



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

Expected antimicrobial susceptibility testing results for the
bacterial strains included in the 2023 EARS-Net EQA exercise

Specific contract no. 3 ECD.14598 ID 25808 under the Framework contract
ECDC/2020/009

Funded by the European Centre for Disease Prevention and Control

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

The 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. 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 DTU Food. The AST profiles were validated by three reference laboratories: the EUCAST Development Laboratory, Sweden; the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), The Doherty Institute, Australia; and the Antimicrobial Resistance Research Center, National Institute of Infectious Diseases (NIID), Japan. Expected results for each antimicrobial and strain combination were determined by the consensus AST results, 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, AMRFinderPlus and CARD RGI (Table 1-6). Finally, a MIC determination was performed at DTU Food after preparation of the agar swab cultures 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 2023¹. 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 applied for the interpretation of the obtained AST results (https://www.eucast.org/clinical_breakpoints/) (Table 1-6). This allowed for categorisation of the test results into three categories: "resistant" (R), "susceptible, increased exposure" (I), and "susceptible, standard dosing regimen" (S).

2023 EARS-Net 1: *Escherichia coli*

Table 1. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2023 EARS-Net 1: *Escherichia coli*

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	<i>aac(6′)-lb-cr</i>
Amoxicillin	8	8	Note***	Note	Easy	>64	R	<i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Amoxicillin-clavulanic acid****	8	8	19	19	Easy	>64/2	R	<i>bla_{OXA-1}</i>
Ampicillin	8	8	14	14	Easy	>32	R	<i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Cefepime	1	4	27	24	Difficult	1	S	<i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Cefotaxime	1	2	20	17	Easy	16	R	<i>bla_{CTX-M-15}</i>
Ceftazidime	1	4	22	19	Difficult	2	I	<i>bla_{CTX-M-15}</i>
Ceftriaxone	1	2	25	22	Easy	32	R	<i>bla_{CTX-M-15}</i>
Ciprofloxacin	0,25	0,5	25	22	Easy	>4	R	<i>aac(6′)-lb-cr</i> , <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> 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	<i>aac(6′)-lb-cr</i> , <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Meropenem	2	8	22	16	Easy	≤0.03	S	ND
Moxifloxacin	0,25	0,25	22	22	Easy	>8	R	<i>aac(6′)-lb-cr</i> , <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Ofloxacin	0,25	0,5	24	22	Easy	>2	R	<i>aac(6′)-lb-cr</i> , <i>gyrA</i> S83L, <i>gyrA</i> D87N, <i>parC</i> S80I, <i>parC</i> E84V, <i>parE</i> I529L
Piperacillin-tazobactam****	8	8	20	20	Difficult	16/4	R	<i>bla_{OXA-1}</i>
Tigecycline	0,5	0,5	18	18	Easy	≤0.25	S	ND

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 <				
Tobramycin	2	2	16	16	Easy	>16	R	<i>aac(6)-Ib-cr</i>

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *mph(A)*, *catB3*, *aadA5*, *sul1*, *dfpA17*. MALDI-TOF by DTU: *Escherichia 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.

Difficulty of AST interpretation for each *E. coli*-antimicrobial combination

Antimicrobial: COLISTIN, ERTAPENEM, GENTAMICIN, IMIPENEM, MEROPENEM, TIGECYCLINE

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: AMIKACIN, CEFEPIME

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: CEFTAZIDIME

Expected interpretation: SUSCEPTIBLE, INCREASED EXPOSURE (I)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: AMOXICILLIN, AMOXICILLIN-CLAVULANIC ACID, AMPICILLIN, CEFOTAXIME, CEFTRIAXONE, CIPROFLOXACIN, LEVOFLOXACIN, MOXIFLOXACIN, OFLOXACIN, TOBRAMYCIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S or I) would be a very major error (VME).

Antimicrobial: PIPERACILLIN-TAZOBACTAM

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as susceptible (S) would be a very major error (VME).

2023 EARS-Net 2: *Klebsiella pneumoniae*

Table 2. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2023 EARS-Net 2: *Klebsiella pneumoniae*

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	<i>aac(6')-Ia</i>
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	<i>bla_{VEB-1}</i> , <i>bla_{SHV-11}</i>
Cefepime	1	4	27	24	Difficult	4	I	<i>bla_{VEB-1}</i> , <i>bla_{SHV-11}</i>
Cefotaxime	1	2	20	17	Easy	8	R	<i>bla_{VEB-1}</i> , <i>bla_{SHV-11}</i>
Ceftazidime	1	4	22	19	Easy	>32	R	<i>bla_{VEB-1}</i> , <i>bla_{SHV-11}</i>
Ceftriaxone	1	2	25	22	Easy	16	R	<i>bla_{SHV-11}</i>
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	<i>ant(2'')-Ia</i>
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	<i>bla_{VEB-1}</i> , <i>bla_{SHV-11}</i> , <i>bla_{OXA-10}</i>
Tobramycin	2	2	16	16	Easy	8	R	<i>aac(6')-Ia</i> , <i>ant(2'')-Ia</i>

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. *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: *Klebsiella 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.

Difficulty of AST interpretation for each *Klebsiella pneumoniae*-antimicrobial combination

Antimicrobial: AMIKACIN, CIPROFLOXACIN, COLISTIN, LEVOFLOXACIN, MEROPENEM, MOXIFLOXACIN, OFLOXACIN

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: IMIPENEM

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: CEFEPIME

Expected interpretation: SUSCEPTIBLE, INCREASED EXPOSURE (I)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: AMOXICILLIN-CLAVULANIC ACID, CEFOTAXIME, CEFTAZIDIME, CEFTRIAZONE, ERTAPENEM, PIPERACILLIN-TAZOBACTAM, TOBRAMYCIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S or I) would be a very major error (VME).

Antimicrobial: GENTAMICIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as susceptible (S) would be a very major error (VME).

2023 EARS-Net 3: *Enterococcus faecalis*

Table 3. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2023 EARS-Net 3: *Enterococcus faecalis*

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	<i>optrA</i>
Vancomycin	4	4	12	12	Easy	2	S	ND
Teicoplanin	2	2	16	16	Easy	≤0.5	S	ND

HLAR: High-level aminoglycoside resistance

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *erm(B)*, *tet(L)*, *tet(M)*, *fxaA*, *str*, *lsa(A)* (intrinsic). MALDI-TOF by DTU: *Enterococcus faecalis* (score 2,35), and MLST: ST-22.

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

Difficulty of AST interpretation for each *Enterococcus faecalis*-antimicrobial combination

Antimicrobial: AMOXICILLIN, AMPICILLIN, GENTAMICIN, TEICOPLANIN, VANCOMYCIN

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: LINEZOLID

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S) would be a very major error (VME).

2023 EARS-Net 4: *Klebsiella pneumoniae*

Table 4. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2023 EARS-Net 4: *Klebsiella pneumoniae*

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	<i>rmtB</i>
Amoxicillin-clavulanic acid***	8	8	19	19	Easy	>64/2	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-1} , <i>bla</i> _{OXA-181} , <i>bla</i> _{SHV-1}
Cefepime	1	4	27	24	Easy	32	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-1} , <i>bla</i> _{OXA-181} , <i>bla</i> _{SHV-1} , <i>bla</i> _{CTX-M-15}
Cefotaxime	1	2	20	17	Easy	>64	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{SHV-1} , <i>bla</i> _{CTX-M-15}
Ceftazidime	1	4	22	19	Easy	>128	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{SHV-1} , <i>bla</i> _{CTX-M-15}
Ceftriaxone	1	2	25	22	Easy	>64	R	<i>bla</i> _{SHV-1} , <i>bla</i> _{CTX-M-15}
Ciprofloxacin	0,25	0,5	25	22	Easy	>4	R	<i>qnrS1</i> , <i>gyrA</i> D87N, <i>gyrA</i> S83F, <i>parC</i> E84K
Colistin	2	2	Note ****	Note	Easy	32	R	<i>mgrB</i> W20R
Ertapenem	0,5	0,5	25	25	Easy	>16	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-181}
Gentamicin	2	2	17	17	Easy	>16	R	<i>rmtB</i>
Imipenem	2	4	22	19	Easy	16	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-181}
Levofloxacin	0,5	1	23	19	Easy	>8	R	<i>qnrS1</i> , <i>gyrA</i> D87N, <i>gyrA</i> S83F, <i>parC</i> E84K
Meropenem	2	8	22	16	Easy	>16	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-181}
Moxifloxacin	0,25	0,25	22	22	Easy	>8	R	<i>qnrS1</i> , <i>gyrA</i> D87N, <i>gyrA</i> S83F, <i>parC</i> E84K
Ofloxacin	0,25	0,5	24	22	Easy	>2	R	<i>qnrS1</i> , <i>gyrA</i> D87N, <i>gyrA</i> S83F, <i>parC</i> E84K
Piperacillin-tazobactam***	8	8	20	20	Easy	>128/4	R	<i>bla</i> _{NDM-5} , <i>bla</i> _{OXA-1} , <i>bla</i> _{OXA-181} , <i>bla</i> _{SHV-1} , <i>bla</i> _{CTX-M-15}
Tobramycin	2	2	16	16	Easy	>16	R	<i>rmtB</i>

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. *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: *Klebsiella 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.

Difficulty of AST interpretation for each *Klebsiella pneumoniae*-antimicrobial combination

Antimicrobial: AMIKACIN, AMOXICILLIN-CLAVULANIC ACID, CEFEPIME, CEFOTAXIME, CEFTAZIDIME, CEFTRIAZONE, CIPROFLOXACIN, COLISTIN, ERTAPENEM, GENTAMICIN, IMIPENEM, LEVOFLOXACIN, MEROPENEM, MOXIFLOXACIN, OFLOXACIN, PIPERACILLIN-TAZOBACTAM, TOBRAMYCIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S or I) would be a very major error (VME).

2023 EARS-Net 5: *Acinetobacter baumannii*

Table 5. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2023 EARS-Net 5: *Acinetobacter baumannii*

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	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Colistin	2	2	Note ***	Note	Easy	0,5	S	ND
Gentamicin	4	4	17	17	Easy	>16	R	<i>ant(2'')-Ia</i>
Imipenem	2	4	24	21	Easy	0,25	S	ND
Levofloxacin	0,5	1	23	20	Easy	8	R	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Meropenem	2	8	21	15	Easy	1	S	ND
Tobramycin	4	4	17	17	Easy	>16	R	<i>ant(2'')-Ia</i>

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. Additional antimicrobial resistance genes or chromosomal point mutations: *bla*_{CARB-2}, *tet(39)*, *tet(B)*, *tet(G)*, *aph(3'')-Ib*, *aph(3'')-Ic*, *aph(6)-Id*, *aadA2*, *sul2*, *bla*_{OXA-51} (intrinsic), *bla*_{ADC-25} (likely intrinsic). The strain appears to harbour multiple copies of genes associated with aminoglycoside resistance. Certain copies of those genes might in fact correspond to other variants able to confer amikacin resistance (e.g. other *aph(3'')* variants). MALDI-TOF by DTU: *Acinetobacter baumannii* (score 2,42), and MLST: ST-1552 (scheme A. *baumannii* #1).

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

Difficulty of AST interpretation for each *Acinetobacter baumannii*-antimicrobial combination

Antimicrobial: COLISTIN, IMIPENEM, MEROPENEM

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: CIPROFLOXACIN, GENTAMICIN, LEVOFLOXACIN, TOBRAMYCIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S or I) would be a very major error (VME).

Antimicrobial: AMIKACIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Difficult. The expected MIC value is less than two dilutions away from the clinical breakpoint (i.e. a two-fold dilution would change the interpretation of S/I/R). A misclassification as susceptible (S) would be a very major error (VME).

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Table 6. EUCAST clinical breakpoints, expected MIC value, level of difficulty in interpretation and interpretation for strain 2023 EARS-Net 6: Enterococcus faecium

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
Vancomycin	4	4	12	12	Easy	64	R	VanHBX
Teicoplanin	2	2	16	16	Easy	1	S	ND

HLAR: High-level aminoglycoside resistance

*The level of difficulty indicates the magnitude of the risk of getting the categorisation wrong, classified as 'Easy' or '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.

** ND: Not detected. 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: *Enterococcus faecium* (score 2,47), and MLST: ST-17.

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

Difficulty of AST interpretation for each Enterococcus faecium-antimicrobial combination

Antimicrobial: GENTAMICIN, LINEZOLID, TEICOPLANIN

Expected interpretation: SUSCEPTIBLE, STANDARD DOSING REGIMEN (S)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: AMOXICILLIN, AMPICILLIN, VANCOMYCIN

Expected interpretation: RESISTANT (R)

Difficulty of interpretation: Easy. The expected MIC value is at least two dilutions away from the clinical breakpoint. A misclassification as susceptible (S or I) would be a very major error (VME).

References

- 1) Antimicrobial resistance (AMR) reporting protocol 2023. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 6066