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TECHNICAL REPORT

External quality assessment (EQA) of performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net), 2019

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Abbreviations

AST	Antimicrobial susceptibility testing
BSAC	British Society for Antimicrobial Chemotherapy
CLSI	Clinical and Laboratory Standards 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
I	'Susceptible, increased exposure'/Intermediate[1]
MIC	Minimum inhibitory concentration
PHE	Public Health England
R	Resistant
S	Susceptible
SFM	Société Française de Microbiologie
UK	United Kingdom
UK NEQAS	United Kingdom National External Quality Assessment Services

[1] In the breakpoint tables for interpreting MICs and zone diameters valid since 1 January 2019, EUCAST changed the definition of 'I' from 'intermediate' to 'susceptible, increased exposure'. The results of the 2019 EQA exercise were interpreted following this guideline. `Intermediate' refers to CLSI results, or EUCAST results prior to 2019.

Executive summary

This report provides an analysis of the external quality assessment (EQA) for the antimicrobial susceptibility testing (AST) performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2019. A total of 952 laboratories (1–95 per country) from 30 EU/EEA countries[2] participated in the EQA exercise. Six bacterial strains were used: *Acinetobacter baumannii* complex, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

For species identification, 87% of laboratories used an automated instrument and 13% used conventional methods. Overall, 99.4% of the identifications were correct and there were no significant issues arising for species identification.

For the determination of AST results, most laboratories used either automated methods, disk diffusion tests or MIC (minimum inhibitory concentration) non-automated methods. For AST, only 51 (5.7%) of the laboratories applied CLSI guidelines, a decline from the previous year when the proportion was 8.6%. EUCAST (or EUCAST-related) guidelines were reported by 94.3% of the laboratories.

Overall, the performance of laboratories participating in the 2019 EQA exercise was very good. The bacterial identification was considered excellent and the AST performance good. Nevertheless, this EQA exercise indicates that both under- and overestimation of antimicrobial resistance percentages in Europe may be possible, although overall the 2019 EQA indicates that underestimation is more frequent. This observation should be kept in mind when interpreting EARS-Net surveillance data.

Specific species-antimicrobial agent combinations were identified as problematic.

The *Acinetobacter baumannii* complex strain (specimen 5582) was resistant (R) to carbapenems (imipenem and meropenem), ciprofloxacin, levofloxacin and gentamicin, but susceptible (S) to amikacin, tobramycin and colistin. There was an excellent or very good concordance achieved for the antimicrobial agents tested, with no method- or interpretative-guidance-related bias detected.

The *Escherichia coli* strain (specimen 5583) was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin and piperacillin-tazobactam. The strain was either 'susceptible, increased exposure' (I, EUCAST) or susceptible (S, CLSI) to ceftazidime and susceptible to cefotaxime, ceftriaxone, ciprofloxacin, levofloxacin, ofloxacin, amikacin, gentamicin, tobramycin, ertapenem, imipenem, meropenem and colistin. There was an excellent or very good concordance with intended AST results for 15 of the antimicrobial agents. There was good concordance for amoxicillin-clavulanic acid; the intended result was resistant (MIC≥128 mg/L) and participants provided the following results: 87.2% resistant, 2.1% 'susceptible, increased exposure' / intermediate (I) and 10.7% susceptible. Participants following CLSI guidelines using automated methods were less likely to report the intended result. For participants following EUCAST guidelines, those performing tests using MIC methods were less likely to report the intended result. There is no 'susceptible, increased exposure' (I) category for amoxicillin-clavulanic acid in the 2019 EUCAST guidelines, so the participants with an 'I' result, who reported that they had followed EUCAST methods, may need to review their methodology. No concordance was achieved for ceftazidime for which the intended result was 'susceptible, increased exposure' according to the EUCAST guidelines (MIC=4 mg/L). The results provided by participants following EUCAST guidelines were: 58.0% susceptible, 32.6% 'susceptible, increased exposure' and 9.4% resistant. Only 32.6% of the participants following EUCAST methods and 94.1% of the participants following CLSI methods provided the correct category.

The *Klebsiella pneumoniae* strain (specimen 5584) was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, gentamicin (CLSI I/R), levofloxacin, ofloxacin and tobramycin (CLSI I). The strain was susceptible/'susceptible, increased exposure' (S/I) to piperacillin-tazobactam (CLSI S) and susceptible to amikacin, cefotaxime, ceftazidime, ceftriaxone, ertapenem, imipenem, meropenem and colistin (CLSI -).

Concordance with intended results was excellent for amikacin, ampicillin, amoxicillin, ciprofloxacin, ofloxacin, ceftazidime, ceftriaxone, cefotaxime, imipenem, levofloxacin, meropenem and colistin, and very good for piperacillin/tazobactam and ertapenem. For gentamicin, concordance was good and for tobramycin it was satisfactory. A low concordance was achieved for amoxicillin-clavulanic acid. For this strain the intended result was resistant (MIC=64 mg/L or >64 mg/L depending on the reference laboratory) and participants provided the following results: 17.3% susceptible; 4.0% 'susceptible, increased exposure'/intermediate (I); and only 78.7% resistant. Participants following CLSI guidelines were more likely to report intermediate due to a difference in breakpoints. Those participants using EUCAST disk method or MIC methods were less likely to report the intended result than those using automated methods.

[2] In 2019, the United Kingdom (UK) participated in the EQA exercise as a Member State of the European Union (EU).

The *Pseudomonas aeruginosa* strain (specimen 5585) was resistant to amikacin (CLSI I/R), gentamicin, tobramycin, ciprofloxacin, levofloxacin, piperacillin-tazobactam, imipenem and meropenem and susceptible to ceftazidime. An excellent concordance was achieved for seven antimicrobial agents, good performance for colistin, and satisfactory for amikacin and ceftazidime. For colistin, the intended result was susceptible or resistant as the MICs (2-4 mg/L) spanned the breakpoint. Participants provided the following results: 86.9% susceptible, 0.5% 'susceptible, increased exposure'/intermediate (I) and 12.7% resistant. EUCAST recommends that this test is only undertaken using broth microdilution. A significant proportion of EUCAST participants claimed to be using a gradient strip method (n=337) or EUCAST disk diffusion method (n=42), even though EUCAST zone diameter breakpoints are not provided for colistin in the 2019 guidelines and gradient strip methods underestimate MIC, resulting in underreporting of resistance to this agent (EUCAST warnings 2016). Similarly, all participants following CLSI guidelines reporting results from an MIC method had used gradient strips. Participants who reported that they followed EUCAST disk diffusion methods or CLSI methods may need to review their methodology.

The *Staphylococcus aureus* strain (specimen 5586) was resistant to benzlypenicillin, cefoxitin, clindamycin, linezolid and tetracycline and susceptible to ciprofloxacin, erythromycin, fusidic acid, gentamicin, rifampicin, teicoplanin and vancomycin. An excellent concordance with intended results was achieved for 11 antimicrobials and satisfactory performance was achieved for linezolid.

For linezolid, the intended result was resistant (MIC=16 mg/L). Participants provided the following results: 84.4% resistant and 15.6% susceptible. Participants using EUCAST or CLSI automated methods were more likely to achieve the intended result of resistant.

The *Streptococcus pneumoniae* strain (specimen 5587) was categorised as 'susceptible, increased exposure'/intermediate (I) to cefotaxime/ceftriaxone. The strain was susceptible to levofloxacin, moxifloxacin and norfloxacin and resistant to clindamycin, erythromycin and penicillin. There was an excellent concordance with intended results for clindamycin, erythromycin, levofloxacin, moxifloxacin, and for cefotaxime/ceftriaxone, when pneumonia breakpoints were applied, and penicillin, when meningitis breakpoints applied. There was a good concordance for penicillin (pneumonia) and a satisfactory concordance for norfloxacin. However, there was low concordance for cefotaxime/ceftriaxone, and cefotaxime/ceftriaxone when applying the breakpoints for meningitis. As in previous years, there were ongoing problems with results for the cephalosporin class antibiotics in a strain of *S. pneumoniae* 'susceptible, increased exposure' (I) to cefotaxime/ceftriaxone (MIC=1-2 mg/L). For each agent, participants found the strain to be more susceptible than the intended result reported by the reference laboratory. Overall, for ceftriaxone, participants reported 66.7% susceptible, 32.5% 'susceptible, increased exposure'/intermediate (I) and 0.8% resistant. EUCAST participants using automated methods were more likely to achieve the intended categorisation of 'susceptible, increased exposure' (I) for ceftriaxone than those using disk or MIC methods.

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. Two such areas that concern several laboratories are the correct categorisation of cephalosporin susceptibility results for *S. pneumoniae* isolates, and ceftazidime resistance for *E. coli* isolates. This report suggests that there is not one overall AST method (EUCAST or CLSI) or other type of method (automated, disk diffusion or MIC) that is likely to resolve all the issues experienced by individual participants during this EQA exercise. Therefore, participants should ensure that they are following their chosen methodology carefully, in particular for species-antimicrobial agent combinations for which they did not achieve the intended results. The observation that some participants are reporting 'susceptible, increased exposure' (I) in cases where their guidelines do not define such a category indicates that methods are not always strictly adhered to and participants should review their reporting practices.

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 not-for-profit organisation with fifty years' experience in delivering an EQA service to more than 1 800 laboratories worldwide. 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 (ECDC) as EARS-Net.

The purpose of the EARS-Net 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 the European Union/European Economic Area (EU/EEA). This report presents an analysis of participants' results for the 2019 EARS-Net EQA exercise.

2. Study design and methods

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. 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: *Acinetobacter baumannii* complex, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* 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 minimum inhibitory concentrations by means of 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 (see 'Results' section of the report). The panel was distributed in October 2019 as UK NEQAS distribution 4677.

A dedicated web page was available on the UK NEQAS website for participants to enter their results. Participants could use the webpage to access instructions for the secure web portal and download the protocol describing the specimen examination process. Detailed instructions were included on how to access the secure website using 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 AST results to be manually recorded prior to 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. The UK NEQAS for Microbiology forwarded the 2018 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. On the date of dispatch, specimens were couriered by air to each national EQA coordinator. The national EQA coordinators were contacted beforehand by email with a final reminder about imminent specimen dispatch and a request to confirm the receipt date by fax using a form enclosed with the shipment. Seven 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.

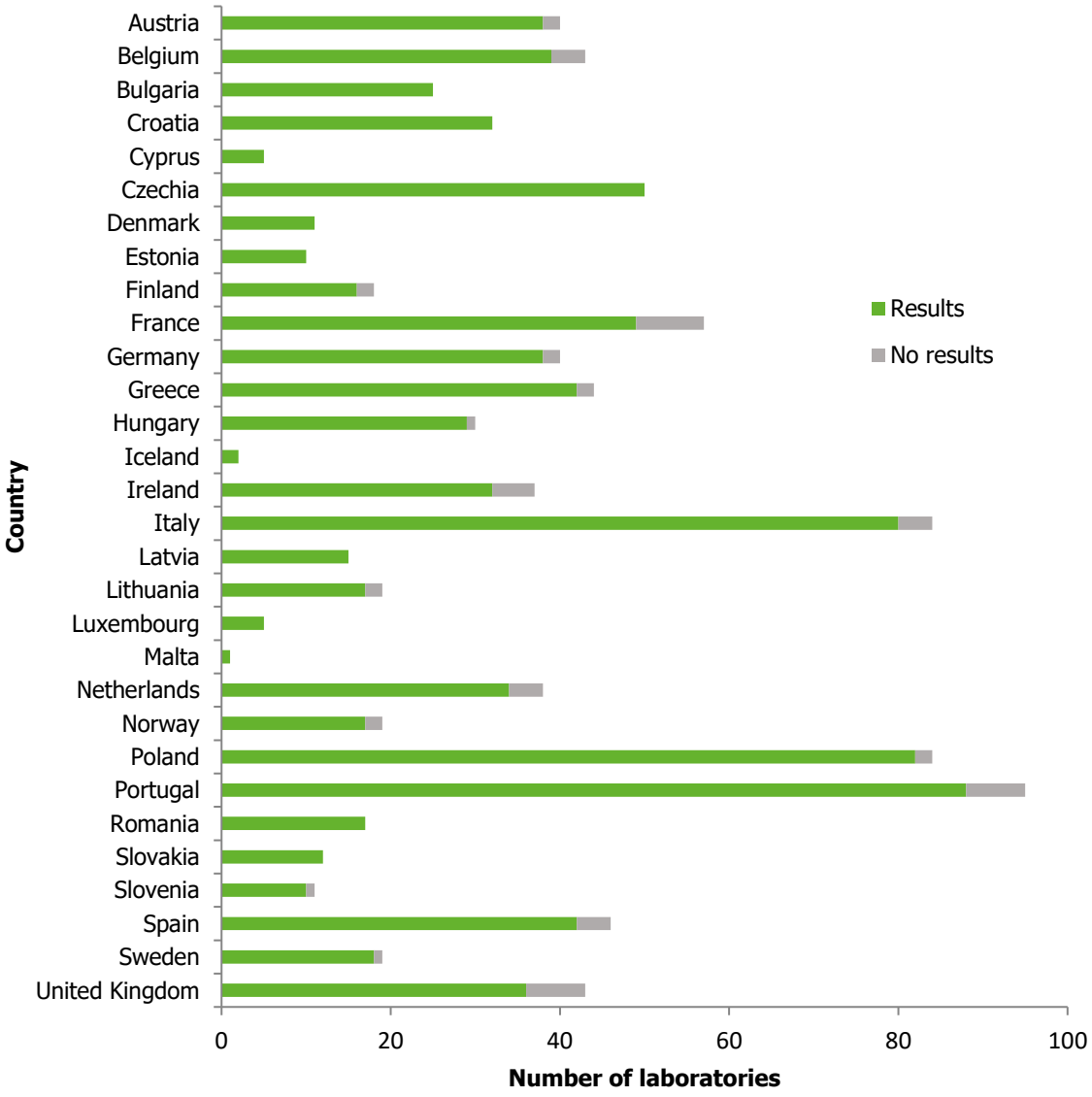
Participants were asked to report the identification of each isolate and antimicrobial susceptibility characterisation – susceptible (S), 'susceptible, increased exposure'/intermediate (I)[3] or resistant (R) – based on clinical breakpoints set out in the guidelines followed at their laboratories. The participants' results were analysed and considered 'concordant' if the reported categorisation agreed with the reference laboratories' interpretation. The concordance for a strain was defined as excellent (≥95% of laboratories obtained the correct result), very good (>90% to <95%), good (>85 - ≤90%) or satisfactory (80 - ≤85%). In addition, information was collected from participants on the methodology used to identify isolates (automated or conventional) and to undertake AST (EUCAST [1], CLSI [2] or other; automated, disk diffusion, MIC or other). MIC options included broth microdilution and gradient diffusion.

[3] In the breakpoint tables for interpreting MICs and zone diameters valid since 1 January 2019, EUCAST has changed the definition of 'I' from 'intermediate' to 'susceptible, increased exposure'. Results of the EQA exercise were interpreted following this guideline. `Intermediate' applies to CLSI results.

3. Results

Six bacterial strains were distributed to 952 laboratories in 30 EU/EEA countries[4] and 892 (93.7%) of them returned reports, including laboratories in all the EU/EEA countries invited to participate. Figure 1 shows the proportion of participating laboratories returning results per country.

Figure 1. Number of participating laboratories returning external quality assessment results, by country, 2019



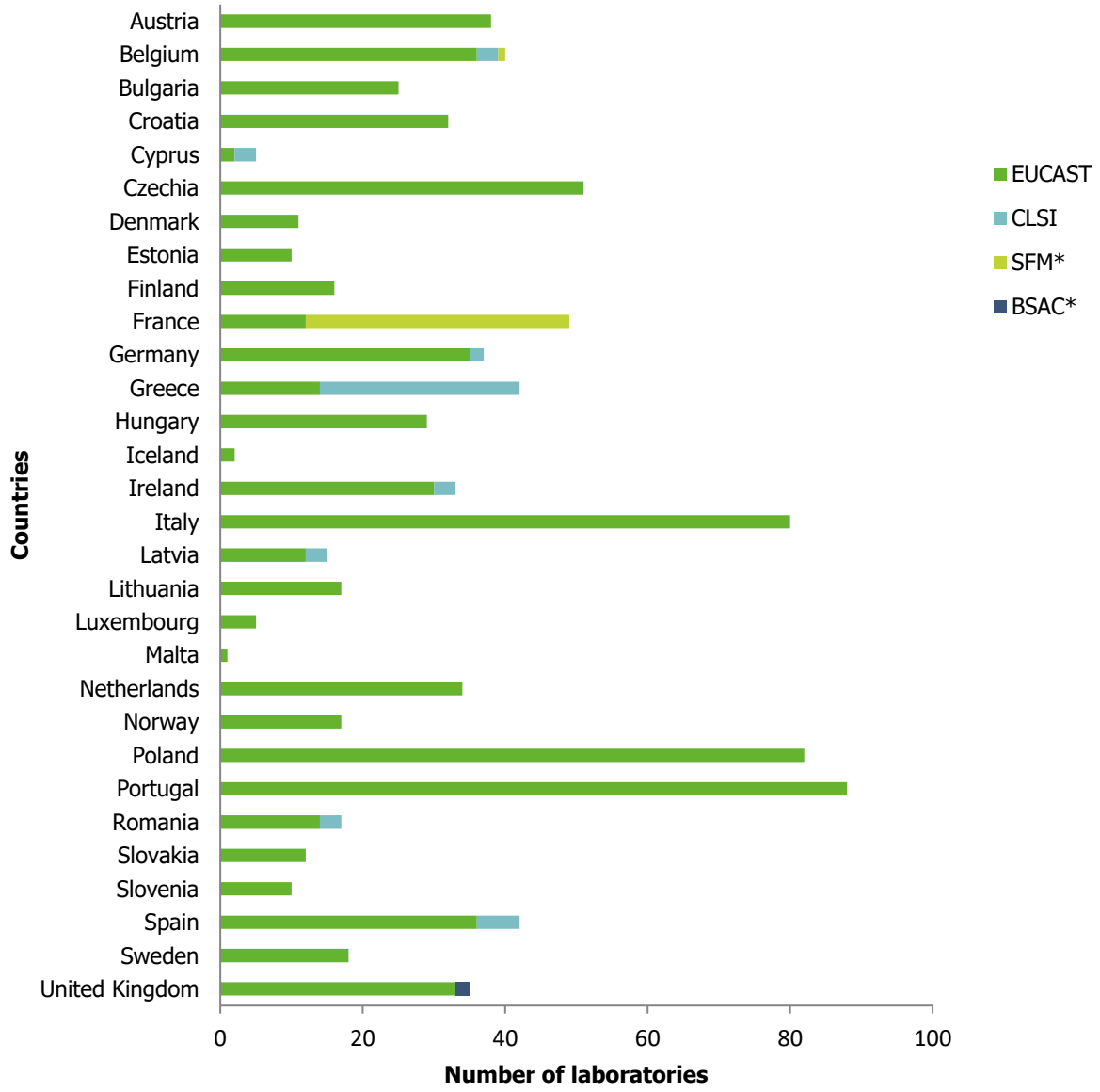
To determine AST results, laboratories used automated methods (50.3%), disk diffusion tests (35.2%), non-automated MIC methods, including broth microdilution and gradient methods, (14.0%), or other methods (0.5%).

Only 51 (5.7%) laboratories applied CLSI guidelines, a decline from the previous year when the proportion was 8.6%. EUCAST (or EUCAST-related) guidelines were reported by 94.3% of laboratories. This represented an increase on 2018, when the reported number applying EUCAST (or EUCAST-related) guidelines was 91.4%. Figure 2 shows the national and international guidelines used by laboratories in different countries.

[4] In 2019, the United Kingdom (UK) participated in the EQA exercise as a Member State of the European Union (EU).

For species identification, 87% of laboratories used an automated instrument and 13% used conventional methods. Overall, 99.4% of the identifications were correct and there were no significant issues arising for species identification. The main problem appeared to be the mixing-up of the six samples in the EQA exercise.

Figure 2. Clinical antimicrobial susceptibility testing guidelines reported as used by laboratories: number of laboratories by country, 2019



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 5582: *Acinetobacter baumannii* complex

This specimen contained a strain of *Acinetobacter baumannii* complex that was resistant to carbapenems (imipenem and meropenem), ciprofloxacin, levofloxacin and gentamicin. Table 1 shows the intended results and concordance for susceptibility testing of this strain.

Table 1. *Acinetobacter baumannii* complex (specimen 5582). Minimum inhibitory concentrations and intended results reported by 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	4	4	S/S	96.5
Ciprofloxacin	32	64	R/R	99.4
Colistin	0.5	0.5	S/S	98.1
Gentamicin	>64	>64	R/R	99.7
Imipenem	32	32	R/R	93.7
Levofloxacin	*	*	R/R	98.4
Meropenem	64	>64	R/R	99.2
Tobramycin	1	1	S/S	95.9

R: resistant
S: susceptible

**There were no reference results for levofloxacin and assigned results were based on participant consensus.*

An excellent or very good concordance of results was achieved for the antimicrobial agents tested. No method- or interpretative-guidance-related bias was detected.

The majority (98.3%) of participating laboratories correctly identified the isolate as *Acinetobacter baumannii* complex, with the vast majority using automated methods (Table 2). Participants using automated methods were more likely to misidentify the isolate. Participants using conventional methods were more likely to unsuccessfully provide full identification to species level.

Table 2. Identification results for specimen 5582

Species	Number of participants responding by identification method	
	Automated	Conventional
Acinetobacter species	2	7
Acinetobacter baumannii complex	750	111
Escherichia coli	1	0
Klebsiella pneumoniae	1	0
Moraxella catarrhalis	1	0
Pseudomonas fluorescens	0	1
Staphylococcus aureus	1	0
Stenotrophomonas maltophilia	1	0
Total	**757**	**119**

The correct result is shaded.

Specimen 5583: *Escherichia coli*

This specimen contained a strain of *Escherichia coli* that was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin and piperacillin-tazobactam. Resistance to the aminopenicillins (ampicillin, amoxicillin), amoxicillin-clavulanic acid and piperacillin-tazobactam was conferred by a hyper-expressed TEM-1 β-lactamase.

There was an excellent or very good concordance with intended AST results for 15 antimicrobial agents and a good concordance for amoxicillin-clavulanic acid, but no concordance was achieved for ceftazidime (Table 3).

Table 3. *Escherichia coli* (specimen 5583). Minimum inhibitory concentrations and intended results reported by 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	2	2	S/S	97.4
Amoxicillin	≥128	≥128	R/R	99.6
Amoxicillin-clavulanic acid	≥128*	≥128*	R/R	87.2
Ampicillin	≥128	≥128	R/R	99.8
Cefotaxime	0.12	0.12	S/S	97.9
Ceftazidime	4	4	I/S	30.6
Ceftriaxone	0.25	0.25	S/S	98.2
Ciprofloxacin	0.015	0.015	S/S	99.9
Colistin	0.5	1	S/†	99.4
Ertapenem	0.008	0.015	S/S	100
Gentamicin	0.5	0.5	S/S	98.1
Imipenem	0.12	0.12	S/S	99.4
Levofloxacin	**	**	S/S	99.8
Meropenem	0.015	0.015	S/S	100
Ofloxacin	**	**	S/S	99.1
Piperacillin-tazobactam	≥128*	≥128*	R/R	91.4
Tobramycin	0.5	1	S/S	97.2

I: 'susceptible, increased exposure'
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 and assigned results were based on participant consensus.*
† No breakpoint provided by CLSI.

For ceftazidime (MIC=4 mg/L) the intended result was 'susceptible, increased exposure' (I) under EUCAST guidelines, with the MIC being close to the breakpoint. Participants provided the following results: 60.4% susceptible; 30.6% 'susceptible, increased exposure'/intermediate (I); and 9.0% resistant. Only 32.6% of participants following EUCAST methods and 94.1% of participants following CLSI methods provided the correct category. EUCAST participants using MIC methods were more likely to obtain the intended result than those using automated methods (Table 4).

Table 4. Susceptibility of *Escherichia coli* (specimen 5583) to ceftazidime reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	254 (69.6%)	97 (26.6%)	14 (3.8%)
	Disk diffusion	127 (52.3%)	76 (31.3%)	40 (16.5%)
	MIC	63 (39.6%)	77 (48.4%)	19 (11.9%)
	Other	5	2	0
	Total	**449 (58.0%)**	**252 (32.6%)**	**73 (9.4%)**
CLSI	Automated	35 (97.2%)	0	1 (2.8%)
	Disk diffusion	9	0	0
	MIC	3	1	1
	Other	1	0	0
	Total	**48 (94.1%)**	**1 (2.0%)**	**2 (3.9%)**

I: 'susceptible, increased exposure', intermediate (CLSI)
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.
Number of participants may not correspond the total, due to some laboratories not testing against this antibiotic.

For amoxicillin-clavulanic acid, the intended result was resistant (MIC≥128 mg/L). Participants provided the following results: 87.2% resistant, 2.1% 'susceptible, increased exposure'/intermediate (I) and 10.7% susceptible. There is no 'susceptible increased exposure' (I) category for amoxicillin-clavulanic acid in the 2019 EUCAST guidelines, so those participants with an 'I' result who reported that they had followed EUCAST methods may need to review their methodology. Participants following CLSI guidelines using automated methods were less likely to report the intended result. For participants following EUCAST guidelines, those performing tests using MIC methods were less likely to report the intended result.

Table 5. Susceptibility of *Escherichia coli* 5583 to amoxicillin-clavulanic acid reported by participants using different guidelines and methods

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	30 (7.8%)	2 (0.5%)	353 (91.7%)
	Disk diffusion	22 (8.9%)	3 (1.2%)	223 (89.9%)
	MIC	15 (12.6%)	0	104 (87.4%)
	Other	0	0	8
	Total	**67 (8.8%)**	**5 (0.7%)**	**688 (90.5%)**
CLSI	Automated	15 (41.7%)	12 (33.3%)	9 (25.0%)
	Disk diffusion	2	1	5
	MIC	1	0	2
	Other	0	0	1
	Total	**18 (37.5%)**	**13 (27.1%)**	**17 (35.4%)**

I: 'susceptible, increased exposure' (EUCAST), intermediate (CLSI)
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.
Number of participants may not correspond the total, due to some laboratories not testing against this antibiotic.

Almost all (99.5%) of the participating laboratories correctly identified the isolate as *Escherichia coli* (Table 6).

Table 6. Identification results for specimen 5583

Species	Number of participants responding by identification method	
	Automated	Conventional
Escherichia coli 0157 toxin negative	1	0
Escherichia coli 0157 toxin not tested	2	1
Escherichia coli	735	134
Total	**738**	**135**

The correct result is shaded.

Specimen 5584: *Klebsiella pneumoniae*

This specimen contained a strain of *Klebsiella pneumoniae* that was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, gentamicin (CLSI I/R), levofloxacin, ofloxacin and tobramycin (CLSI I). The strain was susceptible to cephalosporins and carbapenems. Table 7 shows the intended results and concordance for susceptibility testing of this strain.

Concordance with intended results was excellent for amikacin, ampicillin, amoxicillin, ciprofloxacin, ofloxacin, ceftazidime, ceftriaxone, cefotaxime, imipenem, levofloxacin, meropenem and colistin, and very good for piperacillin/tazobactam and ertapenem. For gentamicin, the concordance was good and for tobramycin it was satisfactory. A low concordance was achieved for amoxicillin-clavulanic acid (Table 7).

Table 7. *Klebsiella pneumoniae* (specimen 5584). Minimum inhibitory concentrations and intended results reported by 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	<0.25	<0.25	S	S	95.9
Amoxicillin	>64	>64	R	R	100
Amoxicillin-clavulanic acid	64*	>64*	R	R	78.7
Ampicillin	>64	>64	R	R	99.9
Cefotaxime	0.5	1	S	S	98.2
Ceftazidime	0.12	0.12	S	S	98.4
Ceftriaxone	0.25	0.25	S	S	98
Ciprofloxacin	32	64	R	R	99.4
Colistin	0.5	0.5	S	-	99.3
Ertapenem	0.25	0.5	S	S	94.2
Gentamicin	8	16	R	I/R	85.6
Imipenem	0.12	0.25	S	S	99.4
Levofloxacin	**	**	R	R	99.4
Meropenem	0.25	0.25	S	S	99.1
Ofloxacin	**	**	R	R	98.7
Piperacillin-tazobactam	4*	16*	S/I	S	92.4
Tobramycin	8	8	R	I	83.7

I: 'susceptible, increased exposure' (EUCAST), intermediate (CLSI)
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 and assigned results were based on participant consensus*

The intended result for amoxicillin-clavulanic acid was resistant (MIC=64 mg/L or >64 mg/L depending on the reference laboratory). Participants provided the following results: 17.3% susceptible; 4.0% 'susceptible, increased exposure'/intermediate (I) and 78.7% resistant.

Participants following CLSI guidelines were more likely to report intermediate due to a difference in breakpoints. Those participants using the EUCAST disk method or MIC methods were less likely to report the intended result than those using automated methods (Table 8). There is no 'susceptible, increased exposure' (I) category for amoxicillin-clavulanic acid susceptibility in the 2019 EUCAST guidelines, so the participants with an 'I' result who reported having followed EUCAST methods may need to review their methodology.

Table 8. Susceptibility of *Klebsiella pneumoniae* (specimen 5584) to amoxicillin-clavulanic acid reported by participants according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	14 (3.7%)	0 (0%)	369 (96.3%)
	Disk diffusion	76 (30.5%)	1 (0.4%)	172 (69.1%)
	MIC	31 (26.7%)	0 (0%)	85 (73.3%)
	Other	2	0	6
	Total	**123 (16.3%)**	**1 (0.1%)**	**632 (83.6%)**
CLSI	Automated	2 (5.7%)	26 (74.3%)	7 (20%)
	Disk diffusion	2	3	2
	MIC	0	0	2
	Other	0	0	1
	Total	**4 (8.9%)**	**29 (64.4%)**	**12 (26.7%)**

I: 'susceptible, increased exposure' (EUCAST), intermediate (CLSI)
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 (99.5%) of the participating laboratories correctly identified the isolate as *Klebsiella pneumoniae* (Table 9).

Table 9. Identification results for specimen 5584

Species	Number of participants responding by identification method	
	Automated	Conventional
Acinetobacter baumannii	1	0
Klebsiella pneumoniae	747	115
Klebsiella species	1	1
Enterococcus species	0	1
Total	**749**	**117**

The correct result is shaded.

Specimen 5585: *Pseudomonas aeruginosa*

This specimen contained a strain of *Pseudomonas aeruginosa* that was susceptible to ceftazidime. Carbapenem resistance was due to reduced porin expression, efflux systems and increased chromosomal AmpC β-lactamase production.

An excellent concordance with intended results was achieved for seven antimicrobial agents, good performance for another agent, and satisfactory performance for the remaining two agents.

Table 10. *Pseudomonas aeruginosa* (specimen 5585). Minimum inhibitory concentrations and intended results reported by 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	32	>64	R	I/R	83.0
Ceftazidime	4	8	S	S	82.0
Ciprofloxacin	4	32	R	R	99.4
Colistin	2	4	S/R	S/R	86.9
Gentamicin	>64	>64	R	R	99.3
Imipenem	32	32	R	R	100
Levofloxacin	*	*	R	R	99.7
Meropenem	16	32	R	R	99.8
Piperacillin-tazobactam	>64	>64	R	R	99.8
Tobramycin	>64	>64	R	R	99.6

I: intermediate (CLSI)
R: resistant
S: susceptible
**: no reference results for levofloxacin – assigned results based on participant consensus*

For colistin, the intended result was susceptible or resistant as the MICs (2-4 mg/L) spanned the breakpoint. Participants provided the following results: 86.9% susceptible, 0.5% 'susceptible, increased exposure'/intermediate (I) and 12.7% resistant. EUCAST recommends that this test is only undertaken using broth microdilution. A significant proportion of EUCAST participants claimed to be using a gradient strip method (n=337) or EUCAST disk diffusion method (n=42), even though no EUCAST zone diameter breakpoints are provided in the 2019 guidelines and gradient strip methods underestimate the MIC, resulting in under-reporting of resistance to this agent (EUCAST warnings 2016). Similarly, all participants following CLSI guidelines reporting results from an MIC method used gradient strips (Table 11). Participants who reported that they followed EUCAST disk diffusion methods or CLSI methods may need to review their methodology.

Table 11. Susceptibility of *Pseudomonas aeruginosa* 5585 to colistin reported by participants using different guidelines and methods

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	162 (95.9%)	0 (0%)	7 (4.1%)
	Disk diffusion	34 (81.0%)	1 (2.4%)	7 (16.7%)
	MIC	306 (83.2%)	1 (0.3%)	61 (16.6%)
	Other	8	1	2
	Total	**510 (86.4%)**	**3 (0.5%)**	**77 (13.1%)**
CLSI	Automated	19 (95.0%)	0	1 (5.0%)
	Disk diffusion	2	0	0
	MIC	14	0	1
	Other	1	0	0
	Total	**36 (94.7%)**	**0 (0%)**	**2 (5.3%)**

I: `susceptible, increased exposure' (EUCAST), intermediate (CLSI)
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 (99.8%) of participating laboratories correctly identified the isolate as *Pseudomonas aeruginosa* (Table 12).

Table 12. Identification results for specimen 5585

Species	Number of participants responding by identification method	
	Automated	Conventional
Pseudomonas aeruginosa	724	128
Pseudomonas species	0	1
Staphylococcus aureus	1	0
Total	**725**	**129**

The correct test result is shaded.

Specimen 5586: *Staphylococcus aureus*

This specimen contained a strain of *Staphylococcus aureus* that was susceptible to ciprofloxacin, erythromycin, fusidic acid, gentamicin, rifampicin, teicoplanin and vancomycin. This strain was resistant to benzylpenicillin, cefoxitin, clindamycin, linezolid and tetracycline. Table 13 shows the intended results and concordance for susceptibility testing of this strain.

An excellent concordance was achieved for 11 antimicrobials and a satisfactory performance was achieved for linezolid.

Table 13. *Staphylococcus aureus* (specimen 5586). Minimum inhibitory concentrations and intended results reported by reference laboratories and the overall concordance of the participating laboratories

Antimicrobial agent	MIC range (mg/L)	Intended interpretation	
	Reference laboratory 1 & 2	EUCAST/CLSI	Overall concordance (%)
Benzylpenicillin	>0.5	R/R	99.7
Cefoxitin	16	R/R	99.6
Ciprofloxacin	0.5	S/S	96.8
Clindamycin	>4	R/R	99.4
Erythromycin	0.5	S/S	97.4
Fusidic acid	≤0.12	S/S	99.7
Gentamicin	0.5	S/S	96.9
Linezolid	16	R/R	84.4
Rifampicin	≤0.008	S/S	99.3
Teicoplanin	0.5	S/S	99.6
Tetracycline	>8	R/R	98.7
Vancomycin	1	S/S	98.7

R: resistant
S: susceptible.

For linezolid, the intended result was resistant (MIC=16 mg/L). Participants provided the following results: 84.4% resistant and 15.6% susceptible. Participants using EUCAST or CLSI automated methods were more likely to achieve the intended result of resistant (Table 14).

Table 14. Susceptibility of *Staphylococcus aureus* 5586 to linezolid reported by participants using different guidelines and methods

Guideline	Method	Number (%) participants responding	
		S	R
EUCAST	Automated	42 (11.4%)	327 (88.6%)
	Disk diffusion	45 (19.1%)	191 (80.9%)
	MIC	33 (21.3%)	122 (78.7%)
	Other	0	8
	Total	**120 (15.6%)**	**648 (84.4%)**
CLSI	Automated	2 (5.7%)	33 (94.3%)
	Disk diffusion	4	2
	MIC	2	2
	Other	1	0
	Total	**9 (19.6%)**	**37 (80.4%)**

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 *Staphylococcus aureus* (Table 15).

Table 15. Identification results for specimen 5586

Species	Number of participants responding by identification method	
	Automated	Conventional
Streptococcus pneumoniae	1	0
Staphylococcus aureus	693	152
Total	**694**	**152**

The correct result is shaded.

Specimen 5587: *Streptococcus pneumoniae*

This specimen contained a *Streptococcus pneumoniae* which was categorised as 'susceptible, increased exposure'/intermediate (I) to cefotaxime/ceftriaxone. The strain was susceptible to levofloxacin, moxifloxacin and norfloxacin and resistant to clindamycin, erythromycin and penicillin.

There was an excellent concordance with intended results for clindamycin, erythromycin, levofloxacin, moxifloxacin, and for cefotaxime/ceftriaxone, when pneumonia breakpoints were applied, and penicillin, when meningitis breakpoints applied. There was a good concordance for penicillin (pneumonia) and a satisfactory concordance for norfloxacin. However, there was low concordance for cefotaxime/ceftriaxone, and cefotaxime/ceftriaxone when applying the breakpoints for meningitis.

Table 16. *Streptococcus pneumoniae* (specimen 5587). Minimum inhibitory concentrations and intended results reported by 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 (%)
Cefotaxime	1	1	I	†	39.7
meningitis			I	I	35.5
pneumonia			I	S	99.3
Ceftriaxone	1	2	I	†	32.5
meningitis			I	I/R	31.8
pneumonia			I	S/I	99.0
Clindamycin	*	*	R	R	96.0
Erythromycin	≥128	≥128	R	R	99.8
Levofloxacin	1	1	S	S	96.9
Moxifloxacin	0.12	0.12	S	S	98.9
Norfloxacin	*	*	S	S	80.9
Penicillin	4	4	R	†	95.0
meningitis			R	R	99.0
pneumonia			R	I	87.9

I: 'susceptible, increased exposure' (EUCAST), intermediate (CLSI)
R: resistant
S: susceptible
** There were no reference results for clindamycin or norfloxacin and assigned results were based on participant consensus.*
† no breakpoint provided by CLSI

As in previous years, there were ongoing problems with susceptibility results for the cephalosporin class of antibiotics in a strain of *S. pneumoniae* 'susceptible, increased exposure' (I) to cefotaxime and ceftriaxone (MIC=1-2 mg/L) by EUCAST categorisation [3]. For each agent, participants found the strain to be more susceptible than the intended result reported by the reference laboratory (Table 17 and Table 18). This was demonstrated for all methods, and irrespective of whether the EUCAST or CLSI guidelines were followed for interpretation of results.

Table 17. Susceptibility of *Streptococcus pneumoniae* (specimen 5587) to cefotaxime reported by participants according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	157 (55.3%)	124 (43.7%)	3 (1.1%)
	Disk diffusion	49 (58.3%)	34 (40.5%)	1 (1.2%)
	MIC	138 (62.2%)	84 (37.8%)	0 (0%)
	Other	2	4	0
	Total	**346 (60.1%)**	**246 (42.7%)**	**4 (0.7%)**

I: 'susceptible, increased exposure' (EUCAST)
R: resistant
S: susceptible
The correct result 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.

Overall, for ceftriaxone, participants reported 66.7% susceptible, 32.5% 'susceptible, increased exposure'/intermediate (I) and 0.8% resistant (Table 16). EUCAST participants using automated methods were more likely to achieve the intended categorisation of 'susceptible, increased exposure' (I) for ceftriaxone than those using disk or MIC methods (Table 18).

Table 18. Susceptibility of *Streptococcus pneumoniae* (specimen 5587) to ceftriaxone reported by participants according to guidelines followed and methods used

Guideline	Method	Number (%) participants responding		
		S	I	R
EUCAST	Automated	129 (57.6%)	92 (41.1%)	3 (1.3%)
	Disk diffusion	74 (69.2%)	32 (29.9%)	1 (0.9%)
	MIC	162 (73.0%)	60 (27.0%)	0 (0%)
	Other	4	2	0
	Total	**369 (66.0%)**	**186 (33.3%)**	**4 (0.7%)**

I: `susceptible, increased exposure' (EUCAST)
R: resistant
S: susceptible
The correct result 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 majority (99.5%) of participating laboratories correctly identified the isolate as *Streptococcus pneumoniae* (Table 19).

Table 19. Identification results for specimen 5587

Species	Number of participants responding by identification method	
	Automated	Conventional
Enterococcus species	0	1
Pseudomonas aeruginosa	1	0
Streptococcus species	1	0
Streptococcus anginosus	1	0
Streptococcus pneumonia	539	301
Total	**542**	**302**

The correct result is shaded.

4. Discussion

Overall, the performance of laboratories participating in the 2019 EQA exercise was very good. There were no significant issues arising in relation to species identification. For AST, ≥95% concordance was achieved for 59 (75.6%) of 78 species-antimicrobial agent combinations tested. Therefore the bacterial identification was considered excellent and the AST performance good.

In recent years, lower concordances in reporting susceptibility results have been seen in previous EQA exercises for species-antimicrobial agent combinations with MIC values close to the breakpoints, or where breakpoints and categorisation of results differed for EUCAST and CLSI guidelines. Species-antimicrobial agent combinations for which recurrent problems have been encountered in previous EARS-Net EQA exercises include:

- *Escherichia coli* with intermediate/resistant or resistant results for piperacillin-tazobactam;
- *Klebsiella pneumoniae* with differing third-generation cephalosporin results;
- *Staphylococcus aureus* with intermediate results for vancomycin; and
- *Streptococcus pneumoniae* with susceptible, increased exposure (EUCAST)/intermediate (CLSI) results for cephalosporins.

In addition, the 2018 EQA exercise reported on the following problematic species-antimicrobial agent combinations:

- *Escherichia coli* with resistant results for colistin; and
- *Streptococcus pneumoniae* with intermediate results for penicillin.

Most of the previous problematic species-antimicrobial agent combinations were not included in this 2019 EQA exercise and other combinations were tested instead.

Specimen 5582 contained a strain of *Acinetobacter baumannii* complex that was resistant to gentamicin, quinolones and carbapenems. An excellent or very good concordance was achieved for all antimicrobial agents. Identification of this strain was excellent, with 98.3% of participants reporting the correct result to species level.

Specimen 5583 contained a strain of *Escherichia coli* exhibiting resistance to beta-lactams, including amoxicillin, amoxicillin-clavulanic acid, ampicillin and piperacillin-tazobactam. An excellent or very good concordance in 15 out of 17 antimicrobial agents was achieved. A good concordance (87.2%) was achieved for amoxicillin-clavulanic acid, but concordance was not achieved (30.6%) for ceftazidime. For ceftazidime, the intended result of 'susceptible, increased exposure' (I) according to EUCAST guidelines, was close to the breakpoint. There were very few methodological issues noted with laboratories reporting susceptible, as opposed to 'susceptible, increased exposure' (I). There was no specific method which appeared to be more likely to yield the correct result. An excellent concordance (99.5%) of participating laboratories reported the correct result to species level. Interestingly, four laboratories reported this isolate as *Escherichia coli* 0157.

Specimen 5584 contained a strain of *Klebsiella pneumoniae* that was resistant to amoxicillin, amoxicillin-clavulanic acid, ampicillin, ciprofloxacin, gentamicin, levofloxacin, ofloxacin and tobramycin. The strain was susceptible to third-generation cephalosporins and carbapenems. An excellent or very good concordance was achieved for 14 out of 17 antimicrobial agents. A low concordance of 78.7% was achieved for amoxicillin-clavulanic acid despite the strain having high level resistance. Among those participants following EUCAST guidelines, automated methods were most likely to achieve the intended result and participants following the EUCAST disk diffusion method were least likely to achieve the intended result. A total of 99.5% of participating laboratories correctly identified the isolate to species level. Two laboratories correctly reported identification to genus level, and a further two laboratories incorrectly reported the identified specimen as *Acinetobacter baumannii* or *Enterococcus* species.

Specimen 5585 contained a strain of *Pseudomonas aeruginosa* susceptible only to ceftazidime following EUCAST and CLSI guidelines. The intended result for colistin was not determined, as it was deemed susceptible by one reference laboratory and resistant by the other. For seven out of 10 antimicrobial agents, an excellent concordance was achieved. Of the remaining three agents, a satisfactory concordance was achieved for amikacin and ceftazidime, and a good concordance for colistin. Almost all (99.8%) of the participating laboratories reported the correct identification to species level. One laboratory incorrectly reported the identified specimen as *Staphylococcus aureus.*

Specimen 5586 contained a strain of *Staphylococcus aureus* resistant to benzylpenicillin, cefoxitin, clindamycin, linezolid and tetracycline. An excellent concordance was achieved for 11 out of 12 antimicrobial agents. The concordance for linezolid attained 84.0%, a significant improvement on 2017 when the same strain of *Staphylococcus aureus* was distributed and only 16.3% of participating laboratories reported the intended result (i.e. resistant) [4]. An excellent concordance of 99.9% was achieved for identification to species level.

Specimen 5587 contained a strain of *Streptococcus pneumoniae* that was only susceptible to levofloxacin, moxifloxacin and norfloxacin by EUCAST and CLSI breakpoints. As in previous years, there was a poor concordance in this EQA exercise for cefotaxime and ceftriaxone results, with an over-reporting of isolates as being susceptible [3]. This was demonstrated by all methods and irrespective of whether EUCAST or CLSI guidelines were followed for the interpretation of results. An improved performance was achieved for penicillin than in previous years [3]. In the 2018 EQA exercise, according to EUCAST categorisation the strain was resistant to penicillin (MIC=4 mg/L), with no concordance achieved (14.6%). In the 2019 EQA exercise 95.0% concordance was reported for a strain with the same MIC (4 mg/L). The reporting of 'penicillin, pneumonia' results also improved in this EQA exercise, with a concordance of 87.9% compared to 64.0% in the 2018 EQA exercise. It is important to note that EUCAST published a warning, stating that gradient strips have a tendency to underestimate benzylpenicillin MIC values in *S. pneumoniae* [5]. An excellent concordance (99.5%) was achieved for identification to species level. However, there were a few incorrect identifications including *Enterococcus* species*, Pseudomonas aeruginosa* and *Streptococcus anginosus.*

Analysis of species-antimicrobial agent combinations, for which the laboratories performed poorly, did not demonstrate any overall advantage of using automated, MIC or disk diffusion methods. In previous years, 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 overestimating resistance.

5. Conclusions

The overall performance of participating laboratories in this EQA exercise was very good.

Nevertheless, this 2019 EARS-Net EQA exercise indicates that the underestimation of antimicrobial resistance percentages may be possible, due to the over-reporting of isolates as susceptible. On the other hand, there is still the possibility of over-reporting resistance, leading to an overestimation of antimicrobial resistance percentages in Europe. There appears to be an overall indication that isolates are under-reported as resistant, which could lead to an underestimation of resistance within Europe.

Several species-antimicrobial agent combinations had already been identified as a recurring or more recent issue in the 2018 EARS-Net EQA exercise. A few of those were included in the 2019 EARS-Net EQA exercise:

- *Streptococcus pneumoniae* with 'susceptible, increased exposure' (I) results for cephalosporins, continued to prove difficult to identify for EQA participants in 2019;
- *Escherichia coli,* with resistant results for piperacillin-tazobactam, was no longer challenging to identify for EQA participants in 2019.

In addition, this 2019 EQA exercise identified an emerging problematic combination:

- *Escherichia coli* with 'susceptible, increased exposure' (I) results for ceftazidime.

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.

As fewer participants report using CLSI methods, it is becoming less relevant to attempt to compare EUCAST and CLSI methods. 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 was similar overall.

6. Recommendations

In this EQA exercise, the overall performance of participating laboratories was excellent for identification to species level and good for AST. Nevertheless, specific areas of difficulty have been highlighted, some previously identified and others emerging (e.g. 'susceptible, increased exposure' (I) results for ceftazidime in *E. coli* isolates, or reporting interpretations for which guidance no longer exists).

This EQA exercise indicates that both under- and overestimation of antimicrobial resistance percentages in Europe may be possible, although overall the 2019 exercise indicates that underestimation is more frequent. This observation should be kept in mind when interpreting EARS-Net surveillance data.

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. Two such areas that concern several laboratories are the correct categorisation of cephalosporin susceptibility results for *S. pneumoniae* isolates, and ceftazidime resistance for *E. coli* isolates.

This report suggests that there is not one overall AST guideline (EUCAST or CLSI) or other type of method (automated, disk diffusion or MIC) that is likely to resolve all the issues experienced by individual participants during the EQA exercise. Therefore, participants should ensure that they are following their chosen methodology carefully, in particular for species-antimicrobial agent combinations for which they did not achieve the intended results.

The observation that some participants are reporting 'susceptible, increased exposure' (I) in cases where their guidelines do not define such a category indicates that methods are not always strictly adhered to and participants should review their reporting practice in these cases.

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.

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