



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 2024 EARS-Net EQA  
exercise**

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## Expected antimicrobial susceptibility testing results for the bacterial strains included in the 2024 EARS-Net EQA exercise

The 2024 EQA focused on species identification and interpretation of the antimicrobial susceptibility testing (AST) of the six strains shared with the participating laboratories (*Acinetobacter baumannii*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*).

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. The expected AST results for each strain were the consensus of AST results from three reference laboratories: DTU Food (performed in triplicate); the EUCAST Development Laboratory, Sweden; and the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), The Doherty Institute, Australia. All reference laboratories used the same AST methodology, specifically the determination of minimum inhibitory concentration (MIC) values for each strain-antimicrobial combination by broth microdilution (or by determining zone diameters through disk diffusion when applicable) according to EUCAST clinical breakpoints tables v14.0. The consensus AST results were reviewed and validated by ECDC and the ECDC EARS-Net Disease Network Coordination Committee. DTU Food performed whole-genome sequencing and bioinformatics analyses of each EQA strain to detect relevant acquired antimicrobial resistance genes (ARG) and chromosomal point mutations (PM). During the preparation of the test swabs, DTU Food performed confirmatory AST of the strains by broth microdilution to confirm that the vials contained the correct strains with the expected AST results.

The EUCAST clinical breakpoints tables v14.0 were applied for the interpretation of the obtained AST results ([https://www.eucast.org/clinical\\_breakpoints/](https://www.eucast.org/clinical_breakpoints/)) (Tables 2-7). This allowed for categorisation of the test results into three categories: “resistant” (R), “susceptible, increased exposure” (I), and “susceptible, standard dosing regimen” (S).

The antimicrobial agents selected for this EQA corresponded to the panel of pathogen and antimicrobial agent combinations under surveillance by EARS-Net, presented in the antimicrobial resistance (AMR) reporting protocol 2024<sup>1</sup>. The exception was testing of ceftazidime-avibactam, cefiderocol, ceftolozane-tazobactam, imipenem-relebactam and meropenem-vaborbactam for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*, which were included in the reporting protocol, but were not part of the 2024 EARS-Net EQA exercise.

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/](https://www.eucast.org/ast_of_bacteria/)).

If the species of the isolate was identified correctly, then the interpretation of AST results were evaluated using the scoring system of the EQA. Conversely, if the species was not identified correctly, the AST results for that isolate were not evaluated further.

The scoring system considered the ‘level of difficulty’ and ‘severity of error’ of every strain-antimicrobial combination. The level of difficulty, classified as ‘Difficult’ or ‘Easy’, reflected the challenge for participating laboratories to report the expected AST interpretation. ‘Difficult’ were situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value was inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. ‘Easy’ were situations where an AST result with a one-fold difference in dilution from the expected MIC value would have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table. The scoring of a result reflected the level of difficulty, with errors in ‘difficult’ results being considered mild and errors in ‘easy’ results being considered severe. The severity of error was divided into three levels: very major error

(VME), major error (ME) and no error. VME is reporting false susceptibility – expecting an R but obtaining an S or I. ME is reporting false resistance – expecting an S or I but obtaining an R. The scoring system penalised VMEs more severely for ‘easy’ results than for ‘difficult’ results, and did not penalise MEs if the test was considered ‘difficult’. The classification of ‘no error’ included situations where one susceptibility category (S or I) was expected, but the other susceptibility category was reported. However, this results in a lower score than if the expected susceptibility category was reported. Table 1 shows the 2024 EARS-Net EQA scoring system.

**Table 1.** Scoring system of the 2024 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	-	-	-	-	-	-

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

## 2024 EARS-Net 1: *Acinetobacter baumannii*

**Table 2.** EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 1: *Acinetobacter baumannii*

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Imipenem	2	4	24	21	Easy	>16	R	<i>bla<sub>OXA-23</sub></i>
Meropenem	2	8	21	15	Easy	>64	R	<i>bla<sub>OXA-23</sub></i>
Ciprofloxacin	0.001	1	50	21	Easy	>8	R	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Levofloxacin	0.5	1	23	20	Easy	16	R	<i>gyrA</i> S81L, <i>parC</i> S84L, <i>parC</i> V104I, <i>parC</i> D105E
Amikacin	8	8	19	19	Easy	128	R	<i>aac(6')-Ib3</i> , <i>aph(3')-Via</i>
Gentamicin	4	4	17	17	Easy	2	S	<i>aph(3')-Via</i>
Tobramycin	4	4	17	17	Difficult	8	R	<i>aac(6')-Ib3</i>
Colistin	2	2	Note ****	Note ****	Easy	0.5	S	ND

\*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. ‘Difficult’ are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty

(ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table.

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and cefoxitin in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*ND: Not detected. Additional ARGs or chromosomal PMs: *sul1*, *dfra7*, *bla<sub>GES-11</sub>*, *bla<sub>OXA-65</sub>* (intrinsic), *bla<sub>ADC-25</sub>* (likely intrinsic). MALDI-TOF by DTU: *Acinetobacter baumannii* (score 2,37). MLST: ST-499 (scheme *A. baumannii* #1) / ST-158 (scheme *A. baumannii* #2).

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

### Difficulty of AST interpretation for each antimicrobial

Antimicrobial: COLISTIN, GENTAMICIN

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, CIPROFLOXACIN, IMPENEM, LEVOFLOXACIN, MEROPENEM

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: TOBRAMYCIN

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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## 2024 EARS-Net 2: *Enterococcus faecium*

**Table 3. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 2: *Enterococcus faecium***

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

\*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed



in, nor added to, the latest EUCAST clinical breakpoint table.

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and ceftazidime in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*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 ARGs or chromosomal PMs: *msr(C)*, *tet(M)*, *gyrA* S83Y, *parC* S80I, *aac(6)-II* (intrinsic). MALDI-TOF by DTU: *Enterococcus faecium* (score 2,42). MLST: ST-17.

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

### Difficulty of AST interpretation for each antimicrobial

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).

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## 2024 EARS-Net 3: *Escherichia coli*

**Table 4. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 3: *Escherichia coli***

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)			EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result*	Expected interpretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Ampicillin	8	8		14	14		Easy	>32	R	<i>bla<sub>OXA-1</sub></i> , <i>bla<sub>CTX-M-15</sub></i>
Amoxicillin	8	8		Note ****	Note ****		Easy	>64	R	<i>bla<sub>OXA-1</sub></i> , <i>bla<sub>CTX-M-15</sub></i>
Amoxicillin-clavulanic acid****	8	8		19	19	19-20	Easy	>64/2	R	<i>bla<sub>OXA-1</sub></i>
Piperacillin-tazobactam** ***	8	8	16	20	20	19	Difficult	16/4	R	<i>bla<sub>OXA-1</sub></i>
Cefepime	1	4		27	24		Difficult	2	I	<i>bla<sub>OXA-1</sub></i> , <i>bla<sub>CTX-M-15</sub></i>
Cefotaxime	1	2		20	17		Easy	>4	R	<i>bla<sub>CTX-M-15</sub></i>
Ceftazidime	1	4		22	19		Difficult	2	I	<i>bla<sub>CTX-M-15</sub></i>
Ceftriaxone	1	2		25	22		Easy	>16	R	<i>bla<sub>CTX-M-15</sub></i>
Ertapenem	0.5	0.5		23	23		Easy	≤0.03	S	ND
Imipenem	2	4		22	19		Easy	≤0.25	S	ND
Meropenem	2	8		22	16		Easy	≤0.03	S	ND



Ciprofloxacin	0.25	0.5	0.5				Easy	>4	R	<i>aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L</i>
Levofloxacin	0.5	1					Easy	>8	R	<i>aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L</i>
Moxifloxacin	0.25	0.25					Easy	>8	R	<i>aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L</i>
Ofloxacin	0.25	0.5					Easy	>2	R	<i>aac(6')-Ib-cr, gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L</i>
Amikacin	8	8					Difficult	8	S	<i>aac(6')-Ib-cr</i>
Gentamicin	2	2					Easy	1	S	ND
Tobramycin	2	2					Easy	>16	R	<i>aac(6')-Ib-cr</i>
Tigecycline	0.5	0.5					Easy	≤0.25	S	ND
Colistin	2	2		Note ****	Note ****		Easy	≤0.25	S	ND

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

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and ceftiofloxacin in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*ND: Not detected. Additional ARGs or chromosomal PMs: *mph(A), catB3, aadA5, sul1, dfrA17*. MALDI-TOF by DTU: *Escherichia coli* (score 2,26). MLST: ST-131 (scheme *E. coli* #1) / ST-43 (scheme *E. coli* #2).

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

\*\*\*\*\*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 antimicrobial

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

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, 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, CEFTRIAZONE, 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).

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## 2024 EARS-Net 4: *Pseudomonas aeruginosa*

**Table 5. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 4: *Pseudomonas aeruginosa***

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≤	R >	ATU				
Piperacillin	0.001	16	50	18	18-19	Difficult	128	R	ND
Piperacillin-tazobactam *****	0.001	16	50	18	18-19	Difficult	≤16/4	I	ND
Cefepime	0.001	8	50	21		Difficult	8	I	ND
Ceftazidime	0.001	8	50	17		Difficult	>8	R	ND
Imipenem	0.001	4	50	20		Easy	>8	R	<i>oprD</i> W339STOP
Meropenem	2	8	20	14		Difficult	8	I	<i>oprD</i> W339STOP
Ciprofloxacin	0.001	0.5	50	26		Easy	>4	R	<i>crpP, gyrA</i> T83I
Levofloxacin	0.001	2	50	18		Easy	8	R	<i>gyrA</i> T83I
Amikacin	16	16	15	15		Easy	4	S	ND
Tobramycin	2	2	18	18		Easy	0.5	S	ND
Colistin	4	4	Note *****	Note *****		Easy	1	S	ND

\*The level of difficulty reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed



in, nor added to, the latest EUCAST clinical breakpoint table.

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and cefoxitin in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*ND: Not detected. Additional ARGs or chromosomal PMs: *aph(3')-IIB*, *fosA* (intrinsic), *catB7* (intrinsic), *bla<sub>PAO</sub>* (intrinsic), *bla<sub>OXA-488</sub>* (likely intrinsic). MALDI-TOF by DTU: *Pseudomonas aeruginosa* (score 2,45). MLST: ST-395.

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

\*\*\*\*\*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 antimicrobial

Antimicrobial: AMIKACIN, COLISTIN, TOBRAMYCIN

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: CEFEPIME, MEROPENEM, PIPERACILLIN-TAZOBACTAM

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: CIPROFLOXACIN, IMIPENEM, LEVOFLOXACIN

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: CEFTAZIDIME, PIPERACILLIN

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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## 2024 EARS-Net 5: *Klebsiella pneumoniae*

**Table 6. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 5: *Klebsiella pneumoniae***

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)			EUCAST zone diameter breakpoints (mm)			Level of difficulty *	Expected result*	Expected interpretation	ARGs and PMs***
	S ≤	R >	ATU	S ≤	R >	ATU				
Amoxicillin-clavulanic acid****	8	8		19	19	19-20	Easy	>64/2	R	<i>bla<sub>VEB-1</sub></i> , <i>bla<sub>SHV-11</sub></i>
Piperacillin-tazobactam**	8	8	16	20	20	19	Easy	>128/4	R	<i>bla<sub>VEB-1</sub></i> , <i>bla<sub>SHV-11</sub></i> , <i>bla<sub>OXA-10</sub></i>
Cefepime	1	4		27	24		Difficult	2	I	<i>bla<sub>VEB-1</sub></i> , <i>bla<sub>SHV-11</sub></i>
Cefotaxime	1	2		20	17		Difficult	4	R	<i>bla<sub>VEB-1</sub></i> , <i>bla<sub>SHV-11</sub></i>





Ceftazidime	1	4		22	19		Easy	>16	R	<i>bla<sub>VEB-1</sub></i> , <i>bla<sub>SHV-11</sub></i>
Ceftriaxone	1	2		25	22		Easy	8	R	<i>bla<sub>SHV-11</sub></i>
Ertapenem	0.5	0.5		23	23		Easy	2	R	ND
Imipenem	2	4		22	19		Difficult	4	I	ND
Meropenem	2	8		22	16		Difficult	2	S	ND
Ciprofloxacin	0.25	0.5	0.5	25	22	22-24	Easy	0.03	S	ND
Levofloxacin	0.5	1		23	19		Easy	0.06	S	ND
Moxifloxacin	0.25	0.25		22	22		Easy	0.06	S	ND
Ofloxacin	0.25	0.5		24	22		Difficult	≤0.25	S	ND
Amikacin	8	8		18	18		Easy	4	S	<i>aac(6')-Ia</i>
Gentamicin	2	2		17	17		Difficult	4	R	<i>ant(2'')-Ia</i>
Tobramycin	2	2		16	16		Easy	8	R	<i>aac(6')-Ia</i> , <i>ant(2'')-Ia</i>
Colistin	2	2		Note ****	Note ****		Easy	0.5	S	ND

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

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and cefoxitin in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*ND: Not detected. *bla<sub>SHV-11</sub>* was an imperfect match (other identified variants: *bla<sub>SHV-40</sub>*, *bla<sub>SHV-56</sub>*, *bla<sub>SHV-79</sub>*, *bla<sub>SHV-85</sub>*, *bla<sub>SHV-89</sub>*). Additional ARGs or chromosomal PMS: *bla<sub>OXA-436</sub>*, *ARR-2*, *aadA1*, *cml*, *cmlA1*, *sul1*, *OqxA* (intrinsic), *OqxB* (intrinsic), *fosA6* (intrinsic), *fosA7* (intrinsic), *ompK36 N49S*, *ompK36 L59V*, *ompK36 G189T*, *ompK36 F198Y*, *ompK36 F207Y*, *ompK36 A217S*, *ompK36 T222L*, *ompK36 D223G*, *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 2,32), and MLST: ST-37.

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

\*\*\*\*\*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 antimicrobial

Antimicrobial: AMIKACIN, CIPROFLOXACIN, COLISTIN, LEVOFLOXACIN, MOXIFLOXACIN

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: MEROPENEM, OFLOXACIN

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, IMIPENEM

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, CEFTAZIDIME, CEFTRIAXONE, 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: CEFOTAXIME, 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).

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## 2024 EARS-Net 6: *Staphylococcus aureus*

**Table 7. EUCAST clinical breakpoints, expected AST results, level of difficulty in interpretation and expected interpretations for strain 2024 EARS-Net 6: *Staphylococcus aureus***

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EUCAST zone diameter breakpoints (mm)		Level of difficulty *	Expected result**	Expected interpretation	ARGs and PMs***
	S ≤	R >	S ≥	R <				
Oxacillin	Note ****	2	Note ****	Note ****	Easy	8	R	ND
Cefoxitin	Note ****	4	22	22	Difficult	27 mm	S	ND
Ciprofloxacin	0.001	2	50	17	Difficult	1	I	ND
Levofloxacin	0.001	1	50	22	Easy	≤0.5	I	ND
Norfloxacin	NA	NA	17	17	Easy	24 mm	S	ND
Vancomycin	2	2	Note ****	Note ****	Easy	1	S	ND
Linezolid	4	4	21	21	Easy	2	S	ND
Daptomycin	1	1	Note ****	Note ****	Easy	≤0.5	S	ND
Rifampicin	0.06	0.06	26	26	Easy	0.015	S	ND

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

\*\*For most organism-antimicrobial combinations the expected value corresponds to the MIC expressed in 'mg/L'. For norfloxacin and oxacillin in *S. pneumoniae* and for norfloxacin and cefoxitin in *S. aureus* the expected value corresponds to the inhibition zone diameter expressed in 'mm', because the latest EUCAST guidelines and/or EARS-Net Reporting Protocol recommend a disk diffusion test instead of broth microdilution.

\*\*\*ND: Not detected. Additional ARGs or chromosomal PMs: *blaZ. fusA* L461K. MALDI-TOF by DTU: *Staphylococcus*

*aureus* (score 2.26). MLST: ST-188.

\*\*\*\*Please refer to notes in the EUCAST clinical breakpoints tables v14.0.

### Difficulty of AST interpretation for each antimicrobial

Antimicrobial: DAPTOMYCIN. LINEZOLID. NORFLOXACIN. RIFAMPICIN. 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 or the expected zone diameter is at least three millimeters away from the clinical breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: CEFOXITIN

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: LEVOFLOXACIN

Expected interpretation: SUSCEPTIBLE. INCREASED EXPOSURE (I)

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

Expected interpretation: SUSCEPTIBLE. INCREASED EXPOSURE (I)

Difficulty of interpretation: Difficult. The EUCAST clinical breakpoint was recently changed and a two-fold dilution variation would change the interpretation of S/I/R if using the previous breakpoint. A misclassification as resistant (R) would be a major error (ME).

Antimicrobial: OXACILLIN

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).

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### References

- 1) Antimicrobial resistance (AMR) reporting protocol 2024. Available at: <https://www.ecdc.europa.eu/sites/default/files/documents/AMR-reporting-protocol-2024.pdf>