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Antimicrobial susceptibility testing of pathogens isolated from blood culture: a performance comparison of Accelerate Pheno™ and VITEK® 2 systems with the broth microdilution method

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Objectives: To compare the performance of the Accelerate Pheno™ system with that of the conventional phenotypic VITEK® 2 system for rapid antimicrobial susceptibility testing (AST) of bacterial pathogens from positive blood culture (PBC) samples, based on the reference broth microdilution (BMD) method.

Methods: Prospectively collected PBCs that represented patient-unique bloodstream infection episodes were included. For PBC samples showing monomicrobial growth ($n = 86$), AST was performed using both Accelerate Pheno™ and VITEK® 2 systems directly from PBC broth. Colony isolates derived from subculture of PBC broth were then used for BMD testing. AST results were interpreted according to 2017 EUCAST breakpoints.

Results: The overall categorical agreement between Accelerate Pheno™ system and BMD was 92.7% (467/504) for Gram-negative organisms and 99.0% (95/96) for Gram-positive organisms, with rates for very major errors of 3.6% (6/166), major errors 2.2% (9/416) and minor errors 3.8% (23/600). The overall categorical agreement between the VITEK® 2 system and BMD was 91.7% (463/505) for Gram-negative organisms and 99.0% (97/98) for Gram-positive organisms, with rates of very major errors of 2.4% (4/169), major errors 1.0% (4/416) and minor errors 5.8% (35/603). Importantly, unlike the VITEK® 2 system, no false-susceptible results occurred with two colistin-resistant organism-growing PBCs tested using the Accelerate Pheno™ system.

Conclusions: Based on these findings, the Accelerate Pheno™ system can be a valid alternative for the rapid AST of Gram-negative and Gram-positive bacteria in bloodstream infections.

Introduction

Bloodstream infections (BSIs) remain a major public health concern because of their high incidence and serious consequences in terms of mortality, morbidity and cost, particularly in the case of nosocomial infection.[1] While timely administration of effective antimicrobial therapy may reduce hospital length of stay and mortality of patients with a BSI,[2–4] delayed (and potentially less effective) treatment often results in more severe stages of BSI-related disease.[5,6] Of note, drug-resistant and MDR organisms are the most frequent trigger for sepsis and septic shock—a particularly serious manifestation of sepsis—thereby requiring initiation of treatment within 1 h of their detection.[7]

Antimicrobial susceptibility testing (AST) of organisms causing BSIs is an undisputed prerequisite for optimal antimicrobial therapy.[8] In contrast to conventional automated methods, such as the widely used VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France),[9,10] recent efforts have led to the development of new-generation methods for AST.[11–16] Among them is the Accelerate Pheno™ system (Accelerate Diagnostics, Tucson, AZ, USA), an automated microscopy platform that uses fluorescence *in situ* hybridization for identification and morphokinetic cellular analysis to provide AST results (i.e. MIC) directly from positive blood cultures (PBCs);[17] blood culture (BC) remains the gold standard for detection of bacterial and fungal BSIs.[18] The Accelerate Pheno™ system obtained US FDA clearance in early 2017. Data from the US clinical trial used to support FDA clearance of the system were recently published.[19]

Until now, there have been few published studies that have evaluated the performance of the Accelerate Pheno™ system;[20–24] these evaluations include an assessment of the Accelerate Pheno™ system for both identification and AST of BSI organisms

(in either fresh or contrived PBCs), and/or restricted evaluations of specific organism groups (e.g. Gram-negative bacteria, MDR Gram-negative bacilli)[20,24] or patient populations (i.e. paediatric oncology).[21] Furthermore, these studies did not use or only partially used the reference broth microdilution (BMD) method as a comparator, in one case to resolve discrepancies between the results of the Accelerate Pheno™ and VITEK® 2 systems. In this prospective study, we assessed the MIC and categorical agreement (susceptible, intermediate and resistant) results between the Accelerate Pheno™ and VITEK® 2 systems by rapid testing of PBCs, in comparison with those obtained with the BMD method using subcultured colony isolates.

Materials and methods

Ethics

The ethics committee of our institution approved this study (approval number 0044603/17) and waived the requirement for informed consent.

Study design, blood samples and microbial isolates

Prospectively collected PBCs that represented single episodes of BSI (i.e. only the first PBC per single patient) at a tertiary-care teaching hospital in Rome, Italy, were evaluated over a 6 month period in 2017 (Table S1, available as Supplementary data at *JAC* Online). We collected blood cultures (BCs) in BacT/ALERT® FA and FN PLUS bottles (bioMérieux, Marcy-l'Étoile, France) using the BacT/ALERT® VIRTUO® system (bioMérieux). For each PBC, organism identification was determined to the species level using a BSI diagnostic algorithm previously described.[25] Gram staining was performed to distinguish PBCs with monomicrobial ($n = 105$) or polymicrobial ($n = 12$) growth, which was followed by direct analysis using the MALDI BioTyper® system (Bruker Daltonics, Bremen, Germany) and supplemented with the FilmArray® BC ID panel (bioMérieux). In parallel, we subcultured PBC broths on standard solid media and incubated them overnight at 35°C to yield colony isolates. MICs were determined by three methods: the Accelerate Pheno™ system; the VITEK® 2 system performed directly from PBC broth (for samples where only one bacterial morphology was seen on Gram stain, i.e. presumed to be monomicrobial) or colony isolates (for samples demonstrating more than one Gram stain morphology, i.e. polymicrobial); and BMD.[26] For VITEK® 2 system testing using PBC broth as inoculum, we subjected PBC broths to a brief pretreatment (i.e. lysis, filtration and centrifugation) as described elsewhere.[9] We also confirmed the initial direct MALDI identification using subcultured colony isolates.

AST by the Accelerate Pheno™ system

According to the manufacturer's instructions, we performed Accelerate Pheno™ system testing on PBC broths within 8 h of growth detection by the BacT/ALERT® VIRTUO® system. A 500 μL aliquot was transferred into the sample vial and immediately loaded onto the system. The analysis software Accelerate Diagnostics Host application version 1.1.0.69 was used.

AST by the VITEK® 2 system and the BMD method

We performed AST with the VITEK® 2 system according to the manufacturer's instructions, using the software version 7.01 and the AST-N201, AST-P632, AST-P586 and AST-ST01 cards for Gram-negative bacteria, staphylococci, enterococci and streptococci, respectively. For direct AST, we selected the VITEK® 2 cards according to the Gram stain results and the direct MALDI organism identification results obtained as described above. We performed AST by the BMD method according to the 2006 ISO 20776-1 procedure, as recommended by EUCAST.[26]

Data analysis and discrepancy resolution

The Accelerate Pheno™ system and VITEK® 2 system AST results were compared with the BMD results. We used the 2017 EUCAST standards to interpret AST results for each of the following antimicrobials: amikacin, ampicillin, ceftazidime, cefepime, ciprofloxacin, colistin, daptomycin, ertapenem, erythromycin, gentamicin, linezolid, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole and vancomycin.[27] Agreement of interpretative results was calculated. The discrepant results were categorized as very major errors [VMEs (false susceptibility)], major errors [MEs (false resistance)] and minor errors [mEs (intermediate result instead of susceptible or resistant)]. Isolates showing VMEs or MEs were retested, and if initial and repeated test results were not the same, the result of repeat testing was used for data analysis. To reproduce the conditions under which blood sample aliquots were originally tested, we performed Accelerate Pheno™ system or VITEK® 2 system retesting on contrived BCs. We subcultured previously frozen isolates to prepare bacterial suspensions, each containing $\sim 5\times10^8$ cfu/mL. We made appropriate dilutions to inoculate BC broths with 1 mL (5×10^2 cfu) of each suspension, along with 10 mL of bank whole-blood samples. After incubation in the BacT/ALERT® VIRTUO® system, contrived PBCs were available for AST discrepancy testing.

Results

Excluding the isolates from species known to be off-panel organisms (Table S1), the Accelerate Pheno™ system correctly identified 115/123 (93.5%) on-panel organisms. Seven of the eight isolates that were not identified were from polymicrobial BCs (data not shown). While Accelerate Pheno™ system AST results were available for 94/115 (81.7%) isolates, we included 86 (62 Gram-negative and 24 Gram-positive) isolates from monomicrobial BCs, resulting in 600 organism/antimicrobial test results. We compared these results with those reported by BMD (416 susceptible, 166 resistant and 18 intermediate results). For the VITEK® 2 system, there were 603 organism/antimicrobial test results—with 3 corresponding to the indeterminate results obtained by the Accelerate Pheno™ system—that could be compared with those reported by BMD (416 susceptible, 169 resistant and 18 intermediate results). Among Gram-negative organisms, the categorical agreement was 92.7% (467/504) for the Accelerate Pheno™ system and 91.7% (463/505) for the VITEK® 2 system (Table 1). Among Gram-positive organisms, the categorical agreement was 99.0% for both the Accelerate Pheno™ (95/96) and the VITEK® 2 (97/98) system (Table 2). The overall rates of VMEs, MEs and mEs were 3.6% (6/166), 2.2% (9/416) and 3.8% (23/600) with the Accelerate Pheno™ system, and 2.4% (4/169), 1.0% (4/416) and 5.8% (35/603) with the VITEK® 2 system (Tables 1 and 2).

As shown in Tables 1 and 2, we analysed the distributions of agreements and errors by individual antimicrobial agent for the species of Gram-negative and Gram-positive organisms, respectively. Overall, we noted that cefepime, meropenem, amikacin, gentamicin and ciprofloxacin showed complete categorical agreement (100%) in the two systems for *Enterobacter* spp. ($n = 4$) and *Serratia marcescens* ($n = 2$), as well as piperacillin/tazobactam, ceftazidime, ertapenem, meropenem, gentamicin and ciprofloxacin for *Proteus* spp. ($n = 3$). The same applied to cefepime and colistin for *Pseudomonas aeruginosa* ($n = 8$) and to meropenem and

Table 1. Performance of the Accelerate Pheno™ system and VITEK® 2 systems compared with BMD for Gram-negative bacterial species ($n = 62$)

	BMD				Accelerate Pheno™ system [% (*n*/*N*)]				VITEK® 2 system [% (*n*/*N*)]			
						errors				errors		
Antimicrobial	*n*	S	I	R	categorical agreement[a]	VMEs	MEs	mEs	categorical agreement[a]	VMEs	MEs	mEs
E. coli	19											
TZP		16	1	2	94.7 (18/19)	0.0 (0/2)	0.0 (0/16)	5.3 (1/19)	89.5 (17/19)	0.0 (0/2)	0.0 (0/16)	10.5 (2/19)
cefepime		13	1	5	78.9 (15/19)	0.0 (0/5)	0.0 (0/13)	21.1 (4/19)	84.2 (16/19)	0.0 (0/5)	0.0 (0/13)	15.8 (3/19)
ceftazidime		11	3	5	89.5 (17/19)	0.0 (0/5)	0.0 (0/11)	10.5 (2/19)	84.2 (16/19)	0.0 (0/5)	0.0 (0/11)	15.8 (3/19)
ertapenem		19	0	0	100 (19/19)	NA (0/0)	0.0 (0/19)	0.0 (0/19)	100 (19/19)	NA (0/0)	0.0 (0/19)	0.0 (0/19)
meropenem		19	0	0	100 (19/19)	NA (0/0)	0.0 (0/19)	0.0 (0/19)	94.7 (18/19)	NA (0/0)	0.0 (0/19)	5.3 (1/19)
amikacin		18	1	0	94.7 (18/19)	NA (0/0)	0.0 (0/18)	5.3 (1/19)	78.9 (15/19)	NA (0/0)	0.0 (0/18)	21.1 (4/19)
gentamicin		16	0	3	100 (19/19)	0.0 (0/3)	0.0 (0/16)	0.0 (0/19)	94.7 (18/19)	0.0 (0/3)	0.0 (0/16)	5.3 (1/19)
ciprofloxacin		10	1	8	94.7 (18/19)	0.0 (0/8)	0.0 (0/10)	5.3 (1/19)	94.7 (18/19)	0.0 (0/8)	0.0 (0/10)	5.3 (1/19)
colistin		19	0	0	94.7 (18/19)	NA (0/0)	5.3 (1/19)	0.0 (0/19)	100 (19/19)	NA (0/0)	0.0 (0/19)	0.0 (0/19)
K. pneumoniae	18											
TZP		6	2	10	83.3 (15/18)	10.0 (1/10)	0.0 (0/6)	11.1 (2/18)	83.3 (15/18)	0.0 (0/10)	0.0 (0/6)	16.7 (3/18)
cefepime		8	0	10	100 (17/17)	0.0 (0/9)	0.0 (0/8)	0.0 (0/17)	88.9 (16/18)	0.0 (0/10)	0.0 (0/8)	11.1 (2/18)
ceftazidime		8	0	10	100 (18/18)	0.0 (0/10)	0.0 (0/8)	0.0 (0/18)	100 (18/18)	0.0 (0/10)	0.0 (0/8)	0.0 (0/18)
ertapenem		9	0	9	94.4 (17/18)	11.1 (1/9)	0.0 (0/9)	0.0 (0/18)	100 (18/18)	0.0 (0/9)	0.0 (0/9)	0.0 (0/18)
meropenem		9	0	9	88.9 (16/18)	22.2 (2/9)	0.0 (0/9)	0.0 (0/18)	100 (18/18)	0.0 (0/9)	0.0 (0/9)	0.0 (0/18)
amikacin		15	3	0	77.8 (14/18)	NA (0/0)	0.0 (0/15)	22.2 (4/18)	72.2 (13/18)	NA (0/0)	13.3 (2/15)	16.7 (3/18)
gentamicin		14	0	4	94.4 (17/18)	0.0 (0/4)	0.0 (0/14)	5.6 (1/18)	72.2 (13/18)	0.0 (0/4)	0.0 (0/14)	27.8 (5/18)
ciprofloxacin		8	0	10	94.4 (17/18)	10.0 (1/10)	0.0 (0/8)	0.0 (0/18)	100 (18/18)	0.0 (0/10)	0.0 (0/8)	0.0 (0/18)
colistin		16	0	2	94.4 (17/18)	0.0 (0/2)	6.3 (1/16)	0.0 (0/18)	94.4 (17/18)	50.0 (1/2)	0.0 (0/16)	0.0 (0/18)
P. aeruginosa	8											
TZP		3	0	5	100 (8/8)	0.0 (0/5)	0.0 (0/3)	0.0 (0/8)	87.5 (7/8)	0.0 (0/5)	33.3 (1/3)	0.0 (0/8)
cefepime		4	0	4	100 (8/8)	0.0 (0/4)	0.0 (0/4)	0.0 (0/8)	100 (8/8)	0.0 (0/4)	0.0 (0/4)	0.0 (0/8)
ceftazidime		3	0	5	62.5 (5/8)	0.0 (0/5)	100 (3/3)	0.0 (0/8)	87.5 (7/8)	0.0 (0/5)	33.3 (1/3)	0.0 (0/8)
meropenem		3	0	5	87.5 (7/8)	0.0 (0/5)	0.0 (0/3)	12.5 (1/8)	87.5 (7/8)	0.0 (0/5)	0.0 (0/3)	12.5 (1/8)
amikacin		3	1	4	87.5 (7/8)	0.0 (0/4)	0.0 (0/3)	12.5 (1/8)	100 (8/8)	0.0 (0/4)	0.0 (0/3)	0.0 (0/8)
gentamicin		2	0	6	100 (8/8)	0.0 (0/6)	0.0 (0/2)	0.0 (0/8)	87.5 (7/8)	16.6 (1/6)	0.0 (0/2)	0.0 (0/8)
ciprofloxacin		1	0	7	87.5 (7/8)	14.3 (1/7)	0.0 (0/1)	0.0 (0/8)	100 (8/8)	0.0 (0/7)	0.0 (0/1)	0.0 (0/8)
colistin		8	0	0	100 (8/8)	NA (0/0)	0.0 (0/8)	0.0 (0/8)	100 (8/8)	NA (0/0)	0.0 (0/8)	0.0 (0/8)
A. baumannii	8											
meropenem		1	0	7	100 (8/8)	0.0 (0/7)	0.0 (0/1)	0.0 (0/8)	100 (8/8)	0.0 (0/7)	0.0 (0/1)	0.0 (0/8)
amikacin		2	1	5	87.5 (7/8)	0.0 (0/5)	0.0 (0/2)	12.5 (1/8)	87.5 (7/8)	0.0 (0/5)	0.0 (0/2)	12.5 (1/8)
ciprofloxacin		1	0	7	100 (8/8)	0.0 (0/7)	0.0 (0/1)	0.0 (0/8)	100 (8/8)	0.0 (0/7)	0.0 (0/1)	0.0 (0/8)
colistin		6	0	2	62.5 (5/8)	0.0 (0/2)	50.0 (3/6)	0.0 (0/8)	87.5 (7/8)	50.0 (1/2)	0.0 (0/6)	0.0 (0/8)
Enterobacter spp.	4											
TZP		3	0	1	75.0 (3/4)	0.0 (0/1)	33.3 (1/3)	0.0 (0/4)	75.0 (3/4)	0.0 (0/1)	0.0 (0/3)	25.0 (1/4)
cefepime		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
ceftazidime		2	1	1	75.0 (3/4)	0.0 (0/1)	0.0 (0/2)	25.0 (1/4)	75.0 (3/4)	0.0 (0/1)	0.0 (0/2)	25.0 (1/4)
ertapenem		3	1	0	100 (4/4)	NA (0/0)	0.0 (0/3)	0.0 (0/4)	75.0 (3/4)	NA (0/0)	0.0 (0/3)	25.0 (1/4)
meropenem		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
amikacin		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
gentamicin		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
ciprofloxacin		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
colistin		4	0	0	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)
Proteus mirabilis	3											
TZP		3	0	0	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)
cefepime		1	1	1	66.6 (2/3)	0.0 (0/1)	0.0 (0/1)	33.3 (1/3)	66.6 (2/3)	0.0 (0/1)	0.0 (0/1)	33.3 (1/3)
ceftazidime		1	0	2	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)
ertapenem		3	0	0	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)
meropenem		3	0	0	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)

Continued

Table 1. *Continued*

	BMD				Accelerate Pheno™ system [% (*n*/*N*)]				VITEK® 2 system [% (*n*/*N*)]			
						errors				errors		
Antimicrobial	*n*	S	I	R	categorical agreement[a]	VMEs	MEs	mEs	categorical agreement[a]	VMEs	MEs	mEs
amikacin		1	1	1	66.6 (2/3)	0.0 (0/1)	0.0 (0/1)	33.3 (1/3)	100 (3/3)	0.0 (0/1)	0.0 (0/1)	0.0 (0/3)
gentamicin		1	0	2	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)
ciprofloxacin		1	0	2	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)
S. marcescens	2											
TZP		1	0	1	100 (2/2)	0.0 (0/1)	0.0 (0/1)	0.0 (0/2)	50.0 (1/2)	0.0 (0/1)	0.0 (0/1)	50.0 (1/2)
cefepime		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
ceftazidime		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
ertapenem		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
meropenem		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
amikacin		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
gentamicin		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
ciprofloxacin		2	0	0	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)	100 (2/2)	NA (0/0)	0.0 (0/2)	0.0 (0/2)
Total	62	332	18	155	92.7 (467/504)	3.9 (6/154)	2.7 (9/332)	4.4 (22/504)	91.7 (463/505)	1.9 (3/155)	1.2 (4/332)	6.9 (35/505)

NA, not applicable; S, susceptible; I, intermediate; R, resistant; TZP, piperacillin/tazobactam.
[a]Values in parentheses are the number of results with same categorical interpretation as the reference BMD results/total number of test results. The Accelerate Pheno™ system did not provide AST results for the cefepime/*K. pneumoniae* combination ($n = 1$).

ciprofloxacin for *Acinetobacter baumannii* ($n = 8$), but only to ertapenem and ceftazidime for *Escherichia coli* ($n = 19$) and *Klebsiella pneumoniae* ($n = 18$), respectively. Except for trimethoprim/sulfamethoxazole [only tested with *Staphylococcus aureus* ($n = 10$)] and vancomycin [when tested with *Enterococcus faecalis* ($n = 5$)], all antibiotics showed 100% agreement in the two systems for the Gram-positive species studied. Among the Gram-negative organisms, the majority of errors (excluding mEs) were accounted for by a high number of MEs for piperacillin/tazobactam, ceftazidime and colistin when tested with the Accelerate Pheno™ system, and for piperacillin/tazobactam, amikacin and ceftazidime when tested with the VITEK® 2 system. Among the Gram-positive organisms, there were no MEs with either system, and only one VME for *E. faecalis* and vancomycin when tested with the VITEK® 2 system.

This study included 51 isolates resistant to one or more antimicrobials, for which 95.2% (158/166) of Accelerate Pheno™ system AST results and 92.9% (157/169) of VITEK® 2 system AST results were in categorical agreement with the reference method (Table 3). When the performance of each method was stratified by type of antimicrobial-resistant organism, categorical agreements of the Accelerate Pheno™ and VITEK® 2 systems were 100% (12/12) and 92.9% (13/14) for Gram-positive bacteria and 94.8% (146/154) and 92.9% (144/155) for Gram-negative bacteria, respectively.

Discussion

Annually, there are 575000–677000 episodes of BSI and 79000–94000 deaths estimated in North America and >1200000 episodes of BSI and 157000 deaths estimated in Europe.[1] In this alarming context, phenotypic AST using emerging technologies would yield rapid information on the susceptibility/resistance status of microbial pathogens directly from PBCs. Therefore, rapid AST would not only ensure the quick administration of the right antimicrobial agent to the patient, but would also avoid subjecting the patient to the expense and toxicity of inefficacious antimicrobial therapy.[28]

Here we report a head-to-head comparison of AST directly from PBCs for both the Accelerate Pheno™ and VITEK® 2 systems against the gold standard BMD method, which may not be practical in most clinical microbiology laboratories. Our study extends what has already been demonstrated on the reliability of performing direct inoculation of Gram-negative and Gram-positive organisms from PBCs to achieve rapid (identification and) AST. Importantly, we show that the Accelerate Pheno™ system performance was overall equivalent (or slightly superior) to that of conventional phenotypic AST methods, such as the VITEK® 2 system. In our study, the Accelerate Pheno™ system performed reliably with both Gram-negative and Gram-positive organisms, with 92.7% and 99.0% categorical agreement, respectively, and 2.2% MEs and 3.8% mEs. However, the rate of VMEs was 3.6%, and the six errors were only observed with Gram-negative organisms; these findings are consistent with other studies.[20–22,24] Most VMEs were detected with *Klebsiella* spp. (*K. pneumoniae*) against various antibiotics (piperacillin/tazobactam, ertapenem, meropenem and ciprofloxacin) (Table 3). Direct testing by the VITEK® 2 system yielded four VMEs, of which two were with colistin (one *K. pneumoniae* and one *A. baumannii*), one with gentamicin (*P. aeruginosa*) and one with vancomycin (*E. faecalis*), but for this latter antimicrobial agent/organism combination no result was available with the Accelerate Pheno™ system (Table 3).

In the light of the reported significant time-to-AST reduction realized with the Accelerate Pheno™ system,[21–23] it is possible to project the impact of using the Accelerate Pheno™ system on the effectiveness of antimicrobial therapy.[29] Of the five VMEs involving *K. pneumoniae* organisms tested with the Accelerate Pheno™ system, two errors (one meropenem and one ciprofloxacin) occurred

Table 2. Performance of the Accelerate Pheno™ system and VITEK® 2 systems compared with BMD for Gram-positive bacterial species ($n = 24$)

	BMD				Accelerate Pheno™ system [% (*n*/*N*)]				VITEK® 2 system [% (*n*/*N*)]			
						errors				errors		
Antimicrobial	*n*	S	I	R	categorical agreement[a]	VMEs	MEs	mEs	categorical agreement[a]	VMEs	MEs	mEs
S. aureus	10											
erythromycin		7	0	3	100 (10/10)	0.0 (0/3)	0.0 (0/7)	0.0 (0/10)	100 (10/10)	0.0 (0/3)	0.0 (0/7)	0.0 (0/10)
SXT		10	0	0	90.0 (9/10)	NA (0/0)	0.0 (0/10)	10.0 (1/10)	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)
daptomycin		10	0	0	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)
linezolid		10	0	0	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)
vancomycin		10	0	0	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)	100 (10/10)	NA (0/0)	0.0 (0/10)	0.0 (0/10)
CoNS	6											
erythromycin		1	0	5	100 (5/5)	0.0 (0/4)	0.0 (0/1)	0.0 (0/5)	100 (6/6)	0.0 (0/5)	0.0 (0/1)	0.0 (0/6)
daptomycin		6	0	0	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)
linezolid		6	0	0	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)
vancomycin		6	0	0	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)	100 (6/6)	NA (0/0)	0.0 (0/6)	0.0 (0/6)
E. faecalis	5											
ampicillin		5	0	0	100 (5/5)	NA (0/0)	0.0 (0/5)	0.0 (0/5)	100 (5/5)	NA (0/0)	0.0 (0/5)	0.0 (0/5)
linezolid		5	0	0	100 (5/5)	NA (0/0)	0.0 (0/5)	0.0 (0/5)	100 (5/5)	NA (0/0)	0.0 (0/5)	0.0 (0/5)
vancomycin		4	0	1	100 (4/4)	NA (0/0)	0.0 (0/4)	0.0 (0/4)	80.0 (4/5)	100 (1/1)	0.0 (0/4)	0.0 (0/5)
Enterococcus faecium	3											
ampicillin		0	0	3	100 (3/3)	0.0 (0/3)	NA (0/0)	0.0 (0/3)	100 (3/3)	0.0 (0/3)	NA (0/0)	0.0 (0/3)
linezolid		3	0	0	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)	100 (3/3)	NA (0/0)	0.0 (0/3)	0.0 (0/3)
vancomycin		1	0	2	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)	100 (3/3)	0.0 (0/2)	0.0 (0/1)	0.0 (0/3)
Total	24	84	0	14	99.0 (95/96)	0.0 (0/12)	0.0 (0/84)	1.0 (1/96)	99.0 (97/98)	7.1 (1/14)	0.0 (0/84)	0.0 (0/98)

NA, not applicable; S, susceptible; I, intermediate; R, resistant. SXT, trimethoprim/sulfamethoxazole.
[a]Values in parentheses are the numbers of results with same categorical interpretation as the reference BMD results over the total numbers of test results. The Accelerate Pheno™ system did not provide AST results for the following antibiotic-pathogen combinations: erythromycin/coagulase-negative *Staphylococcus* sp. ($n = 1$) and vancomycin/*E. faecalis* ($n = 1$).

in one BC sample and two errors (one ertapenem and one meropenem) in another BC sample. Both of these samples were positive for a carbapenemase-producing *K. pneumoniae*, as determined molecularly (Amplex eazyplex® SuperBug CRE; Amplex Diagnostics GmbH, Gars-Bahnhof, Germany). Carbapenems are often used for treatment escalation or combination therapy in cases of septic shock, because they are also effective against Gram-negative ESBL-producing pathogens.[30] Unfortunately, the negative effects of carbapenem false-susceptible phenotypic results cannot be counteracted by supplementing them with a molecular diagnostic test; Amplex eazyplex® is, for example, a PCR-based method for detecting $bla_{\text{CTX-M}}$, bla_{KPC}, $bla_{\text{OXA-48}}$, bla_{NDM} and bla_{VIM} resistance genes. Supplementing phenotypic results would require the Amplex eazyplex® test on all carbapenem-susceptible isolates; however, the PCR-based diagnostic approach is limited to a few frequently occurring β-lactamases and is not useful for 'problem pathogens' other than *E. coli* and *K. pneumoniae*. Failure to detect crucial carbapenemase-producing organisms could be circumvented through parallel phenotypic testing, which would not shorten the time to AST result. Alternatively, the use of both the Accelerate Pheno™ and the VITEK® 2 system may improve the overall AST result but increase laboratory testing costs. Carbapenem-resistant Enterobacteriaceae infections are difficult to treat and salvage therapeutic options are often limited to polymyxins (colistin and polymyxin B).[31] Of the seven multidrug-resistant *A. baumannii* organisms detected in our BC samples, two were also colistin resistant with both BMD and Accelerate Pheno™ system tests, but one of these organisms yielded a VME when tested with the VITEK® 2 system. Another BC sample yielding a VME for colistin with the VITEK® 2 system contained a colistin-resistant organism (*K. pneumoniae*) that was PCR positive for the bla_{KPC} resistance gene. In one recent study, colistin testing of Enterobacteriaceae isolates by the VITEK® 2 system had a VME rate of 36%,[32] which was well in excess of the $\leq$1.5% rate recommended by the CLSI.[33] This was reproduced in our study (2/4, 50%), whereas no VMEs against colistin occurred when the same BCs were tested with the Accelerate Pheno™ system.

The Accelerate Pheno™ system is currently FDA cleared for monomicrobial and polymicrobial BSIs. It is noteworthy that, following FDA clearance, the instructions for use require identification results to be interpreted in conjunction with Gram stain results. In this study, according to a previously implemented BSI diagnostic workflow,[25] we performed AST testing by the VITEK® 2 system on either direct PBCs or subcultured species isolates, depending on the Gram stain information about the type of BSI (i.e. single or multiple). To make our Accelerate Pheno™ system performance evaluation comparable with that of previous studies,[21,23] we undertook direct BC testing for AST with the Accelerate Pheno™

Table 3. Accelerate Pheno™ system and VITEK® 2 system AST results for isolates found to be resistant to at least one antimicrobial agent by the reference BMD method

	Accelerate Pheno™ system			VITEK® 2 system		
Species (*n*)	*n*[a]	categorical agreement, *n* (%)	comment	*n*	categorical agreement, *n* (%)	comment
E. coli (9)	23	21 (91.3)	2 mEs with cefepime	23	20 (86.9)	2 mEs with cefepime, 1 mE with ceftazidime.
K. pneumoniae (11)	63	58 (92.0)	1 VME with TZP, 1 VME with ertapenem, 2 VMEs with meropenem, 1 VME with ciprofloxacin.	64	60 (93.7)	1 VME with colistin, 1 mE with TZP, 2 mEs with cefepime.
P. aeruginosa (8)	36	35 (97.2)	1 VME with ciprofloxacin.	36	34 (94.4)	1 VME with gentamicin, 1 mE with meropenem.
A. baumannii (7)	21	21 (100)	no errors	21	20 (95.2)	1 VME with colistin
Enterobacter species (1)	2	2 (100)	no errors	2	2 (100)	no errors
Proteus mirabilis (2)	8	8 (100)	no errors	8	8 (100)	no errors
S. marcescens (1)	1	1 (100)	no errors	1	0 (0.0)	1 mE with TZP
S. aureus (3)	3	3 (100)	no errors	3	3 (100)	no errors
CoNS (5)	4	4 (100)	no errors	5	5 (100)	no errors
E. faecalis (1)	0	–	–	1	0 (0.0)	1 VME with vancomycin
Enterococcus faecium (3)	5	5 (100)	no errors	5	5 (100)	no errors
Total (51)	166	158 (95.2)	6 VMEs, 2 mEs	169	157 (92.9)	4 VMEs, 8 mEs

VME, very major error; mE, minor error; TZP, piperacillin/tazobactam.
[a]Not including three indeterminate AST results for one isolate of *K. pneumoniae*, one isolate of coagulase-negative *Staphylococcus* species and one isolate of *E. faecalis*.

system blinded to Gram stain results. Consequently, Accelerate Pheno™ system results for polymicrobial BCs (eight species organisms evaluable in total) were not included in the present analysis. Because the Accelerate Pheno™ system AST performance for polymicrobial BSIs may not be optimal,[22,23] a better use of the Accelerate Pheno™ system could be to integrate the system with standard microbiological procedures, such as the microscopic examination of clinical samples in a preliminary diagnostic workflow step.

Potential shortcomings of this study include: (i) the limited number of some organisms tested (e.g. only two *S. marcescens* isolates) and some resistance phenotypes, such as to vancomycin, linezolid and daptomycin; and (ii) the lack of complex resistance phenotypes, such as MDR MRSA [e.g. heterogeneous vancomycin-intermediate *S. aureus* (hVISA, VISA)]. In our study, there were five MRSA organisms correctly detected with the VITEK® 2 system by oxacillin testing, which is not included in the Accelerate Pheno™ system panel. However, the Accelerate Pheno™ system was able to identify the same MRSA organisms using the cefoxitin-induction assay.[19] Further, although Accelerate Pheno™ system results for all organism/antimicrobial combinations were evaluated for research purposes only, we did not use the 2016 EUCAST breakpoints, which are currently employed by the system to automatically interpret AST results. Hence, to mimic real-time experience with the Accelerate Pheno™ system, we manually interpreted MIC data using the 2017 EUCAST breakpoints. Consequently, additional AST testing would have been necessary in a number of cases, particularly for the Gram-positive organisms, for which comparison between the Accelerate Pheno™ and VITEK® 2 systems was possible for only five antimicrobials. Finally, this study does not address the possible implications of rapid AST, such as the effect on laboratory workflow (e.g. time savings, greater technologist autonomy, etc.), or factors outside the laboratory (e.g. time to effective therapy, patient care and outcome, etc.).[34] Future studies will explore these issues in detail.

In conclusion, the Accelerate Pheno™ system provides rapid and accurate AST results for the majority of organism/antimicrobial combinations, which involve the most common bacterial pathogens found routinely in PBCs. It also performs comparably, and in some cases is superior, to the conventional phenotypic VITEK® 2 system. Additional studies including a larger number of Gram-positive organisms, Gram-negative organisms and especially samples containing multiple organisms are necessary before considering the Accelerate Pheno™ system as the standard of care in patients with BSIs.

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Transparency declarations

None to declare.

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Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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