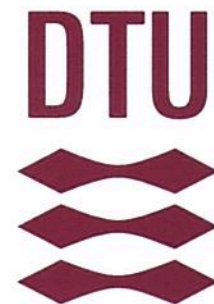


The External Quality Assessment (EQA) of performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2021

National summary report for Austria

Provider of the EARS-Net EQA:
Dept. for Global Surveillance
Global Capacity building
National Food Institute
Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby, Denmark



EARS-Net EQA Coordinator: Birgitte Helwich
earsnet-eqa@food.dtu.dk
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1. Introduction

This report describes and summarises the national results of the external quality assessment (EQA) of performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2021. Participating laboratories are identified by codes known by the corresponding laboratory, the national EQA coordinator and the EQA provider. The correspondence between the codes and laboratories can be found as an Appendix to this report.

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

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

The 2021 EQA focuses on antimicrobial susceptibility testing (AST) of *Escherichia coli* and *Klebsiella pneumoniae*.

In total, 64 laboratories from Austria were invited to participate in the 2021 EARS-Net EQA, 33 laboratories signed up and received the 6 strains for analysis, and 33 laboratories submitted data for evaluation.

2. Materials and Methods

2.1 Strains and antimicrobial susceptibility testing

Three *Escherichia coli* and three *Klebsiella pneumoniae* strains were selected for this EQA from the strain collection at DTU Food based on their antimicrobial resistance profiles. Expected AST results were generated by performing minimum inhibitory concentration (MIC) determinations through broth microdilution (BMD) for all test strains in duplicate at the Technical University of Denmark, National Food Institute (DTU Food). The AST profiles were validated by two reference laboratories: The Centre for Disease Control and Prevention (CDC), Georgia, US and EUCAST Development Laboratory (EUCAST), Uppsala, Sweden. Expected MIC values for each antimicrobial and strain combination were determined by the consensus BMD results obtained by DTU Food and EUCAST and are presented in Table 1 and 2. Subsequently, the results were genotypically compared to acquired resistance genes and chromosomal point mutations by whole genome sequencing and using bioinformatic tool ResFinder v4.1 (Table 3 and 4). Finally, a MIC determination was performed at DTU Food after preparation of the agar stab culture/charcoal swab for shipment to participants to confirm that the vials contained the correct strains with the expected MIC values.

The antimicrobial agents selected for this EQA correspond to the panel of pathogen and antimicrobial agent combinations under surveillance by EARS-Net presented in the antimicrobial resistance (AMR) reporting protocol 2020¹ except for netilmicin that was not included in the 2021 EARS-Net EQA.

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

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

Table 1. EUCAST clinical breakpoints, expected MIC value and interpretation for the *Escherichia coli* strains

Antimicrobial	EUCAST clinical breakpoints MIC (µg/mL)		EARS-Net 2021 EC.1		EARS-Net 2021 EC.2		EARS-Net 2021 EC.3	
	S ≤	R >	Expected MIC value (µg/mL)	Expected interpretation	Expected MIC value (µg/mL)	Expected interpretation	Expected MIC value (µg/mL)	Expected interpretation
Amikacin	8	8	= 2	S	= 2	S	= 1	S
Amoxicillin	8	8	> 32	R	> 32	R	> 32	R
Amoxicillin/ clavulanic acid fixed conc*	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 constant 4*	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

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

**There was no expected interpretation for norfloxacin because in blood stream infections (BSI) norfloxacin is not an appropriate agent since breakpoints refer to uncomplicated urinary tract infections (uUTI) only, and the three *Escherichia coli* strains included in the 2021 EARS-Net EQA were BSI isolates.

Table 2. EUCAST clinical breakpoints, expected MIC value and interpretation for the *Klebsiella pneumoniae* strains

Antimicrobial	EUCAST clinical breakpoints MIC (µg/mL)		EARS-Net 2021 KPN.1		EARS-Net 2021 KPN.2		EARS-Net 2021 KPN.3	
	S ≤	R >	Expected MIC value (µg/mL)	Expected interpretation	Expected MIC value (µg/mL)	Expected interpretation	Expected MIC value (µg/mL)	Expected interpretation
Amikacin	8	8	> 128	R	= 1	S	> 128	R
Amoxicillin/ clavulanic acid fixed conc*	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 constant 4*	8	8	> 64	R	> 64	R	> 64	R
Tobramycin	2	2	> 16	R	= 0.5	S	> 16	R

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

**There was no expected interpretation for norfloxacin because in blood stream infections (BSI) norfloxacin is not an appropriate agent since breakpoints refer to uncomplicated urinary tract infections (uUTI) only, and the three *Klebsiella pneumoniae* strains included in the 2021 EARS-Net EQA were BSI isolates.

Table 3. Antimicrobial resistance genes and chromosomal point mutations detected in the *Escherichia coli* strains through analysis with ResFinder 4.1

Antimicrobial	EARS-Net 2021 EC.1*	EARS-Net 2021 EC.2**	EARS-Net 2021 EC.3***
Amikacin			
Amoxicillin	<i>bla</i> _{TEM-1}	<i>bla</i> _{TEM-1}	<i>bla</i> _{VIM-1}
Amoxicillin/clavulanic acid fixed conc		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Ampicillin	<i>bla</i> _{TEM-1}	<i>bla</i> _{TEM-1}	<i>bla</i> _{VIM-1}
Cefepime		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Cefotaxime		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Ceftazidime		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Ceftriaxone		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Ciprofloxacin	<i>gyrA</i> S83L		
Colistin			
Ertapenem		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Gentamicin	<i>aac(3)-IId</i>		
Imipenem			<i>bla</i> _{VIM-1}
Levofloxacin	<i>gyrA</i> S83L		
Meropenem			<i>bla</i> _{VIM-1}
Moxifloxacin	<i>gyrA</i> S83L		
Norfloxacin	<i>gyrA</i> S83L		
Ofloxacin	<i>gyrA</i> S83L		
Piperacillin/tazobactam constant 4		<i>bla</i> _{OXA-244} , <i>bla</i> _{CTX-M-14b}	<i>bla</i> _{VIM-1}
Tigecycline	ND		
Tobramycin	<i>aac(3)-IId</i>		<i>aac(6')-Ib-cr</i>

* Additional resistance genes detected: *sul2*, *dfrA5*, *tet(A)*, *aph(6)-IId*, *aph(3'')-Ib*,

** Additional resistance genes detected: *catA1*, *sul2*, *dfrA1*, *tet(D)*, *aph(3'')-Ib*, *aph(6)-IId*, *addA1*

*** Additional resistance genes detected: *aadA1*, *tet(39)*

Table 4. Antimicrobial resistance genes and chromosomal point mutations detected in the *Klebsiella pneumoniae* strains through analysis with ResFinder 4.1

Antimicrobial	EARS-Net 2021 KPN.1*	EARS-Net 2021 KPN.2**	EARS-Net 2021 KPN.3***
Amikacin	<i>rmtB</i> , <i>aac(6')-Ib-cr</i>		<i>armA</i> , <i>aac(6')-Ib-cr</i>
Amoxicillin/clavulanic acid fixed conc	<i>bla_{OXA-181}/bla_{OXA-1}</i> , <i>bla_{NDM-5}</i> , <i>bla_{SHV-26}</i>	<i>bla_{SHV-110}</i> , <i>bla_{CMY-2}</i>	<i>bla_{TEM-1B}</i> , <i>bla_{SHV-11}</i> , <i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Cefepime	<i>bla_{OXA-181}/bla_{OXA-1}</i> , <i>bla_{NDM-5}</i> , <i>bla_{CTX-M-15}</i>	<i>bla_{CMY-2}</i>	<i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Cefotaxime	<i>bla_{NDM-5}</i> , <i>bla_{CTX-M-15}</i>	<i>bla_{CMY-2}</i>	<i>bla_{CTX-M-15}</i>
Ceftazidime	<i>bla_{NDM-5}</i> , <i>bla_{CTX-M-15}</i>	<i>bla_{CMY-2}</i>	<i>bla_{CTX-M-15}</i>
Ceftriaxone	<i>bla_{CTX-M-15}</i>	<i>bla_{CMY-2}</i>	<i>bla_{CTX-M-15}</i>
Ciprofloxacin	<i>aac(6')-Ib-cr</i> , <i>qnrS1</i> , <i>gyrA</i> S83F, <i>gyrA</i> D87N, <i>parC</i> E84K		<i>aac(6')-Ib-cr</i> , <i>qnrB1</i> , <i>gyrA</i> D87A, <i>parC</i> S80I
Colistin	ND		
Ertapenem	<i>bla_{OXA-181}</i> , <i>bla_{NDM-5}</i>	<i>bla_{CMY-2}</i>	<i>bla_{CTX-M-15}</i>
Gentamicin	<i>rmtB</i>		<i>armA</i>
Imipenem	<i>bla_{OXA-181}</i> , <i>bla_{NDM-5}</i>	<i>bla_{CMY-2}</i>	
Levofloxacin	<i>aac(6')-Ib-cr</i> , <i>qnrS1</i> , <i>gyrA</i> S83F, <i>gyrA</i> D87N, <i>parC</i> E84K		<i>aac(6')-Ib-cr</i> , <i>qnrB1</i> , <i>gyrA</i> D87A, <i>parC</i> S80I
Meropenem	<i>bla_{OXA-181}</i> , <i>bla_{NDM-5}</i>		
Moxifloxacin	<i>aac(6')-Ib-cr</i> , <i>qnrS1</i> , <i>gyrA</i> S83F, <i>gyrA</i> D87N, <i>parC</i> E84K		<i>aac(6')-Ib-cr</i> , <i>qnrB1</i> , <i>gyrA</i> D87A, <i>parC</i> S80I
Norfloxacin	<i>aac(6')-Ib-cr</i> , <i>qnrS1</i> , <i>gyrA</i> S83F, <i>gyrA</i> D87N, <i>parC</i> E84K		<i>aac(6')-Ib-cr</i> , <i>qnrB1</i> , <i>gyrA</i> D87A, <i>parC</i> S80I
Ofloxacin	<i>aac(6')-Ib-cr</i> , <i>qnrS1</i> , <i>gyrA</i> S83F, <i>gyrA</i> D87N, <i>parC</i> E84K		<i>aac(6')-Ib-cr</i> , <i>qnrB1</i> , <i>gyrA</i> D87A, <i>parC</i> S80I
Piperacillin – tazobactam constant 4	<i>bla_{OXA-181}/bla_{OXA-1}</i> , <i>bla_{NDM-5}</i> , <i>bla_{SHV-26}</i>	<i>bla_{SHV-110}</i> , <i>bla_{CMY-2}</i>	<i>bla_{TEM-1B}</i> , <i>bla_{SHV-11}</i> , <i>bla_{OXA-1}</i> , <i>bla_{CTX-M-15}</i>
Tobramycin	<i>rmtB</i> , <i>aac(6')-Ib-cr</i>		<i>armA</i> , <i>aac(6')-Ib-cr</i>

*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 obtained interpretations for norfloxacin were not scored because in blood stream infections (BSI) norfloxacin is not an appropriate agent since breakpoints refer to uncomplicated urinary tract infections (uUTI) only, and the six strains included in the 2021 EARS-Net EQA were BSI isolates.

For the EARS-Net 2021 EC.1-3 and EARS-Net 2021 KPN.2 strains, reporting S is technically correct but wrong since the breakpoint is only valid for uUTI, and reporting R is technically wrong but could be considered the appropriate response since the breakpoint is not valid. Should you have used norfloxacin to exclude fluoroquinolone resistance, the report should have been converted to an S for ciprofloxacin and/or levofloxacin.

For the EARS-Net 2021 KPN.1 and EARS-Net 2021 KPN.3 strains there are clear and obvious resistance mechanisms to fluoroquinolones, therefore a report of R is technically correct but should have been converted to ciprofloxacin and/or levofloxacin R since norfloxacin should not have been reported for a BSI isolate.

2.3 Procedure

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

All participating laboratories were invited to enter the obtained results into the EARS-Net EQA web-based database using a secure personal login and password.

The deadline for submission of data was 24 August 2021, however it was extended until 9 September 2021. The web tool was closed on 15 September. The results were evaluated using a score algorithm which marked correct interpretation as "correct" and "incorrect" the incorrect interpretation of AST results.

The individual database-generated reports, which contained evaluations of the submitted interpretations, including possible deviations from the expected interpretations, were forwarded to the national EQA coordinators. At the same time the participants received an email with their respective report attached and information on how to retrieve the same report by logging into the webtool.

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

3. Results

3.1 Methods

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). Only the categorisation was evaluated, whereas the MIC values were used as supplementary information.

In total, 33 laboratories from Austria submitted results for the three *E. coli* strains and the three *K. pneumoniae* strains, and all the laboratories used the EUCAST guideline when performing the AST.

For determination of the AST results for the *E. coli* and *K. pneumoniae* strains, the most used

method was Automated system (45.4%) for the *E. coli* strains and Automated system (45.3%) for the *K. pneumoniae* strains (Table 5).

Table 5. Overview of the methods used for determination of the AST results

Austria	EARS-Net EQA <i>Escherichia coli</i> strain 1-3		EARS-Net EQA <i>Klebsiella pneumoniae</i> strain 1-3		Total	
	Number of AST performed	%	Number of AST performed	%	Number of AST performed	%
Automated system	747	45.4	639	45.3	1,386	45.4
Disk/Tablet diffusion	725	44.0	637	45.2	1,362	44.6
Gradient test	76	4.6	42	3.0	118	3.9
MIC – Broth microdilution	67	4.1	68	4.8	135	4.4
MIC – Agar dilution	31	1.9	24	1.7	55	1.8
MIC – Macrobrot h dilution (tubes)	-	-	-	-	-	-
Other	-	-	-	-	-	-
Total	1,646	100.0	1,410	100.0	3,056	100.0

None of the laboratories would send strain EARS-Net 2021 EC.1 to a reference or other laboratory for further testing, and 66.7% and 72.7% would send strain EARS-Net 2021 EC.2 and EARS-Net 2021 EC.3 for further testing, respectively.

In total, 84.8% of the laboratories would send strain EARS-Net 2021 KPN.1 to a reference or other laboratory for further testing, and 33.3% and 39.4% would send strain EARS-Net 2021 KPN.2 and EARS-Net 2021 KPN.3 for further testing, respectively.

3.2 Deviations

Table 6 and 7 present the percentage of AST results in concordance with the expected interpretation per antimicrobial for each strain. The concordance ranged from 89.4% (EARS-Net 2021 EC.1) to 100.0% (EARS-Net 2021 KPN.1).

Table 6. Percentage of correct AST results per antimicrobial for *E. coli* strains

Antimicrobial*	EARS-Net 2021 EC.1		EARS-Net 2021 EC.2		EARS-Net 2021 EC.3	
	Concordance (%)	Number of AST performed	Concordance (%)	Number of AST performed	Concordance (%)	Number of AST performed
Amikacin	100.0%	31	100.0%	31	100.0%	31
Amoxicillin	100.0%	15	100.0%	15	100.0%	15
Amoxicillin/clavulanic acid fixed conc	93.8%	32	100.0%	32	100.0%	32
Ampicillin	100.0%	33	100.0%	33	100.0%	33
Cefepime	100.0%	33	100.0%	33	97.0%	33
Cefotaxime	96.9%	32	100.0%	32	100.0%	32
Ceftazidime	100.0%	32	68.8%	32	100.0%	32
Ceftriaxone	100.0%	19	100.0%	18	100.0%	18
Ciprofloxacin	12.1%	33	100.0%	33	100.0%	33
Colistin	100.0%	22	100.0%	24	95.8%	24
Ertapenem	100.0%	25	96.2%	26	92.0%	25
Gentamicin	100.0%	33	100.0%	33	56.3%	32
Imipenem	100.0%	30	93.3%	30	93.3%	30
Levofloxacin	23.1%	26	100.0%	26	100.0%	26
Meropenem	100.0%	33	66.7%	33	42.4%	33
Moxifloxacin	91.7%	24	100.0%	24	100.0%	24
Ofloxacin	100.0%	8	100.0%	7	100.0%	7
Piperacillin/ tazobactam constant	100.0%	32	100.0%	32	100.0%	32
Tigecycline	87.5%	32	96.9%	32	96.9%	32
Tobramycin	100.0%	24	100.0%	24	100.0%	23
Austria Total	89.4%	549	95.5%	550	92.7%	547

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

Table 7. Percentage of correct AST results per antimicrobial for *K. pneumoniae* strains

Antimicrobial*	EARS-Net 2021 KPN.1		EARS-Net 2021 KPN.2		EARS-Net 2021 KPN.3	
	Concordance (%)	Number of AST performed	Concordance (%)	Number of AST performed	Concordance (%)	Number of AST performed
Amikacin	100.0%	31	100.0%	31	100.0%	31
Amoxicillin/clavulanic acid fixed conc	100.0%	32	100.0%	32	100.0%	32
Cefepime	100.0%	33	72.7%	33	100.0%	33
Cefotaxime	100.0%	32	100.0%	32	100.0%	32
Ceftazidime	100.0%	31	100.0%	32	100.0%	32
Ceftriaxone	100.0%	18	100.0%	18	100.0%	18
Ciprofloxacin	100.0%	33	100.0%	33	100.0%	33
Colistin	100.0%	26	95.7%	23	100.0%	23
Ertapenem	100.0%	25	100.0%	26	100.0%	26
Gentamicin	100.0%	33	100.0%	33	100.0%	33
Imipenem	100.0%	30	27.6%	29	90.0%	30
Levofloxacin	100.0%	26	100.0%	26	100.0%	26
Meropenem	100.0%	32	72.7%	33	60.6%	33
Moxifloxacin	100.0%	24	91.7%	24	100.0%	24
Ofloxacin	100.0%	8	100.0%	7	100.0%	8
Piperacillin/ tazobactam constant	100.0%	32	100.0%	32	100.0%	32
Tobramycin	100.0%	25	95.8%	24	100.0%	25
Austria Total	100.0%	471	90.8%	468	96.6%	471

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

Figure 1 illustrates the overall percentage of deviations in AST results for each participating laboratory. In total, 6.0% of the AST performed by the 33 laboratories deviated from the expected results.

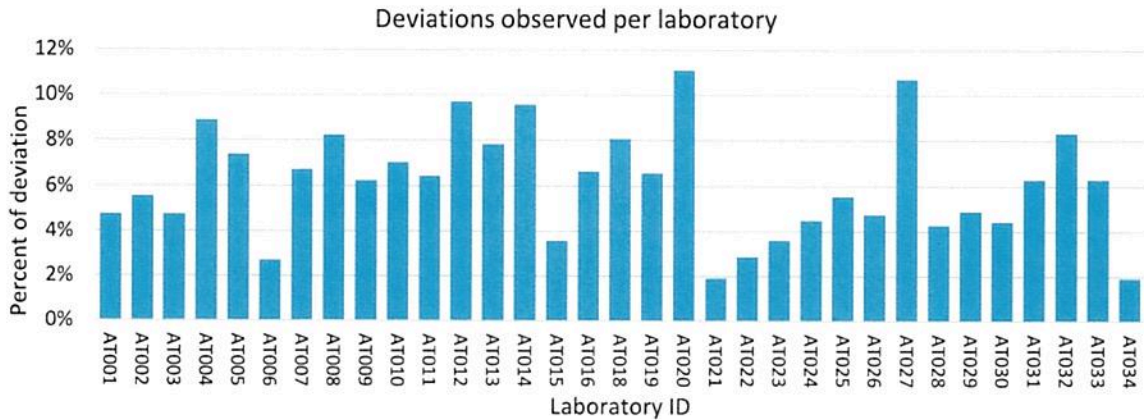


Figure 1. Percentage of deviation from expected interpretation of AST results for all strain/antimicrobial combinations

Figure 2 illustrates the percentage of deviations of AST results for each participating laboratory for *E. coli*. In total, 7.5% of the AST performed by the 33 laboratories deviated from the expected results for *E. coli*.

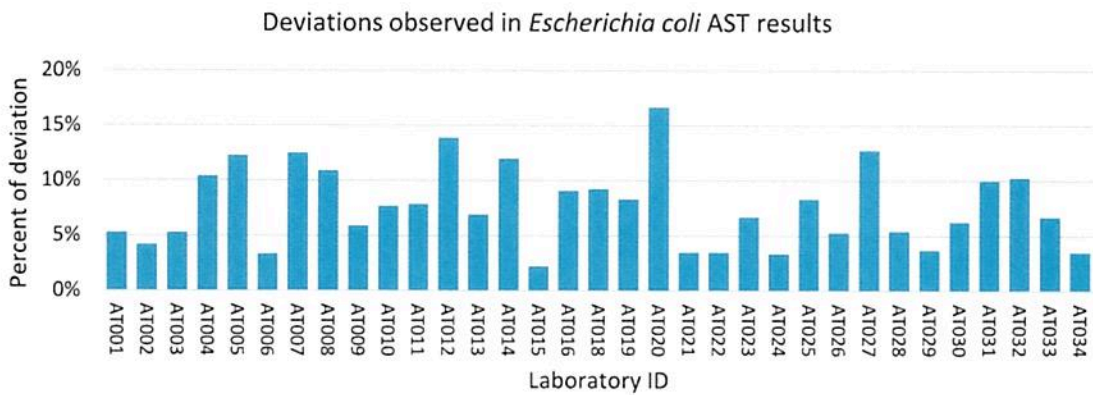


Figure 2. Percentage of deviations observed in all *E. coli* AST results, for each individual participating laboratory

Figure 3 illustrates the percentage of deviations of AST results for each participating laboratory for *K. pneumoniae*. In total, 4.2% of the AST performed by the 33 laboratories deviated from the expected results for *K. pneumoniae*.

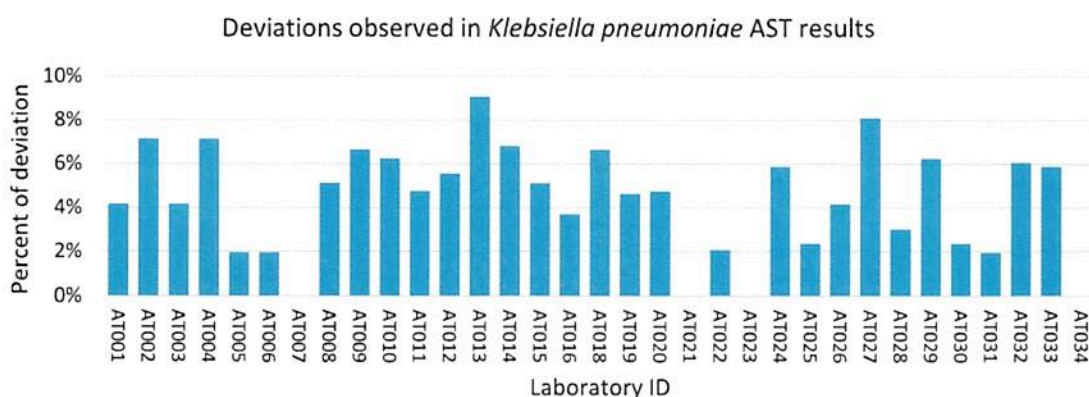


Figure 3. Percentage of deviations observed in all *K. pneumoniae* AST results, for each individual participating laboratory

Table 8 presents the percentage of deviations from the expected interpretations for the different methods used when testing the six strains against the list of antimicrobials.

Results from the 33 laboratories showed the highest level of concordance with the expected interpretation for the EARS-Net 2021 KPN.1 strain (100.0%) and the lowest level of concordance for the EARS-Net 2021 EC.1 strain (89.4%). Further, results showed the lowest level of concordance was reported with using the MIC–Agar dilution (85.5%).

Table 8. Percentage of deviations per method for the six different strains

Method	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	
	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed	Deviation (%)	Number of AST performed
Automated system	10.0%	251	5.6%	248	10.1%	248	0.0%	213	10.8%	212	3.7%	214	6.9%	1,386
Disk/Tablet diffusion	9.9%	242	4.1%	243	3.3%	240	0.0%	212	8.6%	210	3.7%	215	5.0%	1,362
Gradient test	26.9%	26	0.0%	25	8.0%	25	0.0%	14	0.0%	14	0.0%	14	7.6%	118
MIC–Broth microdilution	0.0%	21	0.0%	23	4.3%	23	0.0%	24	4.5%	22	0.0%	22	1.5%	135
MIC–Agar dilution	22.2%	9	9.1%	11	36.4%	11	0.0%	8	10.0%	10	0.0%	6	14.5%	55
Austria Total	10.6%	549	4.5%	550	7.3%	547	0.0%	471	9.2%	468	3.4%	471	6.0%	3,056

4. Conclusions and recommendation for improvement

For the 2021 EARS-Net EQA, the overall deviation level for the six strains was 6.0% out of 3,056 tests performed by the 33 laboratories, with 13 laboratories (39.4%) meeting the level of 95% concordance.

The following methodologies were applied by the laboratories when performing the 3,056 tests: Automated system (45.4%), Disk/Tablet diffusion (44.6%), Gradient test (3.9%), MIC – Broth microdilution (4.4%), MIC – Agar dilution (1.8%), MIC – Broth macrodilution/Tubes (0%), and Other (0%).

In Austria, most deviations from the expected interpretations were observed when testing the following antimicrobials: meropenem and fluoroquinolones for the *E. coli* strains, and meropenem for the *K. pneumoniae* strains.

4.1 *Escherichia coli* strains

For the *E. coli* strains, 0 laboratories were in full concordance with the expected interpretations, 8 laboratories had ≥ 95% concordance with the expected interpretation, 16 laboratories had < 95% and ≥ 90% concordance, and 9 laboratories had < 90% concordance.

Strain EARS-Net 2021 EC.1

Most deviations were observed for ciprofloxacin and levofloxacin and reported when using the Automated system and Disk/Tablet diffusion. While some can be attributed to the inherent method variability and are in the acceptable variation range, they can also be derived from the presence of one point mutation in the *gyrA* gene. This single point mutations confers borderline MIC values and inhibition zone diameters to ciprofloxacin and levofloxacin, which can easily be misread or misinterpreted.

Strain EARS-Net 2021 EC.2

Most deviations were observed for ceftazidime and meropenem. Ceftazidime deviations were exclusively reported when using the Automated system and these seem to be due to the inherent method variability and are in the acceptable variation range. For meropenem, deviations were reported using the Automated system and Disk/Tablet diffusion, and while some can be attributed to the method variability, they can also be derived from the differential expression of the *bla_{OXA-244}* gene harboured by the strain, which can confer difficult to detect low levels of carbapenem resistance.

Strain EARS-Net 2021 EC.3

Most deviations were observed for gentamicin and meropenem. Gentamicin deviations were mainly reported when using the Automated system and Disk/Tablet diffusion, and these seem to be due to the inherent method variability and are in the acceptable variation range. For meropenem, deviations were reported mainly using the Automated system and Disk/Tablet diffusion, and while some can be attributed to the method variability, they can also be derived from the differential expression of the *bla_{VIM-1}* gene harboured by the strain.

4.2 *Klebsiella pneumoniae* strains

For *K. pneumoniae* strains, 4 laboratories were in full concordance with the expected interpretations, 14 laboratories had $\geq 95\%$ concordance with the expected interpretation, and 15 laboratories had $< 95\%$ and $\geq 90\%$ concordance.

Strain EARS-Net 2021 KPN.1

There was 100% concordance with the expected results.

Strain EARS-Net 2021 KPN.2

Most deviations were observed for cefepime, imipenem and meropenem, and reported when using the Automated system and Disk/Tablet diffusion. While some can be attributed to the inherent method variability and are in the acceptable variation range, they can 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 raise the difficulty of proper AST determination.

Strain EARS-Net 2021 KPN.3

Most deviations were observed for meropenem and reported when using the Automated system and Disk/Tablet diffusion. These seem to be due to the inherent method variability and are in the acceptable variation range.

4.3 Recommendations

We recommend the following actions to identify root causes to address the observed deviations:

- Confirm the protocols in use are in accordance with the latest EUCAST recommendations and guidelines and that the most current break points are applied
- Ensure the adequate control strains are being applied and monitored to ensure reliability of results
- Ensure that relevant quality management systems and control measures are in place
- Be aware of method variability when applying the Automated system and Disk/Tablet diffusion methods
- Consider additional training of technical staff to enhance capabilities and performance
- Be aware and potentially seek consultancy on reading fluoroquinolones results when using the Automated system and Disk/Tablet diffusion methodology. There are inherent difficulties associated with reading these results and reading the inhibition zone diameters when performing Disk/Tablet diffusion is notoriously difficult. Thus, special attention should be given to this issue and, if needed, establish the adequate training.
- Be aware and potentially seek consultancy around reading meropenem results when using the Automated system and Disk/Tablet diffusion methodology due to differential expression of carbapenemase genes.