Results from the 2014 Antimicrobial Susceptibility Testing External Quality Assessment (EQA) Exercise Organised for EARS-Net Participants

S Seaton[1], C Lawson[1], EJ Fagan[1],L Quartermain[1], A Thomas[1], A Couchman[1], DFG Brown[2], C Walton[1] EARS-Net participating laboratories

[1]UK NEQAS, Public Health England, Colindale, London, UK, [2]Peterborough, UK

Introduction

The United Kingdom National External Quality Assessment Service for Microbiology (UK NEQAS) provides external quality assessment (EQA) for antimicrobial susceptibility testing for the European Antimicrobial Resistance Surveillance Network (EARS-Net - formerly EARSS) laboratories. In 2014, the annual EQA exercise was the fourth distribution in collaboration with the European Centre for Disease Prevention and Control (ECDC) but the twelfth in succession to EARS-Net laboratories. Antimicrobial resistance is a major topic in the public health arena across Europe.

Methods

An analysis was carried out on the performance of participants in the EQA exercise. Participation was invited from 905 laboratories in 30 countries and results were returned by 838 (92.6%) laboratories. The organisms distributed were single isolates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecium and Acinetobacter baumannii* complex. Participants' results for identification and antimicrobial susceptibility testing were assessed.

Country information

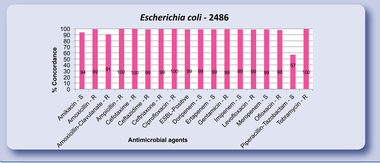
Countries that participated in the EARS-Net 2014 EQA exercise:

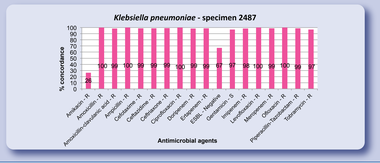
Austria	Finland	Latvia	Romania
Belgium	France	Lithuania	Slovak Republic
Bulgaria	Germany	Luxembourg	Slovenia
Croatia	Greece	Malta	Spain
Cyprus	Hungary	Netherlands	Sweden
Czech Republic	Iceland	Norway	UK
Denmark	Ireland	Poland	
Estonia	Italy	Portugal	

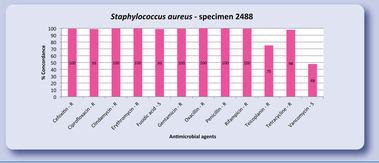
The objective of the EQA exercise:

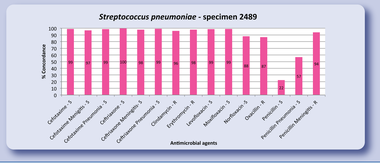
- to assess the ability of participating laboratories to identify antimicrobial resistance of clinical and public health importance
- to determine the accuracy of antimicrobial susceptibility test results reported by individual laboratories
- to assess the comparability of results between laboratories and countries and thus justify the pooling and comparison of routinely collected antimicrobial susceptibility test data across Europe
- education

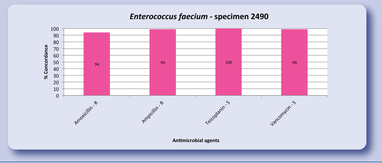
Charts displaying participants' concordance with the intended result for each of the six specimens in the 2014 EQA panel

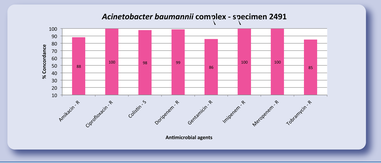












Results

The level of performance with these quality assessment specimens was generally high; concordance with the intended results was 94% for most agent-organism combinations with range from 22% to 100%.

- Data are presented for some of the general findings and some of the discrepancies are highlighted.
- Where country specific data are shown this has been anonymised.

Results for identification were very good. The correct identification was reported by 97 to 100% of laboratories for all the organisms in the exercise.

Chart 1 shows the method used by participants reporting the correct result

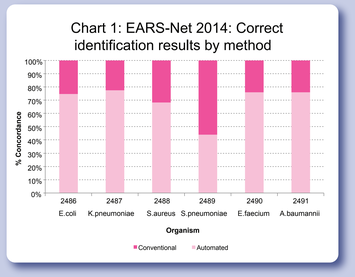
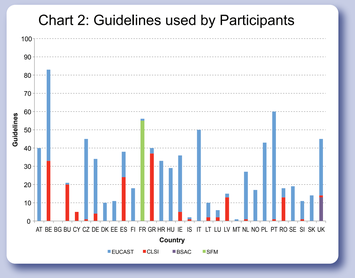


Chart 2 shows the antimicrobial susceptibility testing guidelines followed by the EARS-Net participants by country. Note that BSAC, NWGA, SFM and SRGA guidelines all follow EUCAST.



Specimen 2487 - *Klebsiella pneumoniae* (VIM carbapenemase producer)

This organism produces a VIM carbapenemase and was clearly resistant to carbapenems and all other β-lactam agents tested.

Aminoglycoside susceptibility was typical for an organism producing AAC(6')I in that the organism was susceptible to gentamicin (MIC 1 mg/L), borderline to amikacin (MIC 16 mg/L; intermediate by EUCAST breakpoints and susceptible by CLSI breakpoints); and resistant to tobramycin (MIC 16 mg/L). For amikacin, reporting was variable, with 13.3% of 746 participants reporting susceptible, 60.2% intermediate and 26.5% resistant. Among participants using EUCAST or EUCAST-related guidelines reports of intermediate were common and reports of susceptible uncommon . In line with differences in breakpoints between EUCAST and CLSI, among participants using CLSI guidelines reports of susceptible were more common and reports of intermediate less common than with EUCAST guidelines (Table 1), although despite the susceptible interpretation of reference MICs according to CLSI guidelines 66.7% of CLSI users reported either intermediate or resistant.

Table 1:

Agent	EUCAST and EUCAST related guidelines				CLSI guidelines			
	n	% S	% I	% R	n	% S	% I	% R
Amikacin	590	8.0	63.2	28.8	156	33.3	48.7	18.0

Participants following EUCAST or EUCAST related guidelines were less likely to report the intended amikacin intermediate result if they used disk diffusion methods than if they used automated methods (Table 2). This difference in reporting between participants using disk diffusion or automated methods was also seen among those using CLSI guidelines, but there was less difference than with EUCAST or EUCAST related guidelines (Table2).

Table 2:

Guideline	Disk diffusion methods				Automated methods			
	n	% S	% I	% R	n	% S	% I	% R
EUCAST and EUCAST related	246	13.4	53.7	32.9	289	3.5	73.7	22.8
CLSI	56	23.2	44.6	32.2	94	38.3	51.1	10.6

EUCAST expert rules note that acquired AAC(6')I may not confer phenotypic resistance to amikacin despite modification of amikacin, and that such organisms should be reported intermediate even if they appear susceptible. This rule is under review by EUCAST and may be removed from the next version but, in line with the current expert rule, some participants may have edited susceptible test results to intermediate.

Specimen 2488 *Staphylococcus aureus* (VISA)

This strain exhibited a low level of resistance to vancomycin and teicoplanin (VISA). Of the 819 participants reporting vancomycin susceptibility only 42.1% reported resistant and 9.7% intermediate, whilst 48.2% incorrectly reported susceptible. (Figure 1)

Reduced susceptibility to teicoplanin was more readily detected and of 720 participants reporting teicoplanin susceptibility 75.0% reported resistant, 9.7% intermediate and 15.3% susceptible.

Isolates of *S. aureus* with vancomycin MICs of 4-8 mg/L were originally termed "vancomycin intermediate *S.aureus*" (VISA) because the level of resistance is low and is distinguishable from the high-level resistance displayed by *S. aureus* carrying the *vanA* gene (MICs >8 mg/L). While CLSI have maintained this distinction EUCAST does not have an intermediate category because VISA strains are clinically resistant. A lower proportion of participants incorrectly reported susceptible among the participants following EUCAST or EUCAST-related guidelines than among those following CLSI guidelines. In line with the difference in reporting guidelines, most non-susceptible reports with CLSI guidelines were in the intermediate category while with EUCAST guidelines most were in the resistant category.

MICs of teicoplanin for VISA are usually higher than vancomycin MICs and reduced susceptibility to glycopeptides may be more readily detected with teicoplanin than with vancomycin. While EUCAST breakpoints for teicoplanin are set to report all VISA isolates as teicoplanin resistant (S ≤2 mg/L, R >2 mg/L), CLSI breakpoints are significantly higher (S ≤8 mg/L, R ≥32 mg/L) and VISA isolates are borderline susceptible-intermediate. In line with the differences in breakpoints, participants following EUCAST or EUCAST related guidelines mostly reported teicoplanin resistant while participants following CLSI guidelines were most likely to report susceptible or intermediate.

Reduced susceptibility to glycopeptides in *S. aureus* cannot be reliably detected by disk diffusion methods and EUCAST and CLSI both state that disk diffusion should not be used. In total, 319 participants reported disk diffusion as their method for vancomycin and 79 participants reported disk diffusion zone diameters. However, most of the 319 also reported an MIC value and clearly set up an MIC test instead of or as well as disk diffusion. Of 16 participants apparently using only a disk diffusion method, presumably with historical outdated breakpoints, 14 (87.5%) reported the isolate vancomycin susceptible. Of 370 reporting use of an MIC method or giving an MIC result other than with an automated system, 41.4% reported the isolate vancomycin susceptible. Of 430 reporting use of an automated method, 50.5% reported the isolate vancomycin susceptible. While MIC methods were most reliable in detecting reduced susceptibility to vancomycin and disk diffusion clearly unreliable, none of the methods performed well and overall there are serious concerns regarding the ability of participants to detect vancomycin resistance in VISA isolates.

A breakdown of the results by method and guidelines followed is presented in figures 2 and 3.

Reduced susceptibility to teicoplanin was more readily detected and of 714 participants reporting teicoplanin susceptibility, 75.1% reported resistant, 9.5% intermediate and 15.4% susceptible (figure 4).

A breakdown of the results by method and guidelines followed is presented in figures 5 and 6.

Isolates of *S. aureus* with vancomycin MICs of 4-8 mg/L were originally termed "vancomycin intermediate *S. aureus*" (VISA) because the level of resistance is low and is distinguishable from the high-level resistance displayed by *S. aureus* carrying the *mecA* gene (MICs >8 mg/L). While CLSI have maintained this distinction EUCAST does not have an intermediate category because VISA strains are clinically resistant.

Figure 1: Staphylococcus aureus (VISA) – specimen 2488
Susceptibility results for vancomycin (n = 741)

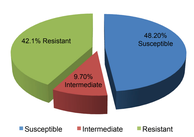


Figure 2: Susceptibility results for vancomycin
Results by method with EUCAST guidelines

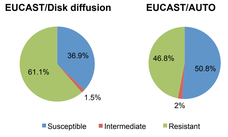


Figure 3: Susceptibility results for vancomycin
Results by method with CLSI guidelines

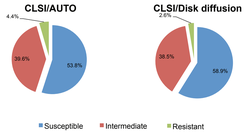


Figure 4. Staphylococcus aureus (VISA) – specimen 2488
Susceptibility results for teicoplanin

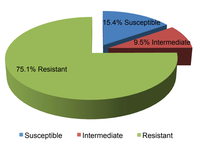


Figure 5. Susceptibility results for teicoplanin
Results by method interpreted with EUCAST guidelines

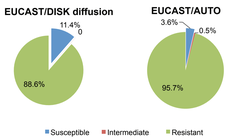
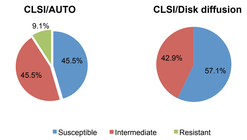
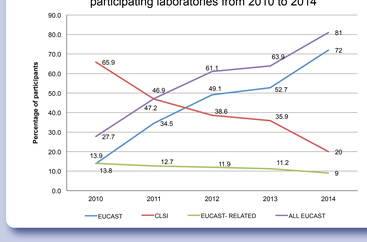


Figure 6: Susceptibility results for teicoplanin
Results by method interpreted with CLSI guidelines



Trends in guidelines used by EARS-Net participating laboratories from 2010 to 2014



Conclusions

- Participant concordance for identification of the organisms was very good, as seen in previous years.
- Clear differences in reporting were seen where breakpoints for interpretation differ between guidelines.
- Variation in interpretation was seen with strain-antimicrobial agent combinations where MIC values were borderline.
- EQA is a valuable tool in the quality assurance of antimicrobial susceptibility testing in the diagnostic laboratory and indicates the validity of comparing collated data between laboratories. In this exercise overall concordance between participating laboratories was high except where there was borderline susceptibility or different guidelines used, revealing remaining discrepancies in susceptibility testing.

ACKNOWLEDGEMENTS:

We are grateful to all the EARS-Net participants for taking part and providing the data for this exercise. In particular we would like to thank the national co-ordinators for their contribution to delivery of the EQA. We also thank the following reference laboratories: EUCAST Laboratory for Antimicrobial Susceptibility Testing (Central Hospital, Växjö, Sweden) and BSAC – Specialist Antimicrobial Chemotherapy Unit (SACU), Public Health Wales (Cardiff, UK) for confirming susceptibilities of the organisms distributed. Finally we extend our gratitude to all the staff at UK NEQAS for their sterling work and dedication.

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