External Quality Assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2023

National summary report for France

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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
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

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1. Introduction

This report describes and summarises national results from the external quality assessment (EQA) of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2023. Participating laboratories are identified by codes known by the corresponding laboratory, the national EQA coordinator and the EQA provider.

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

The report provides a summary of results, including a short conclusion on the capacity of participating laboratories, and if needed, recommendations for improvement.

The 2023 EQA focused on species identification and interpretation of the AST of the six strains shared with the participating laboratories (*Escherichia coli, Klebsiella pneumoniae* (two strains), *Enterococcus faecalis, Acinetobacter baumannii* and *Enterococcus faecium*).

1.1 Participation

Overall, in the 2023 EARS-Net EQA, 957 laboratories from all 30 EU/EEA countries signed up for participation, and 871 laboratories submitted data. This is a little more than in 2022 EARS-Net EQA, when 948 laboratories signed up for participation and 847 submitted data, from all 30 EU/EEA countries.

In France, 68 laboratories signed up for participation in the 2023 EARS-Net EQA and received the six strains for analysis, and 49 laboratories submitted data for evaluation. No results were submitted by 19 laboratories with ID Numbers FRn0217, FRn0218, FRn0399, FRn0614, FRn0625, FRn0756, FR127, FRn0047, FRn0131, FRn0222, FRn0265, FRn0355, FRn0356, FRn0502, FRn0623, FRn0817, FRn0825, FRn0986, FRn1028.

2. Materials and Methods

2.1 Strains and antimicrobial susceptibility testing

The *Escherichia coli, Klebsiella pneumoniae* (two strains)*, Enterococcus faecium, Acinetobacter baumannii* and *Enterococcus faecium* strains were selected for this EQA from the strain collection at the Technical University of Denmark, National Food Institute (DTU Food) based on their antimicrobial resistance profiles and the recommendations from European Centre for Disease Prevention and Control (ECDC).

The expected results were determined by examining the consensus AST results obtained by DTU FOOD through broth microdilution and/or disk diffusion, and results from confirmatory testing provided by three reference laboratories. These were the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory, Uppsala, Sweden; the Microbiological Diagnostic Unit Public Health Laboratory, The Doherty Institute, Australia; and the Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Japan. Subsequently, the consensus phenotypic AST profile was compared with whole-genome sequencing (WGS) data on acquired antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) obtained at DTU FOOD using the bioinformatics tools ResFinder v4.1, AMRFinderPlus and CARD RGI (Tables 1–6). Finally, after the preparation of the agar swab cultures/charcoal swabs for shipment to participants, MIC determinations were performed at DTU FOOD, to confirm that the vials contained the correct strains with the expected AST results.

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 2023[1]. The exception was testing of norfloxacin for *E. coli* and *K. pneumoniae* isolates, which is included in the original table, but is not part of the 2023 EARS-Net EQA exercise because the breakpoints in EUCAST Clinical Breakpoints v13.0 are only applicable to uncomplicated urinary tract infection.

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 v13.0 were used for the interpretation of AST results (https://www.eucast.org/clinical_breakpoints/) (Tables 1-6). This permitted categorisation of the AST results into three categories: "resistant" (R), "susceptible, increased exposure" (I), and "susceptible, standard dosing regimen" (S).

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

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

ND: Not detected.
*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult 'results are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint was new or recently changed.
**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI. Additional antimicrobial resistance genes or chromosomal point mutations: *mph(A), catB3, aadA5, sul1, dfrA17*. MALDI-TOF by DTU: *E. coli* (score 2,24), and MLST: ST-131 (scheme *E. coli* #1).
*** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.
**** Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.

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

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result (mg/L)	Expected interpretation	(ARGs and PMs)**
	S ≤	R >	S ≥	R <				
Amikacin	8	8	18	18	Easy	4	S	aac(6')-Ia
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	*bla*$_{VEB-1}$, *bla*$_{SHV-11}$
Cefepime	1	4	27	24	Difficult	4	I	*bla*$_{VEB-1}$, *bla*$_{SHV-11}$
Cefotaxime	1	2	20	17	Easy	8	R	*bla*$_{VEB-1}$, *bla*$_{SHV-11}$
Ceftazidime	1	4	22	19	Easy	>32	R	*bla*$_{VEB-1}$, *bla*$_{SHV-11}$
Ceftriaxone	1	2	25	22	Easy	16	R	*bla*$_{SHV-11}$
Ciprofloxacin	0,25	0,5	25	22	Easy	0,03	S	ND
Colistin	2	2	Note ****	Note	Easy	0,5	S	ND
Ertapenem	0,5	0,5	25	25	Easy	2	R	ND
Gentamicin	2	2	17	17	Difficult	4	R	ant(2'')-Ia
Imipenem	2	4	22	19	Difficult	2	S	ND
Levofloxacin	0,5	1	23	19	Easy	0,06	S	ND
Meropenem	2	8	22	16	Easy	1	S	ND
Moxifloxacin	0,25	0,25	22	22	Easy	0,06	S	ND
Ofloxacin	0,25	0,5	24	22	Easy	0,125	S	ND
Piperacillin-tazobactam***	8	8	20	20	Easy	>128/4	R	*bla*$_{VEB-1}$, *bla*$_{SHV-11}$, *bla*$_{OXA-10}$
Tobramycin	2	2	16	16	Easy	8	R	aac(6')-Ia, ant(2'')-Ia

ND: Not detected.
*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint was new or recently changed.
**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI. *bla*$_{SHV-11}$ was an imperfect match (other identified variants: *bla*$_{SHV-40}$, *bla*$_{SHV-56}$, *bla*$_{SHV-79}$, *bla*$_{SHV-85}$, *bla*$_{SHV-89}$). Additional antimicrobial resistance genes or chromosomal point mutations: *bla*OXA-436, *ARR-2, aadA1, cml, cmlA1, sul1, OqxA* (intrinsic), *OqxB* (intrinsic), *fosA* (intrinsic), *fosA7* (intrinsic), *ompK36* N49S, *ompK36* L59V, *ompK36* G189T, *ompK36* F198Y, *ompK36* F207Y, *ompK36* A217S, *ompK36* T222L, *ompK36* D223G, *ompK36* Q227_None679del, *ompK36* I228_None229insK, *ompK36* E232R, *ompK36* N304E, *ompK37* I70M, *ompK37* I128M, *acrR* P161R, *acrR* G164A, *acrR* F172S, *acrR* R173G, *acrR* L195V, *acrR* F197I, *acrR* K201M (*ompK36* A217S, *ompK37* I70M and *ompK37* I128M potentially associated with carbapenem resistance). MALDI-TOF by DTU: *K. pneumoniae* (score 2,57), and MLST: ST-37.
*** Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.
**** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.

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

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

HLAR: High-level aminoglycoside resistance
ND: Not detected.
*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'Difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint was new or recently changed.
**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI.
Additional antimicrobial resistance genes or chromosomal point mutations: *erm(B), tet(L), tet(M), fexA, str, lsa(A)* (intrinsic).
MALDI-TOF by DTU: *E. faecalis* (score 2,35), and MLST: ST-22.
*** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.

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

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L)	Expected interpretation	(ARGs and PMs)**
	S ≤	R >	S ≥	R <				
Amikacin	8	8	18	18	Easy	>32	R	rmtB
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{SHV-1}$
Cefepime	1	4	27	24	Easy	32	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{SHV-1}$, *bla*CTX$_{-M-15}$
Cefotaxime	1	2	20	17	Easy	>64	R	*bla*$_{NDM-5}$, *bla*$_{SHV-1}$, *bla*CTX$_{-M-15}$
Ceftazidime	1	4	22	19	Easy	>128	R	*bla*$_{NDM-5}$, *bla*$_{SHV-1}$, *bla*CTX$_{-M-15}$
Ceftriaxone	1	2	25	22	Easy	>64	R	*bla*$_{SHV-1}$, *bla*CTX$_{-M-15}$
Ciprofloxacin	0,25	0,5	25	22	Easy	>4	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Colistin	2	2	Note ****	Note	Easy	32	R	mgrB W20R
Ertapenem	0,5	0,5	25	25	Easy	>16	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Gentamicin	2	2	17	17	Easy	>16	R	rmtB
Imipenem	2	4	22	19	Easy	16	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Levofloxacin	0,5	1	23	19	Easy	>8	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Meropenem	2	8	22	16	Easy	>16	R	*bla*$_{NDM-5}$, *bla*$_{OXA-181}$
Moxifloxacin	0,25	0,25	22	22	Easy	>8	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Ofloxacin	0,25	0,5	24	22	Easy	>2	R	*qnrS1*, *gyrA* D87N, *gyrA* S83F, *parC* E84K
Piperacillin-tazobactam***	8	8	20	20	Easy	>128/4	R	*bla*$_{NDM-5}$, *bla*$_{OXA-1}$, *bla*$_{OXA-181}$, *bla*$_{SHV-1}$, *bla*CTX$_{-M-15}$
Tobramycin	2	2	16	16	Easy	>16	R	rmtB

ND: Not detected.

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

**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI. *bla*$_{SHV-1}$ was an imperfect match (other identified variants: *bla*$_{SHV-26}$, *bla*$_{SHV-78}$, *bla*$_{SHV-98}$, *bla*$_{SHV-145}$, *bla*$_{SHV-179}$, *bla*$_{SHV-194}$, *bla*$_{SHV-199}$). Additional antimicrobial resistance genes or chromosomal point mutations: *bla*$_{TEM-1B}$, *mph(A), catB3, erm(B), tet(A), aph(3')-Ia, aadA2, sul1, dfrA12, fosA5* (intrinsic), *OqxA* (intrinsic), *OqxB* (intrinsic), *ompK36* A217S, *ompK36* N218H, *ompK36* F207W, *ompK36* L191S, *ompK36* T254S, *ompK36* Q227_None679del, *ompK36* L228V, *ompK36* n304_None305insE, *ompK36* N49S, *ompK36* E232R, *ompK36* D224E, *ompK36* L59V, *ompK36* A190_None568del, *ompK37* I70M, *ompK37* I128M, *acrR* G164A, *acrR* F172S, *acrR* P161R, *acrR* R173G, *acrR* L195V, *acrR* K201M, *acrR* F197I (*ompK36* A217S, *ompK36* N218H, *ompK37* I70M and *ompK37* I128M potentially associated with carbapenem resistance). MALDI-TOF by DTU: *K. pneumoniae* (score 2,48), and MLST: ST-16.

*** Reference results for amoxicillin-clavulanic acid MICs relate to test with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to test with a fixed concentration of 4 mg/L tazobactam.

**** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.

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

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty*	Expected result (mg/L)	Expected interpretation	(ARGs and PMs)**
	S ≤	R >	S ≥	R <				
Amikacin	8	8	19	19	Difficult	16	R	ND
Ciprofloxacin	0,001	1	50	21	Easy	>4	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Colistin	2	2	Note***	Note	Easy	0,5	S	ND
Gentamicin	4	4	17	17	Easy	>16	R	ant(2")-Ia
Imipenem	2	4	24	21	Easy	0,25	S	ND
Levofloxacin	0,5	1	23	20	Easy	8	R	*gyrA* S81L, *parC* S84L, *parC* V104I, *parC* D105E
Meropenem	2	8	21	15	Easy	1	S	ND
Tobramycin	4	4	17	17	Easy	>16	R	ant(2")-Ia

ND: Not detected.
*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint was new or recently changed.
**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI. Additional antimicrobial resistance genes or chromosomal point mutations: *bla*$_{\text{CARB-2}}$, *tet(39), tet(B), tet(G), aph(3')-Ib, aph(3")-Ib, aph(6)-Id, aadA2, sul2*, *bla*$_{\text{OXA-51}}$ (intrinsic), *bla*$_{\text{ADC-25}}$ (likely intrinsic). The strain appears to harbour multiple copies of genes associated with aminoglycoside resistance. Certain copies of those genes might in fact correspond to other variants able to confer amikacin resistance (e.g., other *aph(3')* variants). MALDI-TOF by DTU: *A. baumannii* (score 2,42), and MLST: ST-1552 (scheme *A. baumannii* #1).
*** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.

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

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

HLAR: High-level aminoglycoside resistance
ND: Not detected.
*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong. 'Easy' results are far from the clinical breakpoint, where the categorisation is obvious. 'difficult' results are close to the breakpoint, inside the area of technical uncertainty (ATU), or the breakpoint was new or recently changed.
**Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 or CARD RGI. PBP5-R: pbp5 M485A, pbp5 D204G, pbp5 S27G, pbp5 R34Q, pbp5 E525D, pbp5 N496K, pbp5 V24A, pbp5 T324A, pbp5 A499T, pbp5 E100Q, pbp5 L177I, pbp5 E629V, pbp5 A216S, pbp5 A68T, pbp5 P667S, pbp5 E85D, pbp5 G66E, pbp5 K144Q, pbp5 T172A, pbp5 V586L. Additional antimicrobial resistance genes or chromosomal point mutations: tet(M), msr(C), aac(6')-Ii (intrinsic), gyrA S83Y, parC S80I. MALDI-TOF by DTU: E. faecium (score 2,47), and MLST: ST-17.
*** Please refer to notes in the EUCAST clinical breakpoints tables v13.0.

2.2 Procedure

The protocol, test forms and webtool user guide are available on the 2023 EARS-Net EQA website (antimicrobialresistance.dk/ears_net_EQA.aspx).

All participating laboratories were invited to enter results into the EARS-Net EQA web-based database using a secure personal login and password. The deadline for submission of data was 11 August 2023. The results were evaluated using a scoring algorithm considering 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 of improving future EQA exercises. The evaluation questions were provided by ECDC.

2.3 Scoring antimicrobial susceptibility results

In the 2023 EARS-Net EQA, the implemented scoring system for the evaluation of interpretated results 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. The level was considered easy if the expected MIC value was two or more dilutions away from the EUCAST clinical breakpoint, outside the area of technical uncertainty (ATU), and not recently added or changed in EUCAST breakpoint tables. Otherwise, the level was considered difficult. The scoring of a result reflected the level of difficulty (Table 7).

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 (Table 7).

This scoring system is the same as applied in the 2022 EARS-Net EQA, with one exception. Missing results will no longer generate a negative score, but instead the combination will be visible in the laboratory’s individual evaluation report marked with a dash (“-“), to indicate that no result was submitted.

Table 7. Exercise scoring system for reported AST results in the 2023 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
	Not reported	-	-	-	-	-	-

Note: R: resistant, I: susceptible, increased exposure, S: susceptible, standard dosing regimen; VME: very major error, ME: major error; - : no data.

3. Results

3.1 Participation

In the 2023 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 2023 EARS-Net EQA and all six were covered by EARS-Net surveillance. Therefore, results on the species identification and interpretation of AST results should be reported for all six strains. Overall, 49 out of 68 (72.1%) laboratories from France submitted results for one or more of the six strains. No results were submitted by 19 laboratories with ID Numbers FRn0217, FRn0218, FRn0399, FRn0614, FRn0625, FRn0756, FR127, FRn0047, FRn0131, FRn0222, FRn0265, FRn0355, FRn0356, FRn0502, FRn0623, FRn0817, FRn0825, FRn0986, FRn1028.

A total of 49 (100.0%) laboratories submitted AST results for all six strains, and all laboratories provided interpretation for all submitted strains. Providing interpretation of the six strains was a minimum requirement for receiving a certificate of participation.

3.2 Species identification results

For each strain, the species should be identified. In total, 294 out of 294 (100.0%) 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 is given in Table 8. All laboratories reported the correct species identification for all submitted strains .

Only the interpretation of AST results submitted for strains with correct species identification could be evaluated.

Table 8. Number and percentage of laboratories reporting the correct species in the 2023 EARS-Net EQA

France Strain ID	Expected species	No. of labs submitting data with interpretation	No. of labs reporting correct species identification	% of labs reporting correct species identification
2023 EARS-Net 1	*Escherichia coli*	49	49	100.0
2023 EARS-Net 2	*Klebsiella pneumoniae*	49	49	100.0
2023 EARS-Net 3	*Enterococcus faecalis*	49	49	100.0
2023 EARS-Net 4	*Klebsiella pneumoniae*	49	49	100.0
2023 EARS-Net 5	*Acinetobacter baumannii*	49	49	100.0
2023 EARS-Net 6	*Enterococcus faecium*	49	49	100.0

3.3 Antimicrobial susceptibility testing (AST) results

AST results were evaluated for strains with correct species identification. In the 2023 EARS-Net

EQA, the species for the six strains were included in the EARS-Net surveillance.

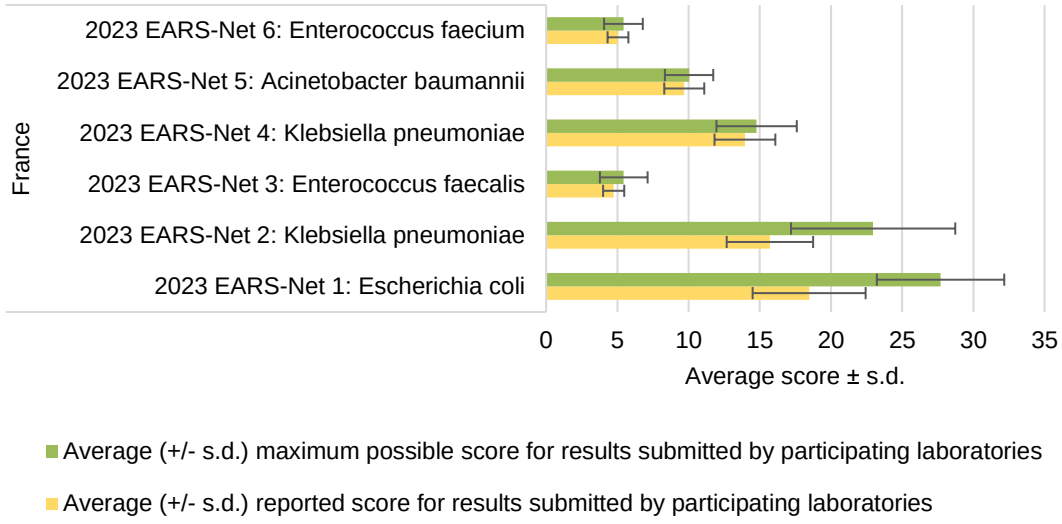
Participants were asked to report AST results, i.e., MIC or zone diameter values and their categorisation as “resistant” (R), “susceptible, increased exposure” (I), and “susceptible, standard dosing regimen” (S) for the species covered by EARS-Net surveillance. Only the categorisation was evaluated,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

For the 2023 EARS-Net EQA, each laboratory could report interpretation for 74 different strain/antimicrobial combinations with a total maximum score of 98.

For the 49 laboratories submitting results with correct species identification results, interpretation of AST results were reported for 3,139 out of the 3,626 possible strain/antimicrobial combinations, and 2,973 (94.7%) were reported with the correct interpretation with an average score for submitted results of 67.6 ± 10.8. The maximum score for the reported results was 86.3 ± 10.2.

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

Figure 1. Average maximum score of reported results ± s.d., and average score of the reported results ± s.d. for each strain, in 2023 EARS-Net EQA



Key: s.d. – standard deviation; Avg – average.

An overview of the methods used for the determination of the antimicrobial resistance for the six strains and the percentage of correct interpretations is given in Tables 9, 10, 11 and 12. The most commonly used method was disk/tablet diffusion (43.5%) (Table 12). The lowest level of concordance with expected interpretations was reported when using the broth microdilution (91.1%) (Table 12).

Table 9. Overview of methods used for determination of the AST results for strains '2023 EARS-Net 1' and '2023 EARS-Net 2'

France	2023 EARS-Net 1 *Escherichia coli*			2023 EARS-Net 2 *Klebsiella pneumoniae*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	-	-	-	1	0.1	100.0
Automated system	360	44.4	87.8	298	41.9	90.3
Broth microdilution	42	5.2	83.3	36	5.1	91.7
Disk/Tablet diffusion	339	41.8	92.0	314	44.2	90.8
Gradient test	69	8.5	95.7	62	8.7	91.9
Macro broth dilution (tubes)	-	-	-	-	-	-
Other	1	0.1	100.0	-	-	-
Total	**811**	100.0	**90.0**	**711**	100.0	**90.7**

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

Table 10. Overview of methods used for determination of the AST results for strains '2023 EARS-Net 3' and '2023 EARS-Net 4'

France	2023 EARS-Net 3 *Enterococcus faecalis*			2023 EARS-Net 4 *Klebsiella pneumoniae*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	-	-	-	-	-	-
Automated system	117	43.8	98.3	311	43.0	100.0
Broth microdilution	11	4.1	90.9	40	5.5	90.0
Disk/Tablet diffusion	95	35.6	97.9	330	45.6	99.7
Gradient test	44	16.5	97.7	43	5.9	93.0
Macro broth dilution (tubes)	-	-	-	-	-	-
Other	-	-	-	-	-	-
Total	**267**	**100.0**	**97.8**	**724**	**100.0**	**98.9**

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

Table 11. Overview of methods used for determination of the AST results for strains '2023 EARS-Net 5' and '2023 EARS-Net 6'

France	2023 EARS-Net 5 *Acinetobacter Baumannii*			2023 EARS-Net 6 *Enterococcus faecium*		
Method	No. of AST performed	% of total AST performed	% correct interpretation	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	1	0.3	100.0	-	-	-
Automated system	97	26.9	97.9	106	39.8	99.1
Broth microdilution	35	9.7	97.1	27	10.2	96.3
Disk/Tablet diffusion	207	57.5	100.0	81	30.5	100.0
Gradient test	19	5.3	100.0	52	19.5	100.0
Macro broth dilution (tubes)	1	0.3	100.0	-	-	-
Other	-	-	-	-	-	-
Total	**360**	**100.0**	**99.2**	**266**	**100.0**	**99.2**

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

Table 12. Overview of methods used for determination of the AST results for all six strains

France	Total		
Method	No. of AST performed	% of total AST performed	% correct interpretation
Agar dilution	2	0.1	100.0
Automated system	1,289	41.1	93.9
Broth microdilution	191	6.1	91.1
Disk/Tablet diffusion	1,366	43.5	95.7
Gradient test	289	9.2	95.8
Macro broth dilution (tubes)	1	0.0	100.0
Other	1	0.0	100.0
Total	**3,139**	**100.0**	**94.7**

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, the participating laboratories could indicate whether they would send that strain to a reference laboratory for further microbiological analysis. Figure 2 provides an overview of the laboratories with very major (VME) that would send the strains for further testing.

For strain '2023 EARS-Net 1' (*E. coli*), 4.1% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain to a reference or other laboratory for further testing. Only 1 (3.8%) of the 26 laboratories reporting VME would send the strain for further analysis.

For strain '2023 EARS-Net 2' (*K. pneumoniae*), 63.3% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain for further testing. Only 9 (52.9%) of the 17 laboratories reporting VME would send the strain for further analysis.

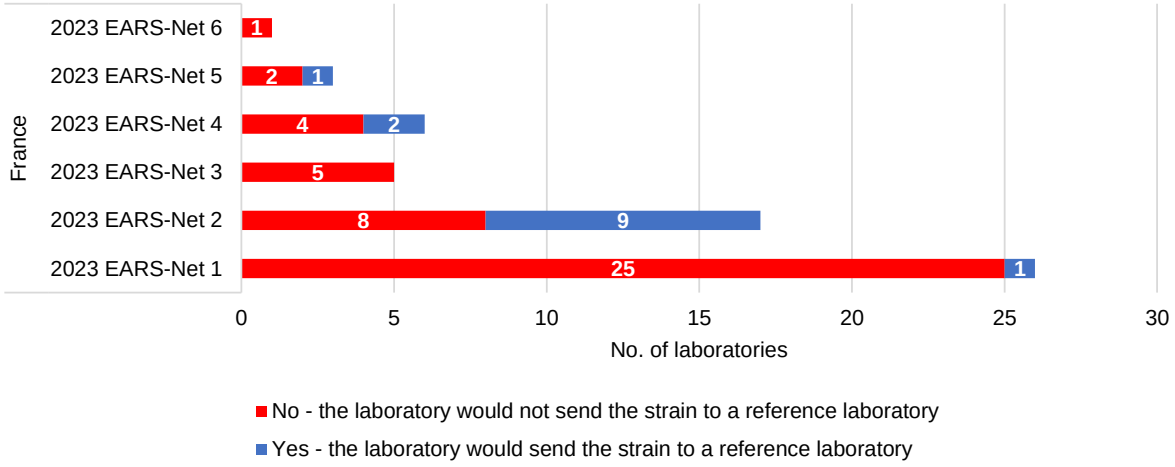
For strain '2023 EARS-Net 3' (*E. faecalis*), 44.9% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain for further testing. None of the 5 laboratories reporting VME would send the strain for further analysis.

For strain '2023 EARS-Net 4' (K. *pneumoniae*), 71.4% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain. Only 2 (33.3%) of the 6 laboratories reporting VME would send the strain for further analysis.

For strain '2023 EARS-Net 5' (*A. baumannii*), 6.1% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain for further testing. Only 1 (33.3%) of the 3 laboratories reporting VME would send the strain for further analysis.

For strain '2023 EARS-Net 6' (*E. faecium*), 59.2% of the 49 laboratories submitting results with correct species identification and providing interpretation would send the strain for further testing. The one laboratory reporting VME would not send the strain for further analysis.

Figure 2. Laboratories with very major errors (VME) intention to send a strain to a reference laboratory for further testing in 2023 EARS-Net EQA, by strain



Antimicrobial agents tested for each EQA strain

The EQA protocol [1] 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 '2023 EARS-Net 1' (*E. coli*), 10 out of 49 laboratories tested all 20 antimicrobials (Figure 5).

For strain '2023 EARS-Net 2' (*K. pneumoniae*), 12 out of 49 laboratories tested all 17 antimicrobials (Figure 7).

For strain '2023 EARS-Net 3' (*E. faecalis*), 29 out of 49 laboratories tested all 6 antimicrobials (Figure 9).

For strain '2023 EARS-Net 4' (K. *pneumoniae*), 14 out of 49 laboratories tested all 17 antimicrobials (Figure 11).

For strain '2023 EARS-Net 5' (*A. baumannii*), 23 out of 49 laboratories tested all 8 antimicrobials (Figure 13).

For strain '2023 EARS-Net 6' (*E. faecium*), 28 out of 49 laboratories tested all 6 antimicrobials (Figure 15).

Figures 4, 6, 8, 10, 12, 14 shows the number of antimicrobial agents tested by the laboratories, for each strain.

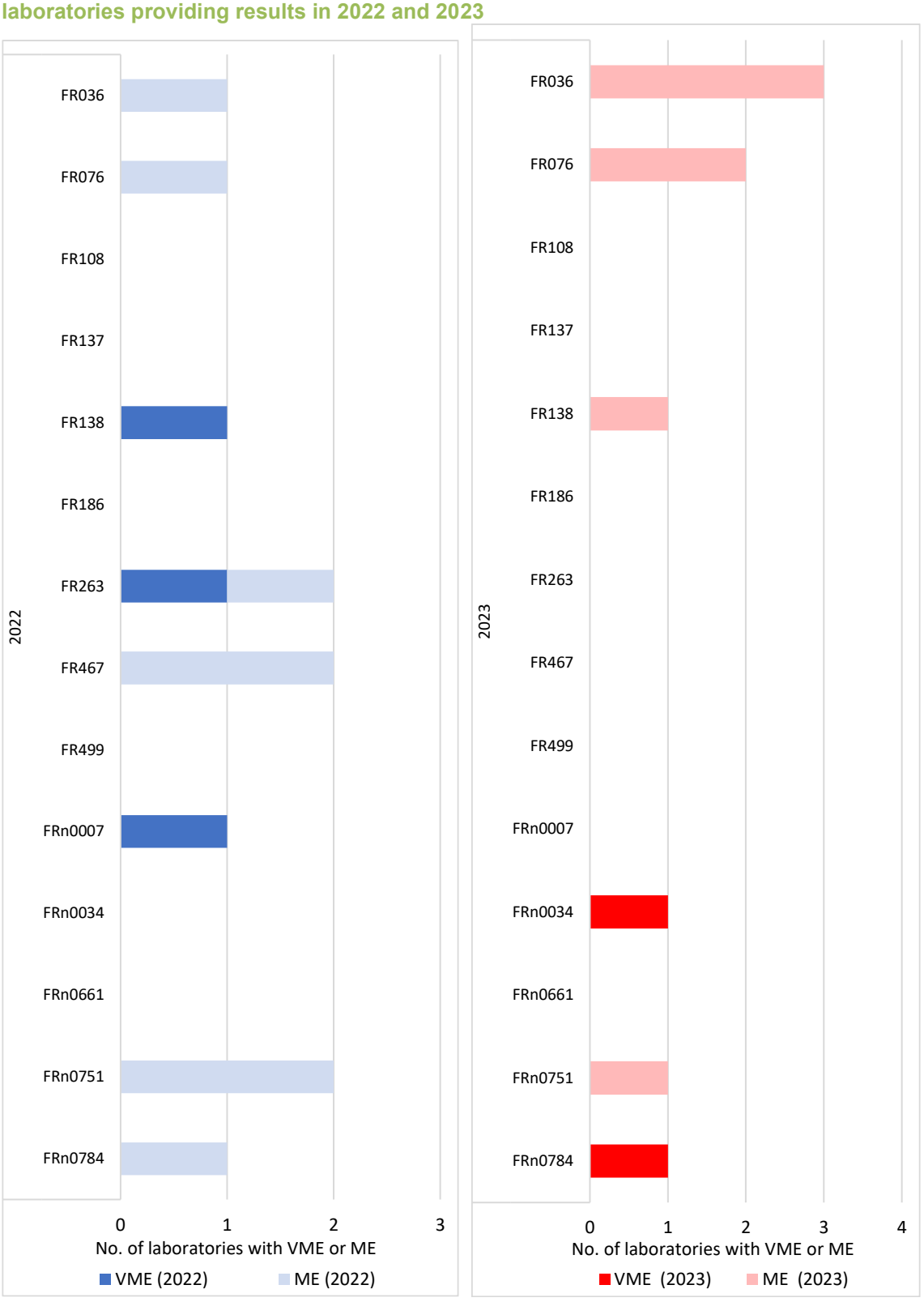
Strain ‘2023 EARS-Net 1’ (*Escherichia coli*)

In the 2022 EARS-Net EQA, the strain ‘2022 EARS-Net 2’ (*E. coli*) was the most challenging for participating laboratories. Therefore, it was decided to include the exact same *E. coli* strain in the panel for the 2023 EARS-Net EQA (strain ‘2023 EARS-Net 1’). To ensure harmonisation between expected results included in the 2023 EQA, the strain was tested by DTU and the reference laboratories under the same conditions as the other strains included in this EQA. The obtained expected results were essentially in agreement with the results obtained and described in the 2022 EARS-Net EQA. However, the obtained consensus for piperacillin-tazobactam (const. 4) for the 2023 EARS-Net EQA was MIC=16/4 mg/L, and therefore received an interpretation as Resistant, whereas for the 2022 EARS-Net EQA the expected result was MIC=8/4 mg/L with an interpretation of Susceptible, standard dosing regimen. Furthermore, the obtained consensus for amikacin for the 2023 EARS-Net EQA was MIC=8 mg/L, and therefore received an interpretation as Susceptible, standard dosing regimen, whereas for the 2022 EARS-Net EQA the expected result was MIC>8 mg/L with an interpretation of Resistant. These results further illustrate the variability of the strain and the difficulty of obtaining concordant AST results.

At the EU/EEA level, when comparing results between the 2022 and the 2023 EARS-Net EQAs, there was little variability of results for this strain (excluding the results obtained for amikacin and for piperacillin-tazobactam). The highest variation was the decrease in ME for cefepime, from 20% of the participating laboratories to 17% in 2023.

In France, 14 laboratories submitted interpretation of AST results both years, and 9 laboratories reported results with VME/ME at least one year (the results on piperacillin-tazobactam (const. 4) and amikacin were excluded). An overview of the laboratories reporting results with VME/ME (leaving out piperacillin-tazobactam (const. 4) and amikacin results) is presented in Figure 3.

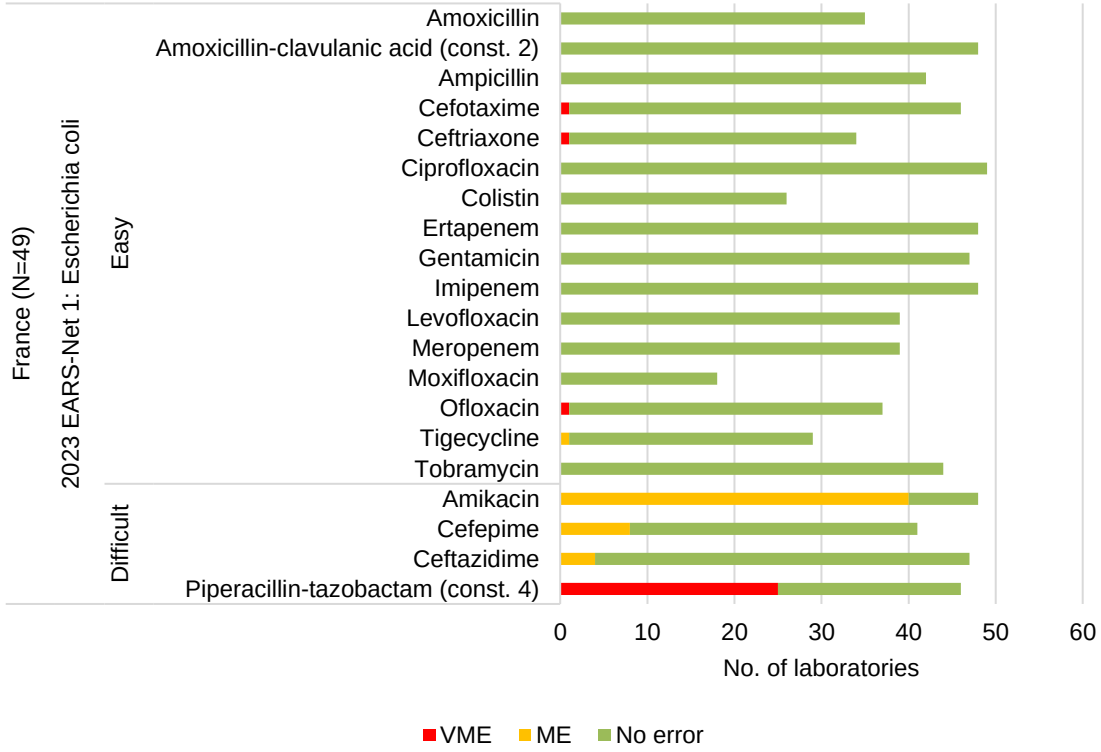
Figure 3. Reported errors of interpretation of AST results (not including piperacillin-tazobactam (const. 4) and amikacin) for strain '2022 EARS-Net 2' and '2023 EARS-Net 1' by laboratories providing results in 2022 and 2023



In the 2023 EARS-Net EQA, the 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ’2023 EARS-Net 1’. Each laboratory could submit results from 20 antimicrobials (Maximum 980 submissions for France). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

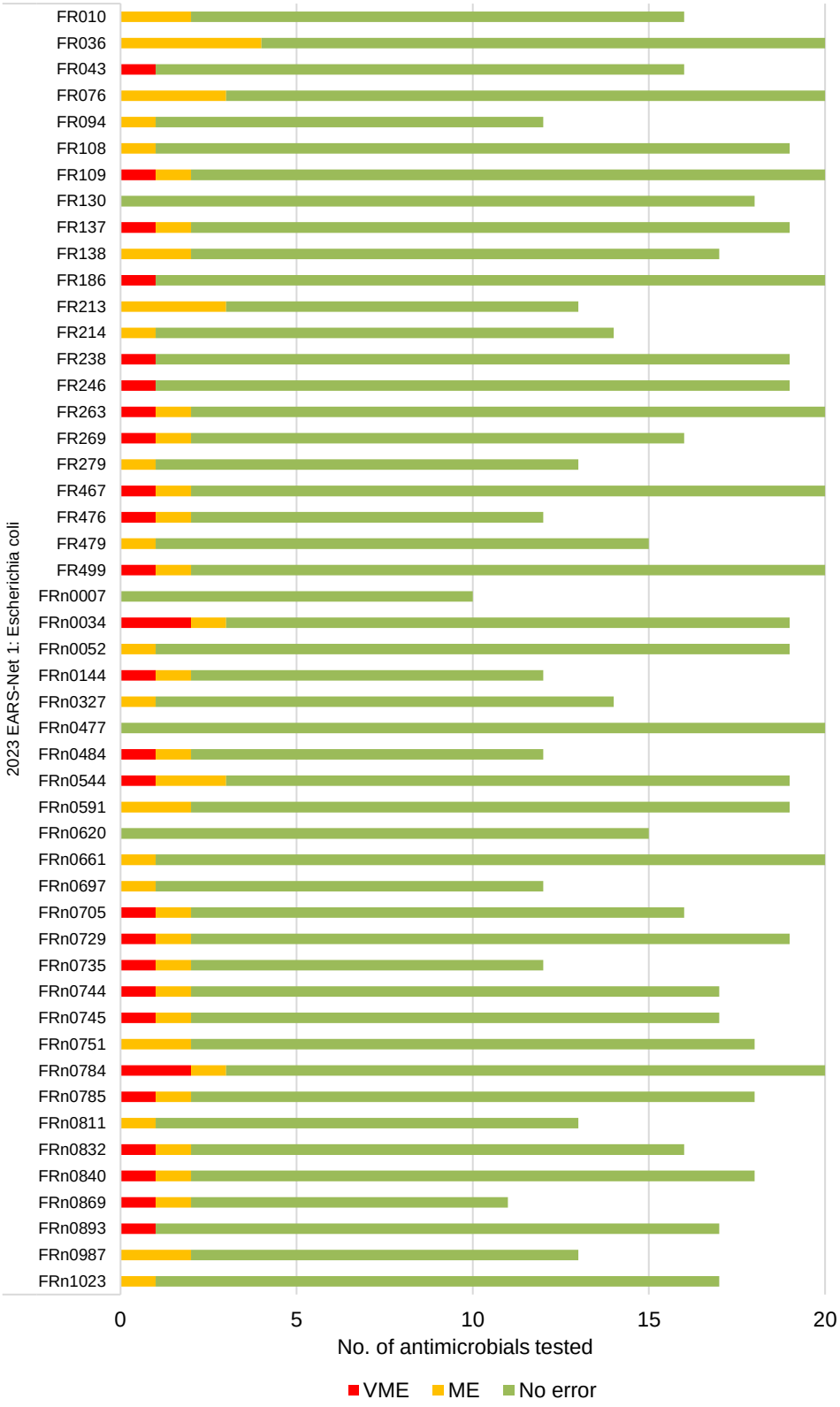
Overall, 811 AST results were submitted and the interpretations were correct for 730 (90.0%) of the results; 53 (6.5%) of the interpretations were ME and 28 (3.5%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 1' were reported for cefotaxime, ceftriaxone, ofloxacin, piperacillin-tazobactam (const. 4) (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 ‘2023 EARS-Net 1’ (*Escherichia coli*) 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 '2023 EARS-Net 1' (*Escherichia coli*) by laboratory



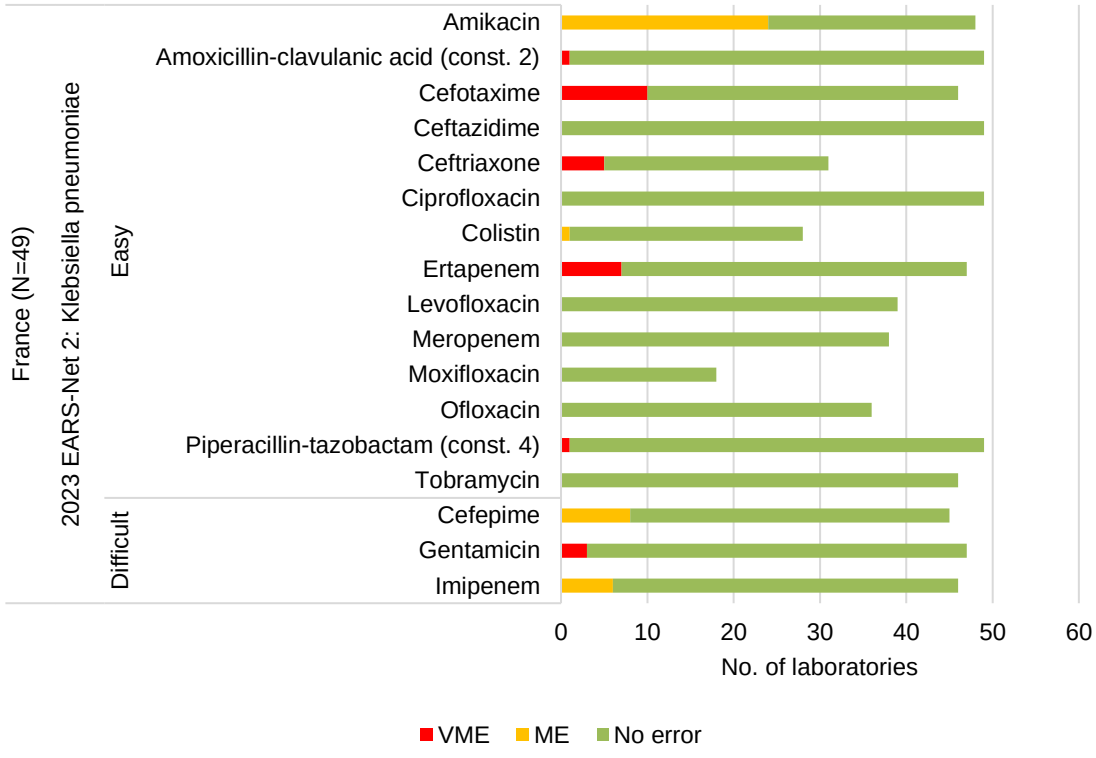
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain ‘2023 EARS-Net 2’ (*Klebsiella pneumoniae*)

The 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ’2023 EARS-Net 2’. Each laboratory could submit results from 17 antimicrobials (Maximum 833 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

Overall, 711 AST results were submitted and the interpretations were correct for 645 (90.7%) of the results; 39 (5.5%) of the interpretations were ME and 27 (3.8%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 2' were reported for amoxicillin-clavulanic acid (const. 2), cefotaxime, ceftriaxone, ertapenem, gentamicin, piperacillin-tazobactam (const. 4) (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 ‘2023 EARS-Net 2’ (*Klebsiella pneumoniae*) 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 '2023 EARS-Net 2' (*Klebsiella pneumoniae*) by laboratory

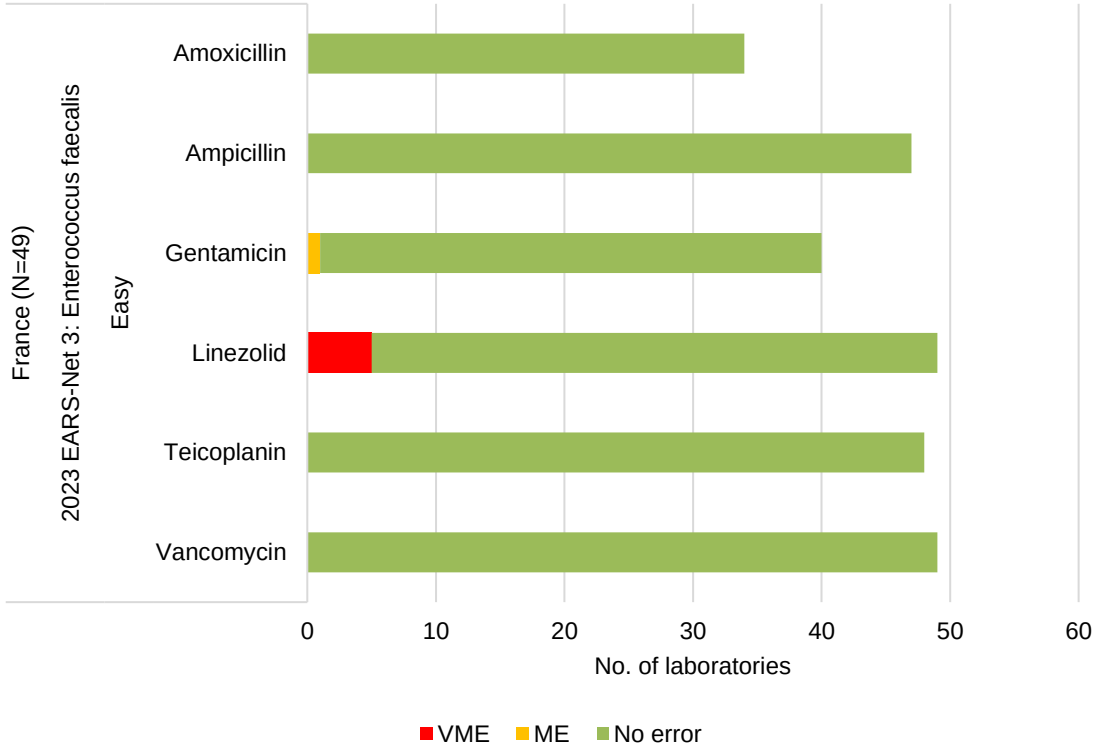
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain ‘2023 EARS-Net 3’ (*Enterococcus faecalis*)

The 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ’2023 EARS-Net 3’. Each laboratory could submit results from 6 antimicrobials (Maximum 294 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

Overall, 267 AST results were submitted and the interpretations were correct for 261 (97.8%) of the results; 1 (0.4%) of the interpretations were ME and 5 (1.9%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 3' were reported for linezolid (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 ‘2023 EARS-Net 3’ (*Enterococcus faecalis*) 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 '2023 EARS-Net 3' (*Enterococcus faecalis*) by laboratory

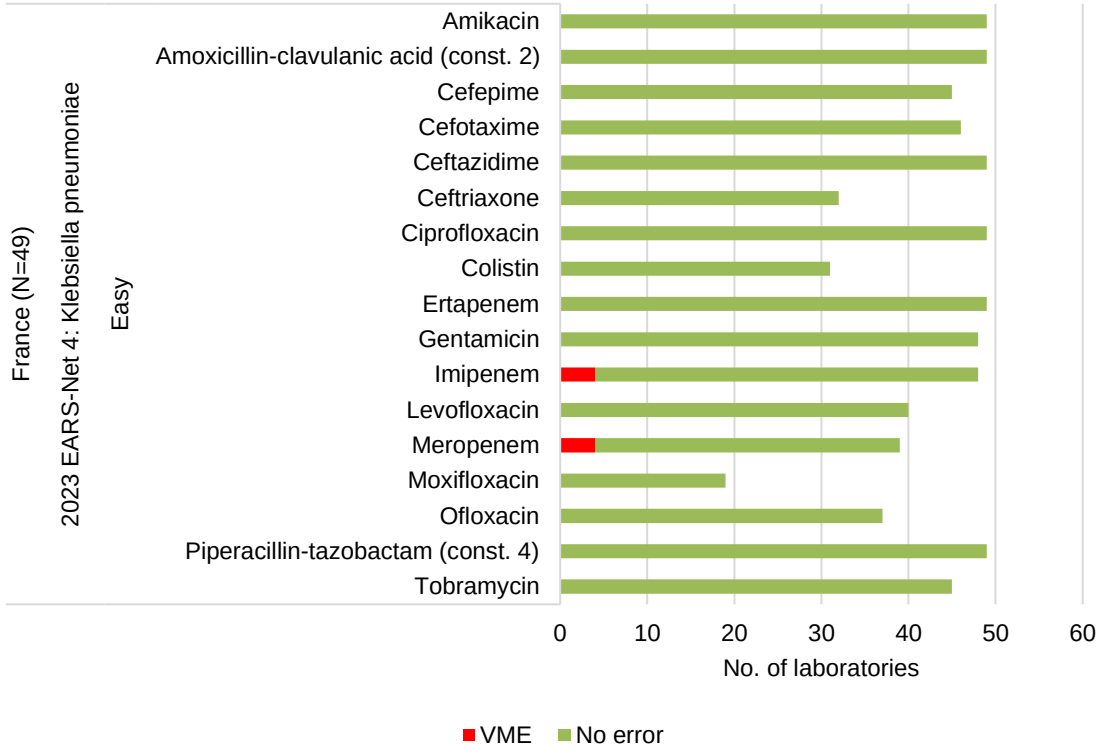
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain ‘2023 EARS-Net 4’ (*Klebsiella pneumoniae*)

The 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ‘2023 EARS-Net 4’. Each laboratory could submit results from 17 antimicrobials (Maximum 833 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

Overall, 724 AST results were submitted and the interpretations were correct for 716 (98.9%) of the results. None of the laboratories reported any ME however 8 (1.1%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 4' were reported for imipenem, meropenem (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 ‘2023 EARS-Net 4’ (*Klebsiella pneumoniae*) 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 '2023 EARS-Net 4' (*Klebsiella pneumoniae*) by laboratory

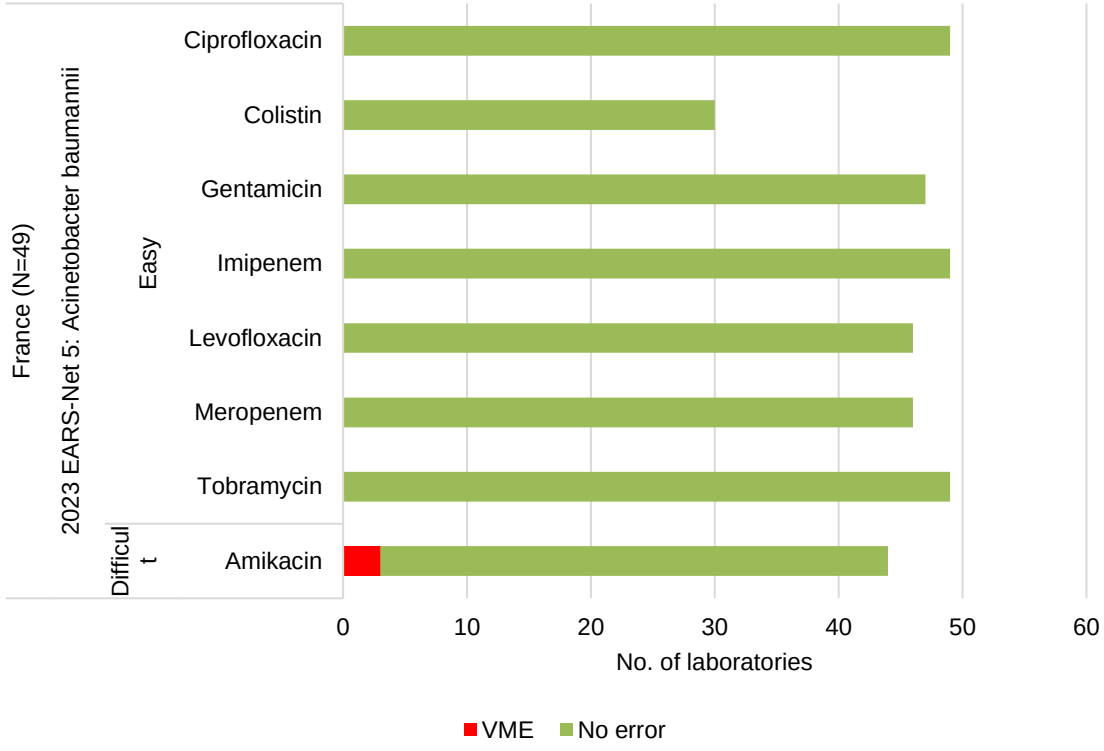
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

Strain ‘2023 EARS-Net 5’ (Acinetobacter baumannii)

The 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ’2023 EARS-Net 5’. Each laboratory could submit results from 8 antimicrobials (Maximum 392 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

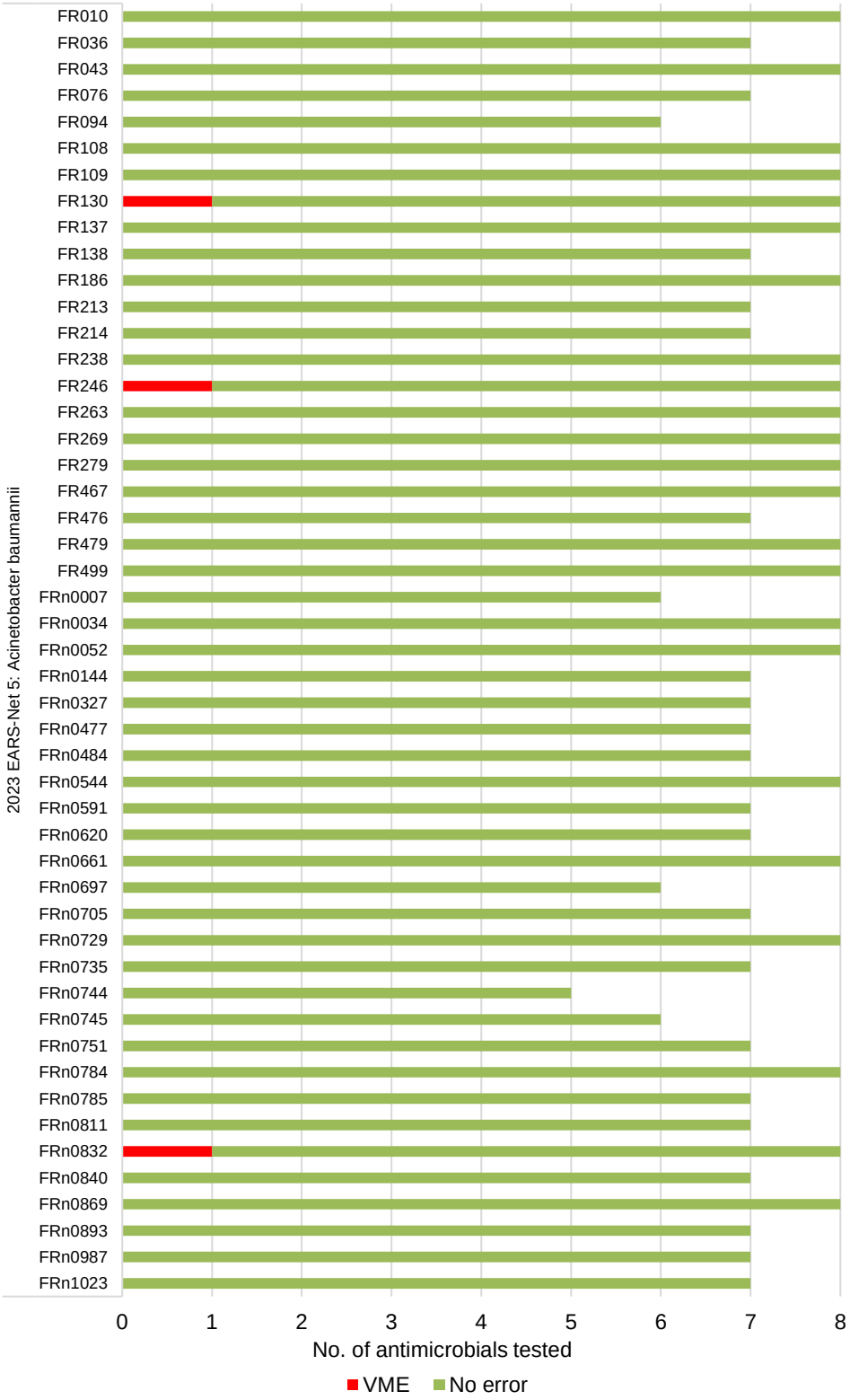
Overall, 360 AST results were submitted and the interpretations were correct for 357 (99.2%) of the results. None of the laboratories reported any ME however 3 (0.8%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 5' were reported for amikacin (Figure 12). An overview of the reported results for all laboratories is presented in Figure 13.

Figure 12. Reported interpretation of AST results for strain ‘2023 EARS-Net 5’ (*Acinetobacter baumannii*) 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 '2023 EARS-Net 5' (*Acinetobacter baumannii*) by laboratory



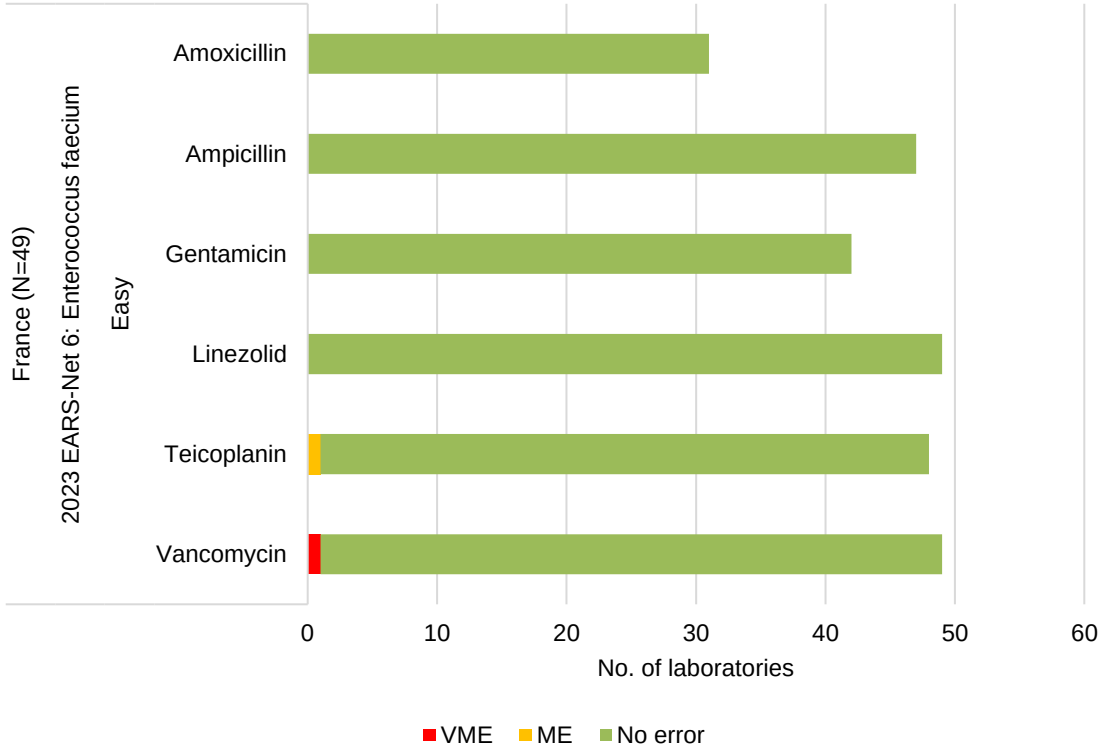
Key: AST – antimicrobial susceptibility testing; VME – very major error; ME – major error

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

The 49 laboratories submitting interpretation of results for further analysis identified the species correctly for the strain ’2023 EARS-Net 6’. Each laboratory could submit results from 6 antimicrobials (Maximum 294 submissions). Annex 1 provides an overview of the antimicrobials included in the EARS-Net reporting protocol 2023.

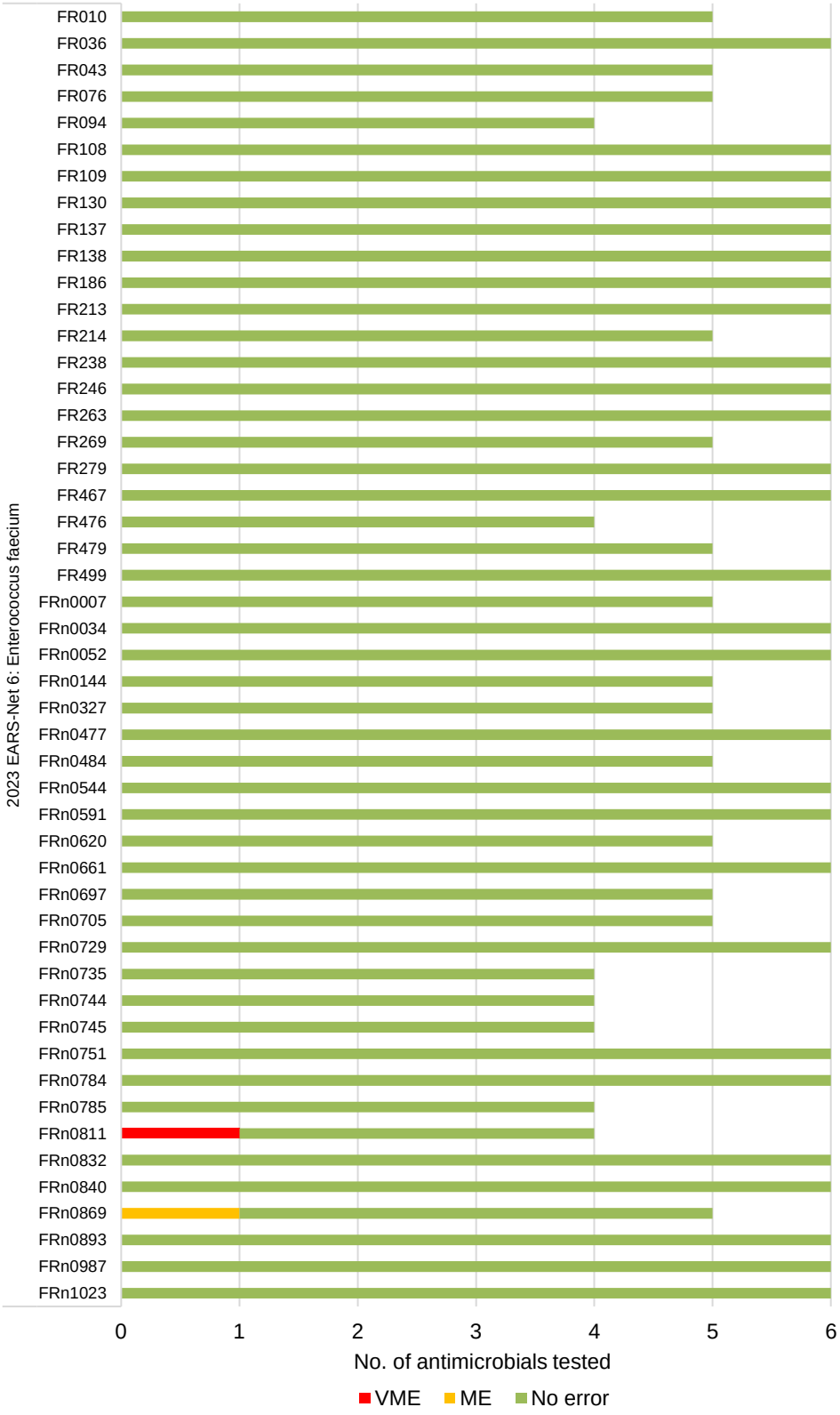
Overall, 266 AST results was submitted and the interpretation were correct for 264 (99.2%) of the results; 1 (0.4%) of the interpretations were ME and 1 (0.4%) of the interpretations were VME. VMEs in the interpretation of AST results for strain '2023 EARS-Net 6' were reported for vancomycin (Figure 14). An overview of the reported results for all laboratories is presented in Figure 15.

Figure 14. Reported interpretation of AST results for strain ‘2023 EARS-Net 6’ (*Enterococcus faecium*) 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 '2023 EARS-Net 6' (*Enterococcus faecium*) by laboratory



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

4. Conclusions and recommendation for improvement

For the 2023 EARS-Net EQA, correct species identification for the six strains was submitted in 294 strains (100.0%) out of the 294 strains submitted by the 49 laboratories in France.

Interpretation of AST results was reported for 3,139 out of the 3,626 possible strain/antimicrobial combinations. Overall, there was very good concordance with the expected interpretations as 2,973 (94.7%) were correct out of the 3,139 tests performed with 23 (46.9%) laboratories meeting the satisfactory level of 95% concordance for the reported interpretations.

The following methodologies were applied by the laboratories when performing the tests: agar dilution (0.1%), automated system (6.1%), broth microdilution (6.1%), disk/tablet diffusion (43.5%), gradient test (9.2%), macro broth dilution (tubes) (0%), and other (0%).

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

For the strain '2023 EARS-Net 1', 4 laboratories were in full concordance with the expected interpretations, 2 laboratories had an excellent concordance with the expected interpretation (≥ 95%), 18 laboratories had a very good concordance (< 95% and ≥ 90%), 15 laboratories had a good concordance (< 90% and ≥ 85%), 9 laboratories had a satisfactory concordance (< 85% and ≥ 80%), and 1 laboratory had < 80% concordance.

In France, for the strain '2023 EARS-Net 1', VMEs were observed for piperacillin-tazobactam, cefotaxime, ceftriaxone and ofloxacin. Deviations in piperacillin-tazobactam corresponded to 54.3% 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, and they can also be derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 1). Deviations in cefotaxime (2.2% of submitted results), ceftriaxone (2.9% of submitted results) and ofloxacin (2.7% of submitted results) were reported when using disk/tablet diffusion. For all three antimicrobials, 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 antimicrobial resistance genes harboured by the strain (Table 1). A high proportion of MEs were observed for amikacin (83.3% of submitted results) and cefepime (19.5% of submitted results) and were reported mainly when using the automated system and disk/tablet diffusion. For both antimicrobials 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 antimicrobial resistance genes harboured by the strain (Table 1).

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

For the strain '2023 EARS-Net 2', 13 laboratories were in full concordance with the expected interpretations, 15 laboratories had a very good concordance (< 95% and ≥ 90%), 10 laboratories had a good concordance (< 90% and ≥ 85%), 8 laboratories had a satisfactory

concordance (< 85% and ≥ 80%), and 3 laboratories had < 80% concordance.

In France, for the strain '2023 EARS-Net 2', VMEs were observed for amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ertapenem, gentamicin and piperacillin-tazobactam. Deviations in cefotaxime corresponded to 21.7% of all submitted interpretations for that antimicrobial and were reported when using the automated system and disk/tablet diffusion. Deviations in ceftriaxone corresponded to 16.1% of all submitted interpretations for that antimicrobial and were reported when using disk/tablet diffusion and gradient test. Deviations in ertapenem corresponded to 10.6% of all submitted interpretations for that antimicrobial and were reported when using the automated system, disk/tablet diffusion and gradient test. Deviations in amoxicillin-clavulanic acid corresponded to 2% of all submitted interpretations for that antimicrobial and were reported when using the automated system. Deviations in piperacillin-tazobactam corresponded to 2% of all submitted interpretations for that antimicrobial and were reported when using the automated system. For the five antimicrobials, 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 antimicrobial resistance genes harboured by the strain (Table 2). Deviations in gentamicin corresponded to 6.4% of all submitted interpretations for that antimicrobial and were reported when using the 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. A high proportion of MEs were observed for amikacin (50% of submitted results) and cefepime (17.8% of sumbitted results). Deviations in amikacin were reported when using the automated system, broth microdilution 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 cefepime 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, and they can also be derived from the differential expression of the antimicrobial resistance genes harboured by the strain (Table 2).

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

For the strain '2023 EARS-Net 3', 43 laboratories were in full concordance with the expected interpretations, and 6 laboratories had a satisfactory concordance (< 85% and ≥ 80%).

In France, for the strain '2023 EARS-Net 3', VMEs were observed for linezolid. These corresponded to 10.2% of all submitted interpretations for that antimicrobial and were reported throughout all methods. 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. No high proportions of MEs were observed for this strain.

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

For the '2023 EARS-Net 4', 43 laboratories were in full concordance with the expected interpretations, and 6 laboratories had a satisfactory concordance (< 85% and ≥ 80%).

In France, for the strain '2023 EARS-Net 4', VMEs were observed for meropenem and imipenem. Deviations in meropenem corresponded to 10.3% of all submitted interpretations for that antimicrobial and were reported when using broth microdilution and gradient test. Deviations in imipenem corresponded to 8.3% of all submitted interpretations for that antimicrobial and were reported when using the same methods and also disk/tablet diffusion. For both antimicrobials, 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 '2023 EARS-Net 5' (*Acinetobacter baumannii*)

For the *'2023 EARS-Net 5'*, 46 laboratories were in full concordance with the expected interpretations, and 3 laboratories had a good concordance (< 90% and ≥ 85%).

In France, for the strain '2023 EARS-Net 5', VMEs were observed for amikacin. These deviations corresponded to 6.8% of all submitted interpretations for that antimicrobial and were reported when using the automated system and 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. No MEs were observed for this strain.

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

For the *'2023 EARS-Net 6'*, 47 laboratories were in full concordance with the expected interpretations, 1 laboratory had a satisfactory concordance (< 85% and ≥ 80%), and 1 laboratory had < 80% concordance.

In France, for the strain '2023 EARS-Net 6', VMEs were observed for vancomycin. These corresponded to 2% of all submitted interpretations for that antimicrobial and were reported when using the automated system. 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. No high proportions of MEs 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;
- 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;
- Be aware and potentially seek consultancy regarding the testing and reading of results for aminoglycosides, due to random and systematic deviations derived from variations in media composition;
- Be aware and potentially seek consultancy regarding the testing and reading of results for cephalosporins and carbapenems, due to differential expression of beta-lactamase genes;
- Consider additional training of technical staff to enhance capabilities and performance.

5. References

1) Antimicrobial resistance (AMR) reporting protocol 2023. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2023

6. Annex 1. Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2023

Copy (adapted) of Table 8 from the EARS-Net reporting protocol 2023: 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- 2023.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 norfloxacin for *Escherichia coli* and *Klebsiella pneumoniae* isolates are included in the original table but are not part of the 2023 EARS- Net EQA exercise. This is due to the breakpoint in the EUCAST clinical breakpoints v13.0 only being applicable to uncomplicated urinary tract infections.

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) Vancomycin (VAN) Teicoplanin (TEC) Linezolid (LNZ)

Microorganism	Antimicrobial agent
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)

Microorganism	Antimicrobial agent
	Ofloxacin (OFX) Moxifloxacin (MFX) 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