	100.0
Total	345	100.0	77.7	775	100.0	86.6
0	-	-	-	-	-	-

Table 9. Overview of methods used for determination of the AST results for strains 2022 EARS-Net 4 and 5

France	2022 EARS-Net 4 *Staphylococcus aureus*			2022 EARS-Net 5 *Pseudomonas aeruginosa*		
Method	Number of AST performed	% of total AST performed	% correct interpretation	Number of AST performed	% of total AST performed	% correct interpretation
Agar dilution	-	-	-	-	-	-
Automated system	147	43.9	88.4	160	33.1	89.4
Broth microdilution	27	8.1	100.0	34	7.0	97.1
Disk/Tablet diffusion	137	40.9	89.8	281	58.2	83.3
Gradient test	18	5.4	100.0	7	1.4	71.4
Other	6	1.8	66.7	1	0.2	100.0
Total	335	100.0	90.1	483	100.0	86.1
0	**-**	**-**	**-**	**-**	**-**	**-**

Table 10. Overview of methods used for determination of the AST results for strains 2022 EARS-Net 6 and total

France	2022 EARS-Net 6 *Acinetobacter Baumannii*			Total		
Method	Number of AST performed	% of total AST performed	% correct interpretation	Number of AST performed	% of total AST performed	% correct interpretation
Agar dilution	-	-	-	**3**	**0.1**	**100.0**
Automated system	94	27.8	75.5	**798**	**35.1**	**86.6**
Broth microdilution	34	10.1	100.0	**139**	**6.1**	**96.4**
Disk/Tablet diffusion	202	59.8	98.5	**1,091**	**47.9**	**87.4**
Gradient test	8	2.4	100.0	**226**	**9.9**	**76.5**
Other	-	-	-	**19**	**0.8**	**78.9**
Total	338	100.0	92.3	**2,276**	**100.0**	**86.5**
0	**-**	**-**	**-**	**-**	**-**	**-**

In total, 39.2% of the laboratories would send strain 2022 EARS-Net 1 to a reference or other laboratory for further testing, and 4.2% would send 2022 EARS-Net 2, 19.6% would send 2022 EARS-Net 4, 10.6% would send 2022 EARS-Net 5, and 51.1% would send 2022 EARS-Net 6 for further testing.

Strain no. 2022 EARS-Net 1 *Streptococcus pneumoniae*

All 51 laboratories with correct species identification submitted interpretation of AST results for further analysis for the strain 2022 EARS-Net 1 *Streptococcus pneumoniae*. Each laboratory should submit results from ten mandatory antimicrobials (Annex 1).

In total, interpretation for 500 AST results should have been provided by the 51 laboratories. Results were submitted for 345 cases and interpretations were correct for 294 (85.2%) of the AST results. Of the reported interpretations, 22 (6.4%) were ME and 29 (8.4%) were VME. VME in the interpretation of AST results were reported for benzylpenicillin (Figure 2). An overview of the reported results for all laboratories is presented in

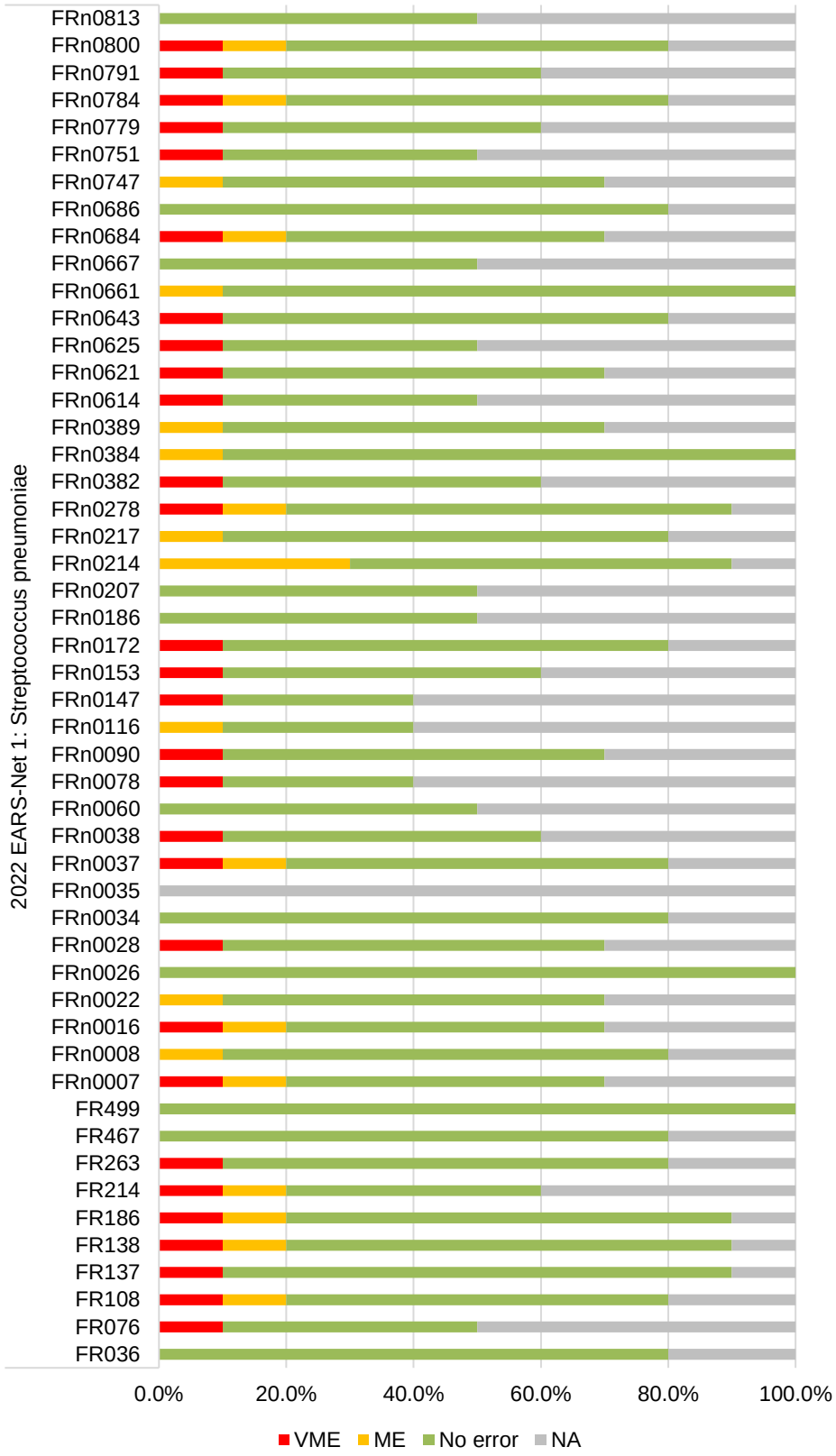
2022 EARS-Net 1: Streptococcus pneumoniae
FRn0813
FRn0800
FRn0791
FRn0784
FRn0779
FRn0751
FRn0747
FRn0686
FRn0684
FRn0667
FRn0661
FRn0643
FRn0625
FRn0621
FRn0614
FRn0389
FRn0384
FRn0382
FRn0278
FRn0217
FRn0214
FRn0207
FRn0186
FRn0172
FRn0153
FRn0147
FRn0116
FRn0090
FRn0078
FRn0060
FRn0038
FRn0037
FRn0035
FRn0034
FRn0028
FRn0026
FRn0022
FRn0016
FRn0008
FRn0007
FR499
FR467
FR263
FR214
FR186
FR138
FR137
FR108
FR076
FR036
0.0%
20.0%
40.0%
60.0%
80.0%
100.0%
VME
ME
No error
NA

Figure 3.

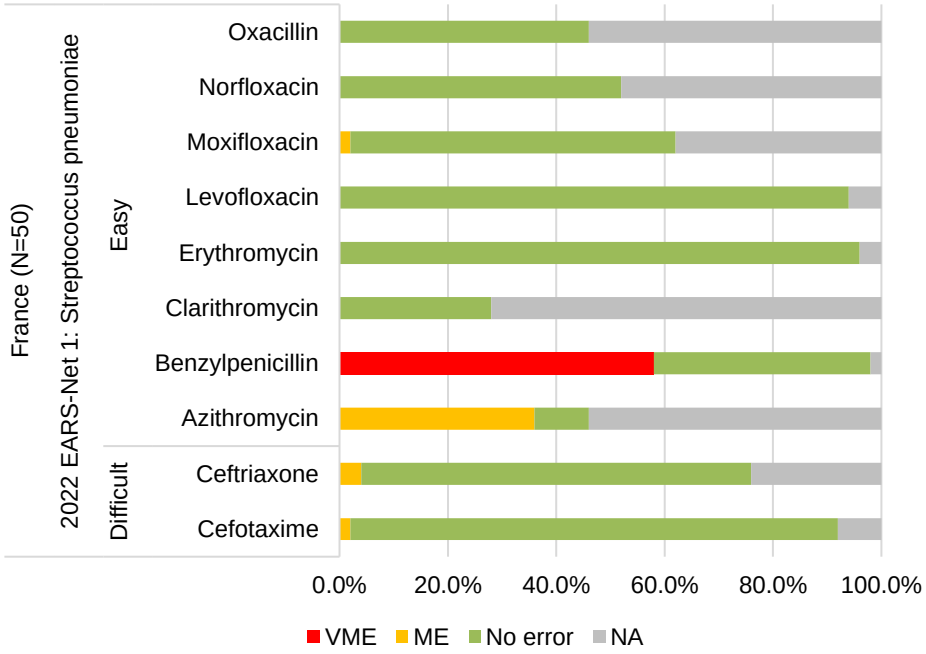

Figure 2. Reported interpretation of AST results for strain 2022 EARS-Net 1: *Streptococcus pneumoniae* per antimicrobial. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

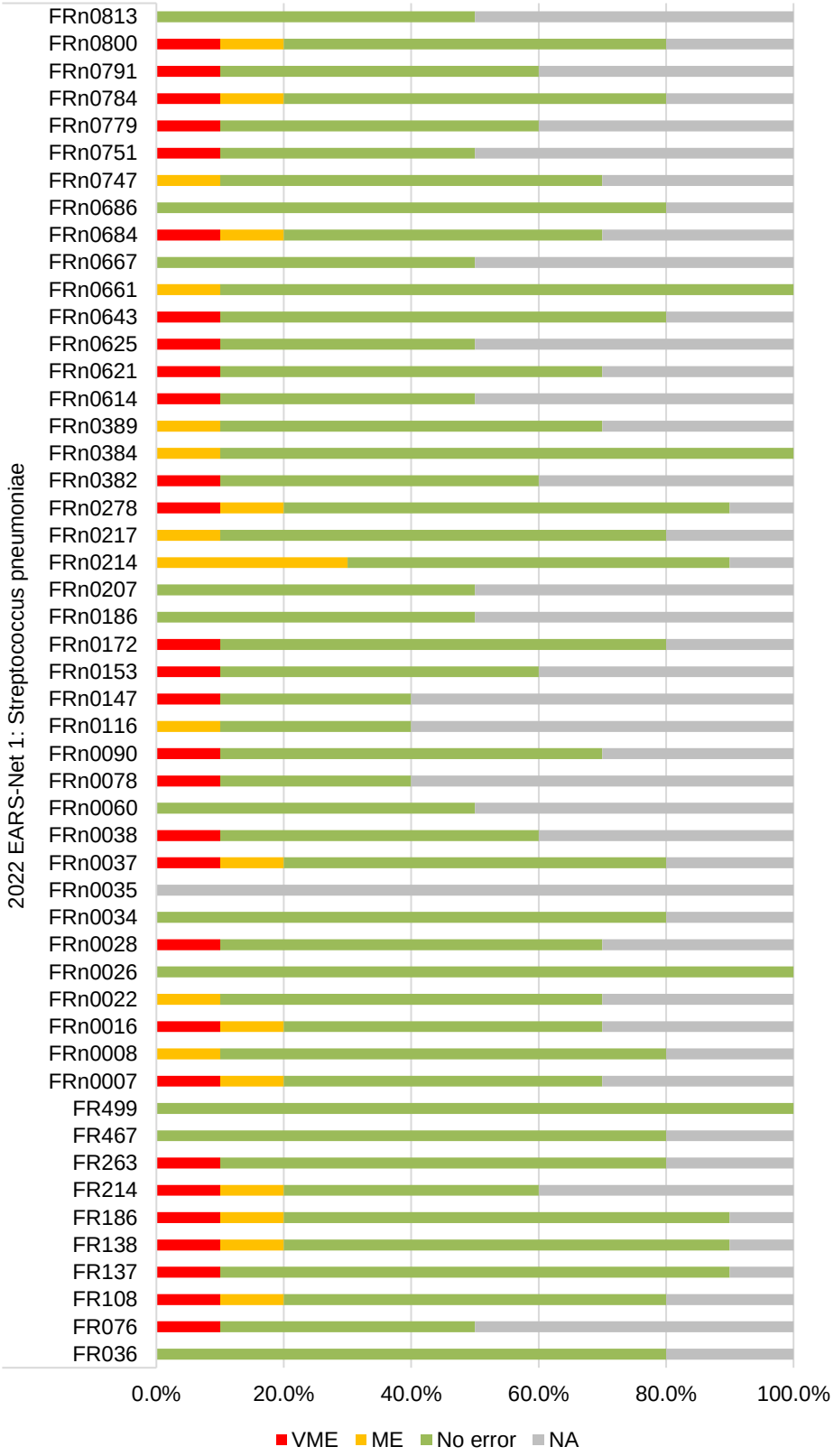

Figure 3. Reported interpretation of AST results for strain 2022 EARS-Net 1: *Streptococcus pneumoniae* per laboratory. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

Strain no. 2022 EARS-Net 2 *Escherichia coli*

All 48 laboratories with correct species identification submitted interpretation of AST results for further analysis for the strain 2022 EARS-Net 2 *Escherichia coli*. Each laboratory should submit results from nineteen mandatory antimicrobials as it was not mandatory to report results on colistin (Annex 1).

In total, interpretation for 855 AST results should have been provided by the 48 laboratories. Results were submitted for 775 cases and interpretations were correct for 725 (93.5%) of the AST results. Of the reported interpretations, 24 (3.1%) were ME and 26 (3.4%) were VME. VME in the interpretation of AST results were reported for amikacin, cefotaxime and ceftriaxone (Figure 4). An overview of the reported results for all laboratories is presented in Figure 5.

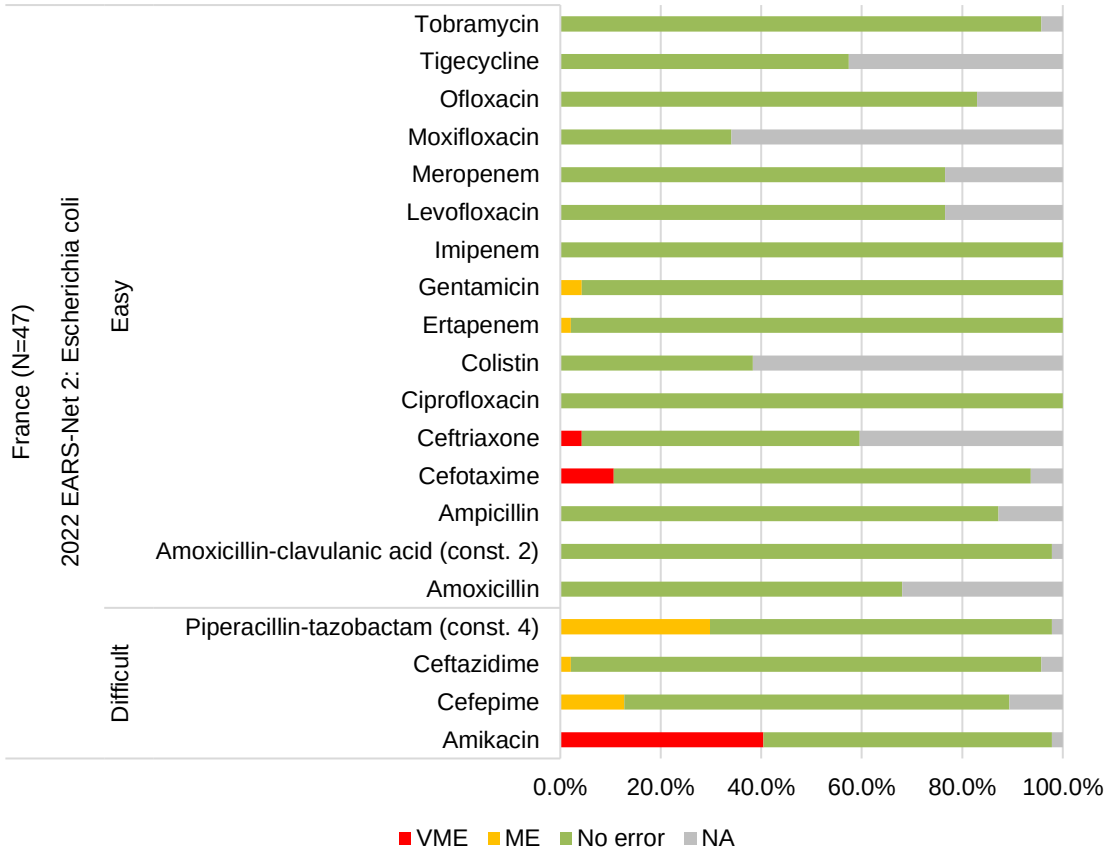

Figure 4. Reported interpretation of AST results for strain 2022 EARS-Net 2 *Escherichia coli* per antimicrobial. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

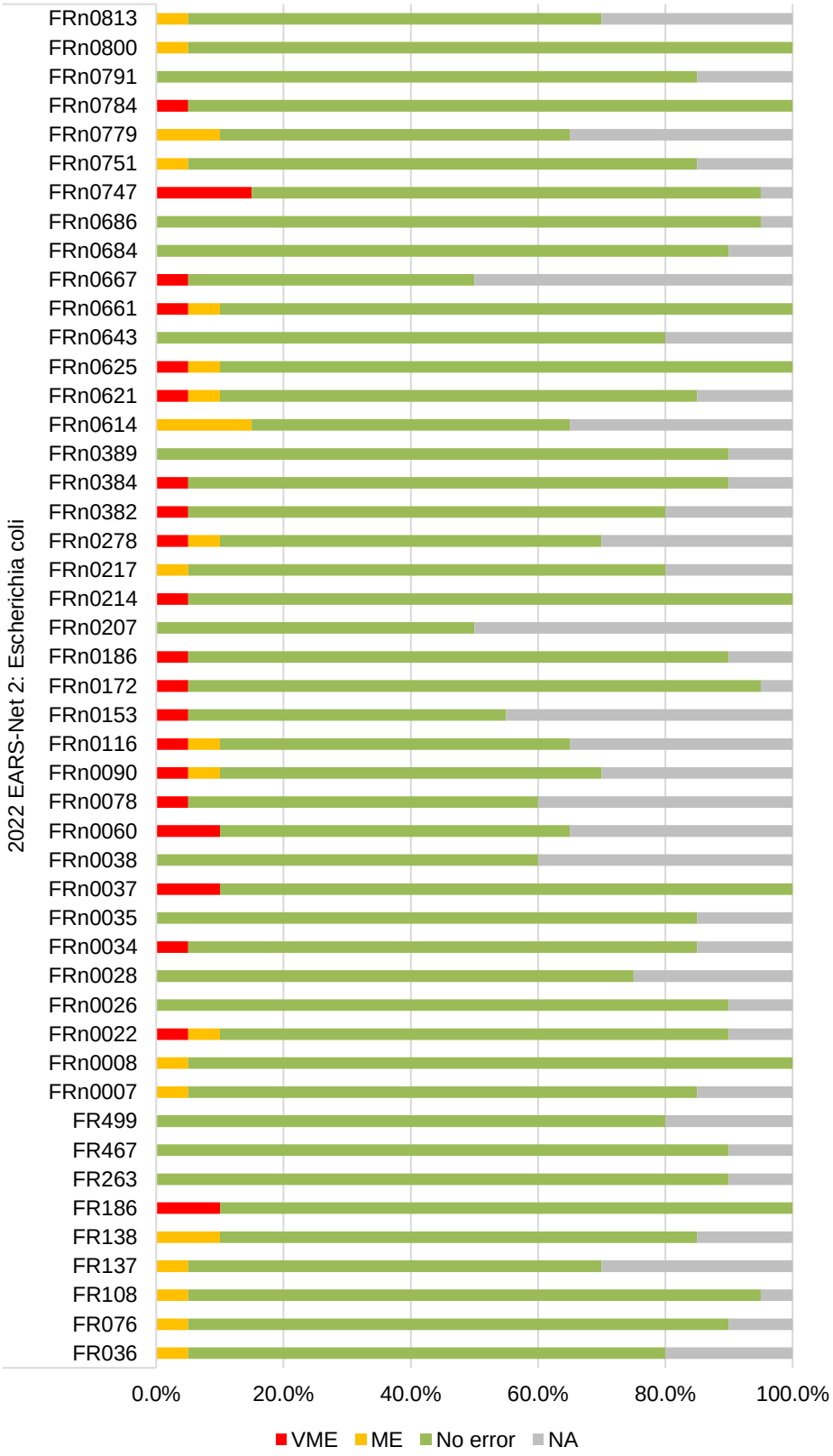

Figure 5. Reported interpretation of AST results for strain 2022 EARS-Net 2 *Escherichia coli* per laboratory. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

Strain no. 2022 EARS-Net 4 *Staphylococcus aureus*

All 47 laboratories with correct species identification submitted interpretation of AST results for further analysis for the strain 2022 EARS-Net 4 *Staphylococcus aureus*. Each laboratory should submit results from nine mandatory antimicrobials (Annex 1).

In total, interpretation for 855 AST results should have been provided by the 47 laboratories. Results were submitted for 335 AST cases and interpretations were correct for 328 (97.9%) of the AST results. . Of the reported interpretations, 1 (0.3%) were ME and 6 (1.8 %) were VME. VME in the interpretation of AST results were reported for oxacillin (Figure 6). An overview of the reported results for all laboratories is presented in Figure 7.

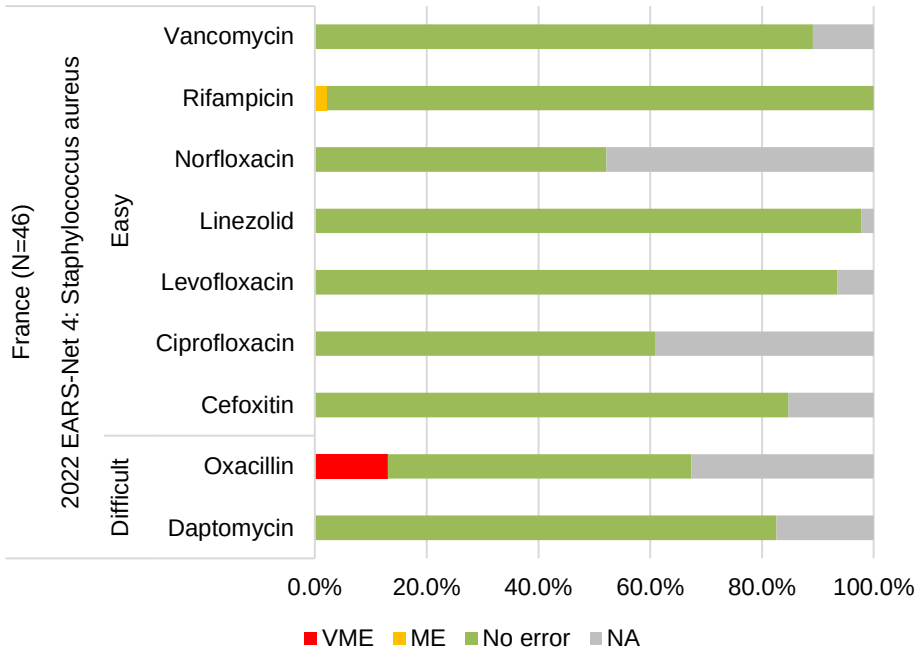

Figure 6. Reported interpretation of AST results for strain 2022 EARS-Net 4 *Staphylococcus aureus* per antimicrobial. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

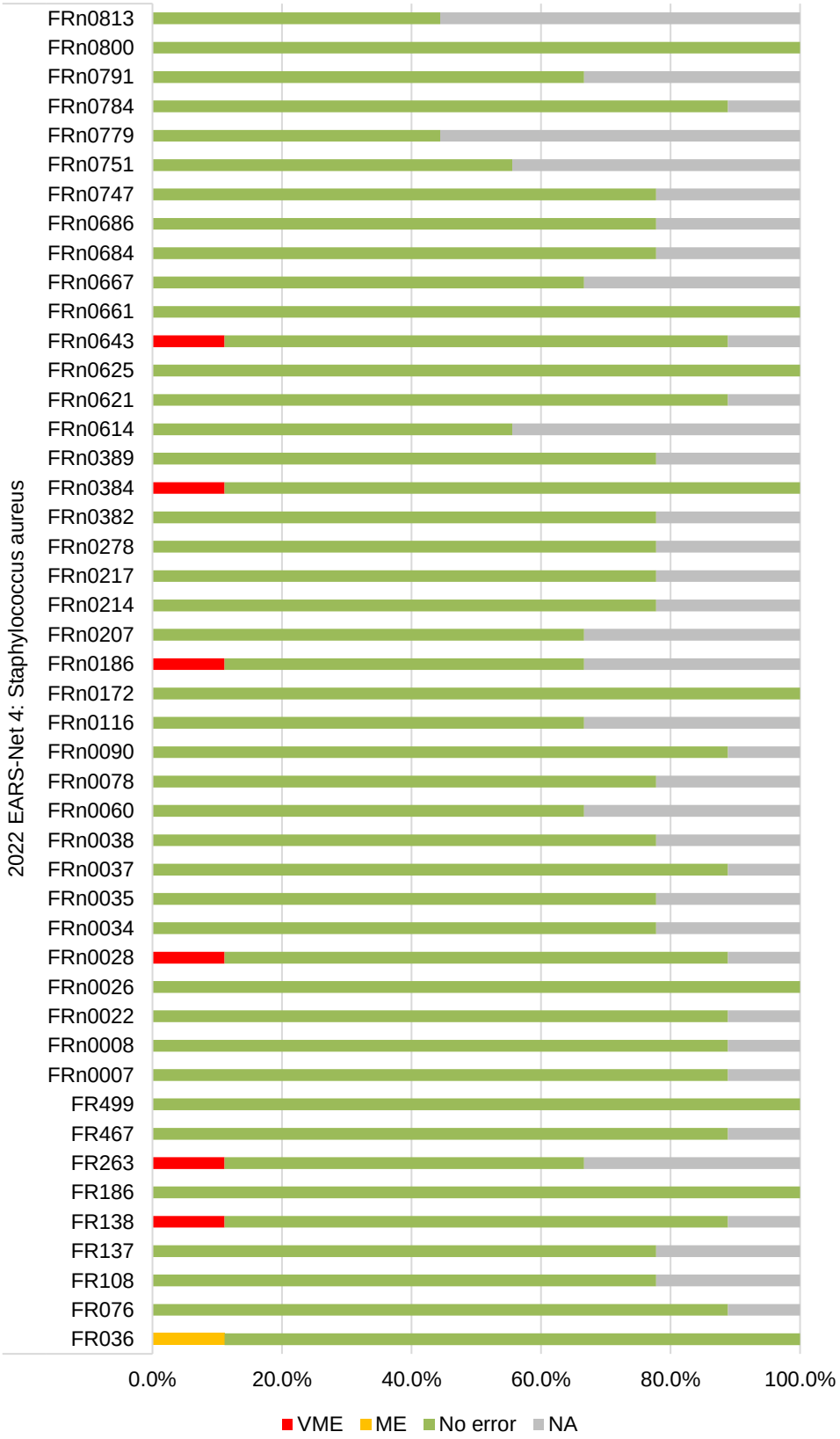

Figure 7. Reported interpretation of AST results for strain 2022 EARS-Net 4 *Staphylococcus aureus* per laboratory. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

Strain no. 2022 EARS-Net 5 *Pseudomonas aeruginosa*

All 47 laboratories with correct species identification submitted interpretation of AST results for further analysis for the strain 2022 EARS-Net 5 *Pseudomonas aeruginosa*. Each laboratory should submit results from ten mandatory antimicrobials as it was not mandatory to report results on colistin (Annex 1).

In total, interpretation for 430 AST results for the mandatory antimicrobials should have been provided by the 47. In addition, 47 results for colistin could have been submitted. Results were submitted for 483 AST cases and interpretations were correct for 433 (89.6%) of the AST results. Of the reported interpretations, 40 (8.3%) were ME and 10 (2.1%) were VME. VME in the interpretation of AST results were reported for ciprofloxacin (Figure 8). An overview of the reported results for all laboratories is presented in Figure 9.

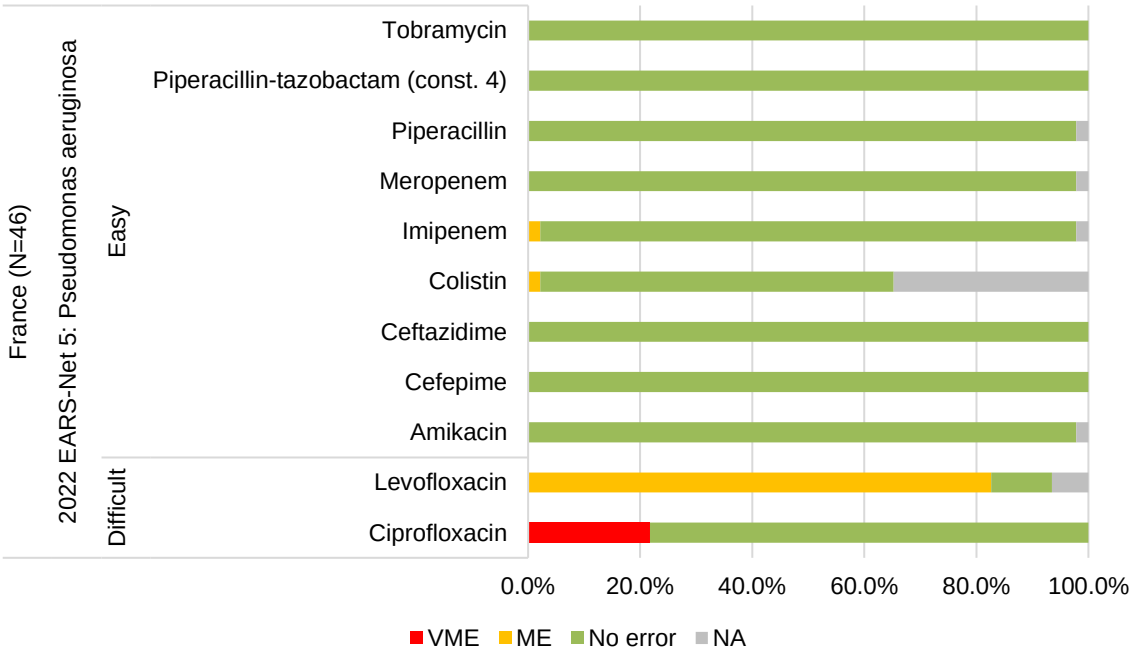

Figure 8. Reported interpretation of AST results for strain 2022 EARS-Net 5 *Pseudomonas aeruginosa* per antimicrobial. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

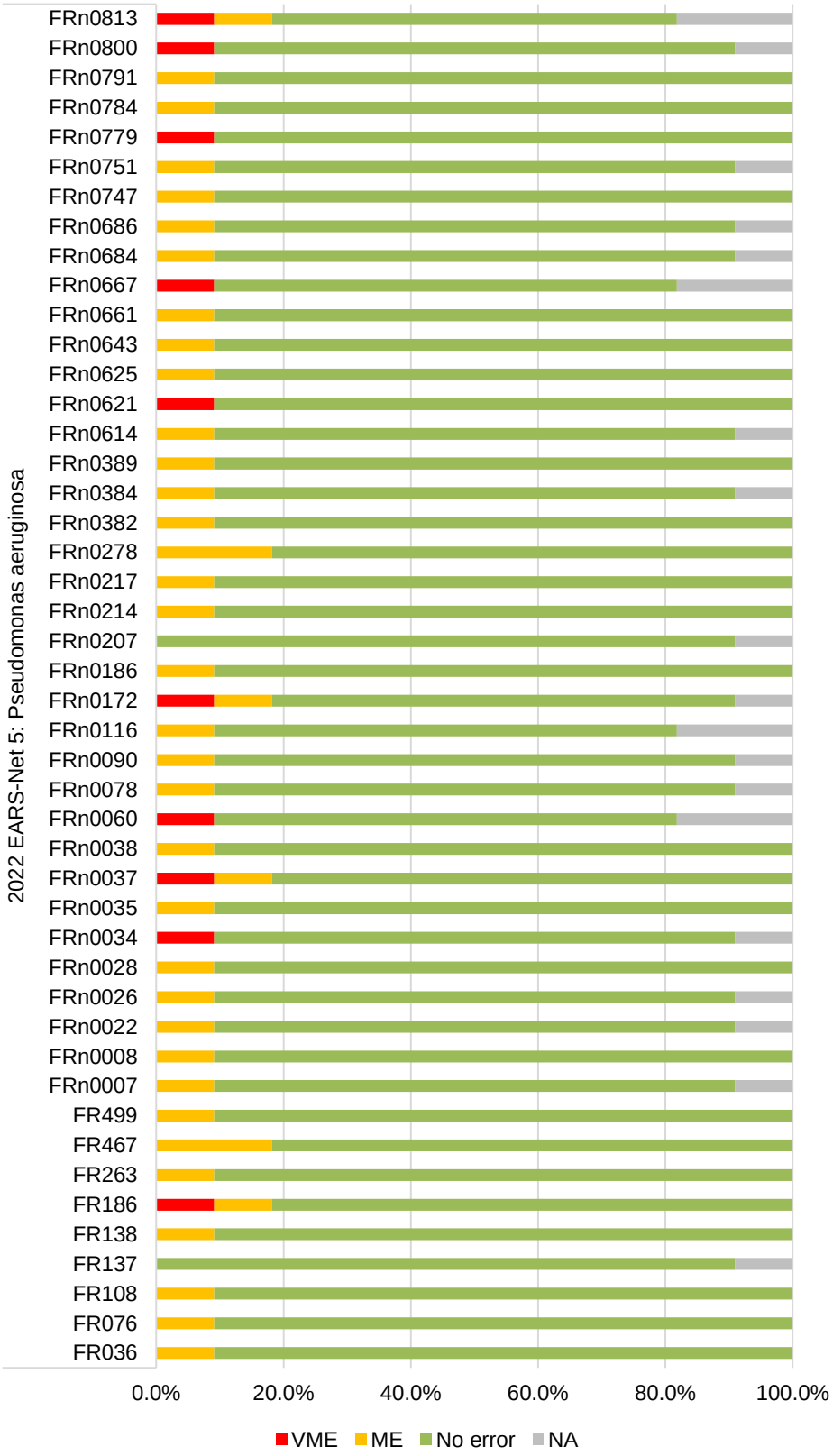

Figure 9. Reported interpretation of AST results for strain 2022 EARS-Net 5 *Pseudomonas aeruginosa* per laboratory. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

Strain no. 2022 EARS-Net 6 *Acinetobacter baumannii*

All 47 laboratories with correct species identification submitted interpretation of AST results for further analysis for the strain EARS-Net 6 *Acinetobacter baumannii*. Each laboratory should submit results from seven mandatory antimicrobials as it was not mandatory to report results on colistin (Annex 1).

In total, interpretation for 301 AST results for the mandatory antimicrobials should have been provided by the 47. In addition, 47 results for colistin may have been submitted. Results were submitted for 338 cases and interpretations were correct for 313 (92.6%) of the AST results. Of the reported interpretations, 1 (0.3%) were ME and 24 (7.1%) were VME. VME in the interpretation of AST results were reported for gentamicin and tobramycin (Figure 10). An overview of the reported results for all laboratories is presented in Figure 11.

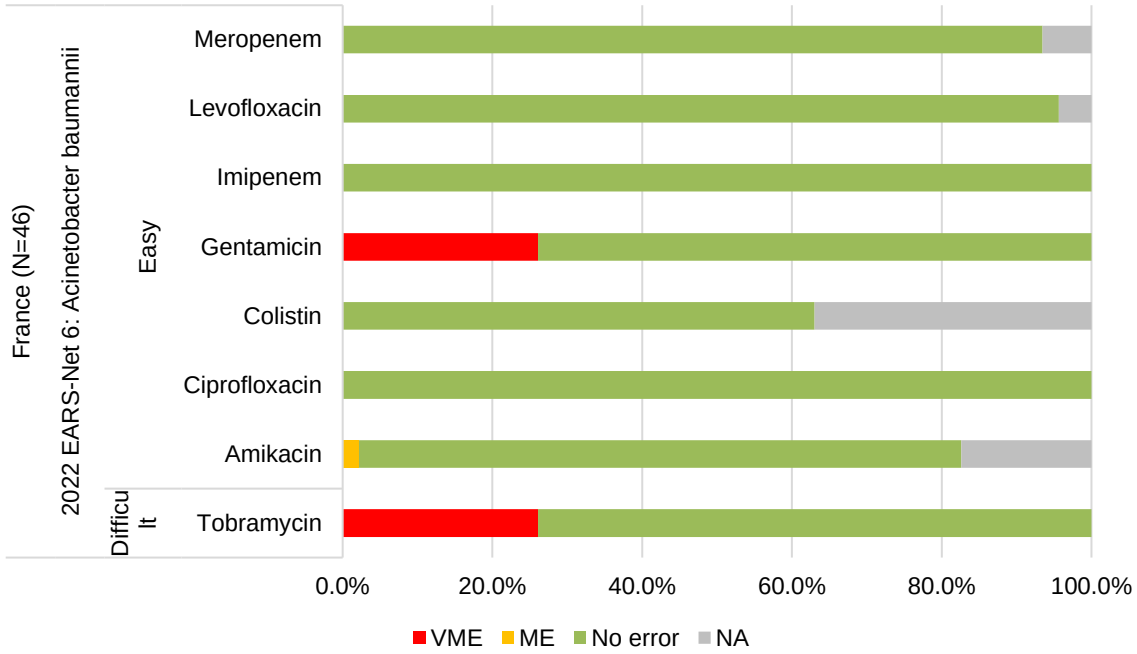

Figure 10. Reported interpretation of AST results for strain 2022 EARS-Net 6 *Acinetobacter baumannii* per antimicrobial. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

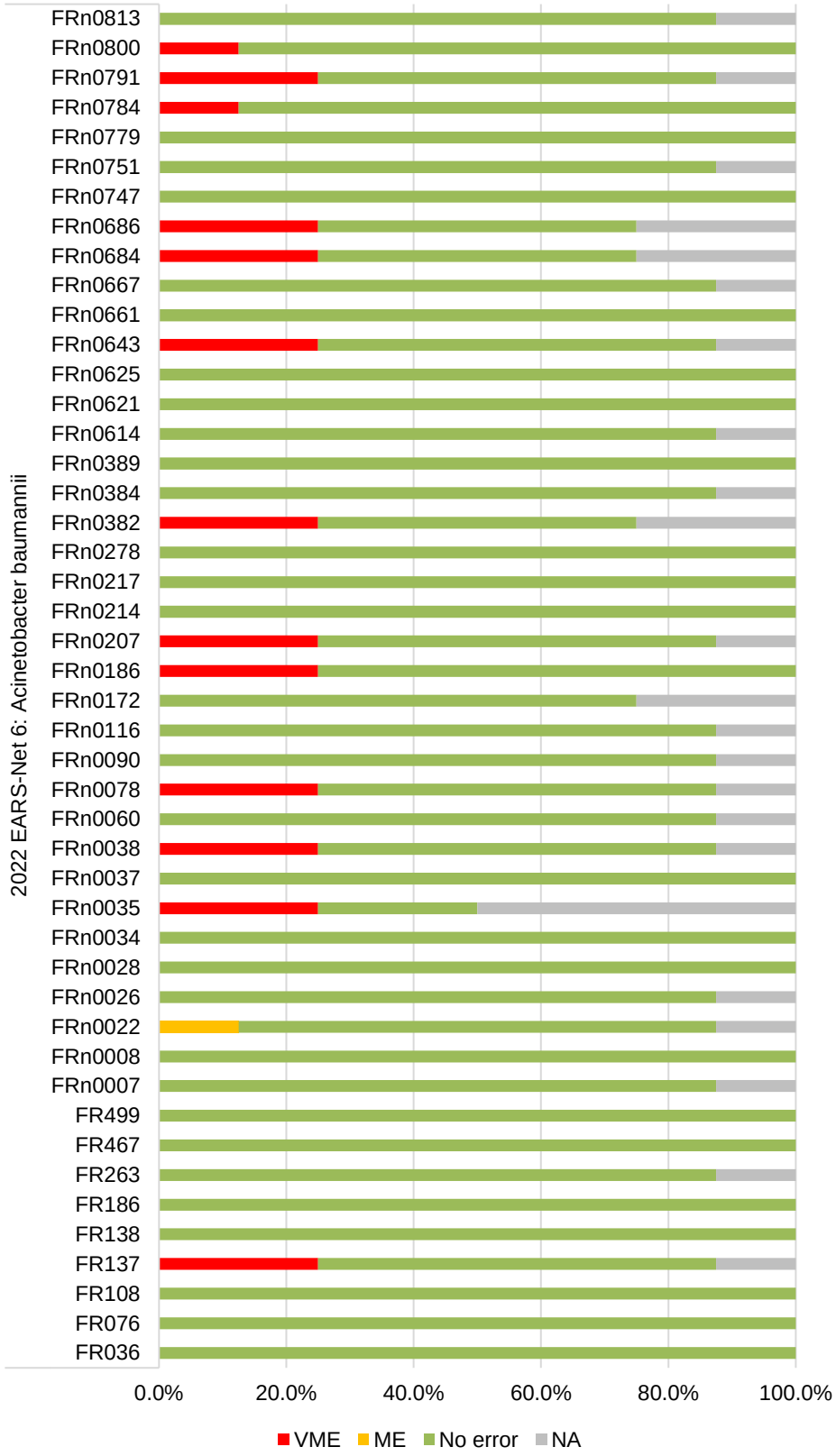

Figure 11. Reported interpretation of AST results for strain 2022 EARS-Net 6 *Acinetobacter baumannii* per laboratory. The distribution is divided into very major errors (VME), major errors (ME), no errors and no data (NA)

4. Conclusions and recommendation for improvement

For the 2022 EARS-Net EQA, correct species identification for the six strains was submitted in 286 cases (94.7%) out of the 302 results submitted by the 51 laboratories in France. The lowest level of concordance of the identified species was reported for strain 2022 EARS-Net 3 *Pseudomonas putida* (90.2%).

Interpretation of AST results was reported for 2,276 out of the 2,604 possible strain/antimicrobial combinations including colistin. It was not mandatory to report interpretation for colistin. Overall, there was a very good concordance with the expected interpretations as 2,093 (92%) were correct out of the 2,276 tests performed although none of the laboratories meet the satisfactory level of 95% concordance for the reported interpretations.

The following methodologies were applied by the laboratories when performing the 2,276 tests: agar dilution (0.1%), automated system (35.1%), broth microdilution (6.1%), disk/tablet diffusion (47.9%), gradient test (9.9%), macro broth dilution (tubes) (-%), and other (0.8%).

Strain no. 2022 EARS-Net 1 *Streptococcus pneumoniae*

For the 2022 EARS-Net 1 *Streptococcus pneumoniae* strain, 9 laboratories were in full concordance with the expected interpretations, none of the laboratories had an excellent concordance with the expected interpretation (≥ 95%), none of the laboratories had a very good concordance (< 95% and ≥ 90%), 9 laboratories had a good concordance (< 90% and ≥ 85%), 6 laboratories had a satisfactory concordance (< 85% and ≥ 80%), and 25 laboratories had < 80% concordance.

In France, for the strain no. 2022 EARS-Net 1 *Streptococcus pneumoniae*, VME were observed for benzylpenicillin. These corresponded to 59.2% of all submitted interpretations for that antimicrobial and were reported throughout most methods except agar dilution and broth microdilution. 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 can likely be attributed to incorrect choice of interpretative criteria, with the selection of the breakpoint referring to indications other than meningitis, instead of the expected breakpoint for meningitis. They might also be attributable to other systematic or random errors in the laboratories' procedures. A high proportion of ME were observed for azithromycin (78.3% of submitted results) and were reported when using the disk/tablet diffusion 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.

Strain no. 2022 EARS-Net 2 *Escherichia coli*

For the 2022 EARS-Net 2 *Escherichia coli* strain, 1 laboratory in full concordance with the expected interpretations, none of the laboratories had an excellent concordance with the expected interpretation (≥ 95%), 11 laboratories had a very good concordance (< 95% and ≥ 90%), 20 laboratories had a good concordance (< 90% and ≥ 85%), 9 laboratories had a satisfactory concordance (< 85% and ≥ 80%), and 6 laboratories had < 80% concordance.

In France, for the strain no. 2022 EARS-Net 2 *Escherichia coli*, VME were observed for amikacin, cefotaxime and ceftriaxone. Deviations in amikacin corresponded to 41.3% of all submitted

interpretations for that antimicrobial and were reported throughout all methods. 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. Deviations in cefotaxime corresponded to 11.4% of all submitted interpretations for that antimicrobial and were reported when using the disk/tablet diffusion. 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, and they can also be derived from the differential expression of the *bla*$_{\text{CTX-M-15}}$ gene harboured by the strain. Deviations in ceftriaxone corresponded to 7.1% of all submitted interpretations for that antimicrobial and were reported when using the disk/tablet diffusion 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, and they can also be derived from the differential expression of the *bla*$_{\text{CTX-M-15}}$ gene harboured by the strain. A high proportion of ME were observed for piperacillin-tazobactam (30.4% of submitted results) and were reported throughout all methods except broth microdilution. 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, and they can also be derived from the differential expression of the *bla*$_{\text{OXA-1}}$ gene harboured by the strain.

Strain no. 2022 EARS-Net 4 *Staphylococcus aureus*

For the 2022 EARS-Net 4 *Staphylococcus aureus* strain, 26 laboratories were in full concordance with the expected interpretations, none of the laboratories had an excellent concordance with the expected interpretation (≥ 95%), none of the laboratories had a very good concordance (< 95% and ≥ 90%), 7 laboratories had a good concordance (< 90% and ≥ 85%), 1 laboratory had a satisfactory concordance (< 85% and ≥ 80%), and 12 laboratories had < 80% concordance.

In France, for the strain no. 2022 EARS-Net 4 *Staphylococcus aureus*, VME were observed for oxacillin. These corresponded to 19.4% of all submitted interpretations for that antimicrobial and were reported when using the automated system 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. No high proportions of ME were observed for this strain.

Strain no. 2022 EARS-Net 5 *Pseudomonas aeruginosa*

For the 2022 EARS-Net 5 *Pseudomonas aeruginosa* strain, 2 laboratories were in full concordance with the expected interpretations, none of the laboratories had an excellent concordance with the expected interpretation (≥ 95%), 22 laboratories had a very good concordance (< 95% and ≥ 90%), 3 laboratories had a good concordance (< 90% and ≥ 85%), 14 laboratories had a satisfactory concordance (< 85% and ≥ 80%), and 5 laboratories had < 80% concordance.

In France, for the strain no. 2022 EARS-Net 5 *Pseudomonas aeruginosa*, VME were observed for ciprofloxacin. These corresponded to 21.7% of all submitted interpretations for that antimicrobial and were reported when using the automated system 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. A high proportion of ME were observed for levofloxacin (88.4% of submitted results) and were reported when using the automated system 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 no. 2022 EARS-Net 6 *Acinetobacter baumannii*

For the 2022 EARS-Net 6 *Acinetobacter baumannii* strain, 31 laboratories were in full concordance with the expected interpretations, none of the laboratories had an excellent concordance with the expected interpretation (≥ 95%), none of the laboratories had a very good concordance (< 95% and ≥ 90%), 4 laboratories had a good concordance (< 90% and ≥ 85%), none of the laboratories had a satisfactory concordance (< 85% and ≥ 80%), and 11 laboratories < 80% concordance.

In France, for the strain no. 2022 EARS-Net 6 *Acinetobacter baumannii*, VME were observed for gentamicin and tobramycin. Deviations in gentamicin corresponded to 26.1% of all submitted interpretations for that antimicrobial and were reported when using the automated system and disk/tablet diffusion. 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. Deviations in tobramycin corresponded to 26.1% of all submitted interpretations for that antimicrobial and were reported when using the automated system 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. No high proportions of ME were observed for this strain.

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:
- Confirm that the most current breakpoints are applied and that clinical manifestation is taken into account when selecting the breakpoints;
- 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 and disk/tablet diffusion methods;
- Consider additional training of technical staff to enhance capabilities and performance.

5. References

1) Antimicrobial resistance (AMR) reporting protocol 2022. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2021

6. Appendix

6.1 Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2022

Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2022: 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- 2022.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." Testing of ofloxacin for *Staphylococcus aureus* and testing of norfloxacin for *Escherichia coli* and *Klebsiella pneumoniae* isolates are included in the original table but are not part of the 2022 EARS- Net EQA exercise. This is due to the lack of a breakpoint in the EUCAST clinical breakpoints v12.0 or due to the breakpoint only being applicable to uncomplicated urinary tract infections, respectively.

Microorganism	Antimicrobial agent
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
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
Enterococcus faecalis **(ENCFAE)**	Ampicillin (AMP) Amoxicillin (AMX) – MIC test Gentamicin-High (GEH)

Microorganism	Antimicrobial agent
	Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)
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) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX) Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Tigecycline (TGC) Colistin (COL) - Broth microdilution
Klebsiella pneumoniae **(KLEPNE)**	Amoxicillin-clavulanic acid (AMC) Piperacillin-tazobactam (TZP) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftriaxone (CRO) Cefepime (FEP) Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Ofloxacin (OFX) Moxifloxacin (MFX)

Microorganism	Antimicrobial agent
	Imipenem (IPM) Meropenem (MEM) Ertapenem (ETP) Colistin (COL) - Broth microdilution
***Pseudomonas aeruginosa* (PSEAER)**	Piperacillin/Tazobactam (TZP) Piperacillin (PIP) Ceftazidime (CAZ) Cefepime (FEP) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution
***Acinetobacter* species (ACISPP)**	Gentamicin (GEN) Tobramycin (TOB) Amikacin (AMK) Ciprofloxacin (CIP) Levofloxacin (LVX) Imipenem (IPM) Meropenem (MEM) Colistin (COL) - Broth microdilution