

## Antimicrobial susceptibility testing in European hospitals: report from the ARPAC study

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### ABSTRACT

This observational study describes the antimicrobial susceptibility testing (AST) methods and interpretive criteria used in European hospitals during 2001, focusing specifically on detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Of 263 hospitals that took part in the ARPAC study, 192 submitted data on AST. Of these, 89% ( $n = 170$ ) routinely used a disk-diffusion AST method, 43% ( $n = 82$ ) used a semi-automated method, and 70% ( $n = 135$ ) routinely determined MICs. Hospitals in southern Europe were less likely to use disk-diffusion, but were more likely to use a semi-automated method ( $p < 0.001$ ). In total, 173 (90%) interpreted AST results using CLSI breakpoints; 30% of these detected MRSA using unmodified CLSI disk-diffusion methods, while 35% used the unmodified CLSI agar-screening method for MRSA; 41% and 30% adhered to unmodified CLSI methodology for disk-diffusion and agar-screening, respectively, to detect VRE. Some of the modifications made may have greatly reduced the ability of the tests to detect MRSA/VRE. For example, 20% of respondents used excessively high incubation temperatures and 13% used inadequate incubation times to detect MRSA by disk-diffusion, and 28% used Mueller–Hinton agar instead of brain–heart infusion agar in VRE screening plates. The majority of respondents stated that they followed CLSI guidelines, but a high proportion had modified the CLSI methods for detecting MRSA and VRE, which may compromise clinical management and antimicrobial resistance surveillance.

**Keywords** Antimicrobial susceptibility testing, ARPAC study, detection, European hospitals, MRSA, VRE

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### INTRODUCTION

The continuing emergence of pathogenic bacteria that are resistant to first-line antibacterial agents poses a challenge to clinical microbiology labor-

atories. Specifically, the choice of methods for identifying resistant phenotypes and for measuring resistance must be sufficiently robust to provide a clinically relevant service. Secondary to fulfilling this objective, laboratory methods must keep abreast of developments in order to enable more meaningful surveillance at all levels, from monitoring resistance in one hospital over time to tracking the epidemiology of resistance both nationally and internationally.

Many organisations exist to provide antimicrobial susceptibility testing (AST) methods and interpretative criteria. The organisation with the greatest global recognition is probably the Clinical and Laboratory Standards Institute (CLSI),

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formerly known as the National Committee for Clinical Laboratory Standards (NCCLS) (<http://www.clsi.org>). Most of the centralised European antimicrobial resistance surveillance systems listed in a recent report [1] adhere to CLSI guidelines, and a large external quality assessment exercise showed that CLSI guidelines are used widely in Europe [2]. Some countries, including the UK [3], France [4], Sweden [5] and Germany [6], have developed their own national AST guidelines, while others are based on CLSI methods and interpretative breakpoints.

The ARPAC (Antibiotic Resistance: Prevention and Control) study was a Concerted Action, funded by DG Research of the European Commission, which aimed to lay the foundations for a better understanding of the emergence and epidemiology of antimicrobial resistance in human bacterial pathogens. The project was conducted by four study groups of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Data from European hospitals concerning AST, antimicrobial resistance prevalence, typing methods, antimicrobial consumption, infection control policies and antibiotic prescribing policies were collated and analysed. The ESCMID Study Group for Antimicrobial Resistance Surveillance (ESGARS) was responsible for investigating the different AST methods and interpretative criteria used across Europe during 2001. The findings of this investigation are the subject of this article.

## MATERIALS AND METHODS

### Hospital recruitment

A recruitment flyer and initial screening questionnaire were circulated during May 2002 to full members of ESCMID. In total, 263 hospitals in Europe and Israel expressed an interest

in participating in the study. Hospitals in Israel were eligible for inclusion because of a bilateral scientific cooperation agreement for EC-funded studies. These 263 hospitals provided data on hospital characteristics for 2001, including teaching status, total number of patient admissions, total number of beds, and numbers of beds in surgical, medical and intensive care unit (ICU) specialties. These data were compared with data for the same characteristics from EU country-specific datasets published by EUROSTAT, the Statistical Office of the European Community (<http://www.euro.who.int>), to assess the representativeness of the recruited ARPAC sample.

### Data collection

A detailed postal questionnaire was designed to capture AST practices during 2001. The questionnaire, to be completed by a medical microbiologist or another appropriate person, included general questions regarding AST, use of breakpoints, participation in quality assurance in the respondent's laboratory, and the methods used for detection of specific resistance phenotypes in *Staphylococcus aureus* and enterococci, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). The questionnaire was developed, piloted and revised by the ARPAC Steering Group before being circulated to the 263 recruited hospitals during January 2003.

### Statistical analysis

Data were entered into Microsoft Access 2000, and an independent validation check was made on a 10% sample of data entered. Statistical analysis was conducted using SPSS v.12.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive analysis was conducted to identify associations between key AST parameters and geographical and hospital factors, including hospital size, teaching status and case-mix variables. Countries were classified into five European geographical regions according to a standard reference system [7], modified to place UK hospitals in western rather than northern Europe (Table 1). Descriptive statistics were conducted using median and inter-quartile range, and statistical testing was conducted using non-parametric, Mann-Whitney *U* and Kruskal-Wallis tests. Assessment of regional representativeness was estimated using 2001 European bed data from EUROSTAT (<http://www.euro.who.int>).

Northern Europe <i>n</i> = 20 (10%)	Western Europe <i>n</i> = 59 (31%)	Central/eastern Europe + Baltic States <i>n</i> = 48 (25%)	South-eastern Europe <i>n</i> = 12 (6%)	Southern Europe + Israel <i>n</i> = 53 (28%)
Denmark ( <i>n</i> = 5)	Austria ( <i>n</i> = 6)	Bulgaria ( <i>n</i> = 8)	Bosnia ( <i>n</i> = 2)	Greece ( <i>n</i> = 11)
The Netherlands ( <i>n</i> = 8)	Belgium ( <i>n</i> = 21)	Czech Republic ( <i>n</i> = 3)	Croatia ( <i>n</i> = 5)	Israel ( <i>n</i> = 3)
Norway ( <i>n</i> = 4)	France ( <i>n</i> = 6)	Estonia ( <i>n</i> = 2)	Macedonia ( <i>n</i> = 1)	Italy ( <i>n</i> = 10)
Sweden ( <i>n</i> = 3)	Germany ( <i>n</i> = 11)	Hungary ( <i>n</i> = 10)	Yugoslavia ( <i>n</i> = 4)	Malta ( <i>n</i> = 1)
	Switzerland ( <i>n</i> = 5)	Latvia ( <i>n</i> = 2)		Portugal ( <i>n</i> = 3)
	UK ( <i>n</i> = 10)	Lithuania ( <i>n</i> = 3)		Spain ( <i>n</i> = 13)
		Poland ( <i>n</i> = 6)		Turkey ( <i>n</i> = 12)
		Romania ( <i>n</i> = 3)		
		Russia ( <i>n</i> = 1)		
		Slovakia ( <i>n</i> = 5)		
		Slovenia ( <i>n</i> = 5)		

**Table 1.** Geographical location of hospitals participating in ARPAC (*n* = 192) that provided data concerning antimicrobial susceptibility testing

## RESULTS

### Response rate

Complete, useable responses concerning AST during the year 2001 were received from 192 hospitals in 32 countries, representing 73% of the hospitals recruited to the ARPAC study (Table 1). The 192 hospitals that provided AST data were more likely to have teaching status than hospitals that failed to provide AST data ( $n = 71$ ;  $p 0.02$ ), but there were no differences by geographical region ( $p 0.13$ ), hospital size ( $p 0.99$ ), and presence or size of ICU ( $p 0.22$ ).

### Characteristics of participating hospitals

Total numbers of beds in hospitals recruited to the ARPAC study were compared with published numbers of hospital beds (acute and non-acute) for 2001 for each region. Estimated regional coverage of acute-care beds ranged from 3% in western Europe to 10% in northern Europe. The majority of participating hospitals that provided AST data were teaching hospitals (146/192; 76%). Median hospital size was 654 beds (inter-quartile range 407, 999), with eight hospitals failing to provide bed data. Ninety-five percent of the hospitals providing AST data had ICU beds, with a median of 26 ICU beds (inter-quartile range 12, 45).

### AST methods

Of the 192 responding hospitals, 89% ( $n = 170$ ) stated that they used a disk-diffusion AST method routinely, with 43% ( $n = 82$ ) using a

semi-automated method, and 70% (135) determining MICs routinely. Hospitals in southern Europe were less likely to use disk-diffusion (Table 2;  $p < 0.01$ ), and were more likely to use a semi-automated method (Table 2;  $p 0.001$ ) compared with other regions. There was no association between methods used and hospital size, case-mix or teaching status. The reported manufacturers of antimicrobial disks were Oxoid ( $n = 95$ , 55.9%), Becton Dickinson ( $n = 71$ , 41.8%), Rosco (18.2%), Sanofi-Diagnostics Pasteur (14.7%) and bioMérieux (11.8%). MICs were determined most frequently using Etest strips ( $n = 119$ , 88.1%) and in-house broth methods ( $n = 16$ , 11.9%). Of the 82 hospitals that used a semi-automated system, 43.9% ( $n = 36$ ) used a Vitek system, 36.6% ( $n = 30$ ) used an ATB system, 9.8% ( $n = 8$ ) used a Walkaway system, and 2.4% ( $n = 2$ ) used an Autoscan system.

### Breakpoints

Regardless of the AST methods used, 173 (90%) of respondents stated that their laboratory interpreted AST results using breakpoints during 2001 (Table 2). In total, 171 hospitals specified which organisation had set the breakpoints used. The majority ( $n = 144$ , 84%), from 27 countries, used breakpoints set by the CLSI. Other breakpoints cited were generally set by national organisations. Eleven (11%) hospitals used breakpoints set by more than one organisation, generally CLSI and national breakpoints. The majority of hospitals ( $n = 169$ , 88%) routinely reported three AST

**Table 2.** Antimicrobial susceptibility testing methods used routinely in 2001 in different European regions

Method used	Number (%) hospitals in indicated region					Total $n = 192$	p value <sup>a</sup>
	Northern $n = 20$	Western $n = 59$	Central/eastern $n = 48$	South-eastern $n = 12$	Southern $n = 53$		
Disk-diffusion							0.004
Yes	19 (95)	52 (88)	47 (98)	12 (100)	40 (75)	170 (89)	
No	1 (5)	7 (12)	1 (2)	0 (0)	13 (25)	22 (11)	
Not stated	0	0	0	0	0	0	
MIC							0.09
Yes	17 (85)	44 (75)	31 (65)	5 (42)	38 (72)	135 (70)	
No	3 (15)	15 (25)	17 (35)	7 (58)	15 (28)	57 (30)	
Not stated	0	0	0	0	0	0	
Semi-automated							0.001
Yes	6 (30)	17 (29)	24 (50)	3 (25)	32 (60)	82 (43)	
No	13 (65)	41 (69)	24 (50)	9 (75)	16 (30)	103 (54)	
Not stated	1 (5)	1 (2)	0	0	5 (9)	7 (4)	
Breakpoints used for interpretation							0.37
Yes	20 (100)	51 (86)	43 (90)	10 (83)	49 (93)	173 (90)	
No	0	8 (14)	4 (8)	2 (17)	4 (7)	18 (9)	
Not stated	0	0	1 (2)	0	0	1 (1)	

<sup>a</sup>Kruskal-Wallis test.

categories, i.e., resistant, intermediate and susceptible.

### Detection of MRSA

Participating hospitals supplied information concerning the methods used to detect MRSA, focusing on disk-diffusion testing and the agar-screening method. In total, 126 hospitals provided information concerning disk-diffusion methods used to detect MRSA. Of these, 94 (75%) stated that they used CLSI breakpoints to interpret AST results, implying that they used CLSI disk-diffusion methods, i.e., Mueller–Hinton agar plates containing no added NaCl, incubated for a full 24 h at 33–35°C, with either 5-µg methicillin disks or 1-µg oxacillin disks [8,9]. Only 28 (30%) of the 94 hospitals followed all of these recommendations. The remaining hospitals used various combinations of the recommended test conditions (Table 3).

Ninety-seven hospitals reported using MRSA screening plates, with 72 (74%) using CLSI methods and breakpoints. During 2000–2001, the CLSI (NCCLS) expressed a preference for the use of oxacillin rather than methicillin plates, but supplied details for the use of both [8,9]. Thus, the CLSI recommended use of Mueller–Hinton agar supplemented with NaCl 4% w/v containing either oxacillin 6 mg/L or methicillin 10 mg/L,

incubated for a full 24 h at 35°C [8,9]. Twenty-five (35%) of the 72 hospitals complied with all of these recommendations. Again, various other combinations of conditions were also used (Table 3).

### Detection of VRE

Ninety-six hospitals reported using disk-diffusion methods to detect VRE, with 71 (74%) using CLSI methods and breakpoints, while 49 reported using vancomycin agar-screening plates, with 40 following CLSI guidelines. The CLSI state that disk-diffusion should be performed on Mueller–Hinton agar with 30-µg disks and incubation for 24 h at 35°C, whereas the agar-screening method should be carried out with brain–heart infusion agar containing vancomycin 6 mg/L, with incubation for 24 h at 35°C [8,9]. Only 41% (29/71) and 30% (12/40) of hospitals adhered to the complete CLSI methodology for disk-diffusion and agar-screening, respectively. Table 4 gives details for the hospitals that adhered to the individual recommendations.

## DISCUSSION

Despite the fact that a previous study concluded that CLSI guidelines are followed widely in Europe [2], it was not established at that time

Method variable	Disk-diffusion method ( <i>n</i> = 94)		Agar-screening method ( <i>n</i> = 72)	
	CLSI recommendation	No. (%) hospitals	CLSI recommendation	No. (%) hospitals
Incubation temperature	33–35°C	57 (61)	35°C	42 (58)
	Other (range 30–37°C)	32 (34)	Other (range 30–37°C)	27 (38)
	Not stated	5 (5)	Not stated	3 (4)
Incubation time	24 h	55 (59)	24 h	36 (50)
	Other (range 17–48 h)	34 (37)	Other (range 17–72 h)	32 (44)
	Not stated	5 (5)	Not stated	4 (6)
Additional NaCl	0%	54 (57)	4%	39 (54)
	Other (range 2–7.5%)	36 (38)	Other (range 2–7.5%)	16 (22)
	Not stated	4 (4)	Not stated	17 (24)
Disk content <sup>a</sup> / antibiotic concentration <sup>b</sup>	Methicillin 5 µg	3 (3)	Methicillin 10 mg/L	0 (0)
	Oxacillin 1 µg	76 (81)	Oxacillin 6 mg/L	50 (69)
	Other <sup>c</sup>	7 (7)	Other <sup>d</sup>	11 (15)
	Not stated	8 (9)	Not stated	11 (15)
	Medium	Mueller–Hinton agar	89 (95)	Mueller–Hinton agar
	Other <sup>e</sup>	2 (2)	Other <sup>f</sup>	15 (21)
	Not stated	3 (3)	Not stated	4 (6)

<sup>a</sup>Six hospitals used only methicillin disks, 85 used only oxacillin disks and three used both.

<sup>b</sup>One hospital used only methicillin disks, 69 used only oxacillin disks and one used both.

<sup>c</sup>Four hospitals cited non-standard methicillin disk contents of 1–29 µg, and three cited non-standard oxacillin disk contents of 5 µg.

<sup>d</sup>One hospital used a non-standard methicillin concentration of 6 mg/L and ten used non-standard oxacillin concentrations of 1–5 mg/L.

<sup>e</sup>Other media cited were IsoSensitest agar, Chapman agar and Natriomkloridplade agar.

<sup>f</sup>Other agar media cited were mannitol salt agar, ORSAB (Oxoid), nutrient agar + anoline, AES methistaph agar, oxacillin screening agar (Becton Dickinson) and Chapman agar.

**Table 3.** Disk-diffusion and agar-screening methods used to detect methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals reporting use of CLSI guidelines

**Table 4.** Disk-diffusion and agar-screening methods used to detect vancomycin-resistant enterococci (VRE) in hospitals reporting use of CLSI guidelines

Method variable	Disk-diffusion method ( <i>n</i> = 71)		Agar-screening method ( <i>n</i> = 40)	
	CLSI recommendation	No. (%) hospitals	CLSI recommendation	No. (%) hospitals
Incubation temperature	35°C	47 (66)	35°C	32 (80)
	Other (range 36–37°C)	22 (31)	Other (range 30–37°C)	6 (15)
	Not stated	2 (3)	Not stated	2 (5)
Incubation time	24 h	48 (68)	24 h	24 (60)
	Other (range 18–48 h)	21 (30)	Other (range 17–72 h)	14 (35)
	Not stated	2 (3)	Not stated	2 (5)
Disk content/ antibiotic concentration	Vancomycin 30 µg	58 (82)	Vancomycin 6 mg/L	30 (75)
	Other (range 5–6 µg)	7 (10)	Other (range 4–30 mg/L)	8 (20)
	Not stated	6 (8)	Not stated	2 (5)
Medium	Mueller–Hinton agar	65 (92)	Brain–heart infusion agar	14 (35)
	Other <sup>a</sup>	5 (7)	Other <sup>b</sup>	21 (52)
	Not stated	1 (1)	Not stated	5 (12)

<sup>a</sup>Other media cited were IsoSensitest agar, Mueller–Hinton agar with blood, chocolate agar, brain–heart infusion agar and 'CNA'.

<sup>b</sup>Other media cited were bile-aesculine agar, aesculine/azide agar, Enterococcus el agar, Enterococcus agar (Difco), VRE agar base (Oxoid) and Enterococcus screen agar (BB).

whether claims made by laboratories were substantiated by the precise details of the specific methods used in routine practice. The ARPAC study found that, despite the majority of participating laboratories claiming to follow CLSI guidelines during 2001, >50% did not follow the recommended methods for the detection of either MRSA or VRE.

The present study is the first pan-European study to assess detailed AST methodology used in clinical microbiology laboratories. The strengths of the study include the large sample of recruited hospitals, the rigorous, piloted data collection methods, and the high-quality data obtained from participating hospitals. It is acknowledged that hospitals were self-selecting, which increases the risk of selection and response bias. Ideally, a full listing of all acute-care hospitals in Europe would provide a sampling frame for random selection of eligible hospitals, but no such list exists. Hence alternative, recognised approaches were used for hospital recruitment [10].

In order to comply with the ARPAC data collection strategy, questionnaires distributed during January 2003 asked about AST methods used during 2001. Thus, there was some potential for recall bias. The response rate of 73% was high within hospitals already willing to participate in ARPAC, with no differences between responders and non-responders, other than the finding that teaching hospitals were more likely to return AST data. However, these hospitals were a self-selecting, motivated sample willing to contribute data to a collaborative research study. Despite this, the data reveal significant inconsistencies when hospitals claimed to follow the same AST guidelines.

In general, the ARPAC study confirmed that European hospitals perform AST by disk-diffusion methods in preference to MIC or (semi)-automated methods [11]. However, when the data were analysed by geographical region, it was apparent that hospitals in southern Europe were less likely to use disk-diffusion and more likely to use a semi-automated method in comparison with other regions. This aligns hospitals in southern Europe more closely with clinical microbiology laboratories in the USA that have determined MICs and used automated instrument-based methods in preference to the disk-diffusion test for some time [12]. The major advantage of automated AST is the efficiency gained in setting-up and reading susceptibility results. Overall, the performance of automated systems correlates well with reference methods, but problems have been reported for some organism–antimicrobial agent combinations, e.g., low-level glycopeptide resistance in enterococci and staphylococci [13]. A full understanding of the limitations of an instrument, together with the use of more appropriate supplementary testing systems, is essential.

There is a growing move towards the surveillance of antimicrobial resistance by collating AST data generated routinely. Raw AST data must be interpreted in order to provide meaningful results for clinicians, and it is the interpreted data that are used for surveillance. Interpretation is based on the application of breakpoints, which are set by various authorities, and which are notoriously diverse [14]. Historically, this has made comparisons among laboratories difficult, especially at an international level. However, more recently,

greater numbers of individual laboratories have opted to follow CLSI breakpoints [2], and two sets of national guidelines have incorporated the CLSI guidelines, or a modified version of CLSI guidelines [15]. The great majority of centralised pan-European, international and pharmaceutical surveillance systems listed in one report [1] use CLSI guidelines, as do most laboratories in North and South America. In the ARPAC study, 84% of participating hospitals from 27 countries stated that they used CLSI guidelines. In the European Antimicrobial Resistance Surveillance System (EARSS), 61% of participants from 19 countries also stated that they used CLSI guidelines [2]. Widespread use of CLSI methods should provide assurance that users are providing rigorous results to clinicians and comparable AST data, ensuring robust surveillance systems. However, this assumption was not supported by the ARPAC study, where detailed analysis revealed considerable deviation from recommended practice.

A review of the literature reveals that alternative AST guidelines for MRSA detection include numerous different media, salt concentrations, incubation temperatures and incubation times. All, or most, of the test conditions can be justified. It is not so much the individual conditions that are important, but rather the specific combinations of conditions, and how they are used in conjunction with specific breakpoints [16]. The CLSI recommend that their breakpoints be used only in conjunction with unmodified CLSI methods, and deviation from prescribed CLSI methodology renders the CLSI breakpoints unusable. This applies to any guidelines on methodology and breakpoints. Thus, the two-thirds of ARPAC participants who have modified the CLSI methods for detection of methicillin resistance in *S. aureus* may be applying CLSI breakpoints inappropriately.

Although the CLSI provides recommendations for testing either oxacillin or methicillin to detect methicillin resistance in *S. aureus*, it is recommended that oxacillin should be used in preference to methicillin, as oxacillin is more resistant to degradation in storage and is more likely to detect heteroresistant isolates [17]. Historically, the reason that many hospitals made the transition to oxacillin may have been the discontinued production of methicillin. More recently, it has been suggested that ceftioxin may replace both meth-

icillin and oxacillin, as ceftioxin detects methicillin resistance in *S. aureus* more reliably, and has the added advantage that no special test conditions are required [16].

Among the ARPAC participants, the disk-diffusion test conditions most likely to be modified were the incubation temperature and the incubation time. Although these modifications may be perceived as minor, they are likely to have a significant impact on AST results [18]. As well as stating that disk-diffusion plates should be incubated at 33–35°C, the CLSI guidelines clearly state that the incubation temperature must not exceed 35°C for detection of MRSA. Despite this, 20% of respondents used temperatures >35°C, and 14% used temperatures <33°C (the latter is less critical). Although 30°C has been generally quoted as being better able to detect heterogeneously resistant MRSA [19,20], the optimal incubation temperature has also been reported to depend on the combination of other test conditions used, e.g., the antibiotic content of the disk [21].

Although the majority of disk-diffusion tests using CLSI methodology should be incubated for 16–18 h, the incubation time for MRSA detection is a full 24 h. However, 13% of respondents incubated tests for <24 h, and 22% for >24 h. A period of 48 h rather than 24 h cannot be assumed to be better for detection of MRSA, e.g., Mouton *et al.* concluded specifically that 24 h is preferable [20]. The concentrations of NaCl used by ARPAC participants also varied considerably, and must be considered in conjunction with intrinsically related test conditions. The effect of changing the salt concentration depends on the agar medium, inoculum size and incubation temperature [16].

In general, test conditions for detection of VRE are less complex and variable than those for MRSA detection. Nevertheless, adherence to CLSI guidelines was poor. Whereas 80% of respondents used the recommended incubation temperature of 35°C for agar-screening, only two-thirds used this temperature for disk-diffusion tests. Conversely, 60% of participants using the agar-screening method, and 68% using disk-diffusion, stated that they used the prescribed incubation time of 24 h. The majority of laboratories deviating from the recommendations incubated plates for <24 h. This may be explained by the fact that the CLSI recommends incubation for 16–18 h for

the detection of most resistance phenotypes, and some laboratories use this incubation period for all AST. The few resistance phenotypes requiring a longer incubation time do so for specific reasons, and such phenotypes may not be detected after incubation for 16–18 h. Thus, it is critically important to follow the guidelines when non-standard test conditions are justified and recommended.

One of the biggest deviations from the CLSI method for the detection of VRE was the use of an agar other than brain–heart infusion agar for agar-screening. Only 35% of respondents used this agar, and 28% used Mueller–Hinton agar inappropriately. Although the original description of the vancomycin screening test reported the use of Mueller–Hinton agar, this was only a preliminary study, which was stated to require further evaluation [22]. The original method was optimised subsequently, and brain–heart infusion agar was found to be superior to Mueller–Hinton agar [23]. Although the specificity and sensitivity of the two agars were similar, there was a slight decrease in sensitivity with Mueller–Hinton agar, depending on the bacterial inoculum used. In addition, results on brain–heart infusion agar were easier to interpret, and the use of this agar in the vancomycin screening test was consistent with the enterococcal aminoglycoside screen test, which also used brain–heart infusion agar; thus its use was adopted by the CLSI [23].

In summary, the results of the pan-European ARPAC study provided a valuable insight into the characteristics of AST methods used by selected hospitals from 32 countries. Despite the fact that the majority of participants stated that their laboratory used CLSI methods, the majority of CLSI laboratories did not use the prescribed methods to detect MRSA and VRE. No single set of recommendations and test conditions can detect every clinically relevant resistance phenotype [16], and this applies to CLSI methods as well as to every other method [24]. However, as demonstrated by the present study, many inappropriate modifications have been made to the MRSA and VRE detection methods, thereby compromising the ability of AST to detect these highly important pathogens. Not only does this hamper institutional and international comparisons of antimicrobial resistance trends, but it impacts adversely on the clinical management and outcome of individual patients. For these

reasons, standard methods should not be modified.

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