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Rapid antimicrobial susceptibility testing by VITEK®2 directly from blood cultures in patients with Gram-negative rod bacteremia

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ABSTRACT

Objective: Optimizing therapy for bacteremia is currently limited by the 1–2-day turnaround time required for antimicrobial susceptibility testing (AST). Here, we assess a rapid AST method with VITEK®2 (bioMérieux, France) directly from positive blood cultures.
Methods: Patient-derived positive blood cultures with Gram-negative rods identified as *Enterobacteriaceae* and *Pseudomonas aeruginosa* were prospectively tested, and other blood culture bottles were spiked with carbapenem-resistant *Enterobacteriaceae* (CRE). Positive cultures were subjected to red blood cell lysis and centrifugation, and setup on VITEK®2.
Results: A total of 109 patient blood cultures and 52 spiked blood cultures were tested. Overall, essential agreement was 97.7% [95% confidence interval (CI) 96.4–99.0], and categorical agreement was 96.8% (95% CI 95.0–98.6). Mean turnaround time from setup to susceptibility results for *Enterobacteriaceae* in the clinical cultures was 9.0 (±1.3) h.
Conclusions: Direct susceptibility testing of blood cultures by VITEK®2 for *Enterobacteriaceae* is an accurate, practical, and inexpensive diagnostic strategy for rapid automated AST.

1. Introduction

Bloodstream infections in North America are a major source of morbidity and mortality, reaching an estimated incidence of over 600,000 episodes and 80,000 deaths a year (Goto and Al-Hasan, 2013). Gram-negative rod bacteremia is a major area of concern due to the increasing antimicrobial resistance profile of these organisms, as well as the association between prolonged time to initiation of appropriate antimicrobial therapy and increased mortality in patients with sepsis (Ferrer et al., 2014). Prompt initiation of appropriate therapy and deescalation of antibiotic coverage from broad to narrow spectrum both depend on availability of antibiotic susceptibility test (AST) results. Despite major advances in the turnaround time of bacterial identification directly from blood cultures by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and multiplex nucleic amplification acid tests (NAATs), conventional phenotypic tests remain slow with a turnaround time of 1 to 2 days due to requirement for pure colonies. Although several NAAT assays can rapidly identify resistance-associated genes from positive blood cultures, testing in Gram-negative bacteria is mostly limited to carbapenemases, which is of limited utility given the low incidence of carbapenemase-producing carbapenem-resistant *Enterobacteriaceae* (CP-CRE) in many centers in North America and the inability to deescalate therapy based on carbapenemase results alone (CDC, 2013; Guh et al., 2015).

A number of novel automated AST systems for rapid testing of positive blood cultures have been recently developed (http://accelerate diagnostics.com/news/accelerate-diagnostics-receives-fda-marketing-authorization-accelerate-pheno-system-accelerate-phenotest-bc-kit; https://www.specificdx.com/fast-dx-; https://www.quantamatrix.com/qmac-drast; https://www.quantamatrix.com/qmac-drast; https://qlinea.com/products/astar). The Accelerate Pheno™ system (Accelerate Diagnostics, Tucson, AZ) was the first of such technologies to become commercially available in the U.S. (Charnot-Katsikas et al., 2018; Lutgring et al., 2018; Marschal et al., 2017; Pancholi et al., 2018; Pantel et al., 2018). Although rapid, the cartridges are expensive and generate large volumes of disposable waste. Furthermore, its throughput of one sample at a time requires larger laboratories to purchase multiple modules. As such, there is an important unmet need for inexpensive, simple, automated and comprehensive AST strategies.

The VITEK®2 (bioMérieux, Marcy-l'Étoile, France) is an automated AST system consisting of instruments, software, and disposable reagent cards. It received FDA approval in 1998–1999 for antimicrobial susceptibility testing of bacteria from pure colonies. Its predecessor, which was based on the same principle, was introduced in the 1970s. The VITEK®2 provides rapid minimal inhibitory concentration (MIC) results as well as

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categorical interpretation (S, I, or R) based on the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria (CLSI, 2018). Here, we investigate the accuracy of a novel inoculum preparation method based on red blood cell lysis and differential centrifugation of bacteria for rapid AST with VITEK®2 (bioMérieux, Marcy-l'Étoile, France) directly from positive blood cultures growing Gram-negative rods.

2. Materials and methods

2.1. Clinical cultures

From August 2017 to January 2018, consecutive adult and pediatric blood culture bottles positive for Gram-negative rods during the day shift were prospectively included in this study. Selection was based on the first blood culture positive per patient with visualization of Gram-negative rods on the Gram stain and later identified as *Enterobacteriaceae* and *Pseudomonas aeruginosa* by MALDI-TOF. Samples with mixed morphologies were excluded. All procedures were completed by licensed technologists working on the blood culture bench.

2.2. Spiked cultures

Frozen stocks of CRE (defined based on the 2015 CDC definition) (Chea et al., 2015) from a repository of well-characterized Stanford Health Care isolates (Senchyna et al., 2018) were subcultured twice on blood agar. Isolates were selected to ensure a broad distribution of organisms and resistance mechanisms. Fresh colonies were emulsified in sterile 0.45% sodium chloride solution to obtain 0.5 McFarland (McF) turbidity. Donor whole blood with citrate anticoagulant was purchased and used within the expiration date. A volume of 20 mL of blood was aseptically removed with a syringe and a needle and aliquoted into sterile conical tubes. Using a sterile pipette tip, 20 μL of the bacterial suspension was added to whole blood, resulting in approximately 10^5 cfu per mL of blood. Ten milliliters of spiked blood was then injected with a syringe into an aerobic and anaerobic blood culture bottle (BD BACTEC™ Plus Aerobic/F and Plus Anaerobic/F). The blood culture bottles were incubated in the BD BACTEC™ FX instrument until flagged positive.

2.3. AST inoculum preparation

2.3.1. Blood culture

After a blood culture bottle flagged positive, 1 mL of bottle content was transferred to a 1.5-mL microcentrifuge tube. Then, 200 μL of lysis buffer [10% (5g/50 mL) sodium dodecyl sulfate >99.5% purity (Invitrogen, Carlsbad, CA) in mass spectrometry (MS)–grade water (CHROMASOLV™ for LC-MS, Honeywell Riedel-de Haën, Seelze, Germany)] was added, and the solution was vortexed for 10 s at 3000 RPM (TALBOYS 120V pulsing vortex mixer, Thorofare, NJ) followed by centrifugation for 2 min at 15,871×*g*. The supernatant was discarded, and the pellet was suspended in 1 mL of wash buffer (MS grade water) by pipetting up and down. The bacteria were sedimented and suspended in 500 μL of sterile 0.45% sodium chloride solution. As needed, an additional washing step with 1 mL of wash buffer (MS grade water) and centrifugation for 2 min at 15,871×*g* was introduced for mucoid bacteria with adherent pellets. The bacterial suspension was added to 3 mL of sterile 0.45% sodium chloride to achieve 0.5 McF turbidity. The inoculum was then used to set up VITEK®2 AST-GN81 cards as per manufacturer procedures. Parallel setup for direct MALDI-TOF extraction was performed using the same initial steps as the direct AST procedure. Direct MALDI-TOF extraction in our laboratory performs with high accuracy for Gram-negative rods [98.1% (51/52) species-level identification; unpublished data].

2.3.2. Colony

Blood cultures were subcultured on blood agar. The next day, fresh colonies were suspended in 3 mL of sterile 0.45% sodium chloride solution to achieve 0.5 McF turbidity which was used to set up VITEK®2 AST-GN81 cards as per manufacturer procedures.

2.3.3. Precision

Isolates with very major errors (VMEs) were tested in quadruplicate using the direct VITEK®2 to assess assay precision. Precision was calculated as per Cumitech 31A recommendations (Clark et al., 2009) as the number of comparisons within one 2-fold dilution of VITEK®2 plate comparator method [precision essential agreement (EA)] or the number of categorical result matches [precision categorical agreement (CA)] over the total number of results multiplied by 100.

2.3.4. Data analysis

VITEK®2 results directly from the blood culture bottles (direct method) were compared to the AST method using colonies from blood agar subculture (comparator method). EA (MIC within one 2-fold dilution of the comparator method) and CA in the susceptible (S), intermediate (I), and resistant (R) categories were calculated using the CLSI M100 S28 as reference (CLSI, 2018). Minor error (MiE) is defined as an S/I, I/S, I/R, or R/I discrepancy. A major error (ME) is defined as an R (direct)/S (comparator) discrepancy, and a VME as an S (direct)/R (comparator) discrepancy. ME rates were calculated using the number of susceptible isolates as denominator, and VME rates using the number of resistant isolates as denominator as per Cumitech 31A recommendations (Clark et al., 2009). Isolates with MEs or VMEs were compared to the Kirby–Bauer disk diffusion susceptibility testing (Becton-Dickinson, Franklin Lakes, NJ) as the referee method or to MicroScan WalkAway *plus* System (Beckman Coulter, Brea, CA) antimicrobial susceptibility results only if disks were not available for the specific antibiotic.

2.4. Statistics

Statistical analysis was performed using Stata 15 (StataCorp, College Station, TX).

3. Results

3.1. Primary analysis

A total of 170 positive blood cultures were included in the study, of which 8 (4.7% of clinical cultures) were excluded due to polymicrobial growth on blood agar subculture at 24 h of incubation and 1 was excluded due to identification as *Clostridium ramosum*. The remaining 161 blood cultures were included in this study. The success rate for completion of the direct AST with VITEK®2 was 100% (161/161). Average procedural hands-on time was approximately 10 min per blood culture including VITEK®2 card setup. For 5 samples (4 *Klebsiella pneumoniae* and 1 *Escherichia coli*), an additional step of washing and centrifuging was required given mucoid consistency and difficulty suspending the bacterial pellet. In addition, 7 samples (3 *E. coli*, 2 *Morganella morganii*, 1 *Klebsiella oxytoca*, and 1 *Proteus mirabilis*) produced a small pellet and thus required preparation of more than 1 bacterial suspension (range 2–5) to achieve a bacterial inoculum for AST within the desired 0.5 McF range. Overall EA was 97.7% [95% confidence interval (CI) 96.4–99.0] and CA was 96.8% (95% CI 95.0–98.6) for the clinical and spiked blood cultures combined. The mean turnaround time (time from VITEK®2 card loading to results) for AST results was 9.0 (±1.3) h among the 99 *Enterobacteriaceae* and 11.8 (±1.0) h among the 10 *P. aeruginosa*. There was no evidence of significant lot-to-lot variability.

3.2. Secondary analysis

A secondary analysis of all 169 blood cultures, including the 8 with mixed organisms, showed high accuracy for the direct VITEK®2 method

with an overall EA of 97.7% (95% CI 96.4–99.0) and CA of 96.8% (95% CI 95.0–98.7).

3.3. Patient blood cultures

A total of 109 nonduplicate clinical blood cultures from 105 patients were collected, for which the majority of organisms were *E. coli* (n=44), *K. pneumoniae* (n=27), and *P. aeruginosa* (n=10), accounting for 74% of cultures tested (Fig. 1). There were 17 antibiotics tested per organism for *Enterobacteriaceae* and 10 antibiotics for *P. aeruginosa*. Some organism–drug combinations are not tested per VITEK®2 procedure given intrinsic resistance (e.g., *K. pneumoniae* and ampicillin) or per laboratory algorithm (e.g., cefepime only tested for *P. aeruginosa*). As such, the total number of tested organisms per antibiotic varied. Overall, EA was 98.3% (95% CI 97.1–99.6) and CA was 97.7% (95% CI 96.1–99.2) between the direct and comparator methods. The highest rates of minor errors for the main antibiotics were observed for cefoxitin (5.9%), piperacillin-tazobactam (4.9%), and amoxicillin-clavulanic acid (4.9%) (Table 1). No MEs were observed. On initial comparison between the direct and colony VITEK®2, there were 3 apparent VMEs in 1 *E. coli*, 1 *K. pneumoniae*, and 1 *Enterobacter cloacae*). However, the *E. coli* isolate was confirmed to be susceptible to piperacillin-tazobactam by disk diffusion testing, and the *K. pneumoniae* to be susceptible to tetracycline by MicroScan. As such, only 1 VME was confirmed in *E. cloacae* and ertapenem.

3.4. Spiked blood cultures

The subgroup of spiked blood cultures included a total of 52 blood culture bottles (26 aerobic and 26 anaerobic) from 26 CRE isolates.

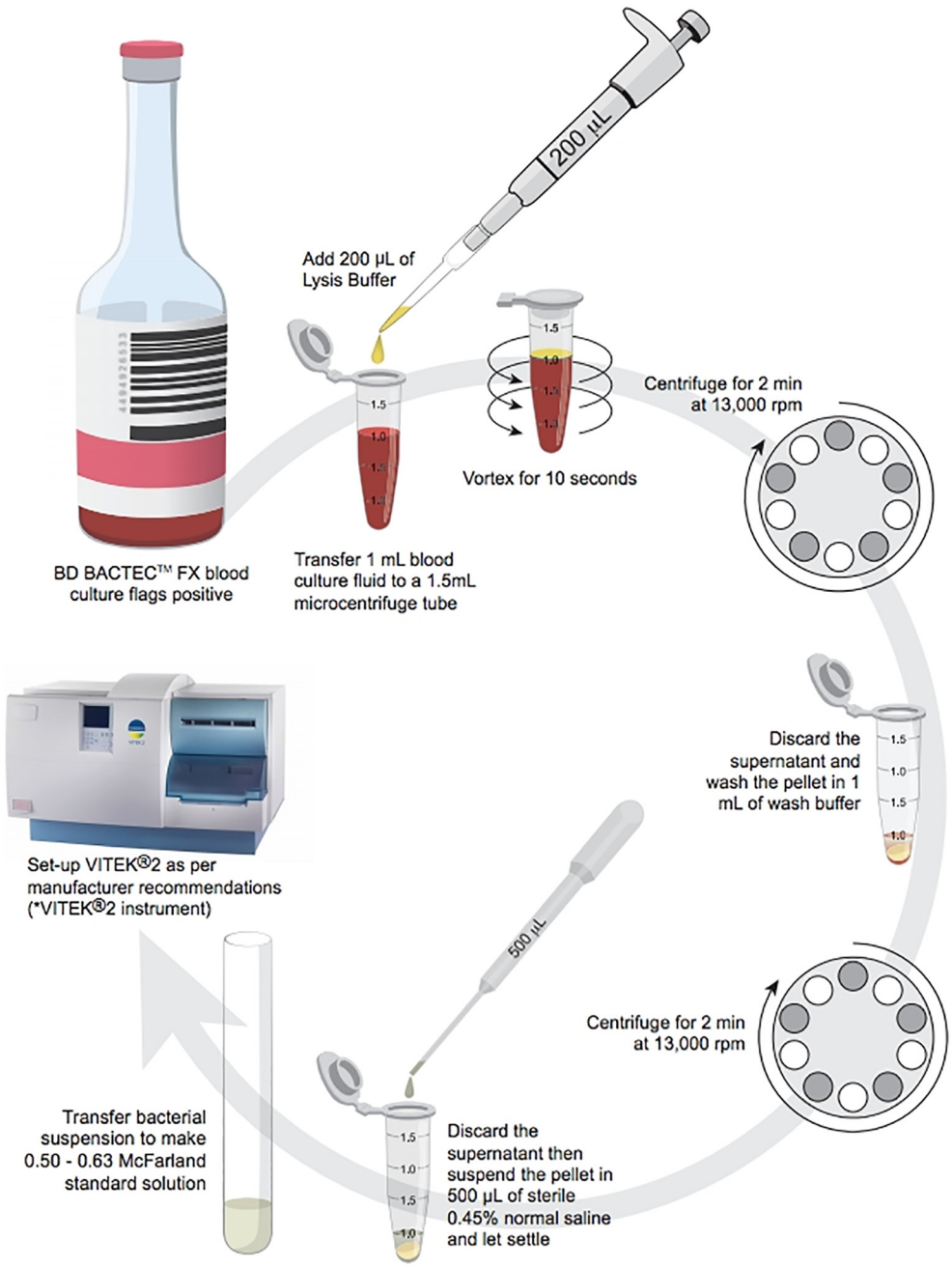

Fig. 1. Inoculum preparation from positive BD BACTEC™ FX blood culture bottles for VITEK®2 AST testing based on novel red blood cell lysis and bacterial centrifugation method.

Table 1
EA and CA for Gram-negative rods in clinical blood cultures (n=109).

	Number tested	S	I	R	EA (%)	CA (%)	MiE (%)	ME (%)	VME (%)
Ampicillin	87	18	0	69	100	100	0	0	0
Amoxicillin-clavulanic acid	102	69	7	26	100	95.1	4.9	0	0
Piperacillin-tazobactam	102	89	5	8	99.0	95.1	4.9	0	0
Cefazolin	109	69	0	40	95.4	100	0	0	0
Cefoxitin	101	72	10	19	99.0	94.1	5.9	0	0
Ceftazidime	108	102	0	6	98.1	99.1	0.9	0	0
Ceftriaxone	101	85	0	16	100	99.0	1.0	0	0
Cefepime	10	9	1	0	90.0	90.0	10	0	ND
Ertapenem	98	94	1	3	99.0	98.0	1.0	0	33.3 (1/3)[a]
Meropenem	109	107	0	2	97.2	100	0	0	0
Amikacin	109	109	0	0	100	100	0	0	ND
Gentamicin	109	103	0	6	100	100	0	0	0
Tobramycin	108	98	8	2	100	98.1	1.9	0	0
Ciprofloxacin	106	94	1	11	100	98.1	0.9	0	0
Levofloxacin	109	95	2	12	97.2	99.1	0.9	0	0
Tetracycline	102	65	1	36	97.1	99.0	0	0	0
TMP-SMX	102	67	0	35	100	100	0	0	0

ND = not determined. Some organism–drug combinations are not tested per VITEK®2 procedure given intrinsic resistance or per laboratory algorithm.
[a] *E. cloacae* complex.

The organisms consisted of *K. pneumoniae* (n=24), *E. coli* (n=10), *E. cloacae* complex (n=6), *Klebsiella aerogenes* (n=6), *Citrobacter freundii* (n=4), and *Serratia marcescens* (n=2) (Fig. 2). Of these, 12 were isolates with carbapenemase genes including OXA-48 [*K. pneumoniae* (n=4), *E. coli* (n=2)], New Delhi metallo-beta-lactamase 1 (NDM-1) [*K. pneumoniae* (n=2), *E. coli* (n=1)], IMP [*K. pneumoniae* (n=1), *E. cloacae* (n=1)], and *Klebsiella pneumoniae* carbapenemase (KPC) [*K. pneumoniae* (n=1)]. Overall, EA was 96.8% (95% CI 94.9–98.8) and CA was 95.3% (95% CI 92.1–98.6) between the direct and comparator methods. The highest rates of minor errors were observed with meropenem (11.5%) and piperacillin-tazobactam (14%) (Table 2). There were no MEs observed. Four VMEs were seen in *C. freundii* with piperacillin-tazobactam (n=2), *K. aerogenes* with ertapenem (n=1), and *K. pneumoniae* with meropenem (n=1), of which 1 isolate harbored a carbapenemase (OXA-48). These 4 isolates were confirmed to be resistant by disk diffusion testing. There was no significant difference overall in test performance in susceptibility results of isolates from the aerobic and anaerobic blood culture broth.

3.5. Precision study

The 4 isolates with VMEs were tested in quadruplicate with 17 antibiotics on the panel. Overall precision EA was 93.5% (95% CI 89.7–96.1%), and precision CA was 95.8% (95% CI 92.6–97.9%). Moreover, none of the 4 VME results were reproducible.

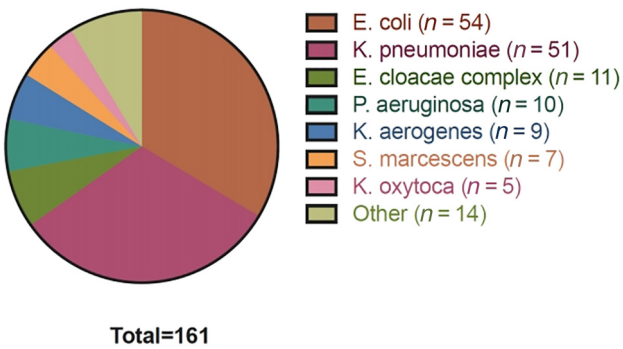

Fig. 2. Distribution of Gram-negative organisms tested in the study (n=161).

4. Discussion

The need for rapid antimicrobial susceptibility results for organisms causing invasive infections such as bloodstream infections has generated much attention recently (Doern, 2018). On-demand *mecA* PCR testing enables rapid identification of methicillin resistance for staphylococci; however, NAATs cannot address the complexity of resistance mechanisms to β-lactams such as cephalosporins in Gram-negative rods. Rapid NAAT-based resistance testing for carbapenemase genes is available; however, in most centers where prevalence of CP-CRE is low, this approach is also of limited utility. Rather, what is most needed from the clinical perspective is rapid identification of antibiotics to which the organism is susceptible, such that therapy can be deescalated, and patients can be switched to oral therapy and safely discharged when appropriate.

In this study, we showed VITEK®2 can be repurposed to provide highly accurate AST results directly from positive blood cultures with *Enterobacteriaceae* and *P. aeruginosa* with average time of 9.0 h for *Enterobacteriaceae*, which may be rapid enough to enable same-day reporting for those cultures that turn positive in early morning hours and allow clinical teams to promptly act on these results. This represents an innovative, pragmatic, and low-cost repurposing strategy of VITEK®2. Although a very simple idea, similar methods have only seldom been tested and published in the literature (Barnini et al., 2016; Machen et al., 2014; Maelegheer and Nulens, 2017; Mauri et al., 2017; Pan et al., 2018; Wattal and Oberoi, 2016) and never before reported by a U.S. laboratory. This approach benefits from the advantage of a more highly purified bacterial suspension from the lysis step compared to a sample with residual red blood cells, potentially leading to very high completion rate and more accurate results (Maelegheer and Nulens, 2017). Furthermore, our method is simpler and faster than previously published procedures (Barnini et al., 2016; Maelegheer and Nulens, 2017; Mauri et al., 2017; Pan et al., 2018; Wattal and Oberoi, 2016), including a recently published study (Pan et al., 2018), as it does not require a preincubation step, lengthy centrifugation or processing, or waiting for the organism identification before phenotypic AST setup. The turnaround time for inoculum preparation and VITEK®2 setup is approximately 10 min, which can be relatively easily integrated into the workflow of low-to-medium volume laboratories. This may be facilitated in settings such as ours, where direct blood culture identification is done by MALDI-TOF, given the initial steps of both procedures are the same, thus improving efficiency and optimizing workflow. However, laboratories that are using NAATs for blood culture identification may also perform inoculum preparation in parallel and benefit from rapid AST with VITEK®2. Importantly, VITEK®2 AST can be initiated without the organism identification; this information can be added once identification is successful.

Among several automated rapid AST systems recently developed, the Accelerate Pheno™ system (Accelerate Diagnostics, Tucson, AZ) was FDA-approved in February 2017 and has since been adopted by some clinical laboratories. The average turnaround time for Gram-negative organisms has been reported at approximately 7 h or less (Pancholi et al., 2018; Pantel et al., 2018), and the instrument provides a full sample-to-answer format for positive blood cultures. Test performance characteristics of the platform are robust, with overall categorical and EA values of ≥94% for Gram-negative organisms, including for more resistant isolates (Pancholi et al., 2018; Pantel et al., 2018). However, there are drawbacks to this technology, including the high expense of instrument acquisition, high single-use cartridge costs (>$200/cartridge), lack of certain Gram-negative rod organisms (80–90% culture identification rate) (Charnot-Katsikas et al., 2018; Lutgring et al., 2018; Marschal et al., 2017), low throughput (single cartridge per instrument), and generation of large volumes of disposable plastic waste. Although competing technologies address most of the shortcomings of the Accelerate Pheno™ system, none are currently commercially available in the U.S.

Table 2
EA and CA for Gram-negative rods in spiked blood cultures (n=52).

	Number tested	S	I	R	EA (%)	CA (%)	MiE % (n/N)	ME % (n/N)	VME % (n/N)
Ampicillin	34	0	0	34	100	100	0	ND	0
Amoxicillin-clavulanic acid	52	0	4	48	98.1	98.1	3.8	ND	0
Piperacillin-tazobactam	50	0	8	42	94.0	82.0	14	ND	4.8 (2/42)[a]
Cefazolin	52	0	0	52	100	100	0	ND	0
Cefoxitin	52	2	0	50	96.2	98.1	1.9	0	0
Ceftazidime	52	2	2	48	96.2	100	0	0	0
Ceftriaxone	52	0	0	52	94.2	100	0	ND	0
Ertapenem	52	12	2	38	96.2	96.2	1.9	0	2.6 (1/38)[b]
Meropenem	52	20	6	26	84.6	86.5	11.5	0	3.8 (1/26)[c]
Amikacin	52	44	2	6	98.1	100	0	0	0
Gentamicin	52	32	0	20	100	100	0 (0/52)	ND	0
Tobramycin	52	24	6	22	98.1	94.2	5.8	0	0
Ciprofloxacin	52	20	2	30	100	96.2	3.8	0	0
Levofloxacin	52	22	2	28	98.1	94.2	5.8	0	0
Tetracycline	52	28	0	24	94.2	94.2	5.8	ND	0
TMP-SMX	52	24	0	28	100	100	0	0	0

Some organism–drug combinations are not tested per VITEK®2 procedure given intrinsic resistance or per laboratory algorithm.
[a] *C. freundii.*
[b] *K. aerogenes.*
[c] *K. pneumoniae.*

The repurposing of VITEK®2 for direct AST on positive blood cultures addresses a number of concerns discussed above. First, the VITEK®2 has been in use for many years, and much has been learned about the capabilities of this system with various drug and bacterial combinations (Funke et al., 1998). Second, the VITEK®2 instrument is already widely used across clinical microbiology laboratories in the world such that the direct AST procedure on the VITEK®2 obviates the need to purchase new costly instruments that take up additional benchtop space. Furthermore, microbiology staff are familiar with the instrument workflow and with result interpretation. Third, the VITEK®2 card cost (approximately $10) is significantly lower, and the volume of disposable waste it generates is attractive given that biohazard waste must be decontaminated before disposal. Fourth, the VITEK®2 AST-GN81 card used in this study provides comprehensive testing with up to 18 antibiotics. However, antibiotics not included on this panel are cefpodoxime, ceftolozane-tazobactam, ceftazidime-avibactam, colistin, and tigecycline. An extension VITEK®2 card (e.g., including cefpodoxime, ceftolozane-tazobactam, ceftazidime-avibactam, and tigecycline) could be used to complement testing. A further limitation of the VITEK®2 cards is that the cefazolin susceptible MIC breakpoint is set at 4 μg/mL, which differs from the susceptible MIC breakpoint of 2 μg/mL recommended for therapy of infections other than uncomplicated urinary tract infections per CLSI (2018).

This study had certain limitations. Firstly, as with other direct methodologies, polymicrobial blood cultures may initially be overlooked through Gram stain identification alone; this occurred in 7% of clinical cultures in this study (5 mixed Gram-negative rods, 2 Gram-negative rods/Gram-positive cocci, and 1 Gram-negative rods/Gram-positive rods). As such, subculture of the blood culture and the AST inoculum is required to ensure pure culture. Nonetheless, errors were infrequent in the mixed samples, leading to limited potential patient harm in reporting these same-day results. Secondly, in 11% of blood cultures, additional processing time for washing and centrifuging or for obtaining sufficient inoculum was required. However, this may have contributed to successful completion of direct VITEK®2 AST testing in all blood cultures. Thirdly, VMEs were observed for piperacillin-tazobactam and carbapenems. The VITEK®2 has been previously recognized to show varying performance based on carbapenem resistance phenotype (Mazzariol et al., 2008). False-susceptible carbapenem results (VME) are an important concern and may result in patient harm. However, where very major carbapenem errors were observed, most of such isolates (4/6) had discrepant carbapenem results, which could serve as trigger for confirmatory testing before reporting of susceptibility results. Finally, the blood culture inoculum preparation method described here for VITEK®2 AST was only evaluated in BD BACTEC™ FX blood culture bottles. Further studies are needed to optimize and evaluate this approach for other blood culture systems.

The question of interest now is whether same-day susceptibility testing leads to improved patient outcomes. Data in support of a mortality benefit with molecular rapid diagnostic tests have largely been mediated by antimicrobial stewardship involvement (Perez et al., 2014; Timbrook et al., 2017), as has evidence of antibiotic deescalation or optimization (Banerjee et al., 2015; Perez et al., 2014; Sothoron et al., 2015). Indeed, in centers where broad-spectrum antimicrobial therapy for sepsis is routinely administered, the main advantage in Gram-negative rod bacteremia may be on a stewardship level, with reduced time to deescalation, reduced length of stay, and reduced antibiotic-related side effects including *C. difficile* infections. These questions will require dedicated study to better inform our quest for more rapid AST. In the meantime, the direct AST procedure described and evaluated in this study for VITEK®2 AST system may enable laboratories to reduce AST turnaround time in a manner that is simple, is cost-effective, and takes advantage of an instrument that is already widely familiar and available.

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Conflicts of interest

There are no conflicts of interest to disclose.

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