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TECHNICAL REPORT

External quality assessment (EQA) of the performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2021

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Abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
BMD	Broth microdilution
BSI	Bloodstream infection
CDC	US Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
DTU FOOD	Technical University of Denmark, National Food Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EARSS	European Antimicrobial Resistance Surveillance System
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
FAO	Food and Agriculture Organization of the United Nations
I	'Susceptible, increased exposure'
IATA	International Air Transport Association
MIC	Minimum inhibitory concentration
R	Resistant
S	'Susceptible, standard dosing regimen'
std	Standard deviation
uUTI	Uncomplicated urinary tract infection
WHO	World Health Organization

Executive summary

This report describes and summarises the national results of the external quality assessment (EQA) of laboratory performance for those laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2021.

The 2021 EARS-Net EQA exercise focused on antimicrobial susceptibility testing (AST) of *Escherichia coli* and *Klebsiella pneumoniae,* and the report provides a summary of results including a short conclusion on the capacity of participating laboratories and recommendations for improvement. Since 2020, only those laboratories using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical guidelines when performing AST may participate in the EARS-Net EQA exercise.

The 2021 EARS-Net EQA exercise aimed to assess the accuracy of quantitative or qualitative antimicrobial susceptibility test results reported by participating individual laboratories, and evaluate the overall comparability of routinely collected test results among laboratories and EU/EEA countries.

For the 2021 EARS-Net EQA exercise, 909 laboratories from 26 countries were sent an invitation and 642 laboratories signed up for the exercise. On 15 June 2021, three *E. coli* strains and three *K. pneumoniae* strains were distributed to the 642 laboratories from 26 EU/EEA countries via the national EARS-Net EQA coordinators. The webtool for submission of results was open from 24 June 2021 until 15 September 2021. AST results from 591 laboratories were evaluated using the categorisation resistant (R), 'susceptible, increased exposure' (I) and 'susceptible, standard dosing regimen' (S), based on the clinical breakpoints set out in the EUCAST guidelines v11.0. The concordance of results was defined as excellent (≥95% of interpretations in concordance with expected results), very good (>90% to <95%), good (>85 - ≤90%) or satisfactory (80 - ≤85%).

Overall, only 21.8% (129) of the 591 laboratories from the 26 EU/EEA countries participating in the 2021 EARS-Net EQA exercise achieved at least 95% concordance with the expected AST results. The results also indicate that both under- and overestimation of AMR may be possible, and that AMR is heterogeneously reported in EU/EEA, with participants reporting both increased susceptibilities (I → S) and decreased susceptibilities (I → R) for the same antimicrobials. This was especially prevalent for carbapenems, as observed, for example, in the strains EARS-Net 2021 EC.3 and EARS-Net 2021 KPN.2. These observations should be kept in mind when interpreting EARS-Net surveillance data.

To determine the AST results, the most commonly used method was automated systems (54.7%) followed by the disk diffusion or tablet diffusion (28.0%) and MIC methods, including broth microdilution and gradient test (16.8%). Overall, the concordance of results varied between very good, good and satisfactory depending on the method. The lowest concordance was observed for agar dilution (84.4%), followed by gradient tests (87.6%). The remaining methods showed a very good concordance: 90.7% for macro broth dilution (tubes), 92.4% for automated systems, 93.7% for disk diffusion or tablet diffusion and 94.2% for broth microdilution.

For each strain-antimicrobial combination, minor errors are defined as classification of a 'susceptible, increased exposure' (I) strain as 'susceptible, standard dosing regimen' (S), or as resistant (R), or vice versa (i.e. I ↔ S or I ↔ R). A major error is the classification of a 'susceptible, standard dosing regimen' (S) strain as resistant (R) (i.e. S → R), and a very major error is the classification of a resistant strain as a 'susceptible, standard dosing regimen' (i.e. R → S).

The **EARS-Net 2021 EC.1** (*E. coli*) strain was resistant to amoxicillin, ampicillin, gentamicin, moxifloxacin, ofloxacin, tigecycline, and tobramycin. Expected MIC values for ciprofloxacin and levofloxacin were in the 'susceptible, increased exposure' (I) range.

In general, the interpretations reported for the strain were in good concordance with those expected. Furthermore, 12.5% (74/590) of laboratories had excellent concordance (with 95% or more correct results), and 67 of these laboratories reported fully concordant results. Overall, all methodologies achieved, as a minimum, a good level of concordance with the expected results.

However, the discordant results revealed that detection of resistant or 'susceptible, increased exposure' profiles towards fluoroquinolones was problematic. While some of the deviations can be attributed to the inherent method variability and are within the acceptable range of variation, they can also be derived from the presence of one point mutation in the *gyrA* gene. This single point mutation confers borderline MIC values and inhibition zone diameters to fluoroquinolones, which can easily be misread or misinterpreted. Most of the deviations were very major errors (R → S) or minor errors (I → S), suggesting that decreased susceptibility to fluoroquinolones can potentially be under-reported in the EU/EEA. Furthermore, the expected MIC value of ciprofloxacin in this strain fell within an area of technical uncertainty (ATU), and results suggest that laboratories should become familiar with the appropriate procedures for reporting such results, as recommended by EUCAST.

Detection of resistant phenotypes was also problematic for tigecycline (R → S). The strain probably harbours genes conferring tigecycline resistance that are currently unknown and may potentially have contributed to the variability of results for this antimicrobial.

The strain was 'susceptible, standard dosing regimen' to amoxicillin-clavulanic acid. However, results presented a high percentage of major errors (S → R), that can be attributed to the inherent method variability, potentially derived from the fact that the expected MIC value corresponds to a borderline concentration, increasing the probability of misclassification.

The **EARS-Net 2021 EC.2** (*E. coli*) strain was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem and piperacillin-tazobactam.

In general, interpretations reported for the strain were in excellent concordance with the expected results. A total of 47.0% (278/591) of laboratories had excellent concordance, and 264 of these laboratories reported fully concordant results. Overall, all methodologies except agar dilution (79.5%) achieved, as a minimum, a very good level of concordance with the expected results.

Nevertheless, discordant results revealed that susceptibility to meropenem and imipenem proved difficult to detect. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, they may also be derived from the differential expression of the *bla*$_{\text{OXA-244}}$ gene harboured by the strain, which can confer low levels of carbapenem resistance that are difficult to detect. Most of the deviations were minor errors (S → I), suggesting that decreased susceptibility to carbapenems may potentially be over-reported in EU/EEA.

Conversely, detection of resistant phenotypes was problematic for ceftazidime (R → I), which presented a borderline expected MIC value, increasing the probability of misclassification.

The **EARS-Net 2021 EC.3** (*E. coli*) strain was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem, imipenem, piperacillin-tazobactam and tobramycin. The expected MIC value for meropenem was in the 'susceptible, increased exposure' (I) range.

In general, interpretations reported for the strain were in good concordance with the expected results. Furthermore, 17.6% (104/591) of laboratories had excellent concordance, and 96 of these laboratories reported fully concordant results. Neither agar dilution nor gradient test achieved a satisfactory level of concordance with the expected results.

In addition, the discordant results showed that detection of the decreased susceptibility to carbapenems was problematic. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, they may also be derived from the differential expression of the *bla*$_{\text{VIM-1}}$ gene harboured by the strain. The deviations included minor errors (R → I and I → S) and very major errors (R → S), suggesting that there is a potential to observe under-reporting of carbapenem resistance in the EU/EEA. Laboratories under-reported resistant and 'susceptible, increased exposure' profiles in 24.6% of all results submitted for the three carbapenems (378/1 538). However, the number of minor errors (I → R) leads us to conclude the opposite - that laboratories classified meropenem as resistant instead of 'susceptible, increased exposure' in 16.3% of all results submitted for the carbapenems (250/1 538), equivalent to 43.9% of the results submitted for meropenem.

Interpretation of results was also problematic for gentamicin (S → R), which presented a borderline expected MIC value, increasing the probability of misclassification.

The **EARS-Net 2021 KPN.1** (*K. pneumoniae*) strain was resistant to amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin.

In general, there was excellent concordance of the reported interpretations with the expected results. A total of 90.5% (534/590) of laboratories reported fully concordant results. Excellent levels of concordance were observed for all methods except for the gradient test.

The **EARS-Net 2021 KPN.2** (*K. pneumoniae*) strain was resistant to amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem and piperacillin-tazobactam. The expected MIC value for imipenem was in the 'susceptible, increased exposure' (I) range.

In general, interpretations reported for the strain were in good concordance with the expected results. Furthermore, 13.7% (81/590) of laboratories had excellent concordance, and all these laboratories reported fully concordant results. Overall, all methodologies except macro broth dilution (tubes) achieved, as a minimum, a satisfactory level of concordance with the expected results.

The discordant results revealed that the detection of resistance to cefepime was unsatisfactory, with a large percentage of minor errors (R → I). Minor errors were also common in imipenem results, for which the strain presented an expected 'susceptible, increased exposure' profile. Most laboratories (41.1%) reported a 'susceptible, standard dosing regimen' profile (I → S), while others reported resistance (I → R) (30.8%). Furthermore, deviations in meropenem results were also observed, and mainly due to minor errors (S → I). These results imply that detection of carbapenem resistance in *K. pneumoniae* strains is complex and not properly harmonised throughout EU/EEA settings. While some deviations can be attributed to the inherent method variability and are in the acceptable variation range, they might also be derived from the differential expression of the *bla*$_{\text{CMY-2}}$ gene harboured by the strain. It has additionally been observed that, in some cases, the *bla*$_{\text{CMY-2}}$ gene can be

accompanied by reduced outer membrane permeability, mediated by decreased porin expression, which can increase the difficulty of correct AST determination.

The **EARS-Net 2021 KPN.3** (*K. pneumoniae*) strain was resistant to amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, levofloxacin, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin.

In general, the interpretations reported were in excellent concordance with the expected results. A total of 57.0% (335/588) of laboratories had excellent concordance, and all these laboratories reported fully concordant results. Overall, all methodologies achieved, as a minimum, a good level of concordance with the expected results.

The discordant results revealed that the detection of the susceptible, standard dosing regimen to meropenem, did not achieve a satisfactory concordance, and there were a large percentage of minor errors (S → I). The expected MIC result for meropenem was a borderline concentration, increasing the probability of misclassification. The same type of errors were also observed for imipenem results, but only when using the automated systems.

An analysis of how the different AST methods performed revealed few differences between methodologies, except for the fact that the gradient test performed less well in the detection of reduced susceptibility towards carbapenems.

Overall, AST of carbapenems was the most problematic issue detected in the 2021 EARS-Net EQA exercise, especially for imipenem and meropenem, and in both bacterial species. Although under-reporting of decreased susceptibility profiles was frequently observed, the dominant problem was the over-reporting of these profiles. In past EARS-Net EQA exercises carbapenem susceptibility testing was identified as a problem, and this issue currently remains unsolved. However, laboratories in the EU/EEA have successfully addressed some of the other issues previously identified and have now achieved excellent concordance with expected phenotypes for piperacillin-tazobactam in *E. coli* strains, and amikacin and third-generation cephalosporins in *K. pneumoniae* strains.

In addition to the deviations seen for carbapenems in the 2021 EARS-Net EQA exercise, over-reporting of decreased susceptibility profiles was also observed for amoxicillin-clavulanic acid and gentamicin in individual *E. coli* strains. Conversely, there was under-reporting of decreased susceptibilities for fluoroquinolones, ceftazidime and tigecycline in individual *E.* coli strains, as well as for cefepime in one *K. pneumoniae* strain.

Of all discordant results detected in this EARS-Net EQA exercise (n=3 662), 52.4% (n=1 918) involved the under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 47.6% (n=1 744) involved over-reporting (S → I, S → R, I → R). In conclusion, there is no exclusive pattern showing the over- or under-reporting of decreased susceptibility profiles in the EU/EEA, and surveillance or control efforts should consider the specific deviations observed for each specific antimicrobial or antimicrobial class.

Laboratories that participate in the EARS-Net surveillance scheme should review their individual performance in this EQA exercise and revisit all areas where they did not achieve the intended results. It would be advisable for several laboratories to review their methodologies relating to the performing, reading and interpreting of AST results for the antimicrobial classes of fluoroquinolones and carbapenems. Moreover, laboratories should confirm that the protocols in use are in accordance with the latest EUCAST recommendations and guidelines, and that the most current breakpoints are applied.

1. Introduction

Since 2010, the European Antimicrobial Resistance System Network (EARS-Net) has organised annual external quality assessment (EQA) exercises for antimicrobial susceptibility testing (AST). From 2000 to 2009, a similar EQA exercise for AST was organised by UK NEQAS and delivered to the European Antimicrobial Resistance Surveillance System (EARSS) which was transferred to the European Centre for Disease Prevention and Control (ECDC) as EARS-Net. In 2021, the EARS-Net EQA exercise was carried out in collaboration with the National Food Institute at the Technical University of Denmark, (DTU FOOD). This report describes and summarises the results of the EQA performance of laboratories participating in EARS-Net in 2021.

The 2021 EARS-Net EQA exercise aimed to assess the accuracy of quantitative or qualitative antimicrobial susceptibility test results reported by participating individual laboratories, and evaluate the overall comparability of routinely collected test results between laboratories and European Union/European Economic Area (EU/EEA) countries.

The 2021 EARS-Net EQA exercise focused on AST of *Escherichia coli* and *Klebsiella pneumoniae* strains.

In 2021, 29 countries were invited to participate in the EARS-Net EQA exercise; however, due to the ongoing COVID-19 pandemic, three countries did not have the resources to participate in this exercise. Therefore, invitations were sent out to 909 laboratories in 26 countries, and 642 laboratories signed up and received the six strains for analysis. Data for evaluation was then submitted by 592 laboratories (Annex 1).

2. Study design and methods

Strains and antimicrobial susceptibility testing

The strains used for the EQA exercise were compatible with the epidemiology of the resistance phenotypes of species under surveillance at ECDC within EARS-Net. Based on their antimicrobial resistance profiles a panel of three *E. coli* and three *K. pneumoniae* strains were selected for this EQA exercise from the strain collection at DTU FOOD. Expected AST results were generated by performing minimum inhibitory concentration (MIC) determinations through broth microdilution (BMD) for all test strains in duplicate at DTU FOOD. The AST profiles were validated by two reference laboratories: The US Centers for Disease Control and Prevention (CDC), Atlanta (Georgia), US, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory, Växjö, Sweden. Expected MIC values for each antimicrobial-strain combination were determined by the consensus BMD results obtained by DTU FOOD and EUCAST and are presented in Table 1 and Table 2. Subsequently, the results were genotypically compared to acquired resistance genes and chromosomal point mutations by whole genome sequencing and using the bioinformatics tool ResFinder v4.1 (Table 3 and Table 4). Finally, MIC determination was performed at DTU FOOD after preparation of the agar stab culture/charcoal swab for shipment to participants, in order to confirm that the vials contained the correct strains with the expected MIC values.

The antimicrobial agents selected for this EQA exercise corresponded to the panel of pathogen and antimicrobial agent combinations under surveillance by EARS-Net presented in the AMR reporting protocol 2020 [1], except for netilmicin which was not included in the 2021 EARS-Net EQA exercise.

Participating laboratories should perform quantitative or qualitative AST according to the laboratory's routine procedures - i.e. automated systems, broth microdilution, agar dilution, disk diffusion or tablet diffusion, gradient diffusion, or others in accordance with EUCAST recommendations[1].

The EUCAST clinical breakpoints v11.0 were applied to interpret the AST results obtained[2] (Table 1 and Table 2). This enabled the test results to be placed into three categories: resistant (R), 'susceptible, increased exposure' (I), and 'susceptible, standard dosing regimen' (S).

[1] EUCAST recommendations: https://www.eucast.org/ast_of_bacteria/

[2] EUCAST clinical breakpoints: https://www.eucast.org/clinical_breakpoints/

Table 1. EUCAST clinical breakpoints, expected MIC value and interpretation for the three *Escherichia coli* strains included in the 2021 EARS-Net EQA exercise

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EARS-Net 2021 EC.1			EARS-Net 2021 EC.2			EARS-Net 2021 EC.3		
	S ≤	R >	Expected MIC value (mg/L)		Expected interpretation	Expected MIC value (mg/L)		Expected interpretation	Expected MIC value (mg/L)		Expected interpretation
Amikacin*	8	8	=	2	S	=	2	S	=	1	S
Amoxicillin	8	8	>	32	R	>	32	R	>	32	R
Amoxicillin-clavulanic acid**	8	8	=	8	S	>	128	R	>	128	R
Ampicillin	8	8	>	32	R	>	32	R	>	32	R
Cefepime	1	4	≤	0.06	S	>	32	R	>	32	R
Cefotaxime	1	2	=	0.06	S	>	64	R	>	64	R
Ceftazidime	1	4	=	0.25	S	=	8	R	>	128	R
Ceftriaxone	1	2	=	0.06	S	>	4	R	>	4	R
Ciprofloxacin	0.25	0.5	=	0.5	I	=	0.03	S	=	0.03	S
Colistin	2	2	≤	0.25	S	≤	0.25	S	≤	0.25	S
Ertapenem	0.5	0.5	=	0.008	S	=	4	R	=	1	R
Gentamicin*	2	2	>	16	R	=	1	S	=	2	S
Imipenem	2	4	=	0.12	S	=	1	S	=	8	R
Levofloxacin	0.5	1	=	1	I	=	0.06	S	≤	0.03	S
Meropenem	2	8	≤	0.015	S	=	0.5	S	=	4	I
Moxifloxacin	0.25	0.25	=	1	R	=	0.06	S	=	0.03	S
Norfloxacin***	0.5	0.5		ND	-		ND	-		ND	-
Ofloxacin	0.25	0.5	=	1	R	≤	0.12	S	≤	0.12	S
Piperacillin-tazobactam**	8	8	=	2	S	>	64	R	>	64	R
Tigecycline	0.5	0.5	=	2	R	=	0.12	S	=	0.12	S
Tobramycin*	2	2	=	8	R	=	0.5	S	=	4	R

** EUCAST clinical breakpoints v11.0 note that the bracketed breakpoints 'can be used to distinguish between organisms with and without acquired resistance mechanisms'. Moreover, aminoglycosides must be used in combination with other active therapy.*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
**** EUCAST clinical breakpoints v11.0 are only available for uncomplicated urinary tract infections (uUTI), and all six strains included in this EQA exercise correspond to blood stream infection (BSI) isolates. Thus, available uUTI breakpoints should not be applied.*

Table 2. EUCAST clinical breakpoints, expected MIC value and interpretation for the three *Klebsiella pneumoniae* strains included in the 2021 EARS-Net EQA exercise

Antimicrobial	EUCAST clinical breakpoints MIC (mg/L)		EARS-Net 2021 KPN.1			EARS-Net 2021 KPN.2			EARS-Net 2021 KPN.3		
	S ≤	R >	Expected MIC value (mg/L)		Expected interpretation	Expected MIC value (mg/L)		Expected interpretation	Expected MIC value (mg/L)		Expected interpretation
Amikacin*	8	8	>	128	R	=	1	S	>	128	R
Amoxicillin-clavulanic acid**	8	8	>	128	R	>	128	R	>	128	R
Cefepime	1	4	>	32	R	=	8	R	>	32	R
Cefotaxime	1	2	>	64	R	>	64	R	>	64	R
Ceftazidime	1	4	=	128	R	=	64	R	>	128	R
Ceftriaxone	1	2	>	4	R	>	4	R	>	4	R
Ciprofloxacin	0.25	0.5	>	8	R	=	0.06	S	>	8	R
Colistin	2	2	>	32	R	≤	0.25	S	≤	0.25	S
Ertapenem	0.5	0.5	>	8	R	=	4	R	>	4	R
Gentamicin*	2	2	>	16	R	≤	0.25	S	>	16	R
Imipenem	2	4	=	16	R	=	4	I	=	0.25	S
Levofloxacin	0.5	1	>	8	R	=	0.12	S	>	8	R
Meropenem	2	8	>	16	R	=	1	S	=	2	S
Moxifloxacin	0.25	0.25	>	8	R	=	0.06	S	>	8	R
Norfloxacin***	0.5	0.5		ND	-		ND	-		ND	-
Ofloxacin	0.25	0.5	>	4	R	=	0.25	S	>	4	R
Piperacillin-tazobactam**	8	8	>	64	R	>	64	R	>	64	R
Tobramycin*	2	2	>	16	R	=	0.5	S	>	16	R

** EUCAST clinical breakpoints v11.0 note that the bracketed breakpoints 'can be used to distinguish between organisms with and without acquired resistance mechanisms'. Moreover, aminoglycosides must be used in combination with other active therapy.*
*** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*
**** EUCAST clinical breakpoints v11.0 are only available for uUTI, and all six strains included in this EQA exercise correspond to BSI isolates. Thus, available uUTI breakpoints should not be applied.*

Table 3. Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 of the three *Escherichia coli* strains included in the 2021 EARS-Net EQA exercise

Antimicrobial	EARS-Net 2021 EC.1*	EARS-Net 2021 EC.2**	EARS-Net 2021 EC.3***
Amikacin			
Amoxicillin	*bla*$_{TEM-1}$	*bla*$_{TEM-1}$	*bla*$_{VIM-1}$
Amoxicillin-clavulanic acid fixed concentration		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Ampicillin	*bla*$_{TEM-1}$	*bla*$_{TEM-1}$	*bla*$_{VIM-1}$
Cefepime		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Cefotaxime		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Ceftazidime		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Ceftriaxone		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Ciprofloxacin	*gyrA* S83L		
Colistin			
Ertapenem		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Gentamicin	*aac(3)-IId*		
Imipenem			*bla*$_{VIM-1}$
Levofloxacin	*gyrA* S83L		
Meropenem			*bla*$_{VIM-1}$
Moxifloxacin	*gyrA* S83L		
Norfloxacin	*gyrA* S83L		
Ofloxacin	*gyrA* S83L		
Piperacillin-tazobactam		*bla*$_{OXA-244}$, *bla*$_{CTX-M-14b}$	*bla*$_{VIM-1}$
Tigecycline	ND		
Tobramycin	*aac(3)-IId*		*aac(6')-Ib-cr*

ND= Not detected
** Additional resistance genes detected: sul2, dfrA5, tet(A), aph(6)-Id, aph(3'')-Ib*
*** Additional resistance genes detected: catA1, sul2, drfA1, tet(D), aph(3'')-Ib, aph(6)-Id, addA1*
**** Additional resistance genes detected: aadA1, tet(39).*

Table 4. Antimicrobial resistance genes and chromosomal point mutations detected through analysis with ResFinder 4.1 of the three *Klebsiella pneumoniae* strains included in the 2021 EARS-Net EQA exercise

Antimicrobial	EARS-Net 2021 KPN.1*	EARS-Net 2021 KPN.2**	EARS-Net 2021 KPN.3***
Amikacin	*rmtB*, *aac(6')-Ib-cr*		*armA*, *aac(6')-Ib-cr*
Amoxicillin-clavulanic acid fixed concentration	*bla*$_{OXA-181}$/*bla*$_{OXA-1}$, *bla*$_{NDM-5}$, *bla*$_{SHV-26}$	*bla*$_{SHV-110}$, *bla*$_{CMY-2}$	*bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefepime	*bla*$_{OXA-181}$/*bla*$_{OXA-1}$, *bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$	*bla*$_{CMY-2}$	*bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Cefotaxime	*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$	*bla*$_{CMY-2}$	*bla*$_{CTX-M-15}$
Ceftazidime	*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$	*bla*$_{CMY-2}$	*bla*$_{CTX-M-15}$
Ceftriaxone	*bla*$_{CTX-M-15}$	*bla*$_{CMY-2}$	*bla*$_{CTX-M-15}$
Ciprofloxacin	*aac(6')-Ib-cr*, *qnrS1*, *gyrA* S83F, *gyrA* D87N, *parC* E84K		*aac(6')-Ib-cr*, *qnrB1*, *gyrA* D87A, *parC* S80I
Colistin	ND		
Ertapenem	*bla*$_{OXA-181}$, *bla*$_{NDM-5}$	*bla*$_{CMY-2}$	*bla*$_{CTX-M-15}$
Gentamicin	*rmtB*		*armA*
Imipenem	*bla*$_{OXA-181}$, *bla*$_{NDM-5}$	*bla*$_{CMY-2}$	
Levofloxacin	*aac(6')-Ib-cr*, *qnrS1*, *gyrA* S83F, *gyrA* D87N, *parC* E84K		*aac(6')-Ib-cr*, *qnrB1*, *gyrA* D87A, *parC* S80I
Meropenem	*bla*$_{OXA-181}$, *bla*$_{NDM-5}$		
Moxifloxacin	*aac(6')-Ib-cr*, *qnrS1*, *gyrA* S83F, *gyrA* D87N, *parC* E84K		*aac(6')-Ib-cr*, *qnrB1*, *gyrA* D87A, *parC* S80I
Norfloxacin	*aac(6')-Ib-cr*, *qnrS1*, *gyrA* S83F, *gyrA* D87N, *parC* E84K		*aac(6')-Ib-cr*, *qnrB1*, *gyrA* D87A, *parC* S80I
Ofloxacin	*aac(6')-Ib-cr*, *qnrS1*, *gyrA* S83F, *gyrA* D87N, *parC* E84K		*aac(6')-Ib-cr*, *qnrB1*, *gyrA* D87A, *parC* S80I
Piperacillin–tazobactam	*bla*$_{OXA-181}$/*bla*$_{OXA-1}$, *bla*$_{NDM-5}$, *bla*$_{SHV-26}$	*bla*$_{SHV-110}$, *bla*$_{CMY-2}$	*bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *bla*$_{OXA-1}$, *bla*$_{CTX-M-15}$
Tobramycin	*rmtB*, *aac(6')-Ib-cr*		*armA*, *aac(6')-Ib-cr*

ND= Not detected.
** Additional resistance genes detected: erm(B), bla*$_{TEM-1B}$*, sul1, oqxA, oqxB, dfrA12, mph(A), qacE, aadA2, tet(A), fosA5, catB3, aph(3')-Ia*
*** Additional resistance genes detected: fosA, cmlA1, catA2, aadA2, sul1, sul2, dfrA15, oqxA, oqxB, qacE*
**** Additional resistance genes detected: aadA1, fosA, oqxA, oqxB, qacE, sul1, sul2, arr-2, cmlA1, catB3, aph(6)- Id, aph(3'')- Ib, ere(A), mphE, msrE, erm(B), mph(A).*

Norfloxacin

The interpretations obtained for norfloxacin were not scored. EUCAST clinical breakpoints v11.0 are only available for uncomplicated urinary tract infections (uUTI), and all six strains included in this EQA exercise correspond to blood stream infection (BSI) isolates. Thus, available uUTI breakpoints should not be applied.

The strains EARS-Net 2021 EC.2, EARS-Net 2021 EC.3 and EARS-Net 2021 KPN.2 presented 'susceptible, standard dosing regimen' profiles towards the other four fluoroquinolones included in this EQA exercise. Therefore, reporting susceptibility to norfloxacin could be considered technically correct. However, since the breakpoint is only valid for uUTI, reporting any interpretation of norfloxacin results is considered incorrect in the context of this EQA exercise which should follow EUCAST guidelines.

The strain EARS-Net 2021 EC.1 presented mixed phenotypes towards fluoroquinolones ('susceptible, increased exposure' or resistant), justified by the presence of one single chromosomal point mutation in the gyrase gene. Reporting the strain as resistant towards norfloxacin could be technically correct, but not valid in the context of this EQA exercise, and potentially not representative of the true phenotype of the strain if uUTI breakpoints were to be applied.

The EARS-Net 2021 KPN.1 and EARS-Net 2021 KPN.3 strains presented clear phenotypical resistance towards fluoroquinolones, which was corroborated by the detection of genetic determinants of resistance. Therefore, reporting them as resistant to norfloxacin could be considered technically correct. However, norfloxacin clinical breakpoints for BSI isolates do not exist and therefore no interpretation should have been reported in the context of this EQA exercise.

Distribution

ECDC provided a list of operational contact points for antimicrobial-resistant pathogens and diseases caused by antimicrobial-resistant microorganisms. Each country appointed a National EARS-Net EQA Coordinator. The National EARS-Net EQA Coordinators were asked to provide a list of possible participating laboratories and all laboratories were invited to sign up to participate in the EQA exercise using a link included in the invitation email. The databases with contact information on the National EARS-Net EQA Coordinators and the participating laboratories were shared with ECDC.

On 15 June 2021, a shipment containing one package for each of the laboratories signed up to participate in the 2021 EARS-Net EQA exercise was sent to the National EARS-Net EQA Coordinator for onward distribution in the country. The National EARS-Net EQA Coordinators were contacted by email with a reminder about imminent specimen dispatch and with a request to confirm the date of receipt by email.

Each package (double pack containers (class UN 6.2)) contained six swabs (Amies agar gel with charcoal; Copan TransystemTM) each containing a pure culture of one of the six strains: three cultures of *E. coli* and three cultures of *K. pneumoniae,* together with a cover letter containing safety instructions and information on how to handle the swabs upon arrival. The shipment (UN3373, biological substances category B) was sent in accordance with International Air Transport Association (IATA) regulations.

Procedure

To submit the EARS-Net EQA data for evaluation of results, a dedicated, password-protected EARS-Net EQA web page was developed and hosted by the Technical University of Denmark. All participating laboratories were invited to enter the results obtained on the web page using a personal login and password provided by email to each contact from the laboratories. The participants were asked to report AST results (i.e. MIC values and their categorisation as resistant (R), 'susceptible, increased exposure' (I), and 'susceptible, standard dosing regimen' (S)), based on the clinical breakpoints set out in EUCAST guidelines. They were also asked to provide information about the standard guideline used. Furthermore, information was collected from participants on the methodology used to undertake AST (automated system, disk diffusion or tablet diffusion, gradient test, MIC, or other), and whether they would send a strain to a reference laboratory for further testing. Reporting of MIC results included BMD and gradient diffusion.

The 2021 EARS-Net EQA protocol, test forms, guidelines and a video tutorial on how to access the password-protected web page were available on the EARS-Net EQA website: https://antimicrobialresistance.dk/ears-net-eqa.aspx

The deadline for submission of results was nine weeks after dispatch of the packages, however due to the COVID-19 pandemic, the submission period was extended for three weeks until 15 September 2021. After submission of results, an email was automatically forwarded to all contacts from the respective laboratory with a report containing the submitted results.

The categorisation R, I, and S was evaluated using a score algorithm which marked a correct interpretation as 'correct' and an incorrect interpretation as 'incorrect', with the MIC values used as supplementary information. Results were considered 'correct' if the reported interpretation was in concordance with the reference laboratories' interpretation.

Only laboratories using the EUCAST guidelines received a laboratory evaluation report and were included in the analysis for the national summary reports and the 2021 EARS-Net EQA Annual Report. The laboratory evaluation reports were released on the password-protected web page. The contacts for each laboratory were notified via email when the report was available for download from the web page using the personal login and password provided. Contacts only had access to the evaluation report from their own laboratory.

The individual laboratory evaluation reports from each country were also shared with the National EARS-Net EQA Coordinators, together with a detailed, country-specific national summary of the performance of the laboratories in the respective country. The national summary reports included an overview of reported results, discussion, and recommendations for improvements, where relevant. Participating laboratories were identified by codes which were known to the corresponding laboratory, the National EARS-Net EQA Coordinator and the EQA provider. A national database with all the reported results and a list connecting the anonymised laboratory ID numbers with the corresponding laboratory was attached to the national report as an appendix. ECDC received the national anonymised summary reports as well as a database containing all submitted results.

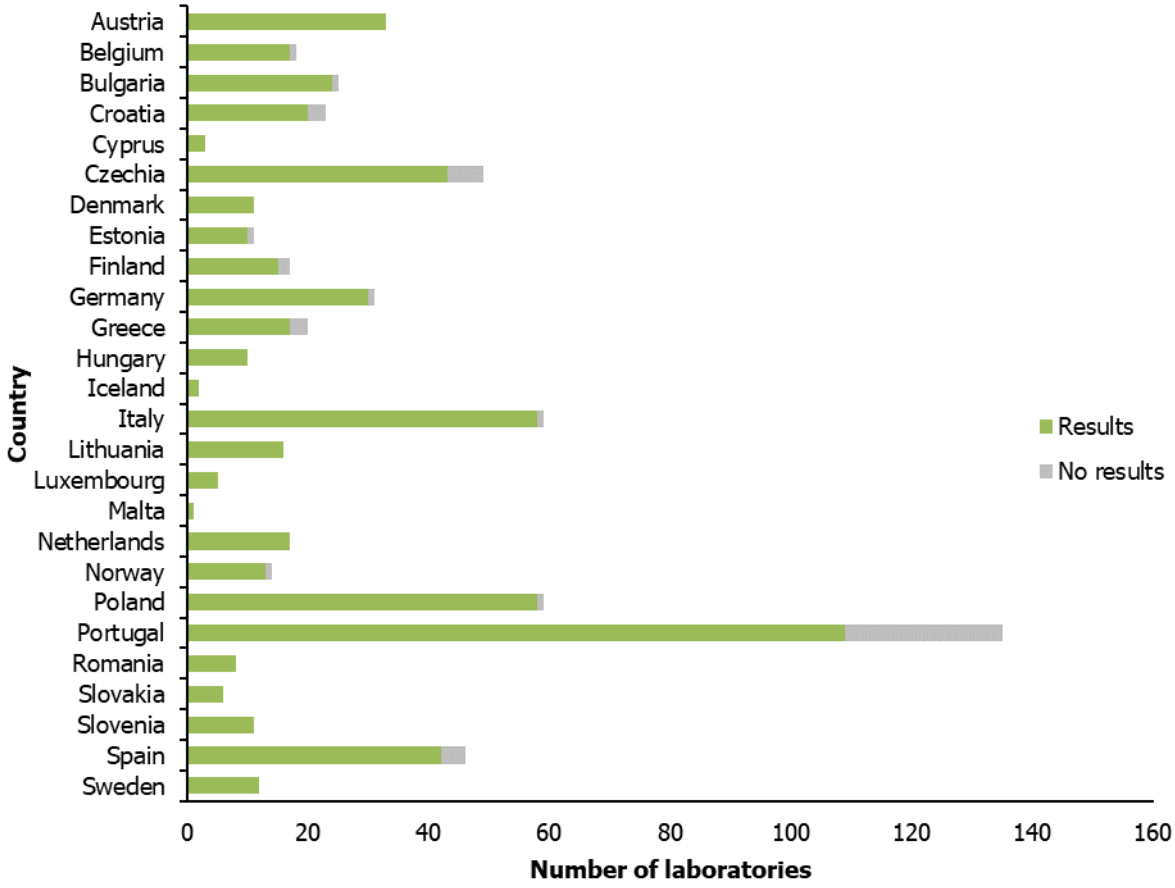
Certificates of participation were shared with the National EARS-Net EQA Coordinators, who were asked to further distribute them.

Participants were also encouraged to complete an electronic evaluation survey using a link sent via email with the aim of improving future EQA exercises. The evaluation questions were provided by ECDC (Annex 2).

3. Results

Six bacterial strains were distributed to 642 laboratories from 26 EU/EEA countries, and 592 (92.2%) laboratories, representing all 26 countries, submitted data for evaluation (Figure 1). Since 2019, only laboratories using the EUCAST guidelines to perform AST can participate in the EARS-Net EQA exercise. One laboratory reported results using the Clinical and Laboratory Standards Institute (CLSI) guidelines and data from this laboratory were therefore not included in the evaluation. Two Norwegian laboratories reported using the NordicAST guidelines, which are based on the EUCAST guidelines, and were therefore included in the analysis. Consequently, results were evaluated for a total of 591 laboratories (92.1% of all laboratories that received the EQA strains).

Figure 1. Number of participating laboratories returning external quality assessment results based on the EUCAST guidelines, by country, 2021

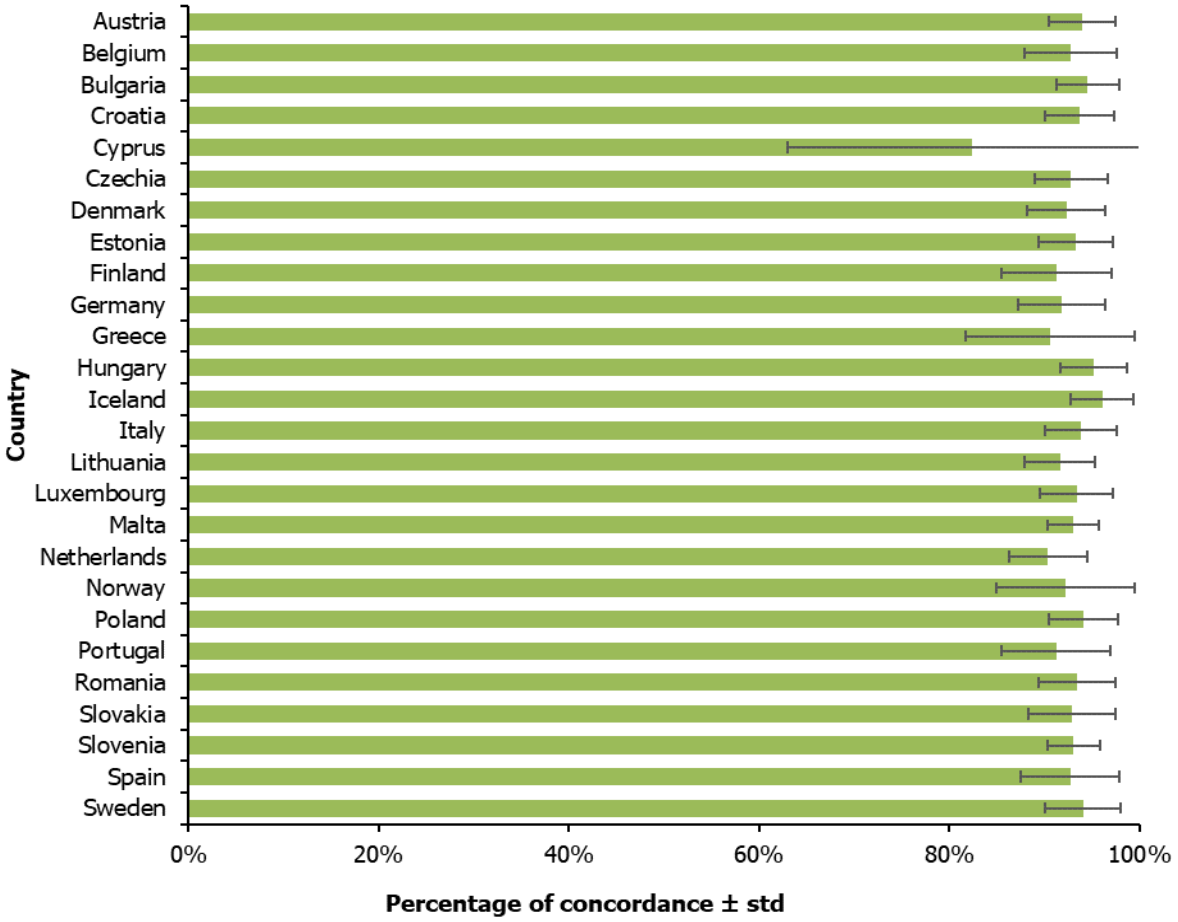


The following countries did not participate in the 2021 EARS-Net EQA: France, Ireland, and Latvia.

The concordance of results was defined as excellent (≥95% of interpretations in concordance with expected results), very good (>90% to <95%), good (>85 - ≤90%) or satisfactory (80 - ≤85%). Overall, the AST interpretations were in very good concordance, with a total of 92.7% correct interpretations (n=46 640) of the 50 302 AST results.

Of the 591 laboratories, 21.8% (n=129) achieved an excellent level of concordance for the reported interpretations compared to the expected interpretations (≥95%). Figure illustrates the mean concordance ± standard deviation (std) of the reported AST interpretations with the expected results, for all six strains, for each of the 26 participating EU/EEA countries.

Figure 2. Mean concordance ± std (%) of the reported AST interpretations with the expected results for all six strains, by participating EU/EEA country, 2021 EARS-Net EQA exercise



For determination of the AST results, the most commonly used method was automated systems (54.7%), followed by disk diffusion or tablet diffusion (28.0%) and MIC–Broth microdilution (11.4%) (Table 5). The distribution of methods was similar for all six strains.

Overall, the concordance of results with the expected interpretations, depending on the method used, varied between very good, good, and satisfactory. The lowest concordance was observed for agar dilution (84.4%), followed by gradient test (87.6%). The remaining methods presented a very good concordance: 90.7% concordance for macro broth dilution (tubes), 92.4% for automated systems, 93.7% for disk diffusion or tablet diffusion and 94.2% for broth microdilution. The option 'Other methods' achieved 93.8% concordance. The detailed results for each strain/antimicrobial combination are presented in Table 6, Table 9, Table 11, Table 13, Table 14 and Table 16.

Table 5. Overview of methods used to determine AST results by strain, 2021 EARS-Net EQA exercise

	EARS-Net 2021 EC.1		EARS-Net 2021 EC.2		EARS-Net 2021 EC.3		EARS-Net 2021 KPN.1		EARS-Net 2021 KPN.2		EARS-Net 2021 KPN.3		Total	
Method	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	4 891	55.2	4 875	54.4	4 864	54.4	4 328	54.9	4 276	54.7	4 282	54.7	**27 516**	**54.7**
Disk/tablet diffusion	2 516	28.4	2 548	28.4	2 540	28.4	2 164	27.4	2 160	27.6	2 165	27.7	**14 093**	**28.0**
Gradient test	425	4.8	435	4.9	438	4.9	390	4.9	388	5.0	386	4.9	**2 462**	**4.9**
Agar dilution	36	0.4	39	0.4	39	0.4	34	0.4	37	0.5	33	0.4	**218**	**0.4**
Broth microdilution	948	10.7	1 016	11.3	1 019	11.4	916	11.6	900	11.5	912	11.6	**5 711**	**11.4**
Macro broth dilution (tubes)	8	0.1	9	0.1	8	0.1	17	0.2	17	0.2	16	0.2	**75**	**0.1**
Other	41	0.5	41	0.5	41	0.5	35	0.4	34	0.4	35	0.4	**227**	**0.5**
Total	**8 865**	**100.0**	**8 963**	**100.0**	**8 949**	**100.0**	**7 884**	**100.0**	**7 812**	**100.0**	**7 829**	**100.0**	**50 302**	**100.0**

Percentage may not total 100% due to rounding.

For each strain/antimicrobial combination the minor, major and very major errors are presented. In this EQA exercise, minor errors are defined as classification of a 'susceptible, increased exposure' (I) strain as 'susceptible, standard dosing regimen' (S), resistant (R), or vice versa (i.e. I ↔ S or I ↔ R). A major error is the classification of a 'susceptible, standard dosing regimen' strain as resistant (i.e. S → R). A very major error is the classification of a resistant strain as 'susceptible, standard dosing regimen' (i.e. R → S).

EARS-Net 2021 EC.1: *Escherichia coli*

The EARS-Net 2021 EC.1 strain was resistant to amoxicillin, ampicillin, gentamicin, moxifloxacin, ofloxacin, tigecycline, and tobramycin (Table 1 and Table 3). Furthermore, expected MIC values for ciprofloxacin and levofloxacin were in the 'susceptible, increased exposure' (I) range.

In total, 8 865 tests were performed, and 7 799 reported interpretations were correct. Thus, the reported interpretations were in good concordance with expected results (88.0%).

Results for the *E. coli* strain EARS-Net 2021 EC.1 were submitted by 590 laboratories. In total, 7.6% of the laboratories (n=45) would have sent the strain to a reference or other laboratory for further testing. In total, 12.5% of laboratories (n=74) had excellent concordance with more than 95% correct results, and for 67 of these laboratories, the results were in full concordance. Furthermore, 30.2% of laboratories (n=178) had very good concordance, 23.4% (n=138) had good concordance, 24.4% (n=144) had satisfactory concordance and 9.5% of the laboratories (n=56) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (55.2%), disk or tablet diffusion (28.4%), broth microdilution (10.7%), gradient test (4.8%), agar dilution (0.4%), macro broth dilution (tubes) (0.1%) and other methods (0.5%) (Table 5). Overall, all methodologies achieved, as a minimum, a good level of concordance with the expected results (Table 6).

Most deviations were observed for fluoroquinolones (Table 6). Ciprofloxacin and levofloxacin deviations were reported across all methods and corresponded to minor errors (Table 7 and Figure 3), with the majority being due to a misclassification of the strain as 'S' instead of the expected 'I'. Specifically, 434 (74.7%) laboratories reported the strain as 'S' to ciprofloxacin and 197 (56.8%) laboratories reported it as 'S' to levofloxacin. Moxifloxacin deviations mainly represented very major errors, (Table 8 and Figure 3) with 38 (22.1%) laboratories submitting an interpretation of 'S'. Deviations in ofloxacin results were equally distributed between minor errors and very major errors (Table 8 and Figure 3), although the overall concordance for this antimicrobial achieved a good level (>85 - ≤90%) (Table 6).

Very major errors were also prevalent in tigecycline results (n=68, 16.5%) (Table 8 and Figure 3) and these deviations were observed throughout all methods. The overall results for tigecycline did not reach a satisfactory level of concordance (Table 6).

Furthermore, deviations were observed for amoxicillin-clavulanic acid and these were mainly reported when using automated systems (Table 6). Most of these were major errors (n=159, 28.9%).

Table 6. Number (n) of antimicrobial susceptibility testing (AST) tests performed and percentage (%) of correct AST interpretations per antimicrobial and method for the *Escherichia coli* EARS-Net 2021 EC.1 strain

	Automated system		Disk/tablet diffusion		Gradient test		Broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	298	98.7	141	93.6	7	100.0	52	100.0	2	100.0	-	-	3	100.0	**503**	**97.4**
Amoxicillin	99	98.0	57	100.0	28	100.0	6	100.0	1	100.0	-	-	2	100.0	**193**	**99.0**
Amoxicillin-clavulanic acid*	330	62.4	170	81.2	15	80.0	31	80.6	3	100.0	-	-	2	100.0	**551**	**70.1**
Ampicillin	299	100.0	174	98.9	10	100.0	34	100.0	1	100.0	-	-	3	100.0	**521**	**99.6**
Cefepime	320	99.4	135	98.5	8	100.0	44	100.0	1	100.0	-	-	3	100.0	**511**	**99.2**
Cefotaxime	328	98.8	139	97.1	13	100.0	47	100.0	1	100.0	-	-	3	100.0	**531**	**98.5**
Ceftazidime	352	99.1	158	97.5	9	100.0	55	100.0	1	100.0	-	-	3	100.0	**578**	**98.8**
Ceftriaxone	115	97.4	145	100.0	37	97.3	12	100.0	1	100.0	-	-	-	-	**310**	**98.7**
Ciprofloxacin	345	22.6	160	26.3	16	0.0	54	13.0	3	0.0	-	-	3	0.0	**581**	**21.9**
Colistin	160	100.0	2	100.0	13	100.0	240	99.6	-	-	8	100.0	1	100.0	**424**	**99.8**
Ertapenem	275	98.9	124	98.4	39	97.4	42	97.6	1	100.0	-	-	1	100.0	**482**	**98.5**
Gentamicin	347	99.1	159	98.1	10	100.0	49	100.0	1	100.0	-	-	3	100.0	**569**	**98.9**
Imipenem	265	98.5	117	98.3	56	100.0	39	100.0	3	100.0	-	-	2	100.0	**482**	**98.8**
Levofloxacin	171	47.4	122	41.0	31	19.4	19	10.5	2	0.0	-	-	2	0.0	**347**	**40.1**
Meropenem	305	99.3	133	99.2	64	100.0	63	100.0	6	100.0	-	-	1	100.0	**572**	**99.5**
Moxifloxacin	46	93.5	106	71.7	12	83.3	7	42.9	1	100.0	-	-	-	-	**172**	**77.3**
Ofloxacin	17	88.2	68	83.8	6	100.0	4	75.0	1	100.0	-	-	2	100.0	**98**	**85.7**
Piperacillin-tazobactam*	334	98.5	164	95.7	8	100.0	58	98.3	3	100.0	-	-	3	100.0	**570**	**97.7**
Tigecycline	218	78.4	101	65.3	34	85.3	56	76.8	3	100.0	-	-	1	100.0	**413**	**75.8**
Tobramycin	267	97.4	141	98.6	9	100.0	36	97.2	1	100.0	-	-	3	100.0	**457**	**97.8**
EARS-NET 2021 EC.1 Total	**4 891**	**88.2**	**2 516**	**86.6**	**425**	**87.5**	**948**	**90.3**	**36**	**86.1**	**8**	**100.0**	**41**	**87.8**	**8 865**	**88.0**

Shaded cells highlight percentages of concordant results below the threshold of satisfactory concordance (80).

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

Table 7. Susceptibility of *Escherichia coli* EARS-Net 2021 EC.1 strain to amoxicillin-clavulanic acid fixed concentration, ciprofloxacin and levofloxacin by method used

Expected interpretation	Amoxicillin-clavulanic acid fixed concentration – S						Ciprofloxacin – I						Levofloxacin – I					
Reported interpretation	S		I		R		S		I		R		S		I		R	
Test strain ID	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	206	62.4	2	0.6	122	37.0	260	75.4	78	22.6	7	2.0	84	49.1	81	47.4	6	3.5
Disk/tablet diffusion	138	81.2	4	2.4	28	16.5	106	66.3	42	26.3	12	7.5	69	56.6	50	41.0	3	2.5
Gradient test	12	80.0	-	-	3	20.0	16	100.0	-	-	-	-	25	80.6	6	19.4	-	-
Agar dilution	3	100.0	-	-	-	-	3	100.0	-	-	-	-	2	100.0	-	-	-	-
Broth microdilution	25	80.6	-	-	6	19.4	46	85.2	7	13.0	1	1.9	15	78.9	2	10.5	2	10.5
Other	2	100.0	-	-	-	-	3	100.0	-	-	-	-	2	100.0	-	-	-	-
EARS-NET 2021 EC.1 Total	**386**	**70.1**	**6**	**1.1**	**159**	**28.9**	**434**	**74.7**	**127**	**21.9**	**20**	**3.4**	**197**	**56.8**	**139**	**40.1**	**11**	**3.2**

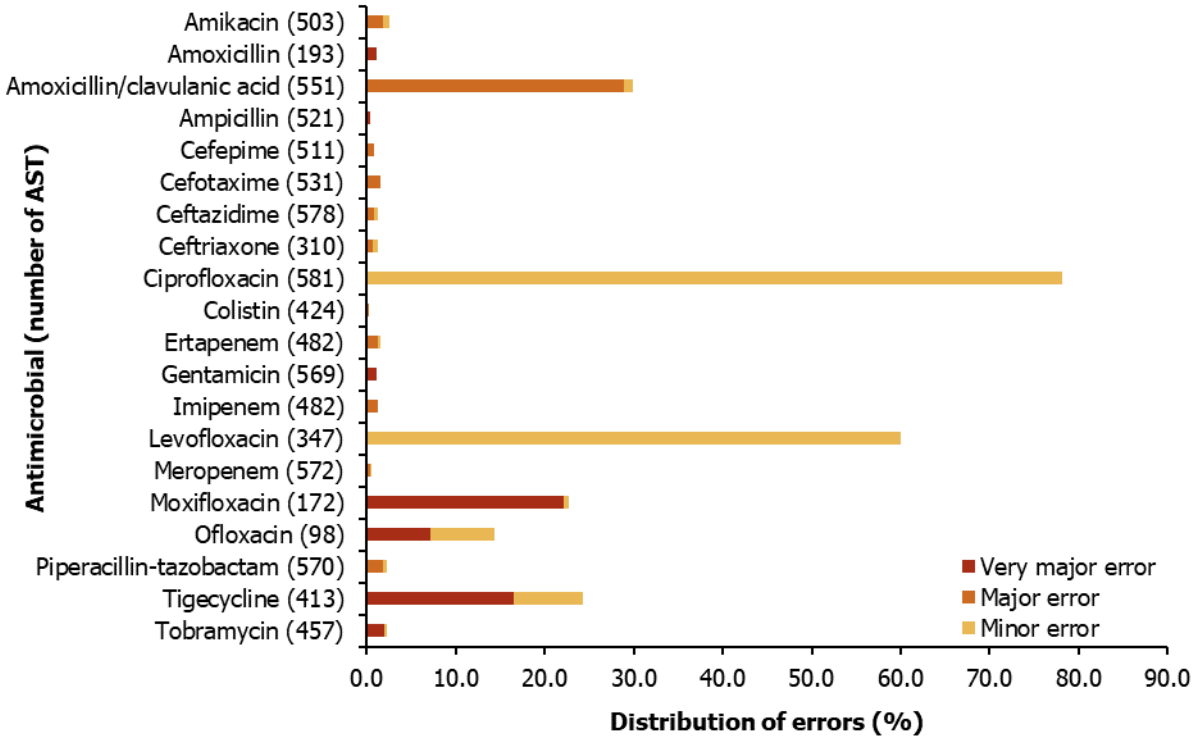
S: 'susceptible, standard dosing regimen'; I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Table 8. Susceptibility of *Escherichia coli* EARS-Net 2021 EC.1 strain to moxifloxacin, ofloxacin and tigecycline by method used

Expected interpretation	Moxifloxacin – R						Ofloxacin – R						Tigecycline – R					
Reported interpretation	S		I		R		S		I		R		S		I		R	
Test strain ID	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	3	6.5	-	-	43	93.5	2	11.8	-	-	15	88.2	19	8.7	28	12.8	171	78.4
Disk/tablet diffusion	29	27.4	1	0.9	76	71.7	4	5.9	7	10.3	57	83.8	34	33.7	1	1.0	66	65.3
Gradient test	2	16.7	-	-	10	83.3	-	-	-	-	6	100.0	5	14.7	-	-	29	85.3
Agar dilution	-	-	-	-	1	100.0	-	-	-	-	1	100.0	-	-	-	-	3	100.0
Broth microdilution	4	57.1	-	-	3	42.9	1	25.0	-	-	3	75.0	10	17.9	3	5.4	43	76.8
Other	-	-	-	-	-	-	-	-	-	-	2	100.0	-	-	-	-	1	100.0
EARS-NET 2021 EC.1 Total	**38**	**22.1**	**1**	**0.6**	**133**	**77.3**	**7**	**7.1**	**7**	**7.1**	**84**	**85.7**	**68**	**16.5**	**32**	**7.7**	**313**	**75.8**

S: 'susceptible, standard dosing regimen'; I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Figure 3. Number of AST tests and distribution of minor, major and very major errors for the *Escherichia coli* EARS-Net 2021 EC.1 strain per antimicrobial



EARS-Net 2021 EC.2: *Escherichia coli*

The EARS-Net 2021 EC.2 strain was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem and piperacillin-tazobactam (Table 1 and Table 3).

In total, 8 963 tests were performed, and 8 516 reported interpretations were correct. Thus, the reported interpretations were in excellent concordance with the expected results (95.0%).

Results for the *E. coli* strain EARS-Net 2021 EC.2 were submitted by 591 laboratories. In total, 59.1% of the laboratories (n=349) would have sent the strain to a reference or other laboratory for further testing. Overall, 47.0% of laboratories (n=278) had excellent concordance, with more than 95% correct results, and 264 of these were in full concordance. Furthermore, 38.4% of laboratories (n=227) had very good concordance, 8.6% (n=51) had good concordance, 4.2% (n=25) had satisfactory concordance and 1.7% of the laboratories (n=10) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (54.4%), disk diffusion or tablet diffusion (28.4%), broth microdilution (11.3%), gradient test (4.9%), agar dilution (0.4%), macro broth dilution (tubes) (0.1%), and other (0.5%) (Table 5). The highest concordance was reported when using the macro broth dilution (tubes) (100%), however this method was only used for colistin analysis nine times. The lowest concordance was reported when using the agar dilution (79.5%). For the other methods the concordance was between 91.0% and 96.7% (Table 9). Overall, all methodologies except agar dilution achieved, as a minimum, a very good level of concordance with the expected results.

Most deviations were observed for ceftazidime and meropenem (Table 9). Ceftazidime deviations were reported across all methods and corresponded mainly to minor errors (Figure 4) where participants reported an interpretation of 'I' instead of 'R' (n=153, 26.5%) (Table 10). Meropenem deviations also mostly corresponded to minor errors, with 127 (22.3%) laboratories submitting an interpretation of 'I' instead of 'S' (Table 10 and Figure 4). These deviations were distributed across most methods for which good and satisfactory levels of concordance were achieved respectively (Table 9). Although the overall concordance for imipenem achieved the 'good' level, the concordance for disk diffusion or tablet diffusion did not reach a satisfactory level (Table 9). Deviations in imipenem interpretation were mostly due to minor errors (n=42, 8.6%) (Table 10 and Figure 4).

Table 9. Number of antimicrobial susceptibility testing (AST) tests performed and percentage of correct AST interpretations per antimicrobial and method used for the *Escherichia coli* EARS-Net 2021 EC.2 strain

	Automated system		Disk/tablet diffusion		Gradient test		Broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Amikacin	300	99.0	143	100.0	7	100.0	56	100.0	2	100.0	-	-	3	100.0	**511**	**99.4**
Amoxicillin	100	99.0	57	100.0	27	100.0	7	100.0	1	100.0	-	-	2	100.0	**194**	**99.5**
Amoxicillin-clavulanic acid*	331	99.7	171	100.0	15	100.0	35	100.0	3	100.0	-	-	2	100.0	**557**	**99.8**
Ampicillin	299	99.7	174	100.0	10	100.0	36	100.0	1	100.0	-	-	3	100.0	**523**	**99.8**
Cefepime	321	99.7	138	100.0	8	100.0	46	100.0	1	100.0	-	-	3	100.0	**517**	**99.8**
Cefotaxime	331	99.7	142	100.0	12	100.0	49	100.0	1	100.0	-	-	3	100.0	**538**	**99.8**
Ceftazidime	350	63.4	159	93.7	9	77.8	56	76.8	1	0.0	-	-	3	33.3	**578**	**73.0**
Ceftriaxone	108	98.1	146	100.0	37	100.0	14	100.0	1	100.0	-	-	0	-	**306**	**99.3**
Ciprofloxacin	346	99.7	166	99.4	14	100.0	56	100.0	2	100.0	-	-	3	100.0	**587**	**99.7**
Colistin	159	100.0	3	100.0	14	100.0	261	99.6	-	-	9	100.0	1	100.0	**447**	**99.8**
Ertapenem	273	99.3	125	98.4	41	97.6	45	95.6	2	100.0	-	-	1	100.0	**487**	**98.6**
Gentamicin	348	98.9	158	99.4	10	100.0	51	98.0	2	100.0	-	-	3	100.0	**572**	**99.0**
Imipenem	260	93.1	118	73.7	62	82.3	44	88.6	5	80.0	-	-	2	100.0	**491**	**86.6**
Levofloxacin	174	97.7	124	100.0	30	100.0	23	100.0	1	100.0	-	-	2	100.0	**354**	**98.9**
Meropenem	301	85.7	131	33.6	66	63.6	64	82.8	7	14.3	-	-	1	0.0	**570**	**69.8**
Moxifloxacin	46	97.8	110	100.0	12	100.0	7	100.0	1	100.0	-	-	-	-	**176**	**99.4**
Ofloxacin	15	93.3	71	98.6	6	100.0	6	100.0	2	100.0	-	-	2	100.0	**102**	**98.0**
Piperacillin-tazobactam*	333	99.7	165	99.4	8	100.0	59	100.0	3	100.0	-	-	3	100.0	**571**	**99.6**
Tigecycline	216	96.3	105	100.0	38	97.4	62	100.0	2	100.0	-	-	1	100.0	**424**	**97.9**
Tobramycin	264	97.7	142	98.6	9	100.0	39	97.4	1	100.0	-	-	3	100.0	**458**	**98.0**
EARS-NET 2021 EC.2 Total	**4 875**	**95.3**	**2 548**	**94.7**	**435**	**91.0**	**1 016**	**96.7**	**39**	**79.5**	**9**	**100.0**	**41**	**92.7**	**8 963**	**95.0**

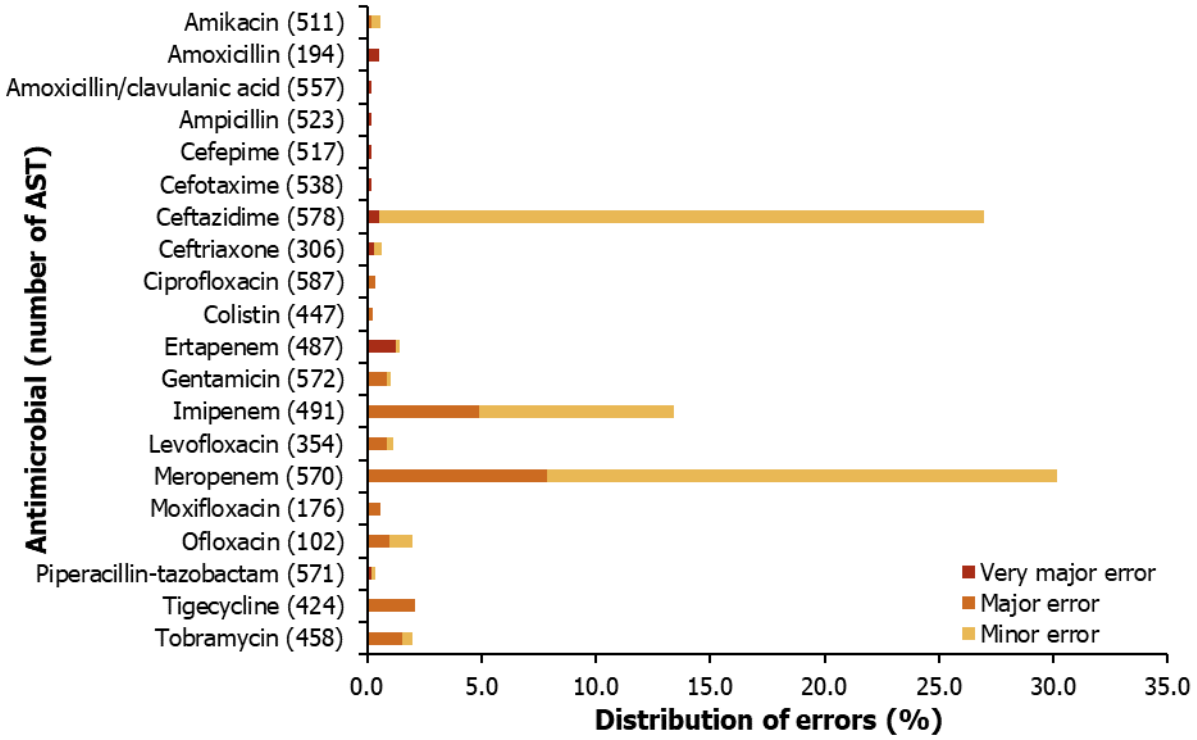
Shaded cells highlight percentages of concordant results below the threshold of satisfactory concordance (80).
**Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 10. Susceptibility of *Escherichia coli* EARS-Net 2021 EC.2 strain to ceftazidime, imipenem and meropenem by method used

Antimicrobial and expected interpretation	Ceftazidime - R						Imipenem - S						Meropenem - S					
Reported interpretation	**S**		**I**		**R**		**S**		**I**		**R**		**S**		**I**		**R**	
Method	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Automated system	2	0.6	126	36.0	222	63.4	242	93.1	10	3.8	8	3.1	258	85.7	31	10.3	12	4.0
Disk/tablet diffusion	1	0.6	9	5.7	149	93.7	87	73.7	22	18.6	9	7.6	44	33.6	70	53.4	17	13.0
Gradient test	-	-	2	22.2	7	77.8	51	82.3	6	9.7	5	8.1	42	63.6	12	18.2	12	18.2
Agar dilution	-	-	1	100.0	-	-	4	80.0	1	20.0	-	-	1	14.3	6	85.7	-	-
Broth microdilution	-	-	13	23.2	43	76.8	39	88.6	3	6.8	2	4.5	53	82.8	8	12.5	3	4.7
Other	-	-	2	66.7	1	33.3	2	100.0	-	-	-	-	-	-	-	-	1	100.0
EARS-NET 2021 EC.2 Total	**3**	**0.5**	**153**	**26.5**	**422**	**73.0**	**425**	**86.6**	**42**	**8.6**	**24**	**4.9**	**398**	**69.8**	**127**	**22.3**	**45**	**7.9**

S: 'susceptible, standard dosing regimen'; I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Figure 4. Number of AST tests and distribution of minor, major and very major errors for the *Escherichia coli* EARS-Net 2021 EC.2 strain per antimicrobial



EARS-Net 2021 EC.3: *Escherichia coli*

The EARS-Net 2021 EC.3 strain was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem, imipenem, piperacillin-tazobactam and tobramycin (Table 1 and Table 3). Furthermore, the expected MIC value for meropenem was in the 'susceptible, increased exposure' (I) range.

In total, 8 949 tests were performed, and 7 978 reported interpretations were correct. Thus, the reported interpretations were in good concordance with the expected results (89.1%).

Results for the *E. coli* strain EARS-Net 2021 EC.3 were submitted by 591 laboratories. In total, 64.1% of the laboratories (n=379) would have sent the strain to a reference or other laboratory for further testing. Overall, 17.6% of laboratories (n=104) had excellent concordance with more than 95% correct results, and 96 of these were in full concordance. Furthermore, 25.9% of laboratories (n=153) had very good concordance, 25.5% (n=151) had good concordance, 20.6% (n=122) had satisfactory concordance and 10.3% of the laboratories (61) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (54.4%), disk diffusion or tablet diffusion (28.4%), broth microdilution (11.4%), gradient test (4.9%), agar dilution (0.4%), macro broth dilution (tubes) (0.1%), and other methods (0.5%) (Table 5). The highest concordance was observed when using the disk diffusion or tablet diffusion method (95.1%). The lowest concordance percentages were observed when using the agar dilution (69.2%) and the gradient test (75.8%), and neither of these two methods achieved a satisfactory level of concordance with expected results (Table 11).

Most deviations were observed for gentamicin and all carbapenems (Table 11). Gentamicin deviations were mainly reported when using automated systems and the highest percentage was due to major errors (Table 12), with participants reporting 'R' instead of the expected 'S' (n=219, 38.5%).

Deviations from expected results for the carbapenems were reported across all methods (Table 11). For ertapenem, deviations were mainly due to very major errors (n=103, 21.5%) (Table 12 and Figure 5) mainly reported using broth microdilution and the gradient test, but also automated systems. Meropenem deviations corresponded to minor errors (Figure 5) with 250 (43.9%) of laboratories reporting 'R' instead of the expected 'I' and 153 (26.9%) laboratories reporting 'S' (Table 12). Deviations in meropenem were more commonly observed with automated systems and the gradient test. Imipenem deviations were also mainly derived from minor errors (n=75, 15.3%) (Table 12 and Figure 5) and more frequent with broth microdilution than other methods.

Table 11. Number of antimicrobial susceptibility testing (AST) tests performed and percentage of correct AST interpretations per antimicrobial and method used for the *Escherichia coli* EARS-Net 2021 EC.3 strain

	Automated system		Disk/tablet diffusion		Gradient test		Broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	298	96.6	143	90.9	7	100.0	55	94.5	2	100.0	-	-	3	66.7	508	94.7
Amoxicillin	101	100.0	58	98.3	28	100.0	7	100.0	1	100.0	-	-	2	100.0	197	99.5
Amoxicillin-clavulanic acid*	331	100.0	171	99.4	15	100.0	35	100.0	3	100.0	-	-	2	100.0	557	99.8
Ampicillin	299	100.0	174	99.4	10	100.0	36	100.0	1	100.0	-	-	3	100.0	523	99.8
Cefepime	320	98.4	138	99.3	8	100.0	46	95.7	1	0.0	-	-	3	100.0	516	98.3
Cefotaxime	330	99.7	142	100.0	12	100.0	49	100.0	1	100.0	-	-	3	100.0	537	99.8
Ceftazidime	353	98.9	159	100.0	9	100.0	56	100.0	1	100.0	-	-	3	100.0	581	99.3
Ceftriaxone	108	99.1	146	99.3	37	100.0	14	100.0	1	100.0	-	-	-	-	306	99.3
Ciprofloxacin	345	99.7	166	98.2	14	100.0	56	100.0	2	100.0	-	-	3	100.0	586	99.3
Colistin	158	99.4	3	100.0	14	92.9	266	98.9	-	-	8	100.0	1	100.0	450	98.9
Ertapenem	267	74.9	123	95.9	42	35.7	44	40.9	2	50.0	-	-	1	100.0	479	73.7
Gentamicin	346	34.7	158	88.6	10	90.0	50	82.0	2	50.0	-	-	3	100.0	569	55.2
Imipenem	259	86.9	117	84.6	63	65.1	44	47.7	5	60.0	-	-	2	100.0	490	79.8
Levofloxacin	175	99.4	123	100.0	30	100.0	22	100.0	1	100.0	-	-	2	100.0	353	99.7
Meropenem	303	16.2	129	60.5	65	15.4	64	45.3	7	0.0	-	-	1	0.0	569	29.2
Moxifloxacin	47	100.0	109	100.0	12	100.0	7	100.0	1	100.0	-	-	-	-	176	100.0
Ofloxacin	14	92.9	71	98.6	6	100.0	6	100.0	2	100.0	-	-	2	100.0	101	98.0
Piperacillin- tazobactam*	333	99.4	165	98.8	8	100.0	59	96.6	3	100.0	-	-	3	100.0	571	98.9
Tigecycline	214	97.2	103	99.0	39	100.0	64	96.9	2	100.0	-	-	1	100.0	423	97.9
Tobramycin	263	97.7	142	95.1	9	100.0	39	94.9	1	100.0	-	-	3	100.0	457	96.7
EARS-NET 2021 EC.3 Total	**4 864**	**87.3**	**2 540**	**95.1**	**438**	**75.8**	**1 019**	**89.5**	**39**	**69.2**	**8**	**100.0**	**41**	**95.1**	**8 949**	**89.1**

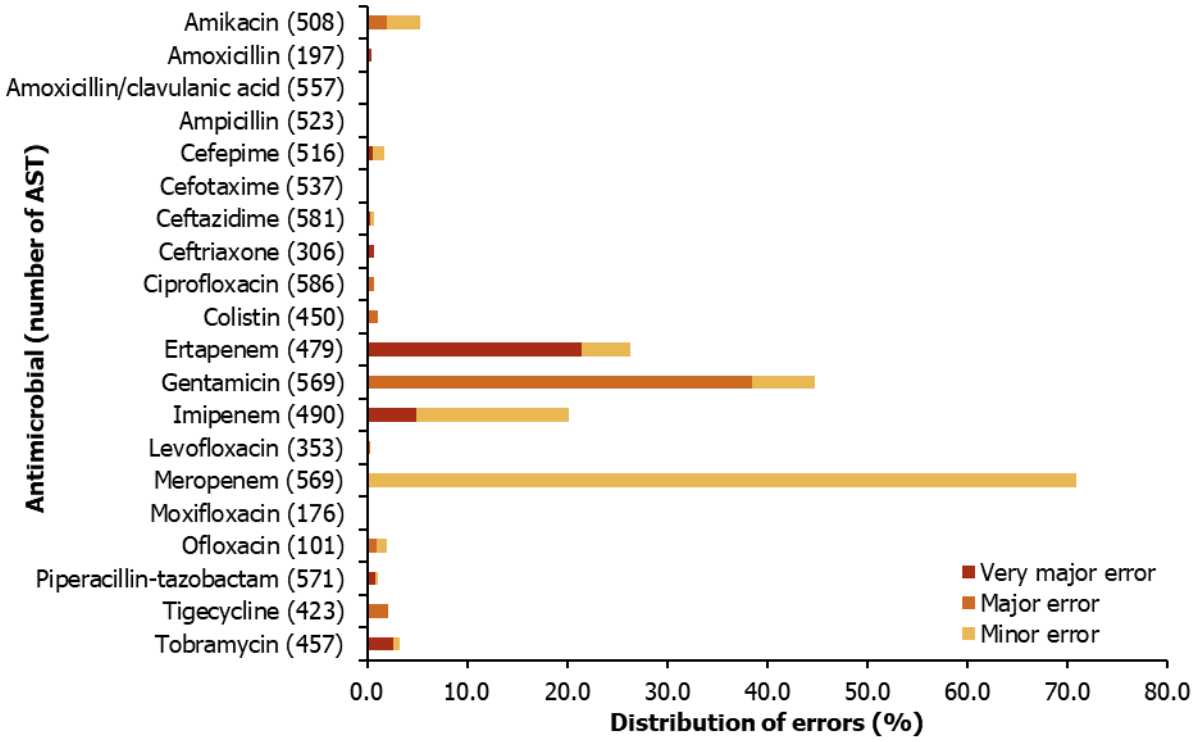
Shaded cells highlight percentages of concordant results below the threshold of satisfactory concordance (80).
**Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 12. Susceptibility of *Escherichia coli* EARS-Net 2021 EC.3 strain to ertapenem, gentamicin, imipenem and meropenem by method

Antimicrobial and expected interpretation	Ertapenem - R						Gentamicin - S						Imipenem - R						Meropenem - I					
Reported interpretation	S		I		R		S		I		R		S		I		R		S		I		R	
Method	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	46	17.2	21	7.9	200	74.9	120	34.7	35	10.1	191	55.2	8	3.1	26	10.0	225	86.9	59	19.5	49	16.2	195	64.4
Disk/tablet diffusion	5	4.1	-	-	118	95.9	140	88.6	-	-	18	11.4	2	1.7	16	13.7	99	84.6	24	18.6	78	60.5	27	20.9
Gradient test	27	64.3	-	-	15	35.7	9	90.0	-	-	1	10.0	8	12.7	14	22.2	41	65.1	42	64.6	10	15.4	13	20.0
Agar dilution	1	50.0	-	-	1	50.0	1	50.0	-	-	1	50.0	1	20.0	1	20.0	3	60.0	5	71.4	-	-	2	28.6
Broth microdilution	24	54.5	2	4.5	18	40.9	41	82.0	1	2.0	8	16.0	5	11.4	18	40.9	21	47.7	23	35.9	29	45.3	12	18.8
Other	-	-	-	-	1	100.0	3	100.0	-	-	-	-	-	-	-	-	2	100.0	-	-	-	-	1	100.0
EARS-NET 2021 EC.3 Total	**103**	**21.5**	**23**	**4.8**	**353**	**73.7**	**314**	**55.2**	**36**	**6.3**	**219**	**38.5**	**24**	**4.9**	**75**	**15.3**	**391**	**79.8**	**153**	**26.9**	**166**	**29.2**	**250**	**43.9**

S: 'susceptible, standard dosing regimen'; I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Figure 5. Number of AST tests and distribution of minor, major and very major errors for the Escherichia coli EARS-Net 2021 EC.3 strain per antimicrobial



EARS-Net 2021 KPN.1: *Klebsiella pneumoniae*

The EARS-Net 2021 KPN.1 strain was resistant to amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin (Table 2 and Table 4).

In total, 7 884 tests were performed, and 7 787 reported interpretations were correct. Thus, there was excellent concordance of the reported interpretations with the expected results (98.8%).

In total, 590 laboratories submitted results for the *K. pneumoniae* strain EARS-Net 2021 KPN.1. In total, 70.2% of the laboratories (n=414) would have sent the strain to a reference or other laboratory for further testing. Overall, 90.5% of laboratories (n=534) had excellent concordance with more than 95% correct results, and all of these were in full concordance. Furthermore, 5.6% of laboratories (n=33) had very good concordance, 1.4% (n=8) had good concordance, 1.5% (n=9) had satisfactory concordance, and 1.0% of the laboratories (n=6) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (54.9%), disk diffusion or tablet diffusion (27.4%), broth microdilution (11.6%), gradient test (4.9%), agar dilution (0.4%), macro broth dilution (tubes) (0.2%), and other methods (0.4%) (Table 5). Excellent levels (≥95%) of concordance were observed for all methods except for the gradient test (94.1%) (Table 13).

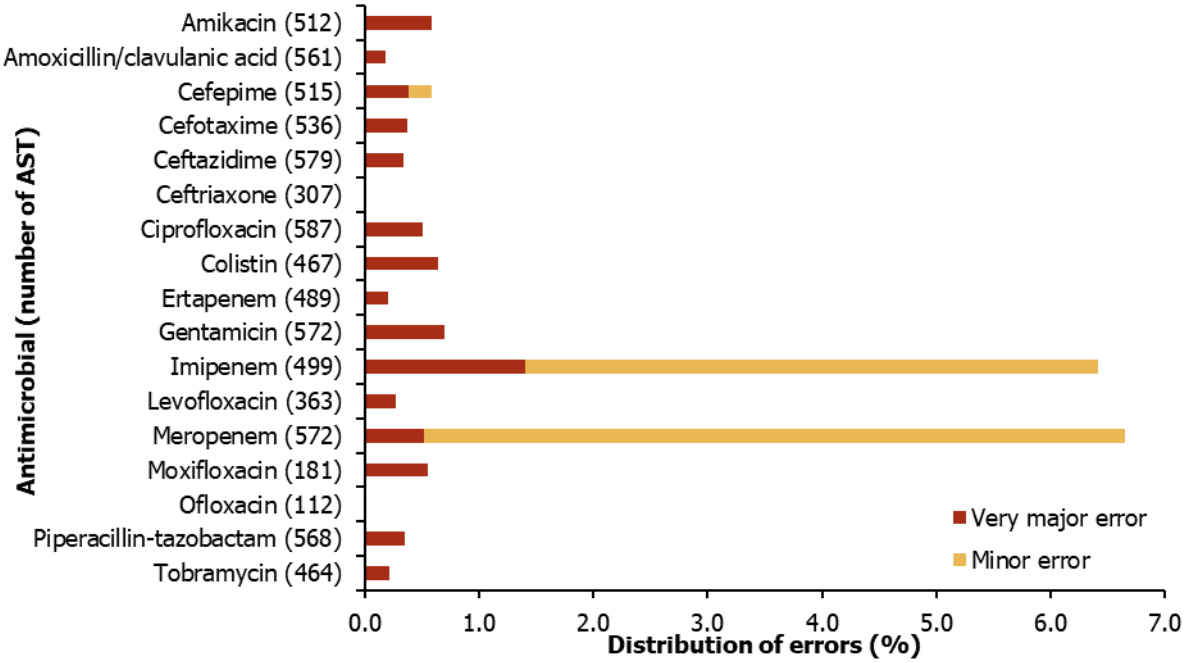
For this strain, results achieved at least a very good level for all antimicrobials included in this EQA exercise (Table 13 and Figure 6).

Table 13. Number of AST tests performed and percentage of correct AST interpretations per antimicrobial and method used for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.1 strain

	Automated system		Disk/tablet diffusion		Gradient test		Broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	301	99.7	140	98.6	9	100.0	57	100.0	1	100.0	1	100.0	3	100.0	512	99.4
Amoxicillin-clavulanic acid*	334	100.0	171	99.4	17	100.0	35	100.0	2	100.0	0	-	2	100.0	561	99.8
Cefepime	320	99.4	135	99.3	10	100.0	45	100.0	1	100.0	1	100.0	3	100.0	515	99.4
Cefotaxime	330	99.7	138	99.3	14	100.0	49	100.0	1	100.0	1	100.0	3	100.0	536	99.6
Ceftazidime	354	99.7	153	99.3	11	100.0	56	100.0	1	100.0	1	100.0	3	100.0	579	99.7
Ceftriaxone	113	100.0	141	100.0	38	100.0	14	100.0	1	100.0	0	-	0	-	307	100.0
Ciprofloxacin	350	99.4	165	99.4	12	100.0	55	100.0	1	100.0	1	100.0	3	100.0	587	99.5
Colistin	165	98.8	7	100.0	17	94.1	269	100.0	1	100.0	7	100.0	1	100.0	467	99.4
Ertapenem	275	100.0	120	99.2	43	100.0	47	100.0	3	100.0	0	-	1	100.0	489	99.8
Gentamicin	349	99.1	158	99.4	10	100.0	50	100.0	1	100.0	1	100.0	3	100.0	572	99.3
Imipenem	265	97.4	115	91.3	66	87.9	44	86.4	7	85.7	0	-	2	100.0	499	93.6
Levofloxacin	182	99.5	124	100.0	29	100.0	25	100.0	1	100.0	0	-	2	100.0	363	99.7
Meropenem	305	95.7	120	96.7	71	80.3	66	89.4	8	100.0	1	100.0	1	100.0	572	93.4
Moxifloxacin	55	98.2	105	100.0	13	100.0	7	100.0	1	100.0	0	-	0	-	181	99.4
Ofloxacin	26	100.0	70	100.0	7	100.0	5	100.0	1	100.0	1	100.0	2	100.0	112	100.0
Piperacillin-tazobactam*	334	99.7	160	99.4	12	100.0	56	100.0	2	100.0	1	100.0	3	100.0	568	99.6
Tobramycin	270	99.6	142	100.0	11	100.0	36	100.0	1	100.0	1	100.0	3	100.0	464	99.8
EARS-NET 2021 KPN.1 Total	**4 328**	**99.2**	**2 164**	**98.9**	**390**	**94.1**	**916**	**98.6**	**34**	**97.1**	**17**	**100.0**	**35**	**100.0**	**7 884**	**98.8**

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

Figure 6. Number of AST tests and distribution of minor, major and very major errors for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.1 strain per antimicrobial



There were no major errors reported for the EARS-Net KPN.1 strain.

EARS-Net 2021 KPN.2: *Klebsiella pneumoniae*

The EARS-Net 2021 KPN.2 strain was resistant to amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ertapenem and piperacillin-tazobactam (Table 2 and Table 4). Furthermore, the expected MIC value for imipenem was in the 'susceptible, increased exposure' (I) range.

In total, 7 812 tests were performed, and 7 018 reported interpretations were correct. Thus, the reported interpretations were in good concordance with the expected results (89.8%).

Results for the *K. pneumoniae* strain EARS-Net 2021 KPN.2 were submitted by 590 laboratories. In total, 50.5% of the laboratories (n=298) would have sent the strain to a reference or other laboratory for further testing. Overall, 13.7% of laboratories (n=81) had excellent concordance with more than 95% correct results, and all of these were in full concordance. Furthermore, 41.7% of laboratories (n=246) had very good concordance, 24.4% (n=144) had good concordance, 15.9% (n=94) had satisfactory concordance and 4.2% of the laboratories (n=25) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (54.7%), disk diffusion or tablet diffusion (27.6%), broth microdilution (11.5%), gradient test (5.0%), agar dilution (0.5%), macro broth dilution (tubes) (0.2%), and other methods (0.4%) (Table 5). The highest concordance was observed when using the broth microdilution (92.9%) (Table 14). The lowest concordance was observed when using the macro broth dilution (tubes) (70.6%), which was the only method not achieving at least a satisfactory level of concordance with expected results.

Most deviations were observed for cefepime and carbapenems. Cefepime deviations were mostly due to minor errors (n=183, 35.9%), with laboratories reporting interpretations of 'I' instead of the expected 'R' (Table 15 and Figure 7.) Deviations were distributed across most methods, except disk diffusion or tablet diffusion and macro broth dilution (tubes). Minor errors were also responsible for the deviations observed for imipenem (Figure 7). Specifically, 204 (41.1%) laboratories classified the strain as being 'S' to imipenem, while 153 (30.8%) classified it as resistant (Table 15). Deviations in meropenem susceptibility testing were prevalent and distributed between minor and major errors. Specifically, 132 (23.1%) laboratories failed to correctly identify the 'S' profile of the strain towards meropenem and reported 'I' results, and 26 (4.5%) laboratories reported it as 'R'. Discordances in carbapenem results were distributed across all methods and more prevalent for imipenem than for meropenem (Table 15). No test method achieved a satisfactory level for imipenem results, and disk diffusion or tablet diffusion results were very poor for meropenem (Table 14).

Table 14. Number of AST tests performed and percentage of correct AST interpretations per antimicrobial and method used for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.2 strain

	Automated system		Disk/tablet diffusion		Gradient test		Broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Amikacin	299	98.0	140	99.3	9	100.0	56	98.2	1	100.0	1	100.0	3	100.0	**509**	**98.4**
Amoxicillin-clavulanic acid*	333	99.7	170	100.0	17	100.0	35	100.0	2	100.0	-	-	2	100.0	**559**	**99.8**
Cefepime	315	49.2	134	92.5	10	80.0	45	64.4	2	50.0	1	100.0	3	66.7	**510**	**62.7**
Cefotaxime	332	100.0	138	100.0	14	100.0	49	100.0	1	100.0	1	100.0	3	100.0	**538**	**100.0**
Ceftazidime	355	100.0	154	100.0	11	100.0	56	100.0	1	100.0	1	100.0	3	100.0	**581**	**100.0**
Ceftriaxone	111	100.0	142	100.0	38	100.0	14	100.0	1	100.0	-	-	-	-	**306**	**100.0**
Ciprofloxacin	349	98.3	165	98.2	12	100.0	55	100.0	1	100.0	1	0.0	3	100.0	**586**	**98.3**
Colistin	153	98.7	5	100.0	17	100.0	258	98.1	1	100.0	7	100.0	1	100.0	**442**	**98.4**
Ertapenem	274	98.2	120	99.2	43	97.7	47	93.6	3	100.0	-	-	1	100.0	**488**	**98.0**
Gentamicin	349	97.7	157	97.5	10	90.0	50	98.0	2	100.0	1	0.0	3	100.0	**572**	**97.4**
Imipenem	262	15.3	115	48.7	66	39.4	44	31.8	7	42.9	-	-	2	0.0	**496**	**28.0**
Levofloxacin	174	98.9	123	97.6	28	100.0	21	100.0	1	100.0	-	-	2	100.0	**349**	**98.6**
Meropenem	306	84.3	120	30.8	70	71.4	66	92.4	8	87.5	1	0.0	1	100.0	**572**	**72.4**
Moxifloxacin	46	97.8	107	95.3	13	100.0	7	100.0	1	100.0	-	-	-	-	**174**	**96.6**
Ofloxacin	17	82.4	70	90.0	7	100.0	5	100.0	1	100.0	1	0.0	1	100.0	**102**	**89.2**
Piperacillin- tazobactam*	333	99.7	160	100.0	12	100.0	56	98.2	3	100.0	1	100.0	3	100.0	**568**	**99.6**
Tobramycin	268	98.5	140	95.0	11	100.0	36	94.4	1	100.0	1	0.0	3	100.0	**460**	**97.0**
EARS-NET 2021 KPN.2 Total	**4 276**	**89.0**	**2 160**	**91.5**	**388**	**83.5**	**900**	**92.9**	**37**	**83.8**	**17**	**70.6**	**34**	**91.2**	**7 812**	**89.8**

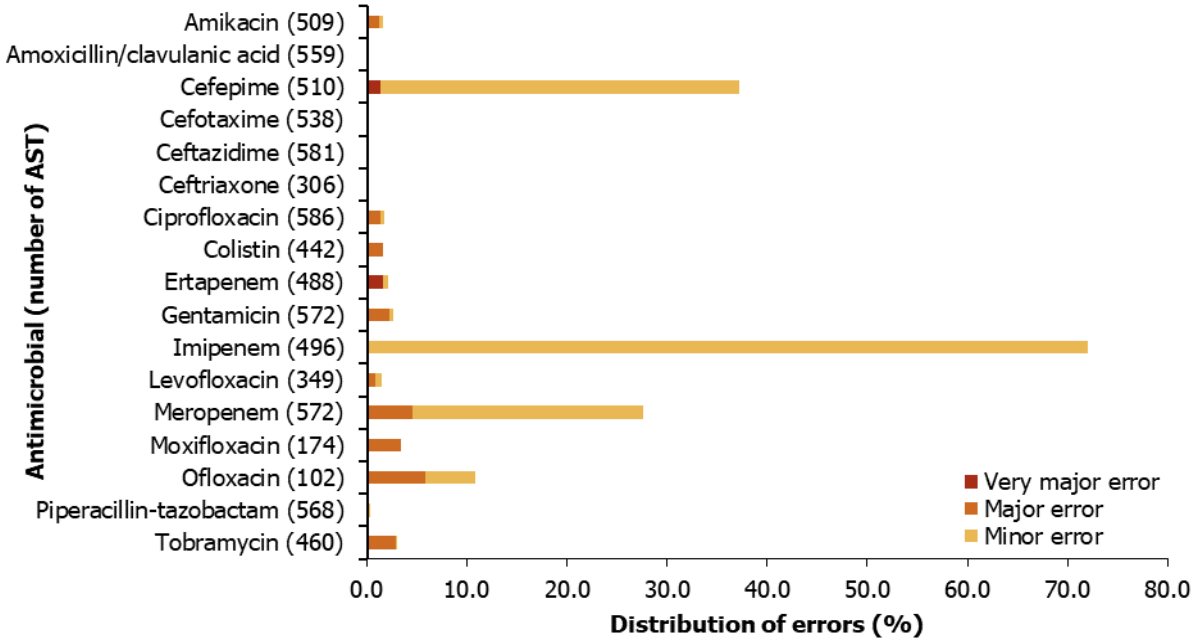
Note: Shaded cells highlight percentages of concordant results below the threshold of satisfactory concordance (80).
**Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 15. Susceptibility of *Klebsiella pneumoniae* EARS-Net 2021 KPN.2 strain to cefepime, imipenem and meropenem by method

Antimicrobial and expected interpretation	Cefepime – R						Imipenem - I						Meropenem - S					
Reported interpretation	S		I		R		S		I		R		S		I		R	
Method	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	2	0.6	158	50.2	155	49.2	156	59.5	40	15.3	66	25.2	258	84.3	42	13.7	6	2.0
Disk/tablet diffusion	2	1.5	8	6.0	124	92.5	13	11.3	56	48.7	46	40.0	37	30.8	68	56.7	15	12.5
Gradient test	-	-	2	20.0	8	80.0	16	24.2	26	39.4	24	36.4	50	71.4	16	22.9	4	5.7
Agar dilution	-	-	1	50.0	1	50.0	-	-	3	42.9	4	57.1	7	87.5	-	-	1	12.5
Broth microdilution	3	6.7	13	28.9	29	64.4	19	43.2	14	31.8	11	25.0	61	92.4	5	7.6	-	-
Macro broth dilution (tubes)	-	-	-	-	1	100.0	-	-	-	-	-	-	-	-	1	100.0	-	-
Other	-	-	1	33.3	2	66.7	-	-	-	-	2	100.0	1	100.0	-	-	-	-
EARS-NET 2021 KPN.2 Total	**7**	**1.4**	**183**	**35.9**	**320**	**62.7**	**204**	**41.1**	**139**	**28.0**	**153**	**30.8**	**414**	**72.4**	**132**	**23.1**	**26**	**4.5**

S: 'susceptible, standard dosing regimen'; I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Figure 7. Number of AST tests and distribution of minor, major and very major errors for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.2 strain per antimicrobial



EARS-Net 2021 KPN.3: *Klebsiella pneumoniae*

The EARS-Net 2021 KPN.3 strain was resistant to amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, levofloxacin, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin (Table 2 and Table 4).

In total, 7 829 tests were performed, and 7 542 reported interpretations were correct. Thus, the reported interpretations were in excellent concordance with the expected results (96.3%).

Results for the *K. pneumoniae* strain EARS-Net 2021 KPN.3 were submitted by 588 laboratories. In total, 51.4% of the laboratories (n=302) would have sent the strain to a reference or other laboratory for further testing. Overall, 57.0% of laboratories (n=335) had excellent concordance with more than 95% correct results, and all of these were in full concordance. Furthermore, 37.1% of laboratories (n=218) had very good concordance, 3.7% (n=22) had good concordance, 1.5% (n=9) had satisfactory concordance and 0.7% of the laboratories (n=4) had concordance below the satisfactory level (<80% correct interpretations).

The following methodologies were applied: automated systems (54.7%), disk diffusion or tablet diffusion (27.7%), broth microdilution (11.6%), gradient test (4.9%), agar dilution (0.4%), macro broth dilution (tubes) (0.2%), and other methods (0.4%) (Table 5).The highest concordance was observed when using the broth microdilution (97.5%) and the lowest concordance was observed when using the macro broth dilution (tubes) (87.5%) (Table 16). Overall, all methodologies achieved at least a good level of concordance with the expected results.

Most deviations were observed for meropenem and were found with all methods (Table 17). These essentially corresponded to minor errors (n=159, 27.8%). Imipenem results, when reported using automated systems, did not reach a satisfactory level, and most deviations observed for this antimicrobial corresponded to minor errors (n=67, 13.5%) (Table 17 and Figure 8).

Table 16. Number of AST tests performed and percentage of correct AST interpretations per antimicrobial and method used for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.3 strain

	Automated system		Disk/tablet diffusion		Gradient test		MIC–broth microdilution		Agar dilution		Macro broth dilution (tubes)		Other		Total	
Antimicrobial	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amikacin	299	99.7	141	99.3	9	100.0	57	100.0	1	100.0	1	100.0	3	100.0	**511**	**99.6**
Amoxicillin-clavulanic acid*	331	100.0	170	100.0	16	100.0	35	100.0	2	100.0	-	-	2	100.0	**556**	**100.0**
Cefepime	317	99.7	135	100.0	10	100.0	45	100.0	1	100.0	1	100.0	3	100.0	**512**	**99.8**
Cefotaxime	330	100.0	137	100.0	14	100.0	49	100.0	1	100.0	1	100.0	3	100.0	**535**	**100.0**
Ceftazidime	353	100.0	154	100.0	11	100.0	56	100.0	1	100.0	1	100.0	3	100.0	**579**	**100.0**
Ceftriaxone	109	100.0	140	100.0	38	100.0	14	100.0	1	100.0	-	-	-	-	**302**	**100.0**
Ciprofloxacin	346	99.4	165	99.4	12	100.0	55	100.0	1	100.0	1	100.0	3	100.0	**583**	**99.5**
Colistin	153	98.0	5	100.0	17	100.0	263	97.7	1	100.0	6	83.3	1	100.0	**446**	**97.8**
Ertapenem	273	99.6	122	100.0	41	97.6	48	97.9	2	100.0	-	-	1	100.0	**487**	**99.4**
Gentamicin	346	99.4	158	99.4	10	100.0	50	98.0	1	100.0	1	100.0	3	100.0	**569**	**99.3**
Imipenem	261	73.2	115	93.9	67	95.5	44	97.7	6	100.0	-	-	2	100.0	**495**	**83.6**
Levofloxacin	183	98.4	124	100.0	28	100.0	26	100.0	1	100.0	-	-	2	100.0	**364**	**99.2**
Meropenem	306	78.8	119	35.3	70	77.1	66	78.8	8	75.0	1	0.0	1	0.0	**571**	**69.2**
Moxifloxacin	53	100.0	107	100.0	13	100.0	7	100.0	1	100.0	-	-	-	-	**181**	**100.0**
Ofloxacin	23	100.0	71	100.0	7	100.0	5	100.0	1	100.0	1	100.0	2	100.0	**110**	**100.0**
Piperacillin-tazobactam*	331	99.4	160	100.0	12	100.0	56	100.0	3	100.0	1	100.0	3	100.0	**566**	**99.6**
Tobramycin	268	99.3	142	100.0	11	100.0	36	100.0	1	100.0	1	100.0	3	100.0	**462**	**99.6**
EARS-NET 2021 KPN.3 Total	**4 282**	**96.5**	**2 165**	**96.0**	**386**	**94.8**	**912**	**97.5**	**33**	**93.9**	**16**	**87.5**	**35**	**97.1**	**7 829**	**96.3**

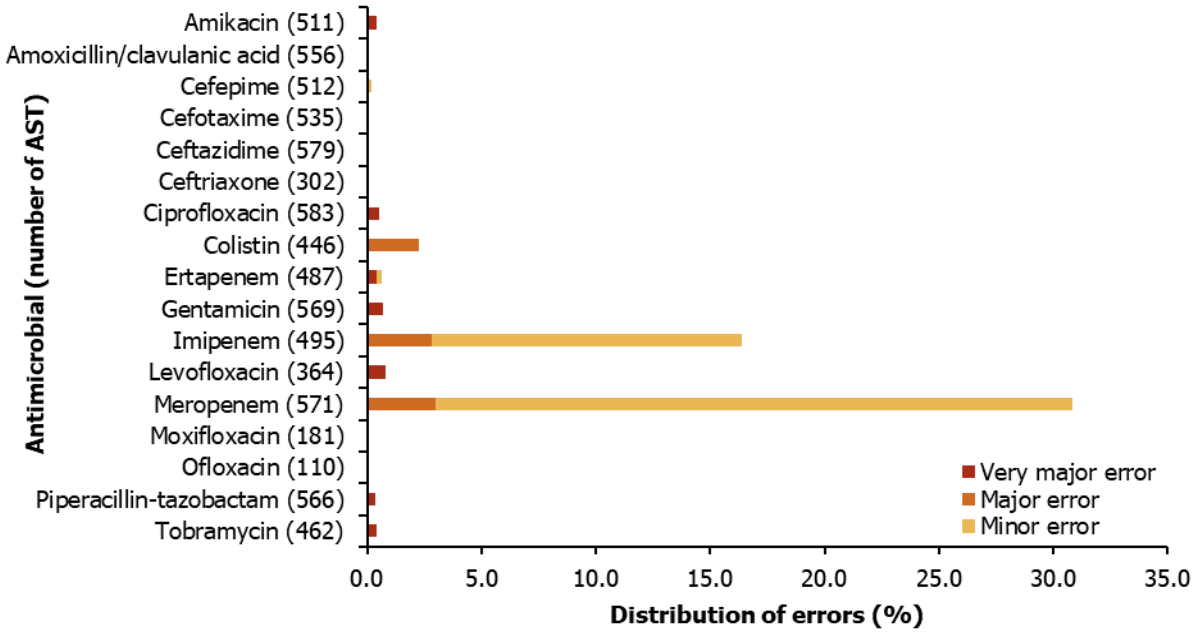
Note: Shaded cells highlight percentages of concordant results below the threshold of satisfactory concordance (80).
**Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, and reference results for piperacillin-tazobactam MICs relate to tests with a fixed concentration of 4 mg/L tazobactam.*

Table 17. Susceptibility of *Klebsiella pneumoniae* EARS-Net 2021 KPN.3 strain to imipenem and meropenem by method

Antimicrobial and expected interpretation	Imipenem - S						Meropenem - S					
Reported interpretation	S		I		R		S		I		R	
Method	n	%	n	%	n	%	n	%	n	%	n	%
Automated system	191	73.2	59	22.6	11	4.2	241	78.8	58	19.0	7	2.3
Disk/tablet diffusion	108	93.9	5	4.3	2	1.7	42	35.3	70	58.8	7	5.9
Gradient test	64	95.5	2	3.0	1	1.5	54	77.1	15	21.4	1	1.4
Agar dilution	6	100.0	-	-	-	-	6	75.0	2	25.0	-	-
Broth microdilution	43	97.7	1	2.3	-	-	52	78.8	13	19.7	1	1.5
Macro broth dilution (tubes)	-	-	-	-	-	-	-	-	1	100.0	-	-
Other	2	100.0	-	-	-	-	-	-	-	-	1	100.0
EARS-NET 2021 KPN.3 Total	**414**	**83.6**	**67**	**13.5**	**14**	**2.8**	**395**	**69.2**	**159**	**27.8**	**17**	**3.0**

S: 'susceptible, standard dosing regimen', I: 'susceptible, increased exposure'; R: resistant. The expected interpretation is highlighted in green.

Figure 8. Number of AST tests and distribution of minor, major and very major errors for the *Klebsiella pneumoniae* EARS-Net 2021 KPN.3 strain per antimicrobial



4. Discussion

In recent EARS-Net EQA exercises (2017, 2018 and 2019), between 952 and 970 laboratories in the EU/EEA signed up for the EQA, and between 860 and 893 laboratories submitted results [2,3,4]. There was no EARS-Net EQA exercise in 2020. However, this year's EQA exercise saw a decline in both the number of laboratories (n=642) and submitted results (n=592), mainly due to the ongoing SARS-CoV-2 virus pandemic (COVID-19) which required the allocation of laboratorial resources, but also due to the fact that the United Kingdom is no longer a Member State of the EU. In addition, since 2020, only laboratories using the EUCAST clinical guidelines when performing AST can participate in the EARS-Net EQA exercise. Nevertheless, in the 2021 exercise, a similar percentage of participating laboratories provided results (92.2%) compared to previous years (90.3% to 93.7%). When interpreting these results, it should be remembered that 909 laboratories from 26 countries were sent an invitation to the 2021 exercise.

The distribution of AST methods used in the 2021 EARS-Net EQA exercise is similar to those observed in 2017, 2018 and 2019: automated systems remain the most common method (54.7% of submitted results in 2021, and 40.7% to 50.8% in previous years), followed by disk diffusion or tablet diffusion (28.0% in 2021 and 35.2% to 47.7% previously) and MIC methods including broth microdilution and gradient test (16.8% in 2021 and 8.3% to 14.0% in previous years) [2,3,4].

Overall, by method the concordance of results with the expected interpretations varied between very good, good, and satisfactory in the 2021 EARS-Net EQA exercise. The lowest concordance was observed for agar dilution (84.4%), followed by the gradient test (87.6%). The remaining methods presented a very good concordance: 90.7% concordance for macro broth dilution (tubes), 92.4% for automated systems, 93.7% for disk diffusion or tablet diffusion and 94.2% for broth microdilution.

For the 2021 EARS-Net EQA exercise, the AST results were in very good concordance with the expected results for the six strains (92.7% out of 50 302 AST), and 129 laboratories (21.8%) reported excellent results, at 95% or higher levels of concordance with the expected interpretation.

Overall, the laboratories from two countries achieved an excellent level of concordance, laboratories from 23 countries achieved a very good level of concordance, and one country only achieved a satisfactory level of concordance (Figure 2). Results by country were not reported in previous EARS-Net EQA annual reports, therefore it is not possible to evaluate the progression over time.

For 89 (80.23%) of the 111 strain-antimicrobial combinations tested, an excellent concordance was observed, with more than 95% of the interpretations being in accordance with the expected interpretations. This represents an increase in the percentage of strain-antimicrobial combinations achieving excellent concordance compared with the EARS-Net EQA exercises from 2018 (80.0%) and 2019 (75.6%) [2,3]. However, it is important to remember that these exercises included other bacterial species which were not included in the 2021 EARS-Net EQA exercise.

The lowest level of concordance was observed for the *E. coli* strain EARS-Net 2021 EC.1, for which only 21.9% of the ciprofloxacin susceptibility test results were correct, followed by the *K. pneumoniae* strain EARS-Net 2021 KPN.2 when tested against imipenem (28.0%) and *E. coli* EARS-Net 2021 EC.3 results for meropenem (29.2%)

The **EARS-Net 2021 EC.1** strain presented resistant or 'susceptible, increased exposure' profiles towards fluoroquinolones, which proved difficult to detect. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, they can also be derived from the presence of one point mutation in the *gyrA* gene. This single point mutation confers borderline MIC values and inhibition zone diameters to fluoroquinolones, which can easily be misread or misinterpreted. Most of these deviations were very major errors (R → S) or minor errors (I → S), suggesting that decreased susceptibility to fluoroquinolones can potentially be under-reported in the EU/EEA. Moreover, the expected MIC value for ciprofloxacin was 0.5 mg/L, which corresponds to the area of technical uncertainty (ATU) for that antimicrobial in *Enterobacterales*. This further complicates proper determination of the susceptibility profiles, requiring repetition of the AST, the use of alternative methods or the downgrading of the susceptibility category.

Detection of resistant phenotypes was also problematic for tigecycline (R → S). The strain probably harbours currently unknown genes conferring tigecycline resistance, which can potentially contribute to the variability of results for this antimicrobial.

The strain was also resistant to amoxicillin, ampicillin, gentamicin, and tobramycin. The concordance of interpretations with these expected resistance profiles was excellent (≥95%).

The strain was 'S' to amoxicillin-clavulanic acid, but results showed a high percentage of major errors (S → R) that can be attributed to the inherent method variability and were potentially derived from the fact that the expected MIC value corresponds to a borderline concentration, increasing the probability of misclassification.

Concordance of results regarding the remaining antimicrobials, with expected 'S' profiles, was excellent (≥95%).

In total, 1 066 deviations were observed for this strain, 75.4% corresponded to under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 24.6% corresponded to over-reporting (S → I, S → R, I → R).

The **EARS-Net 2021 EC.2** strain was 'S' towards two carbapenems included in this EQA exercise. The susceptibility to meropenem and imipenem proved difficult to detect. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, they can also be derived from the differential expression of the $bla_{\text{OXA-244}}$ gene harboured by the strain, which can confer low levels of carbapenem resistance that are difficult to detect. Most of these deviations were minor errors (S → I), suggesting that decreased susceptibility to carbapenems may potentially be over-reported in the EU/EEA.

Conversely, detection of resistant phenotypes was problematic for ceftazidime (R → I), which presented a borderline expected MIC value, increasing the probability of misclassification.

The strain was also resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftriaxone, ertapenem and piperacillin-tazobactam. The concordance of interpretations with these expected resistance profiles was excellent (≥95%). Concordance of results for the remaining antimicrobials with expected 'S' profiles was also excellent (≥95%).

In total, 447 deviations were observed in this strain, 38.5% corresponded to under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 61.5% corresponded to over-reporting (S → I, S → R, I → R).

The **EARS-Net 2021 EC.3** strain was clinically resistant to ertapenem and imipenem and presented a 'susceptible, increased exposure' profile towards meropenem, all of which proved difficult to detect. While some of the deviations can be attributed to the inherent method variability and are within the acceptable variation range, they can also be derived from the differential expression of the $bla_{\text{VIM-1}}$ gene harboured by the strain. The deviations included minor errors (R → I and I → S) and very major errors (R → S), suggesting that there is a potential to observe under-reporting of carbapenem resistance in the EU/EEA. Laboratories under-reported resistant and 'susceptible, increased exposure' profiles in 24.6% of all results submitted for the three carbapenems (378/1 538). However, a not insignificant number of minor errors (I → R) indicates the opposite. Specifically, laboratories classified meropenem as resistant instead of as 'susceptible, increased exposure' in 16.3% of all results submitted for the carbapenems (250/1 538), equivalent to 43.9% of results submitted for meropenem. Therefore, although under-reporting of decreased susceptibility seems to be the most prevalent error, the possibility of over-reporting should not be ignored.

The strain was also resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, piperacillin-tazobactam and tobramycin. The concordance of interpretations with these expected resistance profiles was excellent (≥95%).

Interpretation of results was also problematic for gentamicin (S → R), which presented a borderline expected MIC value, increasing the probability of misclassification.

Concordance of results for the remaining antimicrobials with expected 'S' profiles was excellent for all except amikacin, which presented a very good concordance (>90% to <95%).

In total, 971 deviations were observed in this strain, 43% corresponded to under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 57% corresponded to over-reporting (S → I, S → R, I → R).

The **EARS-Net 2021 KPN.1** strain was clinically resistant to all antimicrobials included in this EQA exercise. The concordance between the submitted interpretations and the expected results was excellent (≥95%) for all antimicrobials.

All deviations observed in this strain (n=97) corresponded to under-reporting of decreased susceptibility profiles.

The **EARS-Net 2021 KPN.2** strain was resistant to cefepime, but concordance of results was not satisfactory, with a large percentage of minor errors (R → I). These types of errors were also common in imipenem results, for which the strain presented an expected 'susceptible, increased exposure' profile. The highest percentage of laboratories (41.1%) reported an 'S' profile (I → S) while others reported resistance (I → R) (30.8%). Furthermore, deviations in meropenem results were also observed, and mainly due to minor errors (S → I). These results imply that detection of carbapenem resistance in *K. pneumoniae* strains is complex and not properly harmonised across all EU/EEA settings. While some deviations can be attributed to the inherent method variability and are within the acceptable variation range, they might also be derived from the differential expression of the bla_{CMY-2} gene harboured by the strain. It has additionally been observed that, in some cases, the bla_{CMY-2} gene can be accompanied by reduced outer membrane permeability, mediated by decreased porin expression, which can increase the difficulty of correct AST determination.

The strain was also resistant to amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, ertapenem and piperacillin-tazobactam. The concordance of interpretations with these expected resistance profiles was excellent (≥95%). Concordance of results for the remaining antimicrobials, with expected 'S' profiles, was also excellent (≥95%), except for ofloxacin for which concordance was good (>85 - ≤90%).

In total, 794 deviations were observed in this strain, 51.3% corresponded to under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 48.7% corresponded to over-reporting (S → I, S → R, I → R).

The **EARS-Net 2021 KPN.3** strain was resistant to amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, ertapenem, gentamicin, levofloxacin, moxifloxacin, ofloxacin, piperacillin-tazobactam and tobramycin. The concordance of interpretations with these expected resistance profiles was excellent (≥95%).

The strain was 'S' to meropenem, but results did not achieve a satisfactory concordance, with a high percentage of minor errors (S → I). The expected MIC result for meropenem was a borderline concentration, increasing the probability of misclassification. The same type of errors were also observed for imipenem results but only when using the automated system. The inability to properly classify the strain as susceptible to these carbapenems points to a potential over-reporting of carbapenem resistance in EU/EEA settings.

Concordance of results for the remaining antimicrobials, with expected 'S' profiles, was excellent (≥95%).

In total, 287 deviations were observed for this strain, 7% corresponded to under-reporting of decreased susceptibility profiles, and 93% corresponded to over-reporting.

For all strains and antimicrobials tested in the 2021 EARS-Net EQA exercise, the gradient test failed to reach a satisfactory level in one strain, with errors largely restricted to carbapenems. Agar dilution results also failed to reach a satisfactory level in two strains. However, the low number of tests performed through agar dilution compared with other methods affects the calculated percentages. Macro broth dilution (tubes) did not reach satisfactory levels in one strain.

Overall, carbapenem susceptibility testing was the most problematic issue detected, especially for imipenem and meropenem, and in both bacterial species. Although under-reporting of decreased susceptibility profiles was frequently observed, the dominant problem was the over-reporting of these profiles.

The discrepancies observed for other antimicrobials were mostly restricted to particular strains and not common between all three strains of the same species. In *E. coli*, decreased susceptibilities towards fluoroquinolones, ceftazidime and tigecycline were under-reported. However, decreased susceptibilities towards amoxicillin-clavulanic acid and gentamicin were over-reported. In *K. pneumoniae*, the only problematic antimicrobial besides carbapenems was cefepime, showing under-reporting of decreased susceptibility profiles.

In total, the 3 662 AST results were reported with incorrect interpretations, 52.4% (n=1 918) corresponded to under-reporting of decreased susceptibility profiles (R → I, R → S, I → S), and 47.6% (n=1 744) corresponded to over-reporting (S → I, S → R, I → R).

In previous years, the problematic issues identified included [2,3,4]:

- *E. coli* with intermediate [past terminology] or R results for piperacillin-tazobactam;
- *E. coli* with R results for colistin;
- *E. coli* with S or R results for amoxicillin-clavulanic acid;
- *E. coli* with I results for ceftazidime;
- *K. pneumoniae* with differing third-generation cephalosporin results;

- *K. pneumoniae* with intermediate [past terminology] results for imipenem and meropenem;
- *K. pneumoniae* with intermediate [past terminology] results for amikacin.

This year, characterisation of piperacillin-tazobactam susceptibility profiles was excellent in all three *E. coli* strains, including two strains presenting resistant phenotypes. Furthermore, although all *E. coli* strains included in the 2021 EARS-Net EQA exercise were 'S' to colistin, one of the *K. pneumoniae* strains was resistant. Colistin susceptibility testing results were excellent for all six strains. Amikacin and third-generation cephalosporins results were in excellent concordance with expected phenotypes for all *K. pneumoniae* strains. However, discrepancies were observed for the fourth-generation cephalosporin (cefepime) in one *K. pneumoniae* strain.

These findings indicate that laboratories in the EU/EEA have successfully addressed some of their past shortcomings and increased their capacity for phenotypic AST. However, as described previously, the problems related to carbapenem, amoxicillin-clavulanic acid and ceftazidime susceptibility testing remain, as well as newly identified issues.

The issues not noted in the previous three years, but noted in the 2021 EARS-Net EQA exercise include:

- *E. coli* with I or R results for fluoroquinolones;
- *E. coli* with R results for tigecycline;
- *E. coli* with S results close to the breakpoint for gentamicin;
- *K. pneumoniae* with R result for cefepime.

Furthermore, minor errors were very prevalent in the 2021 EARS-Net EQA results. Although these deviations may be due to inherent variability associated with the laboratory methods, they may also reflect the fact that some participants do not strictly adhere to European guidelines when performing AST.

The 2021 EARS-Net EQA exercise was limited by not including species isolation. Moreover, by focusing on two species, *E. coli* and *K. pneumoniae*, the EQA does not provide an assessment of the other species included in EARS-Net surveillance. In addition, the 2021 EARS-Net EQA exercise further differs from previous EARS-Net EQA exercises by the exclusion of laboratories not using EUCAST clinical guidelines, the United Kingdom not being included in the exercise as it is no longer part of the EU/EEA, and the fact that the exercise was conducted during the COVID-19 pandemic, with three countries not participating.

5. Conclusions

Overall, only 21.8% of the laboratories participating in the 2021 EARS-Net EQA exercise achieved at least 95% of concordance with the expected AST results. Minor errors were very prevalent in this EARS-Net EQA exercise, and this may suggest that some participants do not always strictly adhere to the most current guidelines. Furthermore, certain antimicrobial classes presented higher percentages of deviations, namely fluoroquinolones and carbapenems. Fluoroquinolone susceptibility testing and interpretation was especially challenging for *E. coli*, while carbapenem susceptibility testing proved difficult for both *E. coli* and *K. pneumoniae*. Other problematic species-antimicrobial combinations were amoxicillin-clavulanic acid, ceftazidime, gentamicin, and tigecycline in *E.coli*, and cefepime in *K. pneumoniae*.

The findings indicate that AMR is heterogeneously reported in the EU/EEA. There was a tendency towards under-detection of reduced susceptibility towards fluoroquinolones, and reporting of carbapenem results showed both under- and overestimation of decreased susceptibility phenotypes, although the dominant problem was the over-reporting of these profiles. However, it should be noted that the strains harboured currently known genetic mechanisms associated with resistance towards these antimicrobial classes. Although genotypic characterisation of the strains was outside of the scope of this exercise, it is possible for the laboratories to screen for AMR determinants. Therefore, when considering both phenotypic and genotypic data, the final reporting of results could present lower proportions of deviations. Specifically, detection of a chromosomal point mutation in the gyrase gene in the EARS-Net 2021 EC.1 strain would probably lead to re-testing or re-evaluation of fluoroquinolone susceptibility test results, and to the potential correct classification of those I/R profiles. Detection of genes encoding extended-spectrum beta-lactamases or carbapenemases (as in EARS-Net 2021 EC.2, EARS-Net 2021 EC.3 and EARS-Net 2021 KPN.2) is also likely to promote increased attention in interpretation of cephalosporin and carbapenem susceptibility test results, or even confirmatory testing using other methods. However, one possible consequence of detecting these genes is the tendency to further over-report decreased susceptibility profiles.

The analysis of the performance of the different AST methods revealed few differences between methodologies, except for a poorer performance of the gradient test in the detection of reduced susceptibility towards carbapenems.

In conclusion, there is no exclusive pattern of over- or under-reporting decreased susceptibility profiles in the EU/EEA.

6. Recommendations

The 2021 EARS-Net EQA concluded that only 21.8% of the laboratories participating in the 2021 EARS-Net EQA exercise achieved at least 95% of concordance with the expected AST results, and specific areas of difficulty have been identified, namely: detection of decreased susceptibility towards fluoroquinolones in *E. coli*, proper characterisation of carbapenem phenotypes in both species, and detection of decreased susceptibility to carbapenems through the gradient test.

Furthermore, results from this EQA exercise indicate that both under- and overestimation of AMR percentages in Europe may be possible. Although genotypic analysis of AMR genes or chromosomal point mutations could potentially solve some of the deviations reported by the laboratories, the focus of this EQA exercise was phenotypic testing and the observed under- and overestimation should be kept in mind when interpreting EARS-Net surveillance data. Overall, surveillance or control efforts should consider the specific deviations observed for each specific antimicrobial or antimicrobial group.

Laboratories that participate in the EARS-Net surveillance scheme should review their individual performance in this EQA exercise and revisit all areas where they did not achieve the intended results. It would be advisable for several laboratories to review their methodologies as follows:

- Performing and reading results for fluoroquinolone susceptibility testing, due to inherent difficulties associated with the reading of these AST results. Reading and interpreting inhibition zone diameters when performing disk diffusion or tablet diffusion is notoriously difficult, thus special attention should be given to this issue and, if necessary, appropriate training established.
- Ensuring that they are familiar with the existence of ATU, and with the suggested EUCAST procedures to resolve AST results that fall within this category.
- Performing and reading results for carbapenem susceptibility testing, since results can vary due to differential expression of carbapenemase genes.

The observation that minor errors were very prevalent may be due to the inherent and acceptable variability of laboratory methods, but it can also suggest that some participants do not always strictly adhere to the most current guidelines. In such cases, laboratories should review their reporting practices and confirm that the protocols in use are in accordance with the latest EUCAST recommendations and guidelines, and that the most current break points are applied.

Furthermore, participants with poor performance, as described in their individual evaluation reports, should ensure that adequate internal quality control strains are being applied and monitored to ensure reliability of results, and that relevant quality management systems and control measures are in place.

Continued regular participation in the annual EQA exercise by the laboratories reporting to EARS-Net is required to evaluate and review their performance. It will also enable the identification and monitoring of those species-antimicrobial agent combinations that may be problematic when performing AST and for which improvement is possible, facilitating the correct interpretation of AST results reported to EARS-Net.

7. Feedback survey

A link to the feedback survey was shared with all contacts for the participating laboratories via email. The survey questions can be found in Annex 2. In total, 137 laboratories provided feedback (23.2% of the 591 laboratories for which results were interpreted).

After receiving the evaluation reports, 71 laboratories reported that they had taken corrective action. The main actions taken were re-testing of isolate(s), verification of reagents, reviews of Standard Operating Procedures, updating/validation of methods and training of laboratory personnel. For 24 laboratories, all EQA analytical test results conformed to expected results and no further action was taken.

Sixty-eight laboratories replied that they would use the results as documentation for accreditation and/or licensing purposes.

Most of the laboratories were satisfied with the individual evaluation report. To improve the report, it was suggested that information should be included on the expected qualitative results when using the disk diffusion method. Some laboratories expressed a desire to receive information on other laboratories, to be able to compare results, and some asked for information about the antimicrobial resistance genes. This information is available in the national summary report shared with the National EARS-Net EQA Coordinators at the same time as the evaluation reports are released.

The main suggestion to further improve the EQA scheme was to allow for more flexibility in the web tool when providing information on the methods used for the AST. A few laboratories suggested that the organisers provide more information on the origin of the strain to ensure correct interpretation of the results and to include more species covered in the EARS-Net surveillance.

A few laboratories reported that they had not received the respective evaluation report, even though the link to the evaluation report was shared in the same email as the link to the feedback survey, and other laboratories asked to receive the evaluation report sooner after the deadline for reporting results.

References

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Annex 1. List of participating countries

Table 1A. Number of laboratories receiving material and submitting results for the 2021 EARS-Net EQA exercise

EU/EEA country	Number of laboratories receiving material for the EQA exercise	Number of participating laboratories
Austria	33	33
Belgium	18	17
Bulgaria	25	24
Croatia	23	20
Cyprus	3	3
Czechia	49	43
Denmark	11	11
Estonia	11	10
Finland	17	15
Germany	31	30
Greece	20	17
Hungary	10	10
Iceland	2	2
Italy	59	58*
Lithuania	16	16
Luxembourg	5	5
Malta	1	1
Netherlands	17	17
Norway	14	13
Poland	59	58
Portugal	135	109
Romania	8	8
Slovakia	6	6
Slovenia	11	11
Spain	46	42
Sweden	12	12
Total	**642**	**591**

** One laboratory reported results using the Clinical and Laboratory Standards Institute (CLSI) guidelines and data from this laboratory were not included in the evaluation.*

Annex 2. Feedback survey questionnaire

Save a backup on your local computer (disable if you are using a public/shared computer)

EARS-Net EQA 2021 feedback survey

Disclaimer

The European Commission is not responsible for the content of questionnaires created using the EUSurvey service - it remains the sole responsibility of the form creator and manager. The use of EUSurvey service does not imply a recommendation or endorsement, by the European Commission, of the views expressed within them.

Dear Participant,
Recently you have participated in an ECDC external quality assessment exercise. To ensure maximum benefit we hereby invite you to answer this short survey. Please note ECDC will receive all your responses anonymised.

Fields marked with * are mandatory.

* **Question 1:** Regarding any of your analytical test results that did not conform to the expected results, can you specify which corrective action(s), if any, was/were taken (e.g. review and adjust SOPs, verify reagents)?

- ☐ Not applicable: all our EQA analytical test results conformed to expected results.
- ☐ No corrective actions for non-conformities were taken.
- ☐ Yes, corrective actions were taken.

Please specify what corrective actions were taken.

* **Question 2:** Are results of this EQA exercise to be used as documentation for accreditation and/or licensing purposes for the method(s) used in your laboratory?

- ☐ Yes.
- ☐ No.
- ☐ Not applicable.

Please specify.

Question 3: Were you satisfied with the EQA report of results specific to your laboratory?

- ☐ Yes.
- ☐ No.

If no, please specify.

Question 4: Do you have any suggestions that would make the EQA scheme more useful?

Question 5: Do you have any suggestions that would make the next EARS-Net EQA exercise better?

On behalf of the ECDC Antimicrobial Resistance and Healthcare-Associated Infections Disease Programme and the Technical University of Denmark (DTU), many thanks for your participation in this EQA exercise and follow-up survey. The anonymised results will be summarised in the final EQA exercise report and aggregated to monitor the Member States' benefits from all EQA exercises commissioned each year by ECDC.

Submit

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