DIRECT DISK DIFFUSION TEST USING EUROPEAN CLINICAL ANTIMICROBIAL SUSCEPTIBILITY TESTING BREAKPOINTS PROVIDES RELIABLE RESULTS COMPARED WITH THE STANDARD METHOD

Sofia Stokkou¹, Gernot Geginat¹, Dirk Schlüter^{1,2} and Ina Tammer^{1,*}

¹Institute of Medical Microbiology, Infection Control and Prevention, Otto-von-Guericke University, Magdeburg, Germany

²Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany

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Sepsis represents a life-threatening infection requiring the immediate start of antibacterial treatment to reduce morbidity. Thus, laboratories use direct antimicrobial susceptibility testing (AST) to rapidly generate preliminary results from positive blood cultures. As the direct AST has not yet been published to be evaluated with EUCAST breakpoints, the purpose of the study was to investigate the reliability of the direct agar diffusion test to correctly produce AST results from positive monobacterial blood cultures compared with the VITEK2-based definitive AST, when current EUCAST breakpoints were used.

A total of 428 isolates from unselected monobacterial routine blood cultures and 110 challenge strains were included. Direct agar diffusion-based and standard VITEK2-based AST of 2803 bacterium–drug combinations yielded a total clinical category agreement of 95.47% with 1.28% very major errors and 3.42% combined major and minor errors. On the species level, very major errors were observed in the species–drug combinations *Enterococcus* spp.–high-level gentamicin (10.87%) and *Staphylococcus* spp.–rifampicin (5%), only. No very major errors occurred with Enterobacteriaceae and *Pseudomonas aeruginosa*.

In most species–drug combinations, the direct agar diffusion test using EUCAST breakpoints precisely predicted the result of the definitive antibiotic susceptibility test and, thus, it can be used to optimize empiric antibiotic therapy until definitive results are available.

Keywords: disk diffusion, AST, blood culture, Vitek2, Gram-positive cocci, Gram-negative rods

Introduction

Clinically suspected septic infections require the prompt analysis and initiation of an empirical antibiotic therapy from positive blood cultures to reduce rates of morbidity and mortality [1–3]. Due to the worldwide increase of antibiotic resistance of bacterial pathogens, empirical antibiotic coverage becomes increasingly difficult. Thus, antimicrobial susceptibility testing (AST) data are required as soon as possible to guide antibacterial therapy.

Standardized protocols for AST from positive blood cultures require subculturing of bacteria on agar plates and subsequent automated AST, which typically takes additional 1–2 days until results become available. To overcome these disadvantages, molecular methods and mass spectrometry were evaluated for rapid identification from positive flagged blood cultures with sometimes conflict-

ing results regarding the sensitivity and the lack of AST results [4]. Among the manufacturers of blood culture systems, only Becton–Dickinson (BD) provides a protocol for direct AST from positive blood culture bottles, which is time-consuming and useful only for Gram-negative rods [5, 6].

The procedure implemented by most of the laboratories is the direct Kirby–Bauer disk diffusion test, which is economical, rapid, and easy to set up with low risk for contamination [7]. Furthermore, the method allows for the detection of heterogeneous resistant isolates, which is impossible with automated methods [8, 9].

Previously published direct AST protocols were validated using interpretative category breakpoints derived from Clinical and Laboratory Standards Institute (CLSI) and from DIN (Deutsches Institut für Normung), respectively [6, 5, 10–12].

* Corresponding author: Ina Tammer; Institute of Medical Microbiology, Infection Control and Prevention, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany; Phone: +49391 67-15859; Fax +4939167-13947; E-mail: ina.tammer@med.ovgu.de

Although EUCAST AST breakpoints are now widely adopted in Europe, direct disk diffusion AST from positive blood cultures has not yet been validated. Thus, the aim of the study was to compare the direct agar diffusion test of positive blood cultures with the standard antimicrobial susceptibility testing by VITEK2 (Biomerieux) using EUCAST AST breakpoints for interpretation of the results (European Committee on Antimicrobial Susceptibility Testing [13]).

Materials and methods

Study design and sample collection

The present study was conducted from July 2012 to October 2014 at a 1146-bed tertiary-care hospital in Germany.

BACTEC blood culture bottles and BACTEC 9240 or 9120 instruments were used (Becton Dickinson, Heidelberg, Germany) which detect bacterial growth by fluorescent sensor technology. Each bottle positive flagged by the BACTEC instrument was initially Gram stained and subcultured.

Only the first positive bottle of individual patients was included. If both the aerobic and the anaerobic bottles were positively detected at the time point and both showed identical Gram staining morphology, the aerobic bottle was processed for AST testing. Bottles with mixed Gram-staining morphologies or polymicrobial subcultures were excluded.

A total of 428 consecutive positive blood culture sets was included during the study period.

Testing of highly resistant challenge strains

As highly resistant strains that are required to detect very major errors were not sufficiently represented in clinical

samples additional 110 challenge strains were tested (Tables 1 and 2). For this purpose, aerobic blood culture bottles were spiked with 5 ml of a bacterial suspension and adjusted to a turbidity of 0.5 Mac Farland using a densitometer (Densichek®; Biomerieux, Marcy l'Etoile, France). The suspension was further three times diluted (1:200 each time) to a final concentration of 2–4 colony forming units per milliliter (cfu/ml). After detecting by the blood culture instrument, positive bottles were processed as described above.

Species identification

The identification of all isolates included in this study was performed after sufficient growth on agar plates by means of mass spectrometry (Vitek MS; Biomerieux) in accordance to the manufacturer's instructions. The results were available at the day after bottles were positive flagged.

Direct disk diffusion test

The direct disk diffusion test was performed as described elsewhere [7] with slight modifications. Briefly, for Gram-negative isolates, 0.3 ml blood culture medium was added to 10 ml 0.9% sterile saline. The suspension was spread over the entire surface of blood-free Mueller Hinton (MH; BD, Heidelberg, Germany) agar plates by swabbing in three directions, and antibiotic disks (Oxoid, Wesel, Germany) were placed on the agar surface.

Depending on the Gram-stain morphology, the following antibiotics were tested:

1. For Gram-positive cocci in clusters suggesting *Staphylococcus* spp.: benzylpenicillin, cefoxitin, vancomycin, rifampicin, and linezolid;

Table 1. Species distribution from the unselected patient population ($n = 428$) and from the challenge collection ($n = 110$)

Gram-negative bacilli ($n = 151$)		Gram-positive cocci ($n = 277$)		Challenge strains ($n = 110$)	
Species	n	Species	n	Species	n
<i>Escherichia coli</i>	90	<i>S. aureus</i>	57	MRSA	28
<i>Enterobacter cloacae</i>	9	MRSA	7	<i>Pseudomonas aeruginosa</i>	49
<i>Enterobacter aerogenes</i>	4	<i>S. lugdunensis</i>	1	<i>Enterococcus faecium</i>	33
<i>Klebsiella pneumoniae</i>	4	<i>S. epidermidis</i>	80	(VRE)	
<i>Proteus mirabilis</i>	3	<i>S. hominis</i>	14	- vanA genotype	16
<i>Morganella morganii</i>	1	<i>S. haemolyticus</i>	8	- vanB genotype	17
<i>Citrobacter freundii</i>	1	<i>S. capitis</i>	4		
<i>Yersinia enterocolitica</i>	1	<i>S. auricularis</i>	1		
		<i>S. warneri</i>	2		
<i>Pseudomonas aeruginosa</i>	38	<i>Enterococcus faecalis</i>	41		
		<i>Enterococcus faecium</i>	62		
		-VRE	4		

n = number; MRSA = methicillin-resistant *S. aureus*; VRE = vancomycin-resistant *E. faecium*

Table 2. Resistance pattern of *Pseudomonas aeruginosa* challenge organisms

Interpretation/Drug	<i>P. aeruginosa</i> (n = 49)				
	TZP	CAZ	ME	CN	CIP
Resistant	40	30	27	13	35
Intermediate	0	0	13	0	5
Susceptible	7	17	7	34	7

TZP = piperacillin-tazobactam; CAZ = ceftazidime; ME = meropenem; CN = gentamicin; CIP = ciprofloxacin

2. For Gram-positive cocci in chains suggesting *Streptococcus/Enterococcus* spp.: benzylpenicillin, ampicillin, imipenem, vancomycin, linezolid, and high-level gentamicin;
3. For Gram-negative rods: piperacillin-tazobactam, cefotaxime, ceftazidime, meropenem, gentamicin and ciprofloxacin were tested.

Agar plates were incubated for 18–24 h at 37 °C in ambient air. After appropriate growth and species identification, inhibition zone diameters were measured and interpreted according to the EUCAST guidance documents and tables of the version 2.0 [13].

To control the accuracy of the direct agar diffusion test, the following quality control strains were weekly tested according to EUCAST recommendations: *Escherichia* (*E.*) *coli* ATCC 25922, *Pseudomonas* (*P.*) *aeruginosa* ATCC 27853, *Staphylococcus* (*S.*) *aureus* ATCC 29213, and *Enterococcus* (*E.*) *faecalis* ATCC 29212. Repeated AST testing with QC strains revealed complete SIR agreement (data not shown).

Standard AST

Bacterial colonies from overnight subcultures on agar plates were used to perform the standard AST testing using the Vitek2 instrument which is the primary test system for AST in our routine laboratory.

The following panels were used: AST-619 (staphylococci), AST-586 (enterococci), AST-263 (Enterobacteriaceae), and AST-248 (*Pseudomonas* spp.). The panels were inoculated as recommended by the manufacturer.

Minimal inhibitory concentration (MIC) values were interpreted according to EUCAST clinical breakpoints (version 2.0; 2012) without the use of the Vitek2 advanced expert system (AES; [13]).

The accuracy of the Vitek2 was controlled using the same quality control strains as described above.

Confirmatory tests

To confirm the resistance to methicillin in *S. aureus*, the Slidex® MRSA detection kit (Biomerieux), which detects the modified penicillin-binding protein 2a, was

used following the recommendations of the manufacturer.

Data analysis

All isolates tested were grouped into the categories susceptible, intermediate, or resistant, according to the EUCAST AST breakpoints version 2.0 [13].

Concordance in both test systems was recorded as agreement. Discrepancies in the agreement of the susceptibility categories determined by direct AST and standard AST were classified into very major errors (VMEs; resistant in standard and susceptible in direct AST = false susceptible), major errors (MEs; susceptible in standard and resistant in direct AST = false resistant), and minor errors (mEs; all other discrepancies) and percentages were calculated. VMEs were determined from the number of resistant strains. MEs and mEs were calculated from the number of susceptible strains [14]. Because of the insufficient number of resistant challenge strains available for the study the resistance to meropenem in Enterobacteriaceae, to vancomycin in staphylococci, and to linezolid in Gram-positive cocci could not be studied.

Results

A total of 428 consecutive positive routine blood cultures were included in this study (Table 1). The Gram-positive isolates were either *Enterococcus* spp. (*n* = 103) or *Staphylococcus* spp. (*n* = 174). The Gram-negative isolates belonged to Enterobacteriaceae (*n* = 113) and *Pseudomonas* spp. (*n* = 38). Among the Enterobacteriaceae, 40 isolates produced extended spectrum β -lactamases (ESBL) and eight isolates expressed the AmpC phenotype, whereas 59 isolates showed the wild type resistance pattern.

The correct detection for the frequency of very major errors requires the testing of at least 35 resistant isolates [14]. Because highly resistant isolates were insufficiently represented among the routine blood culture isolates, resistant challenge strains were included in the analysis (Tables 1 and 2).

Altogether, 2803 organism-drug combinations were tested. Following the criteria by Jorgensen [14], the rate of VMEs should be lower than 3%, and the combined

Table 3. Comparison of the results of direct disk diffusion and standardized AST by automated Vitek2 system of all organisms tested

Drug	No. of tests		sAST (Vitek2)				dDDT				SIR Agreement				ME				VME				ME+mE			
	n		S	I	R		S	I	R		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Piperacillin-tazobactam	200		124	5	71		114	16	70		180	90.00	7	5.65	0	0.00	11	8.87								
Cefotaxime	113		56	0	57		53	1	59		110	97.35	2	3.57	0	0.00	3	5.36								
Ceftazidime	200		116	17	67		104	7	89		169	84.50	6	5.17	1	1.49	13	11.21								
Meropenem	200		148	18	34		142	6	52		176	88.00	2	1.35	0	0.00	6	4.05								
Gentamicin	200		149	0	51		147	1	52		197	98.50	2	1.34	0	0.00	2	1.34								
Ciprofloxacin	200		88	10	102		90	8	102		190	95.00	1	1.14	1	0.98	2	2.27								
Benzylpenicillin	202		50	0	152		38	0	164		190	94.06	12	24.00	0	0.00	12	24.00								
Cefoxitin-screen	202		87	0	115		86	0	116		195	96.53	4	4.60	3	2.61	4	4.60								
Vancomycin	338		301	0	37		302	0	36		337	99.70	0	0.00	1	2.70	0	0.00								
Rifampicin	202		182	0	20		181	0	21		199	98.51	2	1.10	1	5.00	2	1.10								
Linezolid	338		338	0	0		338	0	0		338	100.00	0	0.00	0	nd	0	0.00								
Ampicillin	136		41	1	94		38	0	98		132	97.06	3	7.32	0	0.00	3	7.32								
Imipenem	136		41	0	95		38	2	96		133	97.79	1	2.44	0	0.00	3	7.32								
Gentamicin high-level	136		90	0	46		94	0	42		130	95.59	1	1.11	5	10.87	1	1.11								
All	2803		1811	51	941		1765	41	997		2676	95.47	43	2.37	12	1.28	62	3.42								

n = number; nd = not determined; S = susceptible; I = intermediate; R = resistant; sAST = standard antimicrobial susceptibility testing; dDDT = direct disk diffusion testing; ME = major error; VME = very major error; mE = minor error

Table 4. Comparison of the results of direct disk diffusion and standardized AST by automated Vitek2 system of Gram-negative rods using EUCAST breakpoints

Drug	No. of tests	sAST (Vitek2)				dDDT			SIR agreement		ME		VME		ME+mE		
		<i>n</i>	S	I	R	S	I	R	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
Enterobacteriaceae																	
Piperacillin-tazobactam	113	84	5	24	74	16	23	93	82.30	7	8.33	0	0.00	11	13.10		
Cefotaxime	113	56	0	57	53	1	59	110	97.35	2	3.57	0	0.00	3	5.36		
Meropenem	113	113	0	0	113	0	0	113	100.00	0	0.00	0	nd	0	0.00		
Gentamicin	113	83	0	30	82	1	30	111	98.23	1	1.20	0	0.00	1	1.20		
Ciprofloxacin	113	53	4	56	56	2	55	106	93.81	0	0.00	1	1.79	1	1.89		
Total	678	454	26	198	433	27	218	619	91.30	13	2.86	1	0.51	26	5.73		
<i>Pseudomonas aeruginosa</i>																	
Piperacillin-tazobactam	87	40	0	47	40	0	47	87	100.00	0	0.00	0	0.00	0	0.00		
Ceftazidime	87	51	0	36	49	0	38	83	95.40	3	5.88	1	2.78	3	5.88		
Meropenem	87	35	18	34	29	6	52	63	72.41	2	5.71	0	0.00	6	17.14		
Gentamicin	87	66	0	21	65	0	22	86	98.85	1	1.52	0	0.00	1	1.52		
Ciprofloxacin	87	35	6	46	34	6	47	84	96.55	1	2.86	0	0.00	1	2.86		
Total	435	227	24	184	217	12	206	403	92.64	7	3.08	1	0.54	11	4.85		
Gram-negative rods	1113	681	50	382	650	39	424	1022	91.82	20	2.94	2	0.52	37	5.43		

n = number; nd = not determined; S = susceptible; I = intermediate; R = resistant; sAST = standard antimicrobial susceptibility testing; dDDT = direct disk diffusion testing; ME = major error; VME = very major error; mE = minor error

Table 5. Comparison of the results of direct disk diffusion and standardized AST by automated Vitek2 system of Gram-positive cocci using EUCAST breakpoints

Drug	No. of tests		sAST (Vitek2)			dDDT			SIR agreement			ME		VME		ME+mE	
	<i>n</i>	<i>S</i>	<i>I</i>	<i>R</i>	<i>S</i>	<i>I</i>	<i>R</i>	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>
<i>Staphylococcus</i> spp.																	
Benzylpenicillin	202	50	0	152	38	0	164	190	94.06	12	24.00	0	0.00	12	24.00	12	24.00
Cefoxitin-screen	202	87	0	115	86	0	116	195	96.53	4	4.60	3	2.61	4	4.60	4	4.60
Vancomycin	202	201	0	1	202	0	0	201	99.50	0	0.00	1	nd	0	0.00	0	0.00
Rifampicin	202	182	0	20	181	0	21	199	98.51	2	1.10	1	5.00	2	1.10	2	1.10
Linezolid	202	202	0	0	202	0	0	202	100.00	0	0.00	0	nd	0	0.00	0	0.00
Total	1010	722	0	288	709	0	301	987	97.72	18	2.49	5	1.74	18	2.49	18	2.49
<i>Enterococcus</i> spp.																	
Ampicillin	136	41	1	94	38	0	98	132	97.06	3	7.32	0	0.00	3	7.32	3	7.32
Imipenem	136	41	0	95	38	2	96	133	97.79	1	2.44	0	0.00	3	7.32	3	7.32
Gentamicin high-level	136	90	0	46	94	0	42	130	95.59	1	1.11	5	10.87	1	1.11	1	1.11
Vancomycin	136	100	0	36	100	0	36	136	100.00	0	0.00	0	0.00	0	0.00	0	0.00
Linezolid	136	136	0	0	136	0	0	136	100.00	0	0.00	0	nd	0	0.00	0	0.00
Total	680	408	1	271	406	2	272	667	98.09	5	1.23	5	1.85	7	1.72	7	1.72
Gram-positive cocci	1550	1045	1	504	1032	2	516	1516	97.81	21	2.01	10	1.98	23	2.20	23	2.20

n = number; nd = not determined; S = susceptible; I = intermediate; R = resistant; sAST = standard antimicrobial susceptibility testing; dDDT = direct disk diffusion testing; ME = major error; VRE = very major error; mE = minor error

major and minor errors should be $\leq 7\%$. For all isolates studied, the overall SIR category agreement of the direct disk diffusion test and the standard VITEK2-based AST was 95.47% with 1.28% very major errors and combined minor and major errors of 3.42% (Table 3).

As EUCAST interpretative criteria are defined to the species level, the agreement of direct and standard AST was analyzed separately for the clinically most important species groups (Tables 4 and 5). The percentage of VME for the AST of Enterobacteriaceae was below 3% for all species–drug combinations tested ($n = 678$; Table 4). The combined minor and major errors, however, were above 7% only for the direct AST to piperacillin–tazobactam (combined error 13.1%).

Also, for AST of *P. aeruginosa*, the percentage of VME was below 3% for all species–drug combinations ($n = 436$; Table 4). The combined minor and major errors were above 7% only for the direct AST to meropenem (combined error 17.14%).

Among the 202 isolates belonging to the genus *Staphylococcus* (92 *S. aureus*; 110 coagulase-negative staphylococci), the SIR agreement of the direct and standard VITEK2-based AST was 97.72% (Table 5). Clinically important VMEs were found for the testing of cefoxitin and rifampicin ($n = 1$; VRE = 5%). In the species–drug combination *Staphylococcus* spp.–cefazolin VMEs were observed in three isolates (VME = 2.61%) identified as *Staphylococcus epidermidis* ($n = 2$) and *Staphylococcus hominis* ($n = 1$), respectively. With the exception of benzylpenicillin which yielded combined minor and major errors in 24% of test results, the combined minor and major errors were below 7% for all other species–drug combinations.

For the 136 *Enterococcus* isolates, the results of direct AST corresponded in 98.9% of the tests with the results derived from the reference method. The VME was below 3% for all species drug combinations of enterococci (Table 5) except for high-level gentamicin (VME = 10.87%), which occurred mostly with *E. faecalis* ($n = 4$). Enterococci showed marginally increased combined minor and major errors for the AST against ampicillin and imipenem (combined error 7.32%). Importantly, no errors occurred when enterococci were tested against vancomycin.

Discussion

Direct AST from positive blood cultures is motivated by the considerable time saving of approximately 1 working day until results become available and by the simplicity of the test. To be clinically useful, any AST protocol has to meet certain minimal requirements. Thornsberry et al. (1980) suggested for the evaluation of new automated AST methods that the complete category agreement should be over 90% and that the total of very major errors and major errors, respectively, should not exceed 5% [15].

In the current study, testing of 538 clinical blood cultures and challenge strains in 2803 bacterium–drug com-

binations yielded an agreement of the direct disk diffusion test and standard VITEK2-based AST of 95.47% with a combined rate of VMEs and MEs of 3.65%. Thus, the direct disk diffusion test method fulfills the requirements described above suggesting an acceptable accuracy of the test system.

In 1993, Jorgensen [14] proposed a refined criteria that requires an agreement $>90\%$ of the new test compared with the reference method, a VME $<3\%$, and $\leq 7\%$ combined major and minor errors.

Although the total category agreement in Gram-positive cocci was higher than in Gram-negative rods (97.68% versus 91.85%), which corroborates a previous report [7], in the current study, a VME rate above 3% was observed in the species–drug combinations *Enterococcus* spp.–high-level gentamicin and *Staphylococcus* spp.–rifampicin that yielded VMEs of 10.87% and 5%, respectively.

If AST results are used to optimize empiric antibiotic therapy, VMEs would result in an ineffective antibiotic treatment and, thus, have to be strictly avoided. As both gentamicin and rifampicin, however, are only used as one component in combined therapy regimes, a VME of about 10% might be still acceptable for the preliminary assay as it would not lead to a total treatment failure. On the other hand, it would expose patients to unnecessary side effects of an inactive antimicrobial drug. In this study, false susceptible high-level gentamicin-resistant enterococci were mainly *E. faecalis* (four of five isolates).

The direct AST yielded false susceptible test results only in one out of 20 rifampicin-resistant isolates tested. As the number of resistant isolates was less than the suggested number of 35 resistant strains [14], this result must be considered with caution.

The other requirement of the Jorgensen criteria, i.e., combined major and minor errors $\leq 7\%$, was not met for the species–drug combinations Enterobacteriaceae–piperacillin–tazobactam, *P. aeruginosa*–meropenem, *Staphylococcus* spp.–benzylpenicillin while both *Enterococcus* spp.–ampicillin and *Enterococcus* spp.–imipenem yielded marginal elevated combined errors.

From a clinical point of view, major errors are less critical for the antibiotic treatment of the patients as drugs reported (false) resistant will not be administered to avoid treatment failure. However, they may limit the number of possible treatment options which can be a particular problem for infections caused by highly resistant species such as *P. aeruginosa*, which is intrinsically resistant to various antibiotics.

Among the Enterobacteriaceae tested false resistant to piperacillin–tazobactam, 86% of strains were ESBL-producing *E. coli*. Numerous studies have reported the influence of the inoculum size on the activity of piperacillin–tazobactam against ESBL-producing *E. coli* [16, 17]. For this reason, the Vitek2 Advanced Expert System (AES) translates all piperacillin–tazobactam susceptible test results to intermediate. After application of the Vitek2 AES, the clinical category agreement would increase from 82.3% to 93.8% and the combined

major and minor error rate would be 8.33% instead of 13.1%.

The ability of the Vitek2 system to accurately determine the MICs for meropenem, ceftazidime, and piperacillin–tazobactam is limited. Torres et al. showed that the Vitek2 yielded lower MIC values of piperacillin–tazobactam tested with *P. aeruginosa* compared to the microdilution broth reference method [18]. The recommended MIC range of the quality control strain *E. coli* ATCC25922 for piperacillin–tazobactam is 1–4 mg/l. The lowest level detectable with the Vitek2 system is ≤ 4 mg/l. Thus, the quality control on the Vitek2 is limited because the lower breakpoint is not within the range of the currently available Vitek2 AST panels for Gram-negative bacteria.

The highest rate of major errors was observed when testing the activity of benzylpenicillin against staphylococci because EUCAST MIC breakpoints cannot unequivocally distinguish between penicillinase producers and non-producers. The EUCAST recommends confirmation of the susceptible result by standard disk diffusion test which is more reliable than determination of the MIC [13].

Only in three isolates of coagulase-negative staphylococci, but in no case of *S. aureus*, a false susceptible result was observed with the cefoxitin screen corroborating that cefoxitin disk testing is well-suited for the detection of methicillin-resistant coagulase-negative staphylococci [19].

The VME must be determined exclusively from the resistant population since a susceptible strain cannot contribute false-susceptible results [14]. This requirement is difficult to fulfill for resistance phenotypes that are rarely isolated from blood cultures such as carbapenemase-producing Enterobacteriaceae, linezolid-resistant Gram-positive cocci, or vancomycin-resistant *Staphylococcus* species. Therefore, the VME rate for these resistance phenotypes could not be determined in this study.

In conclusion, despite the inoculum size, which is not strictly defined in direct AST but may strongly influence the AST results [6, 12, 20], the direct disk diffusion testing of blood culture isolates represents a simple and cost-effective method of preliminary antimicrobial susceptibility testing. Using EUCAST guidelines for interpretation, the test system provides reliable results within 24 h. In the age of increasing antibiotic resistance, the direct AST results may be helpful in preventing treatment failure especially in vancomycin-resistant enterococci, ESBL-producing Enterobacteriaceae, multidrug-resistant *Pseudomonas* spp., and MRSA. Due to the limitations of the direct AST, it is important to ensure that definitive antibiotic therapy is guided by the results of definitive AST which seems particularly important for high-level gentamicin-resistant enterococci.

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