TECHNICAL REPORT

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

2018

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External quality assessment (EQA) of performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2018

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Cover picture: Methicillin-resistant *Staphylococcus aureus* bacteria by the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Image licensed under a Creative Commons attribution 2.0 generic license.

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Abbreviations

AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EQA	External quality assessment
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
I	Intermediate[1]
MIC	Minimum inhibitory concentration
R	Resistant
S	Susceptible
UK NEQAS	United Kingdom National External Quality Assessment Services

[1] In the latest breakpoint tables for interpretation of MICs and zone diameters valid since 1 January 2019, EUCAST changed the definition of 'I' from 'intermediate' to 'susceptible, increased exposure'. Since this EQA exercise was conducted in September 2018 (i.e. before the new definition was implemented) EQA results were interpreted according to the previous definition of 'I' (i.e. 'intermediate').

Executive summary

This report provides an analysis of the external quality assessment (EQA) performance with antimicrobial susceptibility testing (AST) of laboratories participating in the European Antimicrobial Resistance Surveillance Network in 2018. A total of 860 laboratories (1 – 114 per country) from 30 EU/EEA countries participated in the EQA exercise. Six bacterial strains were used: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumoniae*.

For species identification, 77.1% laboratories used an automated instrument and 19.4% used conventional methods. Overall, 99.1% identifications were correct and there were no significant issues arising with species identification. For the determination of AST results, most laboratories used either automated methods, disk diffusion or MIC methods. For AST, there was a continuing trend towards increasing use of EUCAST methodology [1] (87.7%) and decreasing use of CLSI guidelines [2] (8.6%, 11 countries) in 2018. Overall, AST performance was satisfactory.

The *E. faecium* strain (specimen 4920) was resistant to amoxicillin/ampicillin, teicoplanin and vancomycin, but did not express high-level gentamicin resistance. A concordance was not achieved for the detection of high-level gentamicin resistance and participants applying the EUCAST guidelines and using disk or gradient diffusion methods were more likely to obtain the intended result than those using automated or broth microdilution methods. An excellent concordance of results was seen for the penicillins and glycopeptides tested.

The *K. pneumoniae* strain (specimen 4921), produced an OXA-48 enzyme. The strain was susceptible to aminoglycosides, fluoroquinolones and colistin, susceptible/intermediate to third-generation cephalosporins, intermediate/resistant to carbapenems and resistant to amoxicillin/ampicillin and inhibitor combinations. There was an excellent concordance of results for 10 antimicrobial agents and a concordance for ceftazidime and ceftriaxone, but a low concordance was achieved for cefotaxime, imipenem and meropenem. For cefotaxime (MIC 2 mg/L), the intended result was intermediate[2] and the MIC was close to the breakpoint. Participants provided the following results: 57.1% susceptible; 31.7% intermediate and 11.3% resistant. Participants using the EUCAST disk diffusion method were more likely to provide the intended results than other participants. For imipenem and meropenem, the intended results (MIC 4 mg/L) were intermediate and were also close to both susceptible and resistant breakpoints. For imipenem, participants provided the following results: 29.4% susceptible; 44.0% intermediate and 26.6% resistant. For meropenem, participants provided the following results: 37.6% susceptible; 44.8% intermediate and 17.6% resistant. For both imipenem and meropenem, participants who used the EUCAST disk diffusion method were the most likely to provide the intended result, with correct results in 65.7% and 66.5% of the participating laboratories, respectively.

The *E. coli* strain (specimen 4922) possessed the *mcr-1* gene, exhibiting resistance to amoxicillin/ampicillin, fluoroquinolones and colistin. The strain was susceptible to other beta-lactams, inhibitor combinations and aminoglycosides. There was an excellent concordance for 11 antimicrobial agents and a very good concordance for ofloxacin and piperacillin-tazobactam, but a low concordance was achieved for amoxicillin-clavulanic acid and colistin. The intended result for amoxicillin-clavulanic acid was susceptible (MIC 8 mg/L), close to the susceptible breakpoint (S≤8, R>8) following EUCAST guidelines. Participants provided the following results: 58.2% susceptible; 2.4% intermediate and 39.5% resistant. Participants following EUCAST disk or gradient diffusion methods were most likely to achieve the intended result and participants following EUCAST automated methods were least likely to achieve the intended result. The intended result for colistin was resistant (MIC 4 mg/L). Participants provided the following results: 30.2% susceptible; 0.6% intermediate and 69.2% resistant. There is no CLSI breakpoint and EUCAST recommends that this test is only undertaken using broth microdilution. Fifty-five participants reported using a EUCAST disk diffusion method although no EUCAST zone diameter breakpoints are provided in the 2018 EUCAST guidelines. There is no intermediate category for either amoxicillin-clavulanic acid or colistin susceptibility in the 2018 EUCAST guidelines, so participants with an intermediate result who reported that they followed EUCAST automated or MIC methodology may need to review their methodology.

The *S. aureus* strain (specimen 4923) was resistant to beta-lactams, fluoroquinolones, clindamycin, erythromycin, gentamicin and rifampicin. It was susceptible to fusidic acid, linezolid, tetracycline and glycopeptides. An excellent concordance was achieved with all 13 antimicrobial agents tested; there were no problems with the AST of this strain.

The *P. aeruginosa* strain (specimen 4924) was susceptible to aminoglycosides, ceftazidime, piperacillin-tazobactam and colistin. It was resistant to imipenem, meropenem, ciprofloxacin and levofloxacin. An excellent concordance was achieved for amikacin, ciprofloxacin, imipenem, levofloxacin, meropenem and tobramycin. A very good

[2] In the latest breakpoint tables for interpretation of MICs and zone diameters valid since 1 January 2019, EUCAST changed its definition of 'I' from 'intermediate' to 'susceptible, increased exposure'. Since this EQA exercise was conducted in September 2018 (i.e. before the new definition was implemented) EQA results were interpreted according to the previous definition of 'I' (i.e. 'intermediate'.)

concordance was achieved for colistin and gentamicin. A low concordance was achieved for ceftazidime and piperacillin-tazobactam. The intended result for ceftazidime was susceptible (MIC 4 mg/L). Participants provided the following results: 74.4% susceptible; 4.3% intermediate and 21.4% resistant. Participants following EUCAST MIC methods were most likely to achieve the intended result and participants following EUCAST automated methods were least likely to achieve the intended result. The intended result for piperacillin-tazobactam was susceptible (MIC 16 mg/L). Participants provided the following results: 47.6% susceptible; 3.7% intermediate and 48.8% resistant. Participants following EUCAST disk diffusion and broth microdilution methods were the most likely to achieve the intended result and participants following EUCAST automated methods were the least likely to achieve the intended result. There is no intermediate category for either ceftazidime or piperacillin-tazobactam susceptibility in the 2018 EUCAST guidelines, so the participants with an intermediate result who recorded that they followed EUCAST methods may need to review their methodology.

The *S. pneumoniae* strain (specimen 4925) expressed an intermediate level of resistance to cefotaxime/ceftriaxone. The strain was susceptible to levofloxacin/moxifloxacin and resistant to clindamycin, erythromycin and penicillin. There was an excellent concordance of results for cefotaxime/ceftriaxone (pneumonia), erythromycin, levofloxacin, moxifloxacin and penicillin (meningitis). There was a very good concordance for norfloxacin and a good concordance for clindamycin. However, there was low concordance for cefotaxime/ceftriaxone, cefotaxime/ceftriaxone (meningitis), penicillin, and penicillin (pneumonia). As in previous years, ongoing problems were seen with results for beta-lactam antibiotics in a strain of *S. pneumoniae* with an intermediate level of resistance to cefotaxime/ceftriaxone (MICs 1-2 mg/L) and resistant to penicillin (MIC 4 mg/L) by EUCAST categorisation. For each agent, participants found the strain to be more susceptible than it was the case. For cefotaxime, 66.8%, 31.3% and 1.9% of participants reported the specimen as susceptible, intermediate and resistant, respectively. For cefotaxime, participants using EUCAST automated methods were more likely to achieve the intended categorisation of intermediate than those using EUCAST disk or MIC methods. Similar results were seen for ceftriaxone. Only 14.6% of participants correctly categorised the strain as resistant to penicillin and 10.3% incorrectly categorised the strain as susceptible. Interestingly, 98.3% participants correctly reported penicillin as resistant in the context of meningitis; however, 36.0% of participants incorrectly reported penicillin as susceptible in the context of pneumonia. For penicillin, participants using EUCAST automated or broth microdilution methods were more likely to achieve the intended categorisation ('resistant') than those using EUCAST disk or gradient diffusion methods.

Laboratories that participate in the EARS-Net surveillance scheme should review their individual performance in this EQA, specifically in all areas where they did not achieve the intended results. Results suggest that there is no one overall AST method (EUCAST or CLSI) or type of method (automated, disk diffusion or MIC) that is likely to resolve all the issues experienced by individual participants during this EQA. Therefore, participants should ensure that they are following their chosen methodology carefully, particularly for species-antimicrobial agent combinations for which they did not achieve the intended results. The observation that some participants are reporting 'intermediate' in cases where their guidelines do not define such categories is an indicator that methods are not always strictly adhered to. Participants should ensure that they are aware of their problem species-antimicrobial agent combination, such as the correct categorisation of beta-lactam resistance in *S. pneumoniae*, and emerging resistance issues, such as colistin resistance in *Enterobacterales* (formerly known as *Enterobacteriaceae*).

Overall, performance in both identification and AST in this EQA was satisfactory. However, specific areas of difficulty (some well-established and some emerging) were identified. The potential for these to cause both under-estimation and over-estimation of antimicrobial resistance in Europe should be noted. Specifically, challenges with correctly identifying beta-lactam resistance in *S. pneumoniae* and plasmid-mediated colistin resistance in *Enterobacterales* may lead to underestimation of the true resistance percentages for these types of resistance in EARS-Net.

1 Introduction

Since 2010, the European Antimicrobial Resistance Surveillance System Network (EARS-Net) has organised annual external quality assessment (EQA) exercises for antimicrobial susceptibility testing (AST), in collaboration with the United Kingdom National External Quality Assessment Services (UK NEQAS). The UK NEQAS for Microbiology division is hosted by Public Health England (PHE) at Colindale, London. UK NEQAS is a non-profit organisation with more than forty years' experience in delivering an EQA service to over 1 800 laboratories globally. Between 2000 and 2009, UK NEQAS delivered similar EQA exercises for AST to the European Antimicrobial Resistance Surveillance System (EARSS), which was then transferred to the European Centre for Disease Prevention and Control as EARS-Net.

The purpose of the EQA exercises is to determine the accuracy of AST results reported by individual laboratories and to allow a comparison of results between laboratories and within countries across Europe. This report presents an analysis of participants' results for the 2018 EARS-Net EQA exercise.

2 Study design and methods

The strains used for the EQA exercise were compatible with epidemiology of the resistance phenotypes of species under surveillance at ECDC within EARS-Net. A panel of six lyophilised specimens containing species of bacteria was prepared. The panel included one strain of each of the following species, as agreed with ECDC: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus pneumoniae*. The strains were characterised and tested for antimicrobial susceptibility by two reference laboratories: the Specialist Antimicrobial Chemotherapy Unit, Cardiff, UK, and the EUCAST Reference and Development Laboratory, Växjö, Sweden. Both reference laboratories confirmed the MICs using broth microdilution and the susceptibility results were interpreted in accordance with established breakpoint criteria (EUCAST and CLSI), as indicated in the summary for each strain outlined in the results section below. The panel was distributed in September 2018 as UK NEQAS distribution 4466.

A dedicated web page was available on the UK NEQAS website for participants to enter their results. Participants were able to consult the web page to access instructions for using the secure web portal and download the protocol describing the process for examining the specimens. Detailed instructions were included on how to access the secure website via a unique user ID and password provided for each participant. The deadline for final submission of results was stated on the instruction sheet and on the secure website. For convenience, there was also a copy of the web reply form available for participants to download, to enable manual recording of antimicrobial susceptibility testing results prior to results submission online. Participants were allowed four weeks from the date of dispatch to examine the EQA specimens and return their results.

ECDC provided a list of operational contact points for antimicrobial-resistant pathogens and diseases caused by antimicrobial-resistant microorganisms. Each country appointed a national EQA coordinator. UK NEQAS for Microbiology forwarded the 2017 EARS-Net participant address databases held for each country to the national EQA coordinator, requesting that the information be checked for accuracy and updated in consultation with the participants. This information was collated for all countries and the updated database was returned to ECDC. On the date of dispatch, specimens were couriered by air to each country. The national EQA coordinators were contacted by email with a final reminder about imminent specimen dispatch and with a request to confirm the date of receipt by fax using a form enclosed with the shipment. Four weeks after the date of dispatch, the results entry was closed and the intended results were published on the secure website. Participants were notified by email that the intended results were available for viewing.

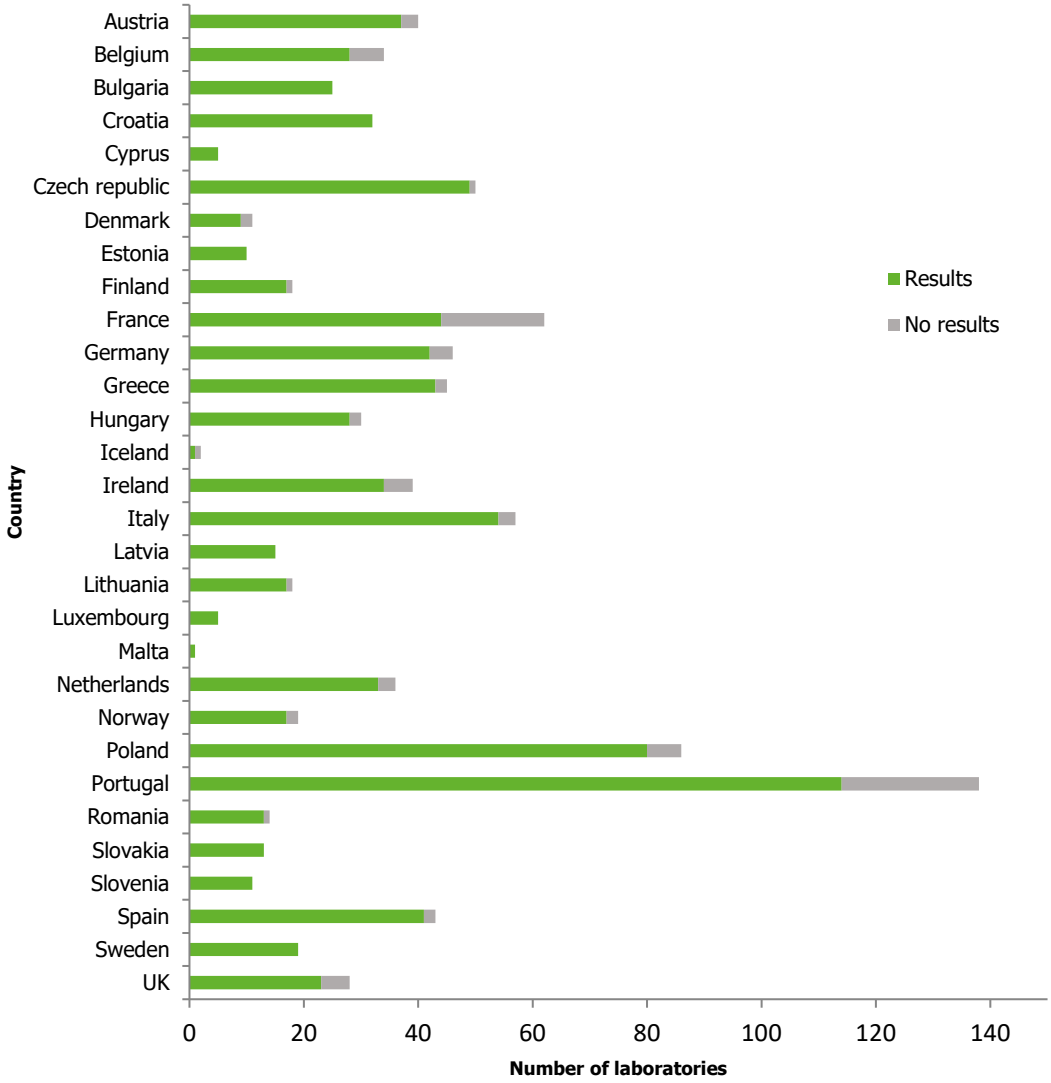
Participants were asked to report the identification of each isolate and antimicrobial susceptibility characterisation – susceptible (S), intermediate (I)[3] and resistant (R) – based on clinical breakpoints according to the guideline followed in their laboratories. Participants' results were analysed and considered 'concordant' if the reported categorisation of participants agreed with the interpretation of the reference laboratories. In addition, information was collected from participants on the methodology used to identify isolates (automated or conventional) and to undertake AST (EUCAST, CLSI or other; automated, disk diffusion, MIC or other). MIC options included broth microdilution and gradient diffusion.

[3] In the latest breakpoint tables for interpretation of MICs and zone diameters valid since 1 January 2019, EUCAST changed the definition of 'I' from 'intermediate' to 'susceptible, increased exposure'. Since this EQA exercise was conducted in September 2018 (i.e. before the new definition was implemented), EQA results were interpreted according to the previous definition of 'I' (i.e. 'intermediate'.)

3 Results

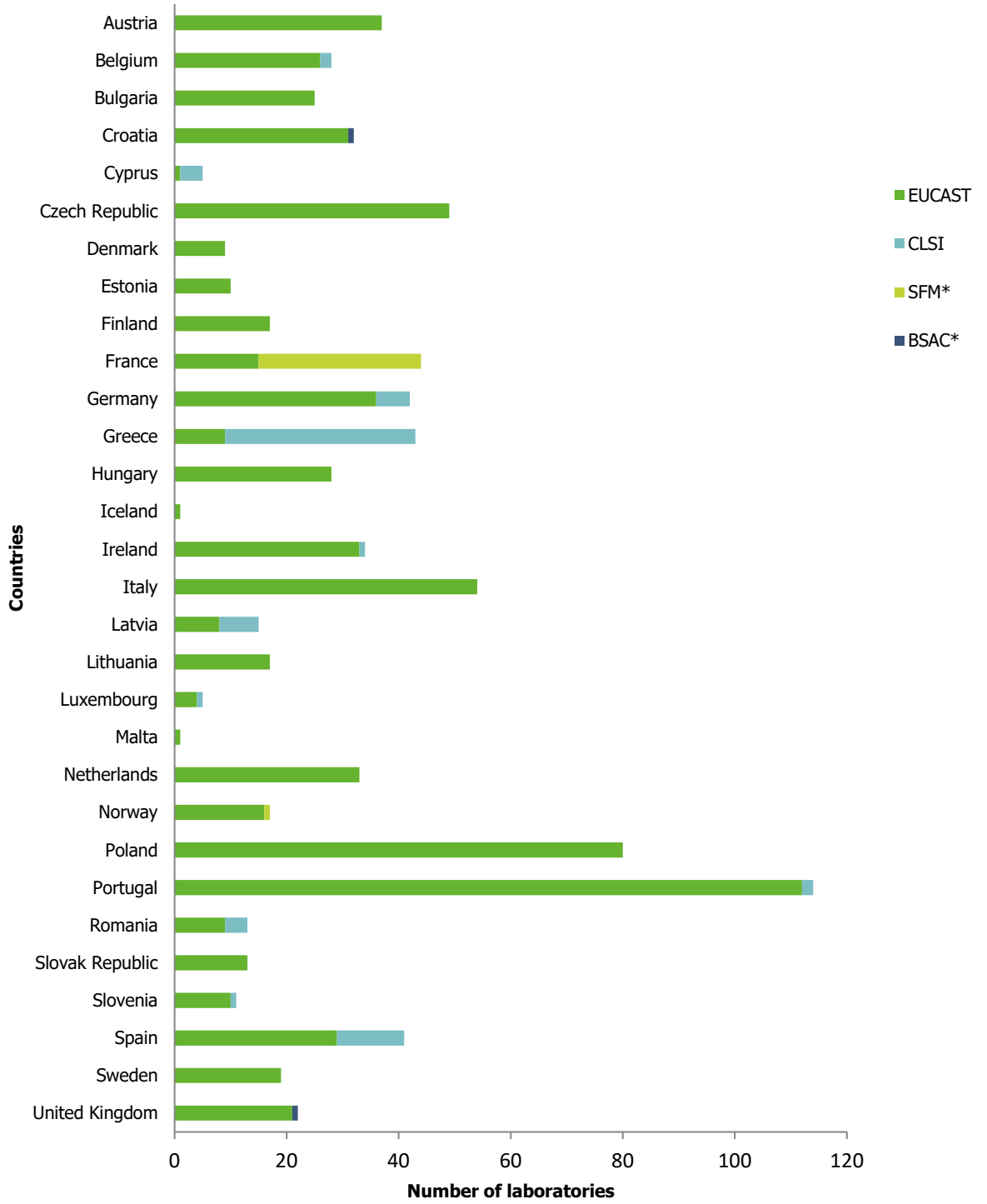
Six bacterial strains were distributed to 952 laboratories in 30 EU/EEA countries and 860 (90.3%) of these, including laboratories in all the invited EU/EEA countries, returned reports. Figure 1 shows the proportion of participating laboratories returning results per country.

Figure 1. Number of participating laboratories returning EQA results, by country, 2018



For the determination of AST results, laboratories used automated methods (50.8%), disk diffusion tests (39.9%), non-automated MIC methods, including broth microdilution and gradient methods, (8.3%), or other methods (0.9%). For species identification, 77.1% used an automated instrument, 19.4% used conventional methods and 3.5% did not report on the method used. In total, 8.6% of laboratories applied CLSI guidelines, a decline from the previous year when the proportion was 10.4%. EUCAST (or EUCAST-related) guidelines were reported by 91.4% of laboratories. This represented an increase of 5.5% compared to 2017. Figure 2 shows the national and international guidelines used by laboratories in different countries.

Figure 2. Clinical antimicrobial susceptibility testing (AST) guidelines reported as used by laboratories: number of laboratories by country, 2018



EUCAST: European Committee on Antimicrobial Susceptibility Testing
CLSI: Clinical and Laboratory Standards Institute
BSAC: British Society for Antimicrobial Chemotherapy
SFM: Société Française de Microbiologie
** National guidelines harmonised with EUCAST*

Specimen 4920: *Enterococcus faecium*

This specimen contained a strain of *Enterococcus faecium* that was resistant to amoxicillin, ampicillin, teicoplanin and vancomycin, but did not express high-level gentamicin resistance. Table 1 shows the intended results and concordance for susceptibility testing of this isolate.

Table 1. *Enterococcus faecium* (specimen 4920). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories

Antimicrobial agent	MIC (mg/L)	Intended interpretation	
	Reference laboratory	EUCAST/CLSI	Overall concordance (%)
Amoxicillin	*	R	99.6
Ampicillin	>8	R	99.9
High-level gentamicin	32	Negative	53.2
Teicoplanin	>32	R	99.1
Vancomycin	>32	R	99.4

R: resistant

**There were no reference results for amoxicillin: assigned results were based on participant consensus.*

An excellent concordance of results was achieved for the antimicrobial agents tested, except for high-level gentamicin resistance, for which concordance was not achieved.

Only 54.7% of participants following EUCAST guidelines and 36.4% of participants following CLSI guidelines correctly reported the result that the isolate did not show high-level gentamicin resistance. EUCAST participants using disk or gradient diffusion methods were more likely to report the intended result than those using automated or broth microdilution methods (Table 2).

Table 2. Susceptibility of *Enterococcus faecium* (specimen 4920) to gentamicin reported by participants, according to guidelines followed and methods used

Guideline	Method	Number participants responding (%)	
		High-level resistance negative	High-level resistance positive
EUCAST	Automated	66 (24.4)	205 (75.6)
	Disk diffusion	189 (75.6)	61 (24.4)
	MIC (all) *Broth microdilution* *Gradient diffusion*	111 (75.0) *28 (59.6)* *83 (82.2)*	37 (25.0) *19 (40.4)* *18 (17.8)*
	Other	4	3
	Total	**370 (54.7)**	**306 (45.3)**
CLSI	Automated	13 (22.8)	44 (77.2)
	Disk diffusion	7	4
	MIC	8	5
	Other	0	0
	Total	**28 (36.4)**	**49 (63.6)**

The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20)
I: intermediate
R: resistant
S: susceptible

The majority (97.7%) of participating laboratories correctly identified the isolate as *Enterococcus faecium* (Table 3).

Table 3. Identification results for specimen 4920

Species	Number of participants responding by identification method	
	Automated	Conventional
Enterococcus faecalis	8	2
Enterococcus faecium	703	120
Enterococcus gallinarum	0	2
Enterococcus species	2	4
Streptococcus species	1	0
Total	**714**	**128**

Specimen 4921: *Klebsiella pneumoniae*

This specimen contained a strain of *Klebsiella pneumoniae* producing an OXA-48 enzyme. The strain was susceptible to aminoglycosides, fluoroquinolones and colistin, susceptible/intermediate to third-generation cephalosporins, intermediate/resistant to carbapenems, and resistant to amoxicillin/ampicillin and inhibitor combinations. There was an excellent concordance of results for 10 antimicrobial agents and a concordance for ceftazidime and ceftriaxone, but a low concordance was achieved for cefotaxime, imipenem and meropenem (Table 4).

Table 4. *Klebsiella pneumoniae* (specimen 4921). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Amikacin	0.5	2	S/S	99.5
Amoxicillin	≥128	≥128	R/R	100
Amoxicillin-clavulanic acid	≥128 (≥128)*	≥128 (≥128)*	R/R	99.9
Ampicillin	≥128	≥128	R/R	99.9
Cefotaxime	2	2	I/I	31.7
Ceftazidime	1	1	S/S	86.5
Ceftriaxone	1	1	S/S	75.6
Ciprofloxacin	0.03	0.03	S/S	99.2
Colistin	<0.25	<0.25	S/†	97.4
Ertapenem	8	64	R/R	98.7
Gentamicin	0.25	0.5	S/S	99.4
Imipenem	4	4	I/R	70.6
Levofloxacin	**	**	S/S	99.0
Meropenem	4	4	I/R	62.4
Ofloxacin	**	**	S/S	98.5
Piperacillin-tazobactam	≥128*	≥128*	R/R	99.5
Tobramycin	0.25	0.25	S/S	99.6

I: intermediate
R: resistant
S: susceptible
** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, while those for piperacillin-tazobactam relate to tests with a fixed concentration of 4 mg/L tazobactam.*
*** There were no reference results for levofloxacin and ofloxacin: assigned results were based on participant consensus*
† No breakpoint provided by CLSI.

For cefotaxime (MIC 2 mg/L) the intended result was intermediate and the MIC was close to the breakpoint. Participants provided the following results: 57.1% susceptible; 31.7% intermediate and 11.3% resistant. Participants using the EUCAST disk diffusion method were most likely to provide the intended result (Table 5).

Table 5. Susceptibility of *Klebsiella pneumoniae* (specimen 4922) to cefotaxime reported by participants, according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	173 (59.9)	84 (29.1)	32 (11.1)
	Disk diffusion	60 (42.6)	63 (44.7)	18 (12.8)
	MIC (all) *Broth microdilution* *Gradient diffusion*	138 (59.0) *29 (67.4)* *109 (56.8)*	75 (32.1) *9 (20.9)* *66 (34.4)*	21 (9.0) *4 (9.3)* *17 (8.9)*
	Other	5	1	0
	Total	**376 (56.1)**	**223 (33.3)**	**71 (10.6)**
CLSI	Automated	12 (37.5)	9 (28.1)	11 (34.3)
	Disk diffusion	6	1	0
	MIC	17 (81.0)	2 (9.5)	2 (9.5)
	Other	0	0	1
	Total	**35 (57.4)**	**12 (19.7)**	**14 (23.0)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20)
Percentages may not total 100% due to rounding.

For imipenem and meropenem, the intended results (MIC 4 mg/L) were intermediate and were also close to both susceptible and resistant breakpoints. For imipenem, participants provided the following results: 29.4% susceptible; 44.0% intermediate and 26.6% resistant. For meropenem, participants provided the following results: 37.6% susceptible; 44.8% intermediate and 17.6% resistant. For both imipenem and meropenem, participants who used the EUCAST disk diffusion method were most likely to provide the intended result (Tables 6 and 7).

Table 6. Susceptibility of *Klebsiella pneumoniae* (specimen 4921) to imipenem reported by participants, according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	88 (33.5)	99 (37.6)	76 (28.9)
	Disk diffusion	34 (18.1)	125 (66.5)	29 (15.4)
	MIC (all) *Broth microdilution* *Gradient diffusion*	58 (45.0) *21 (58.3)* *37 (39.8)*	48 (37.2) *12 (33.3)* *36 (38.7)*	23 (17.8) *3 (8.3)* *20 (21.5)*
	Other	3	2	1
	Total	**183 (31.2)**	**274 (46.8)**	**129 (22.0)**
CLSI	Automated	0	15 (31.9)	32 (68.1)
	Disk diffusion	2	4	6
	MIC	2	3	7
	Other	0	0	0
	Total	**4 (5.6)**	**22 (31.0)**	**45 (63.4)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20).

Table 7. Susceptibility of *Klebsiella pneumoniae* (specimen 4921) to meropenem reported by participants, according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	154 (46.8)	132 (40.1)	43 (13.1)
	Disk diffusion	32 (14.8)	142 (65.7)	42 (19.4)
	MIC (all) *Broth microdilution* *Gradient diffusion*	85 (51.8) *32 (61.5)* *53 (47.3)*	52 (31.7) *12 (23.1)* *40 (35.7)*	27 (16.5) *8 (15.4)* *19 (17.0)*
	Other	4	2	2
	Total	**275 (38.4)**	**328 (45.7)**	**114 (15.9)**
CLSI	Automated	6 (14.3)	20 (47.6)	16 (38.1)
	Disk diffusion	3	6	7
	MIC	4	4	5
	Other	0	0	0
	Total	**13 (18.3)**	**30 (42.3)**	**28 (39.4)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method is ≥20)
Percentages may not total 100% due to rounding

Almost all (99.6%) of participating laboratories correctly identified the isolate as *Klebsiella pneumoniae* (Table 8).

Table 8. Identification results for specimen 4921

Species	Number of participants responding by identification method	
	Automated	Conventional
Escherichia coli	0	1
Klebsiella oxytoca	1	1
Klebsiella pneumoniae	715	128
Total	**716**	**130**

Specimen 4922: *Escherichia coli*

This specimen contained a strain of *Escherichia coli* possessing the *mcr-1* gene, exhibiting resistance to amoxicillin, amoxicillin-clavulanic acid, colistin and quinolones. The strain was susceptible to other beta-lactams, inhibitor combinations and aminoglycosides. Table 9 shows the intended results and concordance for susceptibility testing of this organism.

There was an excellent concordance for 13 antimicrobial agents and a very good concordance for ofloxacin and piperacillin-tazobactam, but a low concordance was achieved for amoxicillin-clavulanic acid and colistin.

Table 9. *Escherichia coli* (specimen 4922). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories

Antimicrobial agent	MIC (mg/L)	Intended interpretation	
	Reference laboratory	EUCAST/CLSI	Overall concordance (%)
Amikacin	≤4	S/S	99.6
Amoxicillin	>32	R/R	99.6
Amoxicillin-clavulanic acid	8*	S/S	58.2
Ampicillin	>32	R/R	99.5
Cefotaxime	≤0.5	S/S	99.1
Ceftazidime	≤0.5	S/S	99.3
Ceftriaxone	0.12	S/S	98.8
Ciprofloxacin	>2	R/R	99.5
Colistin	4	R/†	69.2
Ertapenem	≤0.12	S/S	99.7
Gentamicin	≤0.5	S/S	100
Imipenem	≤0.5	S/S	100
Levofloxacin	**	R/R	99.2
Meropenem	≤0.12	S/S	99.6
Ofloxacin	**	R/R	97.2
Piperacillin-tazobactam	4*	S/S	97.3
Tobramycin	≤2	S/S	99.4

I: intermediate
R: resistant
S: susceptible
** Reference results for amoxicillin-clavulanic acid MICs relate to tests with a fixed concentration of 2 mg/L clavulanic acid, while those for piperacillin-tazobactam relate to tests with a fixed concentration of 4 mg/L tazobactam.*
*** No reference results for levofloxacin and ofloxacin: assigned results were based on participant consensus*
† No breakpoint provided by CLSI.

The intended result for amoxicillin-clavulanic acid was susceptible (MIC 8 mg/L), but close to the susceptible breakpoint. Participants provided the following results: 58.2% susceptible; 2.4% intermediate and 39.5% resistant. Participants following EUCAST disk or gradient diffusion methods were most likely to achieve the intended result and participants following EUCAST automated methods were least likely to achieve the intended result (Table 10). There is no intermediate category for amoxicillin-clavulanic acid susceptibility in the 2018 EUCAST guidelines, so the participants with an intermediate result who reported that they followed EUCAST methods may need to review their methodology.

Table 10. Susceptibility of *Escherichia coli* (specimen 4922) to amoxicillin-clavulanic acid reported by participants, according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	145 (41.4)	6 (1.7)	199 (56.9)
	Disk diffusion	190 (73.1)	2 (0.8)	68 (26.2)
	MIC (all) *Broth microdilution* *Gradient diffusion*	58 (67.4) *30 (60)* *28 (77.8)*	2 (2.3) *0* *2 (5.6)*	26 (30.2) *20 (40)* *6 (16.7)*
	Other	1	0	4
	Total	**394 (56.2)**	**10 (1.4)**	**297 (42.4)**
CLSI	Automated	29 (65.9)	4 (9.1)	11 (25.0)
	Disk diffusion	14	3	2
	MIC	4	2	1
	Other	0	0	0
	Total	**47 (67.1)**	**9 (12.9)**	**14 (20.0)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20
Percentages may not total 100% due to rounding.

The intended result for colistin was resistant (MIC 4 mg/L). Participants provided the following results: 30.2% susceptible 0.6% intermediate and 69.2% resistant. There is no CLSI breakpoint and EUCAST recommend that this test is only undertaken using broth microdilution. Participants using an automated method were least likely to obtain the intended result. Fifty-five participants claimed to be using a EUCAST disk diffusion method (Table 11), although no EUCAST zone diameter breakpoints are provided in the 2018 guideline. There is no intermediate category for colistin susceptibility in the 2018 EUCAST guidelines, so participants with an intermediate result who reported that they followed EUCAST automated or MIC methodology may also need to review their methodology.

Table 11. Susceptibility of *Escherichia coli* (specimen 4922) to colistin reported by participants, according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	82 (46.1)	2 (1.1)	94 (52.8)
	Disk diffusion	12 (21.8)	0	43 (78.2)
	MIC (all) *Broth microdilution* *Gradient diffusion*	44 (19.8) *37 (19.5)* *7 (21.9)*	1 (0.5) *0* *1 (3.1)*	177 (79.7) *153 (80.5)* *24 (75)*
	Other	1	0	3
	Total	**139 (30.3)**	**3 (0.7)**	**317 (69.1)**

I: intermediate
R: resistant
S: susceptible
The correct result for the guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20
Percentages may not total 100% due to rounding.

Almost all (99.9%) of the participating laboratories correctly identified the isolate as *Escherichia coli* (Table 12).

Table 12. Identification results for specimen 4299

Species	Number of participants responding by identification method	
	Automated	Conventional
Citrobacter freundii	1	0
Escherichia coli	676	141
Escherichia coli O157 toxin negative	2	0
Escherichia coli O157 toxin not tested	2	0
Total	**681**	**141**

Specimen 4923: *Staphylococcus aureus*

This specimen contained a strain of *Staphylococcus aureus* that was resistant to beta-lactams, fluoroquinolones, clindamycin, erythromycin, gentamicin and rifampicin. It was susceptible to fusidic acid, linezolid, tetracycline and glycopeptides. An excellent concordance was achieved for all 13 antimicrobial agents tested and there were no problems with susceptibility testing of this organism. Table 13 shows the intended results and concordance for susceptibility testing of this organism.

Table 13. *Staphylococcus aureus* (specimen 4923). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Cefoxitin	>64	>64	R/R	99.6
Ciprofloxacin	≥128	≥128	R/R	99.7
Clindamycin	≥128	≥128	R/R	99.7
Erythromycin	≥128	≥128	R/R	99.9
Fusidic acid	0.06	0.12	S/†	99.4
Gentamicin	64	64	R/R	99.4
Linezolid	1	2	S/S	99.4
Oxacillin	*	*	R/R	99.4
Penicillin	64	64	R/R	99.7
Rifampicin	≥128	≥128	R/R	99.7
Teicoplanin	≤0.25	0.5	S/S	97.7
Tetracycline	≤0.125	0.25	S/S	99.0
Vancomycin	1	1	S/S	98.7

S: susceptible
R: resistant
** No reference results for clindamycin or norfloxacin – assigned results based on participant consensus*
† No breakpoint provided by CLSI.

Almost all (99.8%) of participating laboratories correctly identified the isolate as *Staphylococcus aureus* (Table 14).

Table 14. Identification results for specimen 4923

Species	Number of participants responding by identification method	
	Automated	Conventional
Staphylococcus aureus	662	150
Coagulase-negative staphylococcus	1	1
Total	**663**	**151**

Specimen 4924: *Pseudomonas aeruginosa*

This specimen contained a strain of *Pseudomonas aeruginosa* that was susceptible to aminoglycosides, ceftazidime, piperacillin-tazobactam and colistin. The strain was resistant to imipenem, meropenem, ciprofloxacin and levofloxacin. Table 15 shows the intended results and concordance for susceptibility testing of this organism.

An excellent concordance was achieved for amikacin, ciprofloxacin, imipenem, levofloxacin, meropenem and tobramycin. A very good concordance was achieved for colistin and gentamicin. A low concordance was achieved for ceftazidime and no concordance was achieved for piperacillin-tazobactam.

Table 15. ***Pseudomonas aeruginosa*** **(specimen 4924). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories**

Species	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Amikacin	4	4	S/S	97.9
Ceftazidime	4	4	S/S	74.4
Ciprofloxacin	8	16	R/R	99.3
Colistin	1	2	S/S	95.6
Gentamicin	2	2	S/S	96.8
Imipenem	16	16	R/R	99.6
Levofloxacin	**	**	R/R	100
Meropenem	16	16	R/R	98.1
Piperacillin-tazobactam	16*	16*	S/S	47.6
Tobramycin	1	1	S/S	99.6

R: resistant
S: susceptible
** Reference results for piperacillin-tazobactam relate to tests with a fixed concentration of 4 mg/L tazobactam.*
*** No reference result for levofloxacin: assigned results were based on participant consensus.*

The intended result for ceftazidime was susceptible (MIC 4 mg/L). Participants provided the following results: 74.4% susceptible; 4.3% intermediate and 21.4% resistant. Participants following EUCAST MIC methods were most likely to achieve the intended result and participants following EUCAST automated methods were least likely to achieve the intended result (Table 16). There is no intermediate category for ceftazidime susceptibility in the 2018 EUCAST guidelines, so the participants with an intermediate result who reported that they followed EUCAST automated methodology may need to review their methodology.

Table 16. Susceptibility of ***Pseudomonas aeruginosa*** **(specimen 4924) to ceftazidime reported by participants, according to guidelines followed and methods used**

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	241 (65.8)	18 (4.9)	107 (29.2)
	Disk diffusion	196 (79.7)	1 (0.4)	49 (19.9)
	MIC (all) *Broth microdilution* *Gradient diffusion*	104 (85.2) *50 (87.7)* *54 (83.1)*	0	18 (14.8) *7 (12.3)* *11 (16.9)*
	Other	6	0	1
	Total	**547 (73.8)**	**19 (2.6)**	**175 (23.6)**
CLSI	Automated	31 (66.0)	13 (27.7)	3 (6.4)
	Disk diffusion	13	2	2
	MIC	6	2	0
	Other	0	0	0
	Total	**50 (69.4)**	**17 (23.6)**	**5 (6.9)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded;
Percentages are only provided where the total number of participants using a method was ≥20
Percentages may not total 100% due to rounding

The intended result for piperacillin-tazobactam was susceptible (MIC 16 mg/L). Participants provided the following results: 47.6% susceptible; 3.7% intermediate and 48.8% resistant. Participants following EUCAST disk diffusion and broth microdilution methods were most likely to achieve the intended result and participants following EUCAST automated methods were least likely to achieve the intended result (Table 17). There is no intermediate category for piperacillin-tazobactam susceptibility in the 2018 EUCAST guidelines, so the participants with an intermediate result who reported that they followed EUCAST methods may need to review their methodology.

Table 17. Susceptibility of *Pseudomonas aeruginosa* (specimen 4924) to piperacillin-tazobactam reported by participants, according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	99 (27.4)	0	262 (72.6)
	Disk diffusion	175 (65.8)	2 (0.8)	89 (33.5)
	MIC (all) *Broth microdilution* *Gradient diffusion*	64 (62.1) *41 (68.3)* *23 (53.5)*	2 (1.9) *2 (3.3)* *0*	37 (35.9) *17 (28.3)* *20 (46.5)*
	Other	3	0	3
	Total	**341 (46.3)**	**4 (0.5)**	**391 (53.1)**
CLSI	Automated	19 (42.2)	23 (51.1)	3 (6.7)
	Disk diffusion	11	2	4
	MIC	7	2	0
	Other	0	0	0
	Total	**37 (52.1)**	**27 (38.0)**	**7 (9.9)**

I: intermediate
R: resistant
S: susceptible
The correct result for each guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20)
Percentages may not total 100% due to rounding.

Almost all (99.9%) of participating laboratories correctly identified the isolate as *Pseudomonas aeruginosa* (Table 18).

Table 18. Identification results for specimen 4924

Species	Number of participants responding by identification method	
	Automated	Conventional
Pseudomonas species	0	1
Pseudomonas aeruginosa	666	143
Total	**666**	**144**

Specimen 4925: *Streptococcus pneumoniae*

This specimen contained a strain of *Streptococcus pneumoniae* that had an intermediate level of resistance to cefotaxime/ceftriaxone. The strain was susceptible to levofloxacin/moxifloxacin and resistant to clindamycin, erythromycin and penicillin. Table 19 shows the intended results and concordance for susceptibility testing of this organism.

There was an excellent concordance of results for cefotaxime/ceftriaxone (pneumonia), erythromycin, levofloxacin, moxifloxacin and penicillin (meningitis). There was a very good concordance for norfloxacin and a good concordance for clindamycin. However, there was no concordance achieved for cefotaxime/ceftriaxone, cefotaxime/ceftriaxone (meningitis), penicillin and penicillin (pneumonia).

Table 19. ***Streptococcus pneumoniae*** **(specimen 4925). Minimum inhibitory concentration (MIC) and intended results reported by the reference laboratories and the overall concordance of the participating laboratories**

Agent	MIC range (mg/L) ref. lab		Intended interpretation	
	From	to	EUCAST/CLSI	Overall concordance (%)
Cefotaxime meningitis pneumonia	1	1	I/† I/I I/S	31.3 26.1 98.4
Ceftriaxone meningitis pneumonia	1	2	I/† I / I/R I / S/I	26.7 28.1 97.9
Clindamycin	*	*	R/R	93.8
Erythromycin	≥128	≥128	R/R	98.0
Levofloxacin	1	1	S/S	99.6
Moxifloxacin	0.12	0.12	S/S	100
Norfloxacin	*	*	S/S	95.7
Penicillin meningitis pneumonia	4	4	R/† R/R R/I	14.6 98.3 64.0

I: intermediate
R: resistant
S: susceptible
** No reference results for clindamycin or norfloxacin: assigned results were based on participant consensus.*
† No breakpoint provided by CLSI.

As in previous years, ongoing problems were seen with results for beta-lactam antibiotics in a strain of *S. pneumoniae* with an intermediate level of resistance to cefotaxime/ceftriaxone (MICs 1-2 mg/L) and resistant to penicillin (MIC 4 mg/L) by EUCAST categorisation. For each agent, participants found the strain to be more susceptible than was the case. For cefotaxime, 66.8%, 31.3% and 1.9% of participants reported the specimen as susceptible, intermediate and resistant, respectively. For cefotaxime, EUCAST participants using automated methods were more likely to achieve the intended categorisation of intermediate than those using disk or MIC methods (Table 20). Similar results were seen for ceftriaxone.

Table 20. Susceptibility of *Streptococcus pneumoniae* (specimen 4925) to cefotaxime reported by participants, according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	107 (44.0)	129 (53.1)	7 (2.9)
	Disk diffusion	78 (77.2)	22 (21.8)	1 (1.0)
	MIC (all) *Broth microdilution* *Gradient diffusion*	188 (79.3) *30 (71.4)* *158 (81.0)*	46 (19.4) *11 (26.2)* *35 (17.9)*	3 (1.3) *1 (2.4)* *2 (1.0)*
	Other	3	3	0
	Total	**376 (64.1)**	**200 (34.1)**	**11 (1.9)**

I: intermediate
R: resistant
S: susceptible
The correct result for the guideline is shaded
Percentages are only provided where the total number of participants using a method was ≥20
Percentages may not total 100% due to rounding.

Only 14.6% of participants correctly categorised the strain as resistant to penicillin and 10.3% incorrectly categorised the strain as susceptible. Interestingly, 98.3% of participants did correctly report penicillin as resistant in the context of meningitis; however, 36.0% of participants incorrectly reported penicillin as susceptible in the context of pneumonia. For penicillin, EUCAST participants using automated or broth microdilution methods were more likely to achieve the intended categorisation of resistant than those using disk or gradient diffusion methods (Table 21).

Table 21. Susceptibility of *Streptococcus pneumoniae* (specimen 4925) to penicillin reported by participants, according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	21 (9.1)	152 (65.5)	59 (25.4)
	Disk diffusion	12 (9.5)	98 (77.8)	16 (12.7)
	MIC (all) *Broth microdilution* *Gradient diffusion*	13 (5.3) *3 (8.8)* *10 (4.7)*	216 (87.8) *25 (73.5)* *191 (90.1)*	17 (6.9) *6 (17.6)* *11 (5.2)*
	Other	0	3	2
	Total	**46 (7.6)**	**469 (77.0)**	**94 (15.4)**

I: intermediate
R: resistant
S: susceptible
The correct result for the guideline shaded
Percentages are only provided where the total number of participants using a method was ≥20
Percentages may not total 100% due to rounding

The majority (98.5%) of participating laboratories correctly identified the isolate as *Streptococcus pneumoniae* (Table 8).

Table 22. Identification results for specimen 4925

Species	Number of participants responding by identification method	
	Automated	Conventional
Erysipelothrix rhusiopathiae	1	0
Streptococcus species	2	1
Streptococcus mitis	3	0
Streptococcus mutans	1	0
Streptococcus pneumoniae	495	295
Streptococcus salivarius	3	0
Streptococcus sanguis	1	0
Total	**506**	**296**

4 Discussion

Overall, the performance of laboratories participating in the 2018 EQA was satisfactory. There were no significant issues concerning species identification. For AST, ≥95% concordance was achieved for 56 (80.0%) of 70 species-antimicrobial agent combinations tested. In recent years, lower concordances in reporting susceptibility results have been seen for species-antimicrobial agent combinations with borderline MIC values and where breakpoints and categorisation of results differed between EUCAST and CLSI guidelines. Species-antimicrobial agent combinations for which recurrent problems have been encountered included:

- *Escherichia coli* with intermediate/resistant or resistant piperacillin-tazobactam results;
- *Klebsiella pneumoniae* with differing third-generation cephalosporin results;
- *Klebsiella pneumoniae* with intermediate/resistant imipenem and meropenem results;
- *Klebsiella pneumoniae* with susceptible/intermediate amikacin results;
- *Staphylococcus aureus* with intermediate vancomycin results; and
- *Streptococcus pneumoniae* with intermediate penicillin results.

Specimen 4920 contained a strain of *Enterococcus faecium* that was resistant to amoxicillin, ampicillin, teicoplanin and vancomycin, but did not express high-level gentamicin resistance. An excellent concordance of results was achieved for all antimicrobial agents tested except for high-level gentamicin resistance, for which concordance was not achieved. Potentially, this could lead to an over-estimation of the percentages of high-level gentamicin resistance in invasive *E. faecium* isolates in Europe.

Specimen 4921 contained a strain of *Klebsiella pneumoniae* producing an OXA-48 enzyme. The strain was susceptible/intermediate to third-generation cephalosporins, intermediate/resistant to carbapenems and resistant to amoxicillin/ampicillin and beta-lactamase inhibitor combinations. There was an excellent concordance of results for 10 antimicrobial agents and a concordance for ceftazidime and ceftriaxone, but a low concordance was achieved for cefotaxime, imipenem and meropenem. In previous years, participants had achieved a poor concordance of results for *K. pneumoniae* strains for which third-generation cephalosporin results had differed, or there had been either intermediate or resistant carbapenem results. For cefotaxime, imipenem and meropenem, the MICs were close to the breakpoints. Participants using EUCAST methods were most likely to provide the intended results if they used a disk diffusion method and were most likely to under-estimate resistance if they used a broth microdilution method. Potentially, this could lead to an under-estimation of resistance to third-generation cephalosporins and carbapenems in invasive *K. pneumoniae* isolates in Europe.

Specimen 4922 contained a strain of *Escherichia coli* possessing the *mcr-1* gene and expressing resistance to amoxicillin, amoxicillin-clavulanic acid, colistin and quinolones. There was an excellent concordance for 13 antimicrobial agents and a very good concordance for ofloxacin and piperacillin-tazobactam, but a low concordance was achieved for amoxicillin-clavulanic acid and colistin. The MIC for amoxicillin-clavulanic acid was close to the susceptible breakpoint. Participants following EUCAST methods were most likely to achieve the intended result if they used a disk or gradient diffusion and least likely to achieve the intended result using an automated method. Potentially, this could lead to an over-estimation of resistance to amoxicillin-clavulanic acid in invasive *E. coli* isolates in Europe. There is no CLSI breakpoint for colistin and EUCAST recommend that colistin susceptibility testing is only undertaken using broth microdilution. Participants using an automated method were least likely to obtain the intended result. Fifty-five participants claimed to be using a EUCAST disk diffusion method, although no EUCAST zone diameter breakpoints are provided in the 2018 guideline. This is the second EQA distribution that contained a colistin-resistant strain of *E. coli* and participants again experienced difficulty in reporting the intended result. Potentially, this could lead to an under-estimation of resistance to colistin in invasive *E. coli* isolates in Europe.

Specimen 4923 contained a strain of *Staphylococcus aureus* that was resistant to beta-lactams, fluoroquinolones, clindamycin, erythromycin, gentamicin and rifampicin. An excellent concordance was achieved for all 13 antimicrobial agents tested and there were no problems with AST of this strain.

Specimen 4924 contained a strain of *Pseudomonas aeruginosa* that was resistant to imipenem, meropenem, ciprofloxacin and levofloxacin. An excellent concordance was achieved for amikacin, ciprofloxacin, imipenem, levofloxacin, meropenem and tobramycin. A very good concordance was achieved for colistin and gentamicin. A low concordance was achieved for ceftazidime and concordance was not achieved for piperacillin-tazobactam. For ceftazidime, participants following EUCAST methods were most likely to achieve the intended result using MIC methods, and least likely to achieve the intended result using an automated method. For piperacillin-tazobactam, participants following EUCAST methods were most likely to achieve the intended result using disk diffusion or broth microdilution, and least likely to achieve the intended result using automated methods. Potentially, this could lead to an over-estimation of resistance to ceftazidime and to piperacillin-tazobactam in invasive *P. aeruginosa* isolates in Europe.

As in previous years [3], there was a poor consensus in this EQA exercise for results of penicillin susceptibility testing of *S. pneumoniae* (specimen 4925). There was a bias towards under-reporting resistance to penicillin and to the third-generation cephalosporins cefotaxime and ceftriaxone. For cefotaxime, EUCAST participants using automated methods were more likely to achieve the intended categorisation of intermediate than those using disk diffusion or MIC methods. For penicillin, EUCAST participants using automated or broth microdilution methods were more likely to achieve the intended results than those using disk or gradient diffusion methods. This clearly remains a difficult area for participants. Potentially, this could lead to an underestimation of beta-lactam resistance in Europe for invasive isolates of *S. pneumoniae*.

Analysis of species-antimicrobial agent combinations, for which the laboratories performed poorly, did not show any overall advantage of using automated, MIC or disk methods. In 2018, we looked in more detail at the two most commonly used MIC methods (broth microdilution and gradient diffusion), to identify areas where the performance of the two methods differed. All methods performed well for some combinations, but poorly for others. Similarly, there was no consistent bias noted in terms of under- or over-estimating resistance.

5 Conclusions

The overall performance of participating laboratories in this EQA was satisfactory.

Several species-antimicrobial agent combinations that were already known to be problematic when performing AST again proved difficult for participants in 2018:

- *Streptococcus pneumoniae* with intermediate penicillin results;
- *Klebsiella pneumoniae* with differing third-generation cephalosporin results; and
- *Escherichia coli* with resistant colistin results.

For these species-antimicrobial agent combinations, there is a potential risk of under-estimating antimicrobial resistance percentages in Europe. In particular, it is important that laboratories are able to identify the emergence of new, or unexpected, resistance such as colistin resistance. For a similar number of other species-antimicrobial agent combinations, there is a potential risk that antimicrobial resistance percentages are over-estimated.

Analysis of species-antimicrobial agent combinations for which laboratories performed poorly did not show any overall advantage of using automated, MIC or disk diffusion methods. Nevertheless, there were clear differences in the performance of different methods for specific species-antimicrobial agent combinations and participants should look at these details when investigating areas where they did not achieve the intended results.

As fewer participants report using CLSI methods, it is becoming less relevant to attempt to compare EUCAST and CLSI methods overall. However, it is worth noting that in the areas where participants experienced difficulties, the number of species-antimicrobial agent combinations for which either EUCAST or CLSI methods performed better overall is similar. For future EQA exercises, we will only report by AST method for participating laboratories that follow EUCAST methods.

6 Recommendations

Overall in this EQA exercise, the performance of laboratories for both identification and AST was satisfactory. However, specific areas of difficulty – some well-established and some emerging - have been highlighted. There is a potential for this to cause both under-estimation or over-estimation of antimicrobial resistance percentages in reports on antimicrobial resistance in Europe.

Laboratories that participate in the EARS-Net surveillance scheme should review their individual performance in this EQA exercise and review all areas where they did not achieve the intended results.

This report suggests that there is no one overall AST guideline (EUCAST or CLSI) or type of method (automated, disk diffusion or MIC) that is likely to resolve issues experienced by individual participants during the EQA exercise. Therefore, participants should ensure that they are following their chosen methodology carefully, particularly with species-antimicrobial agent combinations for which they did not achieve the intended results.

The observation that some participants are reporting 'intermediate' in cases where their guideline does not define such a category is an indicator that methods are not always strictly adhered to and participants should review their reporting practice in these cases.

Finally, participants should ensure that they are aware of problem species-antimicrobial agent combination, such as the correct categorisation of beta-lactam resistance in *S. pneumoniae*, as well as emerging resistance issues, such as colistin resistance in *Enterobacterales*.

Support to participating laboratories will be available from 2018 to 2020 through the carbapenem- and/or colistin-resistant Enterobacteriaceae (CCRE) project of the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net), a new ECDC network for genomic-based surveillance of multidrug-resistant bacteria. The CCRE project includes national capacity assessment and systematic reviews and the development of guidance and training on the detection of carbapenem- and/or colistin-resistant *Enterobacterales*.

Regular participation of the laboratories that report to EARS-Net in the annual EQA exercise is required to evaluate and review the performance of these laboratories, identify species-antimicrobial agent combinations that may represent a problem when performing AST and for which improvement is possible and facilitate the correct interpretation of AST results reported to EARS-Net.

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