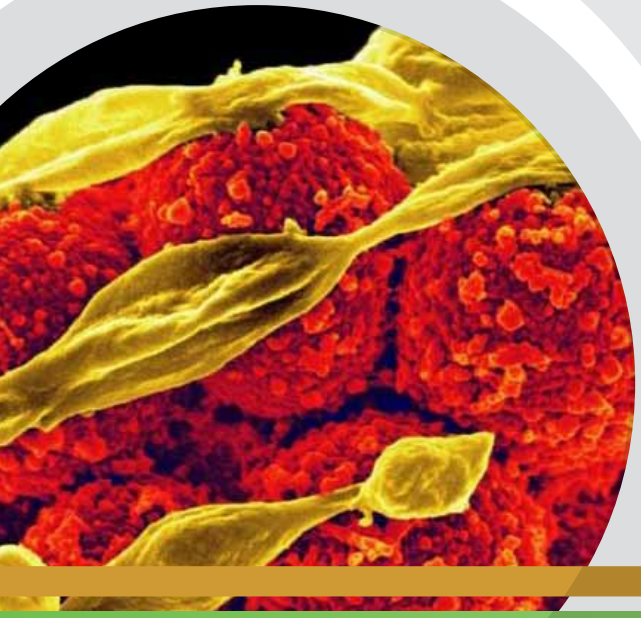

TECHNICAL REPORT

External quality assessment of laboratory performance – European Antimicrobial Resistance Surveillance Network (EARS-Net), 2017

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Author
Paul Chadwick, UK NEQAS Bacteriology Scheme Organiser

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Abbreviations

AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
EARS-Net	European Antimicrobial Resistance Surveillance Network
EU/EEA	European Union/European Economic Area
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MIC	Minimum inhibitory concentration
UK NEQAS	United Kingdom National External Quality Assessment Scheme for Microbiology

Executive summary

This report provides an analysis of the external quality assessment (EQA) performance of laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2017. A total of 893 laboratories participated in the EQA exercise. Six bacterial strains were used: *Acinetobacter baumannii* complex, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

For species identification, 58.2% laboratories used an automated instrument and 39.8% used conventional methods. There were no significant issues regarding species identification. For the determination of antimicrobial susceptibility testing (AST) results, most laboratories used either automated methods or disk diffusion tests. For AST, there was a continuing trend towards increasing use of European Committee on Antimicrobial Susceptibility Testing (EUCAST) or EUCAST-related guidelines (85.9% in 2017) and a decreasing use of the Clinical and Laboratory Standards Institute (CLSI) guideline (10.4% in 2017). The overall AST performance was satisfactory.

For the *S. pneumoniae* strain, a very good concordance of susceptibility results was seen for seven out of eight antimicrobial agents tested. However, only 72.1% of participants correctly categorised the isolate as having an intermediate level of resistance to penicillin and 17.6% incorrectly categorised the isolate as penicillin-susceptible. The ECDC report for 2016 noted that *S. pneumoniae* with intermediate resistance to penicillin represented a problem for EARS-Net participants and this clearly remained an issue of concern in 2017.

For the *S. aureus* strain, an excellent concordance of susceptibility results was seen for 11 of 12 antimicrobial agents tested, but only 16.3% of participants identified linezolid resistance. This was the first year that linezolid susceptibility testing was included as part of the EQA exercise. It is important that laboratories are able to identify the emergence of new or unexpected resistance, such as linezolid resistance in *S. aureus*.

For the *E. faecium* strain, a good concordance of susceptibility results was seen for all five antimicrobial agents tested.

For the *E. coli* strain, an excellent concordance of susceptibility results was seen for 15 of 17 antimicrobial agents tested. However, many participants did not achieve the intended results (i.e. resistant for amoxicillin/clavulanic acid and colistin). This was the first year that colistin susceptibility testing for Enterobacteriaciae was included in the EQA exercise.

For the *K. pneumoniae* strain, a good concordance of susceptibility results was seen for 13 of 17 agents tested. For amikacin, only 38.8% participants reported the intended result, i.e. intermediate (EUCAST) or susceptible (CLSI). Only 82.7% and 76.5% of participants provided the intended result, i.e. susceptible, for ceftazidime and ceftriaxone respectively. *Klebsiella pneumoniae* with susceptible/intermediate amikacin results and with differing third-generation cephalosporin results have also been noted to represent a problem for EARS-Net participants in previous years. For meropenem, 89.6% of participants correctly reported the intended result (i.e. susceptible).

For the *Acinetobacter baumannii* complex strain, a good concordance of susceptibility results was seen for all eight antimicrobial agents tested.

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

Several species/antimicrobial agent combinations that were already known as presenting a problem when performing AST have again proven difficult for participants in 2017:

- *Streptococcus pneumoniae* with intermediate penicillin results
- *Klebsiella pneumoniae* with differing third-generation cephalosporin results; and
- *Klebsiella pneumoniae* with susceptible/intermediate amikacin results.

Two new species/antimicrobial agent combinations that may present a problem when performing AST were identified after introducing new tests into the 2017 EQA exercise:

- *Staphylococcus aureus* with linezolid resistance; and
- *Escherichia coli* with colistin resistance.

An analysis of species/antimicrobial agent combinations with poor performance did not show any overall advantage of using automated, minimum inhibitory concentration (MIC) or disk methods.

Participating laboratories that report to EARS-Net have been provided feedback that allows them to assess their individual performance in this EQA exercise and review all areas where they did not achieve the intended results.

The findings demonstrate the importance of laboratories ensuring that they follow their chosen methodology carefully, in particular for species/antimicrobial agent combinations for which they did not achieve the intended results.

The findings also emphasise the need for laboratories to ensure that they are aware of species/antimicrobial agent combinations that represent a problem when performing AST and of potential new resistance issues.

Annual EQA exercises are needed to evaluate and review the performance of laboratories that report to EARS-Net. The EQA exercise identifies species/antimicrobial agent combinations that may represent problems for AST due to existent or emerging resistance. In this way, it defines targets for training and improvement in testing methodology and interpretation of results, eventually leading to more reliable surveillance outputs.

1 Introduction

The United Kingdom National External Quality Assessment Service (UK NEQAS) is a not-for-profit organisation hosted by Public Health England (PHE) at Colindale, London with more than 40 years' experience in delivering external quality assessment (EQA) service to microbiological laboratories worldwide. Between 2000 and 2009, UK NEQAS delivered EQA exercises for antimicrobial susceptibility testing (AST) to the European Antimicrobial Resistance Surveillance System (EARSS). Since 2010, UK NEQAS has provided EQA services for the European Centre for Disease Prevention and Control (ECDC), offering this service to laboratories participating in the European Antimicrobial Resistance Surveillance Network (EARS-Net) through a framework contract between ECDC and UK NEQAS.

The purpose of the EQA exercises is to determine the accuracy of AST results reported by individual laboratories and allow comparison of results between laboratories and within countries across Europe. This report presents an analysis of participant results for the 2017 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 by EARS-Net at ECDC. 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, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *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 EUCAST Reference and Development Laboratory, Växjö, Sweden. Both reference laboratories confirmed the MICs by broth microdilution and MIC gradient methods and the susceptibility results were interpreted in accordance with established breakpoint criteria (CLSI and EUCAST) as indicated in the summary for each species outlined in the results section below. The panel was distributed in September 2017 as UK NEQAS distribution 4261.

A dedicated web page was available on the UK NEQAS website for participants to enter their results. Participants could access instructions through the web page 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 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 test results prior to submission online. Participants were allowed four weeks from the date of dispatch to examine the EQA specimens and return their results.

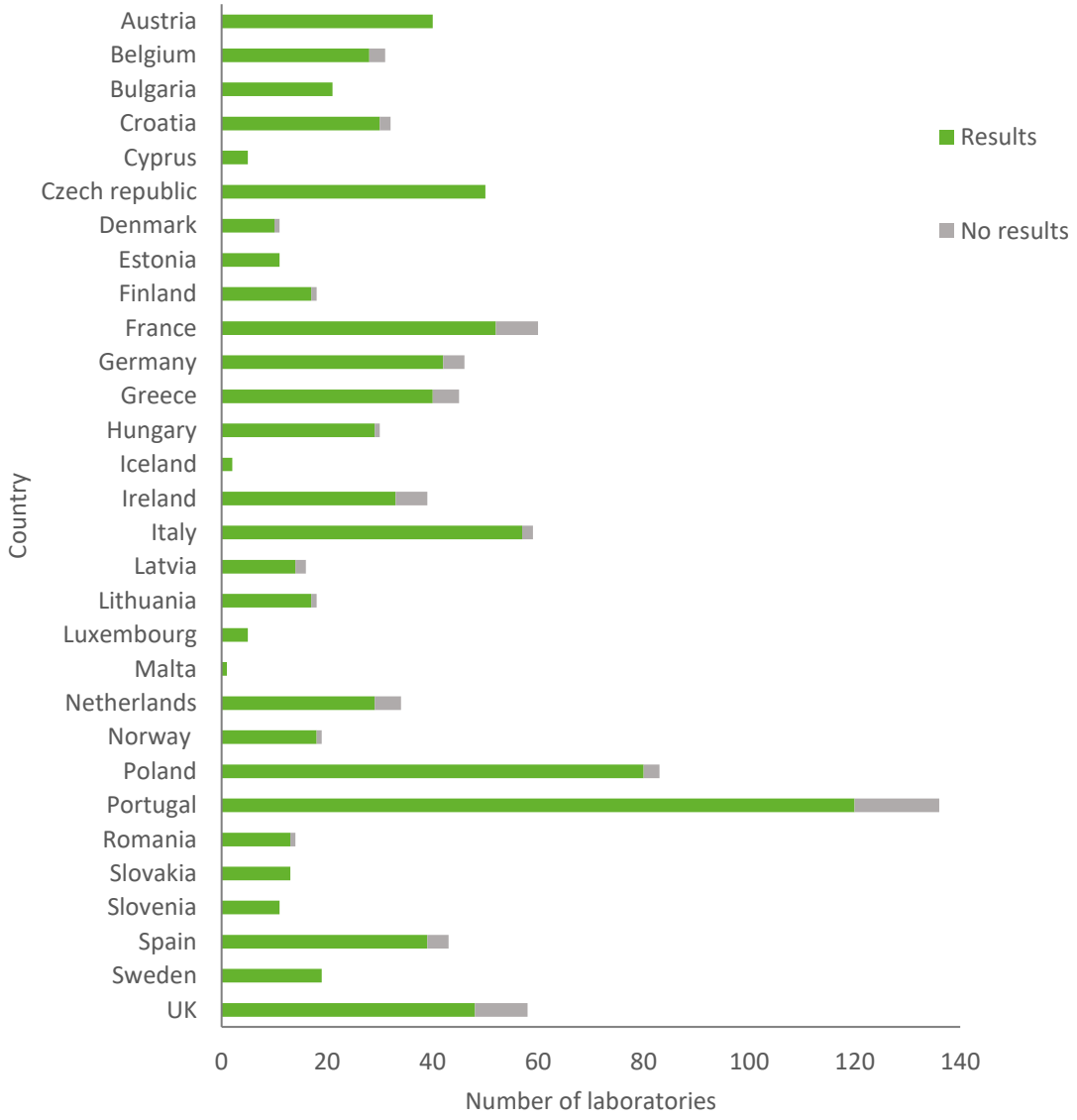
ECDC provided a list of operational contact points for antimicrobial-resistant pathogens and diseases caused by antimicrobial-resistant microorganisms. Each country appointed a national EQA coordinator. UK NEQAS for Microbiology forwarded the 2016 EARS-Net participant address databases for each country 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 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) or resistant (R) – based on clinical breakpoints according to the guideline followed in their laboratories. Participant results were analysed and considered 'concordant' if the reported categorisation agreed with the interpretation of the reference laboratories (Tables 1–6).

3 Results

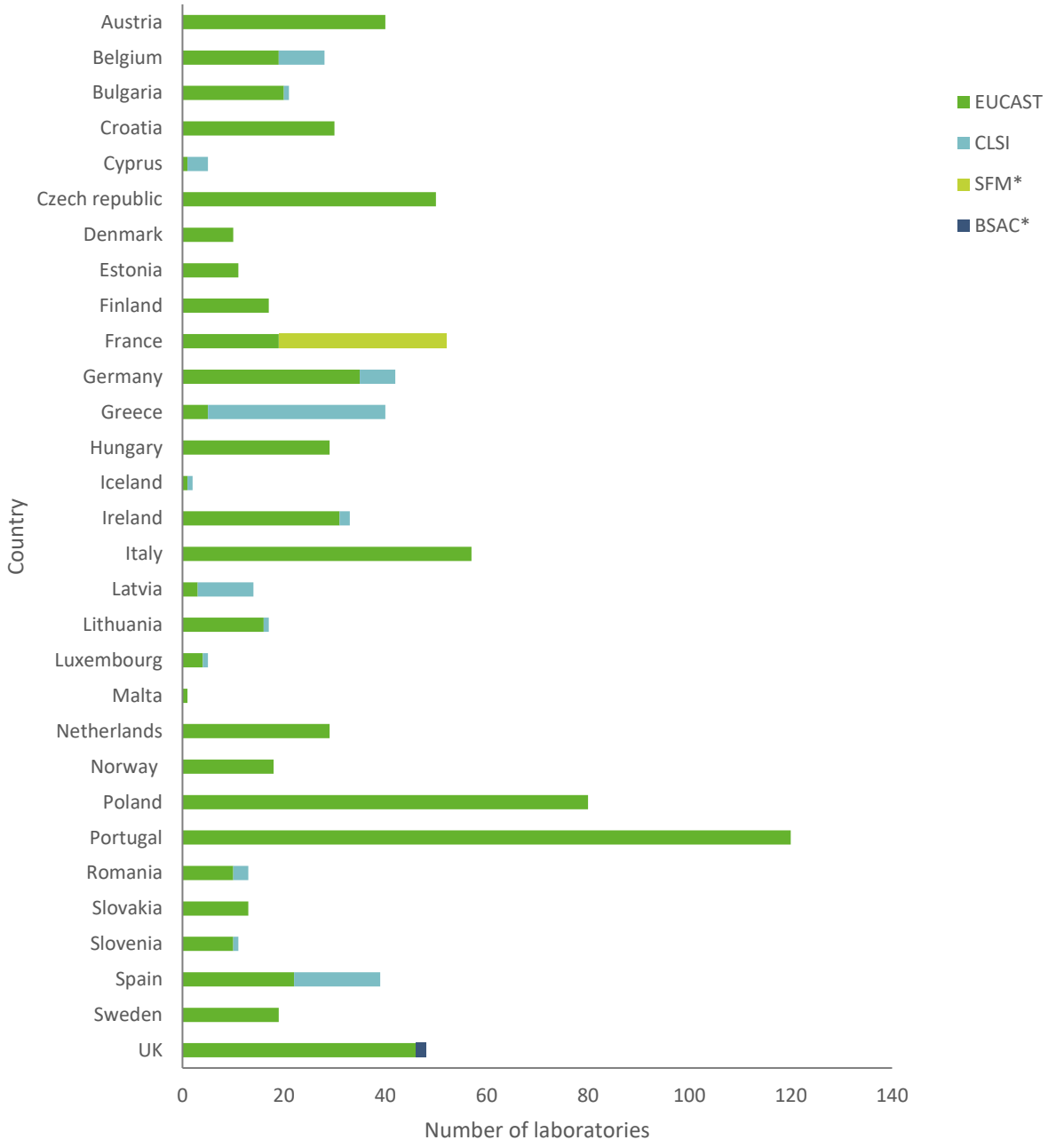
Six bacterial strains were distributed to 970 laboratories in 30 EU/EEA countries and 893 (92.1%) returned results. Figure 1 shows the proportion of participating laboratories returning results per country.

Figure 1. Number of participating laboratories returning EQA results by country, 2017



For the determination of AST results, laboratories used automated methods (40.7%), disk diffusion tests (47.7%), non-automated MIC methods (10.1%), or a combination of methods (1.5%). For species identification, 58.2% used an automated instrument, 39.8% used conventional methods, and 2.0% did not report on the method used. The greatest use of conventional methods was associated with the identification of *S. pneumoniae*: 10.4% of laboratories applied CLSI guidelines, a decline from 2016, when the proportion was 12%. EUCAST or EUCAST-related guidelines were reported by 85.9% of laboratories. This represented an increase of 3.6% compared with 2016. Figure 2 shows 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 per country, 2017



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 4317: *Streptococcus pneumoniae*

This isolate was a strain of *Streptococcus pneumoniae* that expressed an intermediate level of resistance to penicillin and resistance to erythromycin, but susceptibility to clindamycin. Table 1 shows the intended results and concordance for susceptibility testing of this isolate.

Table 1. *Streptococcus pneumoniae* (specimen 4317). Minimum inhibitory concentration (MIC) and intended results reported by reference laboratories and overall concordance of participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Cefotaxime meningitis pneumonia	0.12	0.25	 S S	99.1% 98.5% 98.9%
Ceftriaxone meningitis pneumonia	0.25	0.5	 S S	98.9% 98.5% 98.9%
Clindamycin	*	*	S*	98.0%
Erythromycin	4	8	R	98.1%
Levofloxacin	1	1	S	98.3%
Moxifloxacin	0.12	0.12	S	98.8%
Norfloxacin	*	*	S*	96.7%
Penicillin meningitis pneumonia	0.25	0.25	 R S	72.1% 93.9% 44.0%

R resistant
S: susceptible: no reference results for clindamycin or norfloxacin – assigned results based on participant consensus.*

Only 72.1% of participants correctly categorised the isolate as having an intermediate level of resistance to penicillin and 17.6% incorrectly categorised the isolate as susceptible. Interestingly, 93.9% participants correctly reported the isolate as resistant to penicillin in the context of meningitis, although the reason for this apparent discrepancy (compared to the categorisation above) is unclear. Only 44% of participants correctly reported the isolate as penicillin-susceptible in the context of pneumonia. Participants that followed the CLSI breakpoints were more likely to report false susceptible results, with 46.6% categorising the isolate as susceptible compared to 15.6% of participants that followed EUCAST breakpoints (Table 2).

A very good concordance of susceptibility results was achieved with all of the other antimicrobial agents tested and 99.4% of participating laboratories correctly identified the isolate as *S. pneumoniae* (Table 3).

Table 2. Susceptibility of *S. pneumoniae* (specimen 4317) to penicillin reported by participants according to guidelines followed and methods used

Guideline	Method	Numberof participants responding (%)		
		S	I	R
EUCAST	Automated	30 (13.9%)	156 (72.2%)	30 (13.9%)
	Disk diffusion	24 (16.8%)	108 (75.5%)	11 (7.7%)
	MIC	40 (15.8%)	190 (75.1%)	23 (9.1%)
	Other	3 (27.3%)	7 (63.6%)	1 (9.1%)
	Total	**97 (15.6%)**	**461 (74.0%)**	**65 (10.4%)**
CLSI	Automated	10 (37.0%)	12 (44.4%)	5 (18.5%)
	Disk diffusion	3 (42.9%)	3 (42.9%)	1 (14.3%)
	MIC	13 (56.5%)	10 (43.5%)	0
	Other	1 (100%)	0	0
	Total	**27 (46.6%)**	**25 (43.1%)**	**6 (10.3%)**

Correct result for each guideline shaded.

Table 3. Identification results for specimen 4317

Species	Number of participants responding by identification method	
	Automated	Conventional
Streptococcus species	1	0
Streptococcus pneumoniae	517	353
Streptococcus mitis	0	1
Streptococcus anginosus	1	0
Erysipelothrix rhusiopathiae	0	1
Enterococcus faecium	0	1
Total	**519**	**356**

Specimen 4318: *Staphylococcus aureus*

The isolate was a strain of *Staphylococcus aureus* that was resistant to beta-lactam agents, clindamycin (but not erythromycin), linezolid and tetracycline. Table 4 shows the intended results and concordance for susceptibility testing of this isolate.

Table 4. *Staphylococcus aureus* (specimen 4318). Minimum inhibitory concentration (MIC) and intended results reported by reference laboratories and overall concordance of participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Cefoxitin	16	16	R	99.4%
Ciprofloxacin	0.5	0.5	S	98.6%
Clindamycin	>4	>4	R	98.9%
Erythromycin	0.5	0.5	S	97.5%
Fusidic acid	≤0.12	≤0.12	S/†	99.9%
Gentamicin	0.5	0.5	S	98.3%
Linezolid	16	16	R	16.3%*
Penicillin	>0.5	>0.5	R	99.9%
Rifampicin	≤0.008	≤0.008	S	98.2%
Teicoplanin	0.5	0.5	S	99.2%
Tetracycline	>8	>8	R/I/R	98.9%
Vancomycin	1	1	S	98.8%

I: intermediate
R: resistant
S: susceptible
**: presence of heteroresistant population*
†: no breakpoint provided by CLSI.

Most participants did not achieve the intended result for linezolid, with only 16.3% correctly identifying linezolid resistance. This result should be interpreted with caution due to the presence of a heteroresistant population. The reference MIC for this strain was 16 mg/L, which is resistant by the EUCAST and CLSI breakpoints of >4 mg/L and >8 mg/L respectively. Participants using EUCAST breakpoints were more likely to report resistant results if they used a non-automated MIC method, but there were no other clear differences between methods (Table 5).

An excellent concordance of susceptibility results was achieved with all of the other antimicrobial agents tested and 99.9% of participating laboratories correctly identified the isolate as *S. aureus* (Table 6).

Table 5. Susceptibility of *S. aureus* (specimen 4318) to linezolid reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	282 (87.0%)		42 (13.0%)
	Disk diffusion	234 (82.7%)		49 (17.3%)
	MIC	76 (70.4%)	2 (1.9%)	30 (27.8%)
	Other	13 (100%)		0
	Total	**605 (83.1%)**	**2 (0.3%)**	**121 (16.6%)**
CLSI	Automated	46 (85.2%)		8 (14.8%)
	Disk diffusion	18 (90.0%)		2 (10.0%)
	MIC	13 (92.9%)		1 (7.1%)
	Other	1 (100%)		0
	Total	**78 (87.6%)**		**11 (12.3%)**

Correct result for each guideline shaded.
I: intermediate
R: resistant
S: susceptible.

Table 6. Identification results for specimen 4318

Species	Number of participants responding by identification method	
	Automated	Conventional
Staphylococcus aureus	671	210
Staphylococcus haemolyticus	1	0
Total	**672**	**210**

Specimen 4319: *Enterococcus faecium*

This isolate was a strain of *Enterococcus faecium* resistant to amoxicillin and ampicillin and expressed high-level gentamicin resistance, but was susceptible to vancomycin and teicoplanin. Table 7 shows the intended results and concordance for susceptibility testing of this isolate.

Table 7. *Enterococcus faecium* (specimen 4319). Minimum inhibitory concentration (MIC) and intended results reported by reference laboratories and overall concordance of participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Amoxicillin	32	32	R	98.2%
Ampicillin	32	64	R	99.5%
High-level gentamicin	>512	>512	Positive	91.6%
Teicoplanin	1	1	S	99.6%
Vancomycin	1	1	S	99.3%

R: resistant
S: susceptible.

A good concordance of susceptibility results was achieved with all of the antimicrobial agents tested and no significant issue noted. The isolate was correctly identified as *E. faecium* by 97.0% of the participating laboratories (Table 8).

Table 8. Identification results for specimen 4319

Species	Number of participants responding by identification method	
	Automated	Conventional
Enterococcus faecalis	5	9
Enterococcus faecium	695	148
Enterococcus gallinarum	4	2
Enterococcus species	3	3
Total	**707**	**162**

Specimen 4320: *Escherichia coli*

This isolate was a strain of *Escherichia coli* possessing the mobilised colistin resistance (mcr-1) gene exhibiting resistance to amoxicillin, amoxicillin/clavulanic acid, colistin and fluoroquinolones. Table 9 shows the intended results and concordance for susceptibility testing of this isolate.

Table 9. *Escherichia coli* (specimen 4320). Minimum inhibitory concentration (MIC) and intended results reported by reference laboratories and overall concordance of participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Amikacin	2	4	S	99.7%
Amoxicillin	>32	>32	R	98.5%
Amoxicillin/clavulanic acid	32*	32*	R	33.0%
Ampicillin	>32	>32	R	99.6%
Cefotaxime	0.5	0.5	S	99.0%
Ceftazidime	0.5	1	S	99.2%
Ceftriaxone	0.25	0.25	S	98.2%
Ciprofloxacin	>4	>4	R	99.8%
Colistin	4	4	R/†	50.1%
Ertapenem	0.03	0.03	S	98.6%
Gentamicin	1	1	S	99.7%
Imipenem	0.12	0.12	S	100.0%
Levofloxacin	-	-	R**	99.2%
Meropenem	0.03	0.03	S	99.6%
Ofloxacin	-	-	R**	98.8%
Piperacillin/tazobactam	8*	8*	S	97.6%
Tobramycin	0.5	0.5	S	99.4%

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

Most participants did not achieve the intended result for amoxicillin/clavulanic acid: only 33.0% correctly identified amoxicillin/clavulanic acid resistance. The reference MIC for this strain was 32 mg/L and tested with a fixed clavulanic acid concentration of 2 mg/L, which is resistant by EUCAST and CLSI breakpoints of >8 mg/L and ≥32 mg/L respectively. Participants that followed the EUCAST guideline were more likely to achieve the intended result than participants following the CLSI guideline, potentially due the strain's MIC being close to the (higher) CLSI breakpoint. Participants following the EUCAST guideline were more likely to achieve the intended result if they used an automated method rather than a disk diffusion or non-automated MIC method (Table 10).

Table 10. Susceptibility of *E. coli* (specimen 4320) to amoxicillin/clavulanic acid reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	165 (52.7%)	4 (1.3%)	144 (46.0%)
	Disk diffusion	217 (71.6%)	7 (2.3%)	79 (26.1%)
	MIC	49 (59.0%)	4 (4.8%)	30 (36.1%)
	Other	10 (90.9%)	0	1 (9.1%)
	Total	**441 (62.1%)**	**15 (2.1%)**	**254 (35.8%)**
CLSI	Automated	37 (77.1%)	6 (12.5%)	5 (10.4%)
	Disk diffusion	18 (78.3%)	1 (4.3%)	4 (17.4%)
	MIC	9 (69.2%)	3 (23.1%)	1 (7.7%)
	Other	1 (100%)	0	0
	Total	**65 (76.5%)**	**10 (11.8%)**	**10 (11.8%)**

Correct result for each guideline is shaded.
I: intermediate
R: resistant
S: susceptible.

There was a poor consensus of reported results for colistin susceptibility testing. The intended result was resistant (reference MIC 4mg/L, EUCAST breakpoint >2 mg/L), but only 50.1% of participants reported the isolate as resistant. Participants following the EUCAST guideline were more likely to achieve the intended result than those who followed the CLSI guideline. There is no CLSI colistin breakpoint for *E. coli*. EUCAST breakpoint tables state that an MIC method should be used. Despite this recommendation, 66 of 421 participants (15.7%) that reported following the EUCAST guideline stated they used a disk diffusion method and it is unclear which criteria they used to categorise the susceptibility result. Participants that followed both EUCAST and CLSI guidelines were less likely to achieve the intended result if they used an automated method (Table 11).

Table 11. Susceptibility of *E. coli* (specimen 4320) to colistin reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	102 (61.4%)	0	64 (38.6%)
	Disk diffusion	24 (36.4%)	0	42 (63.6%)
	MIC	74 (42.8%)	0	99 (57.2%)
	Other	4 (25%)	0	12 (75%)
	Total	**204 (48.5%)**	**0**	**217 (51.5%)**
CLSI	Automated	19 (59.4%)	0	13 (40.6%)
	Disk diffusion	4 (50%)	0	4 (50%)
	MIC	5 (50%)	0	5 (50%)
	Other	3 (100%)	0	0
	Total	**31 (58.5%)**	**0**	**22 (41.5%)**

Correct result for the EUCAST guideline shaded.

An excellent concordance of susceptibility results was achieved with all other antimicrobial agents tested and 99.9% of participating laboratories correctly identified the isolate as *E. coli* (Table 12).

Table 12. Identification results for specimen 4320

Species	Number of participants responding by identification method	
	Automated	Conventional
Escherichia coli	700	169
Klebsiella oxytoca	0	1
Total	**700**	**170**

Specimen 4321: *Klebsiella pneumoniae*

This isolate was a strain of *Klebsiella pneumoniae* producing both oxacillinase (OXA-1) and sulfhydryl-variable extended-spectrum beta-lactamase (SHV-1) enzymes expressing dissociated resistance to third-generation cephalosporins with an intermediate/resistant phenotype to cefotaxime and susceptibility to ceftazidime and ceftriaxone. The strain also expressed dissociated resistance to carbapenems, resistance to ertapenem and susceptibility to imipenem and meropenem. The strain was resistant to ciprofloxacin, colistin, amoxicillin/ clavulanic acid, piperacillin/tazobactam, gentamicin and tobramycin, but susceptible/intermediate to amikacin. Table 13 shows the intended results and concordance for susceptibility testing of this isolate.

Table 13. *Klebsiella pneumoniae* (specimen 4321). Minimum inhibitory concentration (MIC) and intended results reported by reference laboratories and overall concordance of participating laboratories

Antimicrobial agent	MIC range (mg/L)		Intended interpretation	
	Reference laboratory 1	Reference laboratory 2	EUCAST/CLSI	Overall concordance (%)
Amikacin	16	16	I/S	38.8%
Amoxicillin	>32	>32	R	100.0%
Amoxicillin/clavulanic acid	>64*	>128*	R	99.9%
Ampicillin	>32	>64	R	99.9%
Cefotaxime	2	4	I/R	91.1%
Ceftazidime	1	1	S	82.7%
Ceftriaxone	1	1	S	76.5%
Ciprofloxacin	>4	>8	R	99.9%
Colistin	32	32	R/†	95.6%
Ertapenem	2	4	R	90.2%
Gentamicin	>16	>32	R	99.5%
Imipenem	0.5	1	S	91.8%
Levofloxacin	*	*	R**	99.8%
Meropenem	0.5	0.5	S	89.6%
Ofloxacin	*	*	R**	100.0%
Piperacillin/tazobactam	64*	>64*	R/I/R	99.4%
Tobramycin	>16	>32	R	100.0%

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

The reference MIC to amikacin was 16 mg/L, which is intermediate/susceptible by EUCAST/CLSI breakpoints respectively. A poor consensus was achieved for the intended result, with only 38.8% participants reporting intermediate (EUCAST) or susceptible (CLSI). Participants following the CLSI guideline were more likely to report the intended result than those following the EUCAST guideline (Table 14). Regardless of whether the CLSI or EUCAST guideline was followed, participants using non-automated MIC methods performed better than those using automated or disk diffusion methods.

Table 14. Susceptibility of *K. pneumoniae* (specimen 4321) to amikacin reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	4 (1.5%)	67 (25.8%)	189 (72.7%)
	Disk diffusion	21 (7.5%)	85 (30.2%)	175 (62.3%)
	MIC	7 (6.9%)	38 (37.6%)	56 (55.4%)
	Other	2 (15.4%)	5 (38.5%)	6 (46.1%)
	Total	**34 (5.2%)**	**195 (29.8%)**	**426 (65.0%)**
CLSI	Automated	13 (29.5%)	10 (22.7%)	21 (47.7%)
	Disk diffusion	3 (12.0%)	10 (40.0%)	12 (48.0%)
	MIC	6 (40.0%)	6 (40.0%)	3 (20.0%)
	Other	0	0	1 (100%)
	Total	**22 (25.9%)**	**26 (30.6%)**	**37 (43.5%)**

Correct result for each guideline shaded.
I: intermediate
R: resistant
S: susceptible.

The reference MICs to third-generation cephalosporins were cefotaxime 2-4 mg/L (intermediate, EUCAST/resistant, CLSI); ceftazidime 1 mg/L (susceptible); and ceftriaxone 1 mg/L (susceptible). Although a good consensus was achieved for cefotaxime, this was not the case for ceftazidime and ceftriaxone. Only 82.7% and 76.5% of participants respectively provided the intended responses of susceptible for ceftazidime and ceftriaxone. Participants following the EUCAST guideline were more likely to provide correct results than those following the CLSI guideline. More participants provided responses for ceftazidime (n=866) than for ceftriaxone (n=395). Regardless of whether the CLSI or EUCAST guideline was followed, participants using disk diffusion methods for ceftazidime performed better than those using automated or non-automated MIC methods (Table 15).

Table 15. Susceptibility of *K. pneumoniae* (specimen 4321) to ceftazidime reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	267 (80.7%)	43 (13%)	21 (6.3%)
	Disk diffusion	254 (89.8%)	17 (6%)	12 (4.2%)
	MIC	92 (82.1%)	11 (9.8%)	9 (8.0%)
	Other	12 (85.7%)	1 (7.1%)	1 (7.1%)
	Total	**625 (84.5%)**	**72 (9.7%)**	**43 (5.8%)**
CLSI	Automated	28 (53.8%)	9 (17.3%)	15 (28.8%)
	Disk diffusion	17 (85.0%)	1 (5%)	2 (10.0%)
	MIC	11 (73.3%)	0	4 (26.7%)
	Other	0	0	1 (100%)
	Total	56 (63.6%)	10 (11.4%)	22 (25.0%)

Correct result for each guideline shaded.

The reference MICs to carbapenems were ertapenem 2-4 mg/L (resistant), imipenem 0.5-1 mg/L (susceptible) and meropenem 0.5 mg/L (susceptible). Reduced susceptibility (intermediate or resistant) to ertapenem was detected by 97.5% of participants. Although there was a good consensus (90.2%, 91.8% and 89.6% respectively) for reporting the intended results for all three agents, participants following the EUCAST guideline were more likely to report susceptibility to imipenem/meropenem correctly than those following the CLSI guideline. Regardless of whether the CLSI or EUCAST guideline was followed, participants using disk diffusion or non-automated MIC methods for meropenem performed better than those using automated methods (Table 16).

Table 16. Susceptibility of *K. pneumoniae* (specimen 4321) to meropenem reported by participants according to guidelines followed and methods used

Guideline	Method	Number of participants responding (%)		
		S	I	R
EUCAST	Automated	267 (85.6%)	40 (12.8%)	5 (1.6%)
	Disk diffusion	234 (93.6%)	12 (4.8%)	4 (1.6%)
	MIC	154 (96.3%)	6 (3.8%)	0
	Other	12 (100%)	0	0
	Total	**667 (90.9%)**	**58 (7.9%)**	**9 (1.2%)**
CLSI	Automated	35 (68.6%)	6 (11.8%)	10 (19.6%)
	Disk diffusion	15 (83.3%)	2 (11.1%)	1 (5.6%)
	MIC	12 (92.3%)	0	1 (7.7%)
	Other	0	0	1 (100%)
	Total	**62 (74.7%)**	**8 (9.6%)**	**13 (15.7%)**

Correct result for each guideline is shaded.

A good concordance of susceptibility results was achieved for the other antimicrobial agent tested and 99.7% of participating laboratories correctly identified the isolate as *K. pneumoniae* (table 17).

Table 17. Identification results for specimen 4321

Species	Number of participants responding by identification method	
	Automated	Conventional
Acinetobacter baumannii	1	0
Enterobacter aerogenes	1	0
Klebsiella pneumoniae	703	150
Total	705	150

Specimen 4322: *Acinetobacter baumannii* complex

This isolate was a strain of *Acinetobacter baumannii* complex producing a GES-12 carbapenemase that was susceptible to colistin, but resistant to the other antimicrobial agents tested. Table 18 shows the intended results and concordance for susceptibility testing of this isolate.

Table 18. *Acinetobacter baumannii* complex (specimen 4322). 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	≥128	≥128	R	98.6%
Ciprofloxacin	64	≥128	R	99.8%
Colistin	0.5	1	S	97.0%
Gentamicin	32	64	R	95.6%
Imipenem	32	64	R	99.6%
Levofloxacin	-	-	R*	100.0%
Meropenem	64	≥128	R	99.4%
Tobramycin	32	32	R	93.5%

**: no reference results for levofloxacin – assigned results based on participant consensus.*

A good concordance of results was achieved for all of the antimicrobial agents tested without any significant issue being noted and 97.1% of participating laboratories correctly identified the isolate as *A. baumannii* complex (table 19).

Table 19. Identification results for specimen 4322

Species	Number of participants responding by identification method	
	Automated	Conventional
Acinetobacter baumannii complex	702	131
Acinetobacter species	11	8
Enterobacter aerogenes	1	0
Klebsiella pneumoniae	2	0
Pseudomonas aeruginosa	2	1
Total	**718**	**140**

4 Discussion

Overall, the performance of laboratories participating in the 2017 EQA was satisfactory. There were no significant issues arising for species identification and for AST, there was a very good (≥95%) overall concordance for most species/antimicrobial agent combinations tested. In recent years, lower concordances in reporting AST results were seen for species-antimicrobial agent combinations with borderline MIC values and for which breakpoints and categorisation of results have differed between EUCAST and CLSI guidelines. Species/antimicrobial agent combinations that encountered problems in more than one EQA exercise 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.

There was a poor consensus in the 2017 EQA with *S. pneumoniae* (specimen 4317) for results of penicillin susceptibility testing. It was noted in the ECDC summary report for 2013 to 2016 that *S. pneumoniae* with intermediate resistance to penicillin caused problems for participants and this clearly remains a difficult area.

Linezolid susceptibility testing was assessed for the first time in 2017 as part of the EARS-Net EQA and a linezolid-resistant strain of *S. aureus* (specimen 4318) was included. It is important that laboratories are able to identify the emergence of new or unexpected resistance. Only 16.3% of participants correctly identified the linezolid resistance. However, this result should be interpreted with caution as investigations after the EQA identified the presence of a heteroresistant population in this specimen.

The other new drug resistance phenotype of public health concern included in the 2017 EQA exercise was colistin for the two Enterobacteraciae strains. There was a poor consensus of reported results for colistin susceptibility testing with *E. coli* (specimen 4320), with only 50.1% of participants correctly reporting the isolate as resistant. Interestingly, 15.7% participants who reported using EUCAST methods recorded that they had used a disk diffusion method despite EUCAST breakpoint tables stating that an MIC method should be used. It is therefore unclear which criteria these participants used to categorise the result. It is also noteworthy that only 36.1% participants that used an MIC method correctly reported the isolate as colistin resistant. Further investigation of which MIC methodology and quality control strain were used would be of interest. For the same *E. coli* strain, only 33.0% of participants correctly identified amoxicillin/clavulanic acid resistance. Participants using EUCAST methodology were more likely to achieve the intended result than participants using CLSI methodology, potentially due the strain's MIC being close to the (higher) CLSI breakpoint.

A poor consensus of reported results was observed with *K. pneumoniae* (specimen 4321) for amikacin, ceftazidime and ceftriaxone. Only 38.8% participants reported the intended result of intermediate/susceptible for amikacin (by EUCAST/CLSI breakpoints respectively). Despite a good consensus for cefotaxime, only 82.7% and 76.5% of participants respectively correctly reported susceptibility to ceftazidime and ceftriaxone.

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. For *E. coli*/amoxicillin/clavulanic acid, participants following EUCAST methodology were more likely to achieve the intended result if they used an automated method. For *E. coli*/colistin, participants were less likely to achieve the intended result if they used an automated method. For *K. pneumoniae*/amikacin, participants using non-automated MIC methods performed better. For *K. pneumoniae*/ceftazidime, participants using disk diffusion methods for ceftazidime performed better. Finally for *K. pneumoniae*/imipenem or *K. pneumoniae*/meropenem, participants using disk diffusion or non- automated MIC methods for meropenem performed better than those using automated methods.

5 Conclusions

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

Several species/antimicrobial agent combinations that were already known as representing a problem when performing AST again proved difficult for participants in 2017:

- *Streptococcus pneumoniae* with intermediate penicillin results
- *Klebsiella pneumoniae* with differing third-generation cephalosporin results; and
- *Klebsiella pneumoniae* with susceptible/intermediate amikacin results.

Two new species/antimicrobial agent combinations that may represent a problem when performing AST were identified after introducing new tests into the 2017 EQA exercise:

- *Staphylococcus aureus* with linezolid resistance and
- *Escherichia coli* with colistin resistance.

It is important that laboratories are able to identify the emergence of new or unexpected resistance patterns.

6 Recommendations

Participating laboratories that report to EARS-Net have been provided with feedback that allows them to assess their individual performance in this EQA exercise and review all areas where they did not achieve the intended results. The findings demonstrate the importance of laboratories ensuring that they follow their chosen methodology carefully, particularly for species/antimicrobial agent combinations that did not achievethe intended results. The findings also emphasise the need for laboratories to ensure that they are aware of species/antimicrobial agent combinations that represent a problem when performing AST and of potential new resistance issues.

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 Enterobacteriaceae.

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 data reported to EARS-Net.

European Centre for Disease Prevention and Control (ECDC)

Address:
Gustav III:s boulevard 40, SE-169 73 Solna, Sweden

Tel. +46 858601000
Fax +46 858601001
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