Diagnostic Microbiology and Infectious Disease 95 (2019) 114841

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Bacteriology

Direct antimicrobial susceptibility testing of positive blood cultures: a comparison of the Accelerate Pheno™ and VITEK® 2 systems

Jack G. Schneider [a,*], James B. Wood [a], Nathan W. Smith [b], Christopher L. Emery [c], Thomas E. Davis [c], John J. Manaloor [a], Brittany Bocian [d], Bryan H. Schmitt [c]

[a] Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis, Indiana, USA
[b] Accelerate Diagnostics, Inc., Tucson, AZ, USA
[c] Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA
[d] Indiana University Health Methodist and University Hospitals, Indianapolis, Indiana, USA

ARTICLE INFO

Article history:
Received 15 March 2019
Received in revised form 14 May 2019
Accepted 18 May 2019
Available online 29 May 2019

ABSTRACT

Objectives: To compare the performance and time-to-result (TTR) for antimicrobial susceptibility testing (AST) of positive blood cultures (PBC) using the Accelerate Pheno™ system (AXDX) and both a direct VITEK® 2 card inoculation workflow (DV2) and traditional FDA-approved VITEK® 2 workflow using subcultured isolates (V2). Methods: Patient samples with monomicrobial Gram-negative rod bacteremia were tested on AXDX and DV2 in tandem and compared to V2 AST results. Categorical agreement (CA) errors were adjudicated using broth microdilution. Instrumentation times and AST TTR were compared. Results: AXDX and DV2 had a CA of 93.4% and 97.4%, respectively, compared to V2. Postadjudication, AXDX, DV2, and V2 had CA of 94.7%, 95.7%, and 96.5%, respectively. Instrument run times were 6.6 h, 9.4 h, and 9.2 h, and AST TTR were 8.9 h, 12.9 h and 35.5 h, respectively. Conclusions: AXDX and DV2 ASTs are fast and reliable, which may have significant antimicrobial stewardship implications.

1. Introduction

Early recognition of sepsis and initiation of targeted antibiotic therapy is crucial in the treatment of Gram-negative rod (GNR) bacteremia, as mortality increases for each hour of delay in effective antibiotic therapy (Kang et al. 2005; Kumar et al. 2006). Additionally, the increasing incidence and complexity of GNR antimicrobial resistance mechanisms (Peleg and Hooper 2010; Meropol et al. 2018) have necessitated urgent improvement in fast antimicrobial susceptibility testing (AST).

Traditional laboratory methods for AST often take up to 48 h from detection of a pathogen in blood culture. This process generally involves utilizing commercial systems to incubate blood in liquid medium, Gram staining, then subculturing signal-positive samples onto solid media until visible colonies form (Chen et al. 2008; Romero-Gómez et al. 2012; Schneiderhan et al. 2013; Machen et al. 2014). Automated systems, such as the VITEK® 2 system, expedite the reporting of AST profiles, but the traditional U.S. Food and Drug Administration (FDA) cleared VITEK® 2 system workflow (V2) still requires isolation of bacterial colonies for testing (Funke et al. 1998). However, without isolation of bacteria, utilizing a direct card inoculation VITEK® 2 workflow (DV2) for AST testing (Ling et al. 2003; Romero-Gómez et al. 2012; Machen et al. 2014; Barnini et al. 2016; Jo et al. 2016; Bazzi et al. 2017) improves reporting times for microbe identification (ID) and AST results. Despite this potential advantage, the DV2 method is understudied, remains labor intensive, and is not cleared in the United States for in vitro diagnostic use by the FDA.

Additionally, in an effort to improve ID/AST reporting times, there have been an increasing number of novel, culture-independent fast technologies developed. The Accelerate Pheno™ system (AXDX) is one such technology that uses morphokinetic cellular analysis to provide fast AST results directly from positive blood cultures (PBC) (Accelerate Diagnostics, Inc., 2018). The performance and timing of AST results compared to the VITEK® 2 system, including V2 and DV2 workflows, have previously not been directly evaluated.

Thus, the aim of this study was to compare the performance of AXDX and the VITEK® 2 system, including both V2 and DV2 workflows, for AST results of clinical samples and isolates from patients with GNR bacteremia. Secondarily, we assessed the total instrumentation times for testing commonly isolated GNRs among all 3 methods, along with time to results (TTR) for AST.

2. Materials and methods

2.1. Study design and population

A prospective study comparing AST performance and TTR of AXDX (software version 1.3.1.15, Accelerate Diagnostics, Inc., Tucson, AZ)

* Corresponding author. Tel.: +1-317-274-7936; fax: +1-317-274-1587.
E-mail address: jgschnei@iu.edu (J.G. Schneider).

https://doi.org/10.1016/j.diagmicrobio.2019.05.013
0732-8893/

with both V2 and DV2 (VITEK® 2 XL system, BioMérieux, Marcy-l'Étoile, France) workflows for samples of GNR bacteremia was conducted at the Indiana University Health Pathology Laboratory (Indianapolis, IN). Patients seen at any of the Indiana University Health Hospitals, including Riley Hospital for Children, from September to December 2017 were eligible for inclusion if PBC indicated monomicrobial GNR bacteremia. Exclusion criteria for the performance evaluation were polymicrobial samples, AXDX or VITEK® 2 AST nonreport, monomicrobial Gram-positive or off-panel organisms, unconfirmed/mismatched ID results, replicate samples, or nonviable samples.

2.2. Sample testing

Patient blood samples were collected using either aerobic, anaerobic, or pediatric blood culture bottles and incubated in the BACTEC® FX blood culture monitoring system (Becton Dickinson, Franklin Lakes, NJ). All PBC demonstrating monomicrobial GNRs by Gram stain were tested on the AXDX system with the Accelerate PhenoTest™ BC kit and by DV2 for AST in parallel. Three AXDX testing modules were made available for use during this investigation such that a maximum of 3 positive blood cultures could be tested simultaneously. For DV2, bacterial cell suspensions were made by pelleting bacteria from broth in serum separator tubes and resuspending the pellets in saline. VITEK® 2 GN73 cards were inoculated with cell suspensions adjusted to a turbidity approximating a 0.5 McFarland standard. All AXDX and DV2 AST outcomes were compared with V2 results obtained from testing isolated colonies of bacteria per standard laboratory protocol (Fig. 1). All potential ESBL-producing isolates were initially flagged on the VITEK® 2 system and confirmed using the CLSI ESBL disk diffusion test from isolated colonies (Clinical and Laboratory Standards Institute 2016).

2.3. Performance comparison & adjudication testing

Identification was carried out using our available laboratory ID methods: MALDI Biotyper® system (Bruker Daltonics, Fremont, CA), VERIGENE® system (Luminex Corporation, Northbrook, IL), and AXDX. Monomicrobial GNR samples with concordant species identification between these identification modalities were included in AST performance analysis. However, ID performance analysis was outside the scope of this study. Samples reporting AST results for each of the 3 methods under investigation were included in performance analysis: AXDX, DV2, and V2. Using V2 as the initial comparator, calculations of essential agreement (EA), categorical agreement (CA), minor errors (MiEs), major errors (MEs), and very major errors (VMEs) for AXDX and DV2 were performed. CA was defined as results of susceptible, intermediate, or resistant that matched between compared methods based on 2016 CLSI minimum inhibitory concentration (MIC) breakpoints (Clinical and Laboratory Standards Institute 2016) for the identified species. EA was defined as agreement of MICs within ±1 doubling dilution between compared methods. A VME occurred when a sample was called resistant by the comparator and susceptible by the test method. An ME occurred when a sample was called susceptible by the comparator and resistant by the test method. An MiE occurred when a sample was intermediate by one method and susceptible or resistant by the other method. Samples that did not have the same categorical result (S, I, or R) across all 3 of our laboratory methods (AXDX, DV2, and V2) were adjudicated in a reference laboratory using broth microdilution (BMD) as a gold standard method for AST (Food and Drug Administration 2009; van Belkum and Dunn 2013). Frozen isolates were shipped to a reference laboratory for BMD testing once initial laboratory testing was completed, and technologists were blinded to all results. BMD discrepancy testing was performed in triplicate, with the modal MIC reported as the adjudicating result. These results were

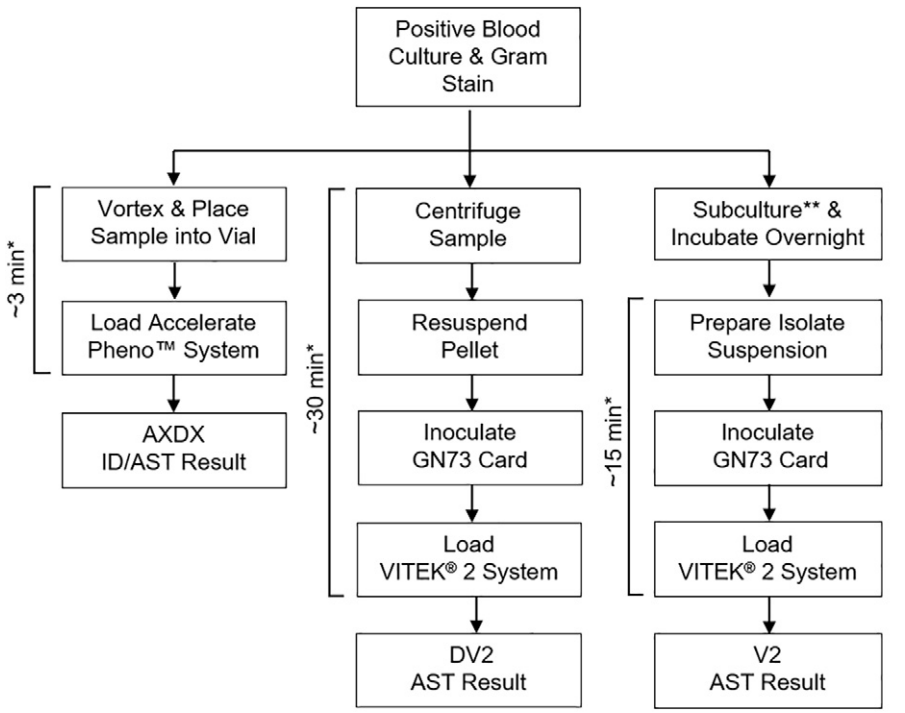


Fig. 1. Laboratory AST methodologies, including AXDX, DV2, and V2 workflows.

used to generate a hybrid adjudication set (V2/BMD), which was also compared to AXDX, DV2, and original V2 results.

2.4. Timing comparison

Total instrumentation times for commonly isolated GNRs among all 3 methods were compared, along with TTR for AST from time of blood culture positivity. Technologist hands-on time was included in TTR from time of blood culture positivity but not included in instrumentation run time calculations (Fig. 1). Additionally, adjustments for delays in results reporting from the laboratory to clinicians were not included in the timing calculations.

2.5. Statistical analysis

Statistical analyses were performed with SciPy (Jones et al. 2001) using the Python programming language (Oliphant 2007). Statistical analyses comparing independent continuous variables (e.g., instrument run time to AST between all 3 modalities) were performed using the 2-sided Mann–Whitney *U* test (Mann and Whitney 1947). Fisher's exact testing was performed when comparing discrete contingency tables (e.g., counts of errors in CA between modalities). Computed probability (P) of less than a significance level (α) of 0.05 was considered statistically significant. Where relevant, mean (μ) and standard deviation (σ) were noted.

3. Results

3.1. Microbiology results

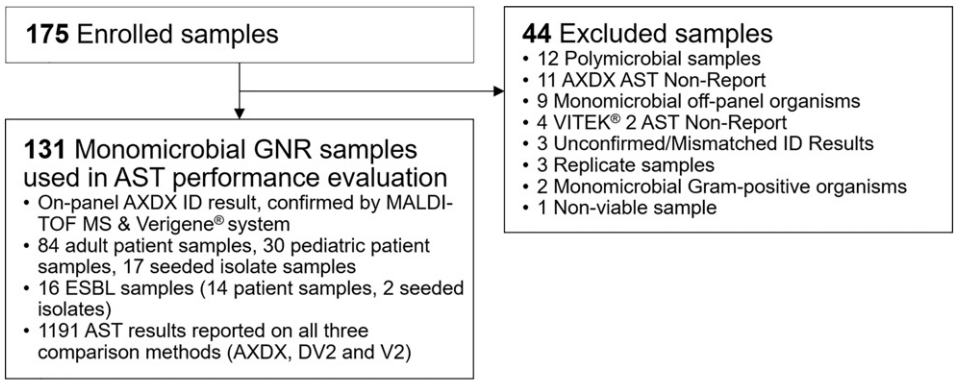
A total of 175 samples were collected. After excluding 44 samples (12 polymicrobial, 15 AST nonreports [11 AXDX, 4 V2], 9 off-panel organisms, 3 mismatched IDs, 3 replicate samples, 2 Gram-positive organisms, and 1 nonviable sample), a total of 131 samples including 17 seeded isolates (nonpatient samples), 30 pediatric (age ≤ 21) clinical samples, and 84 adult (age > 21) clinical samples from patients with monomicrobial GNR bacteremia were included in the AST performance evaluation (Fig. 2). *E. coli* and *Klebsiella* spp. were the most frequently isolated GNRs (63 and 29 isolates, respectively). Twelve percent (16/131) of the organisms were ESBL producers. Nine organisms not present on the AXDX ID panels made up 5.5% of total monomicrobial GNR cultures tested (prior to their exclusion in the analyzed dataset) and included 1 each of the following organisms: *Bacteroides fragilis*, *Salmonella* spp., *Shewanella* spp., *Dialister* spp., *Bacteroides thetaiotaomicron*, *Burkholderia gladioli*, *Pantoea calida*, *Achromobacter xylosoxidans*, and *Aeromonas* spp.

3.2. Comparison results

A total of 131 pathogens were isolated and produced AST results on all 3 modalities. A total of 1191 AST combinations were analyzed, with AXDX having an overall CA of 93.4% compared to V2. AXDX had 3 VMEs (1 ampicillin-sulbactam, 1 piperacillin-tazobactam, 1 cefepime) compared to V2 (Table S1). When comparing DV2 to V2, there was 97.4% categorical agreement, with 7 VMEs, including 3 each for cefepime and ceftazidime (Table S1). Of the 232 resistant combinations adjudicated by BMD, DV2 called 13 of these susceptible (5.6% VME rate; 7 cefepime, 5 ceftazidime, 1 aztreonam) and V2 called 6 susceptible (2.6% VME rate; 1 piperacillin-tazobactam, 2 cefepime, 2 ceftazidime, 1 aztreonam) compared to 2 on AXDX (0.9% VME rate; 2 piperacillin-tazobactam) (Table S2). For MEs, AXDX had 12 MEs compared to V2. AXDX MEs decreased to 4 when compared to BMD adjudicated results.

On further analysis, there were 63 errors (5.3%) on AXDX compared to BMD adjudicated results (Table S2). Of these, AXDX called 14 more susceptible than adjudicated (i.e., AXDX called intermediate when adjudication called resistant, or AXDX called susceptible when adjudication called resistant or intermediate), while the remaining 49 were called more resistant than adjudicated (Fig. 3c). AXDX was 3.5 times (49/14) more likely to have an error on the side of resistant if an actual error was determined. Conversely, V2 (using isolated colonies) had 42 errors (3.5%) compared to BMD adjudicated results. Of these, 29 were called more susceptible than adjudicated, while the remaining 13 were called more resistant. V2 was observed to be 2.2 times (29/13) more likely to have an error on the side of susceptible if an error was determined. Overall, AXDX was observed to have a 1.8% higher rate of errors compared to V2 (or 1.5 times the number of V2 errors). When in error, AXDX is more likely to overcall resistance compared to V2, which tended to overcall susceptibility ($P < 0.001$) from our dataset (Fig. 3).

When comparing AXDX with DV2, errors from AXDX remained the same as previously listed, while DV2 had a total of 51 errors (4.3% of all AST combinations) compared to BMD adjudicated results (Table S2). Of these, 46 were called more susceptible than adjudicated results, while the remaining 5 were called more resistant. DV2 was observed to be 9.2 times (46/5) more likely to have an error on the side of susceptible if an error was determined. Overall, AXDX was observed to have a 1.0% higher rate of errors compared to DV2 (or 1.3 times the number of DV2 errors). Therefore, however, AXDX is more likely to overcall resistance compared to DV2 which tended to overcall susceptibility ($P < 0.001$) (Fig. 3c). Finally, DV2 was observed to have a 0.8%



Abbreviations: AST=antimicrobial susceptibility testing; AXDX=Accelerate Pheno™ system; DV2=direct inoculation VITEK® 2; ESBL=extended spectrum beta-lactamase; ID=microbe identification; MALDI=matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; V2=traditional VITEK® 2.

Fig. 2. Flow diagram of study design and population inclusivity criteria.

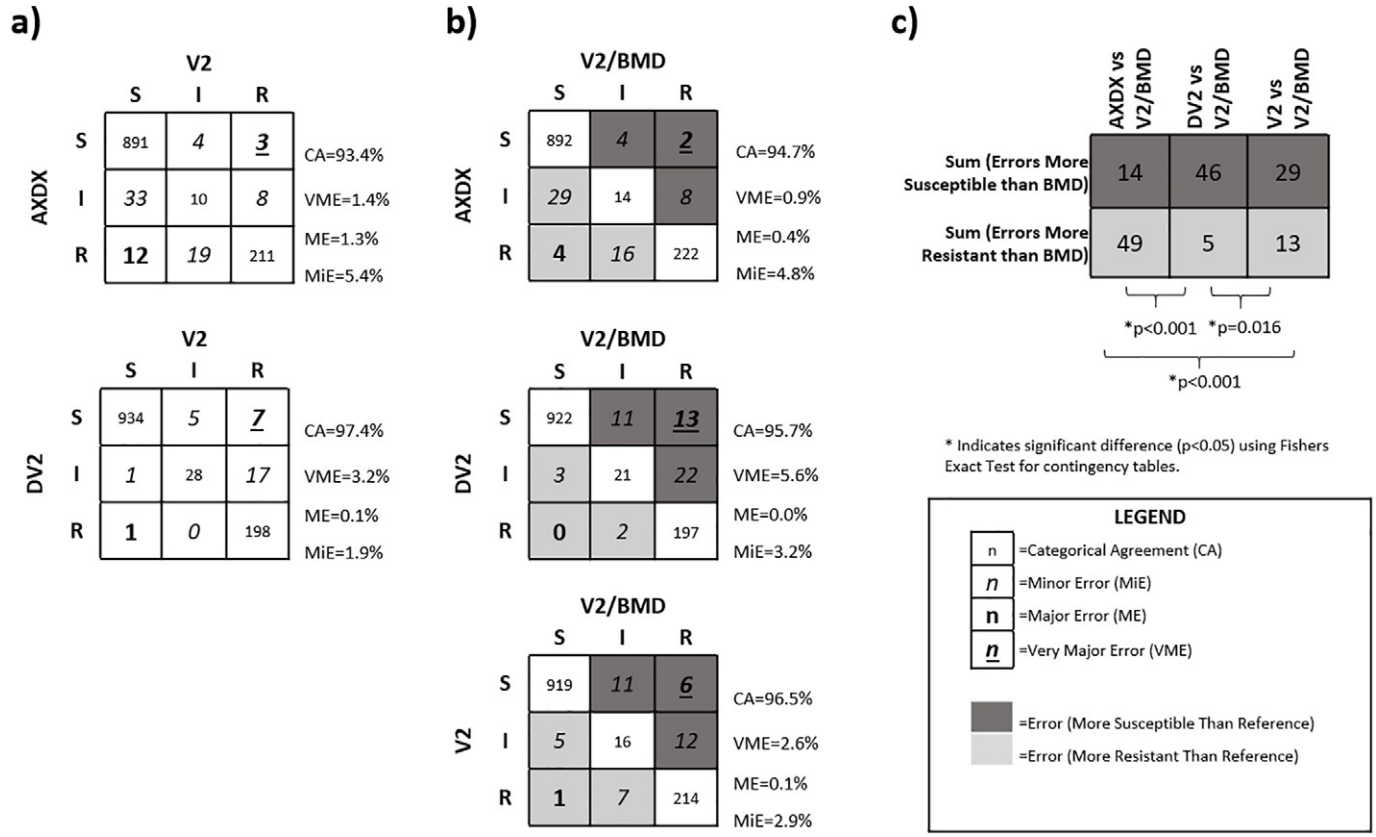


Fig. 3. Analysis of categorical agreement. (a) Comparison between initial laboratory method results for AXDX and DV2 against V2. (b) Comparison between laboratory results rescored against V2/BMD adjudicated results. (c) Comparison of errors tending more resistant or more susceptible than V2/BMD adjudicated results.

higher rate of errors compared to V2 (or 1.1 times the number of V2 errors). As stated before, DV2 is 9.2 times (46/5) and V2 is 2.2 times (29/13) more likely to have an error on the side of susceptible if an error is determined. While both methods tended to overcall susceptibility, there was a significant difference between the ratio of errors between these 2 modalities ($P = 0.016$) (Fig. 3).

3.3. ESBL-producing organisms

Twelve percent (16/131) of samples were found to be ESBL producers (2 from seeded isolate samples, 14 from fresh patient samples). For these isolates, AXDX had an overall CA of 89.9%, while DV2 had a CA of 90.6% compared to V2 (Fig. S2a). CA was 85.5%, 91.2%, and 92.5% for DV2, V2, and AXDX, respectively, against BMD adjudicated discrepancy results (Fig. S2b). Overall, there was still a tendency for DV2 to overcall susceptibility compared to AXDX ($P = 0.003$). The effect of V2 to overcall susceptibility against AXDX was less pronounced ($P = 0.09$) (Fig. S1c).

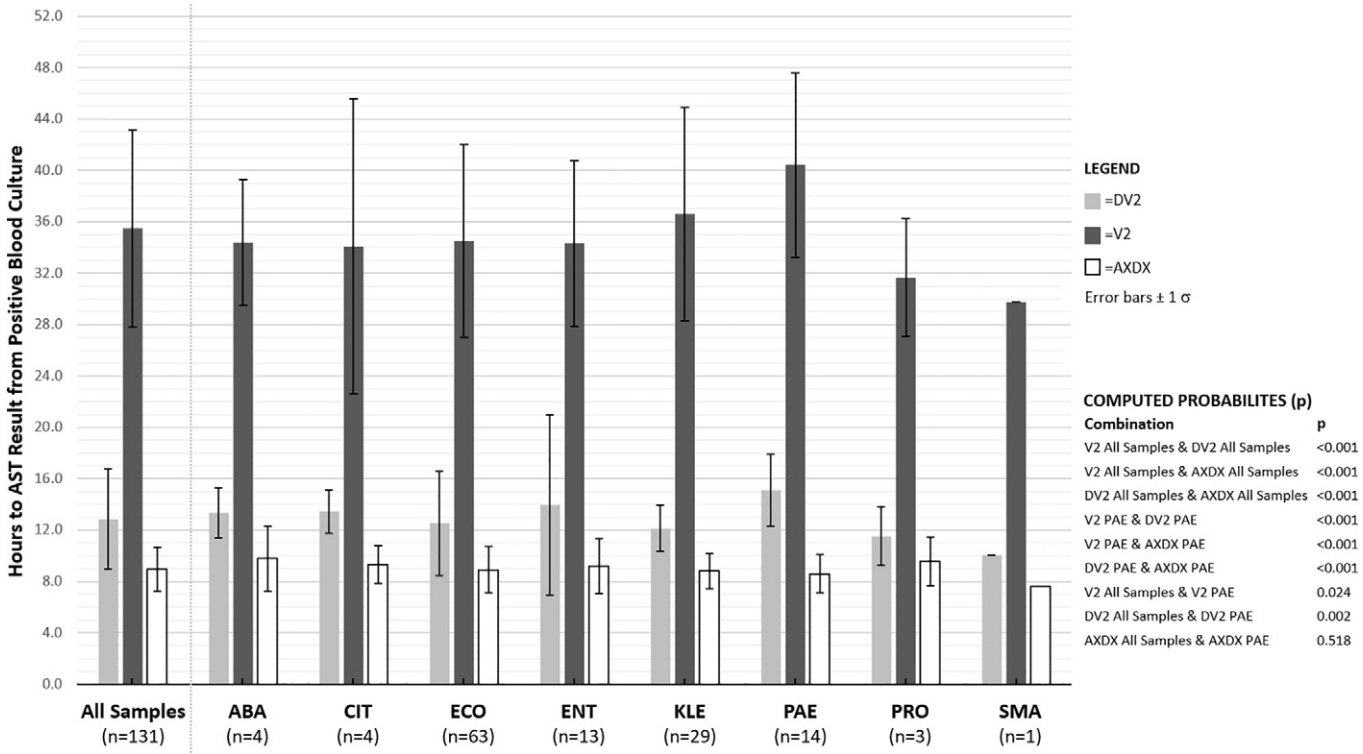
3.4. Timing/workflow results

Mean time to AST result from positive blood culture was 8.9 h, 12.9 h, and 35.5 h for AXDX, DV2, and V2, respectively (Fig. 4). Mean instrument run time for these methods for all samples, from time of setup to time of AST result, was 6.6 h, 9.4 h, and 9.2 h for AXDX, DV2, and V2, respectively (Fig. 5). For *Pseudomonas aeruginosa*, mean instrument run time for AXDX was 6.5 h, with longer run times for DV2 and V2, at 12.2 h and 12.0 h, respectively. Despite highly variable organism growth time from time of positivity as shown in the culture-dependent V2 workflow ($\sigma = 7.65$ h for all samples, Fig. S1), AXDX instrument run time for AST was also consistent ($\sigma = 0.05$ h) compared to DV2 ($\sigma = 1.5$ h) and V2 ($\sigma = 1.6$ h) (Fig. 5). Finally, DV2 and V2 workflows collectively required approximately 45 min of hands-on technologist preparation time compared to AXDX workflow which was approximately 3 min (Fig. 1).

4. Discussion

Relative to traditional modalities such as the V2 workflow, both AXDX and DV2 are fast and reliable for testing GNR's from positive blood cultures. When compared against reference BMD, AXDX tends to overcall resistance, while the VITEK® 2 system (both DV2 and V2 workflows) tends to overcall susceptibility. Additionally, AST instrument run time by the VITEK® 2 system (both DV2 and V2 workflows) is variable, especially with *Pseudomonas aeruginosa,* compared to AXDX, which has more consistent AST instrument run times.

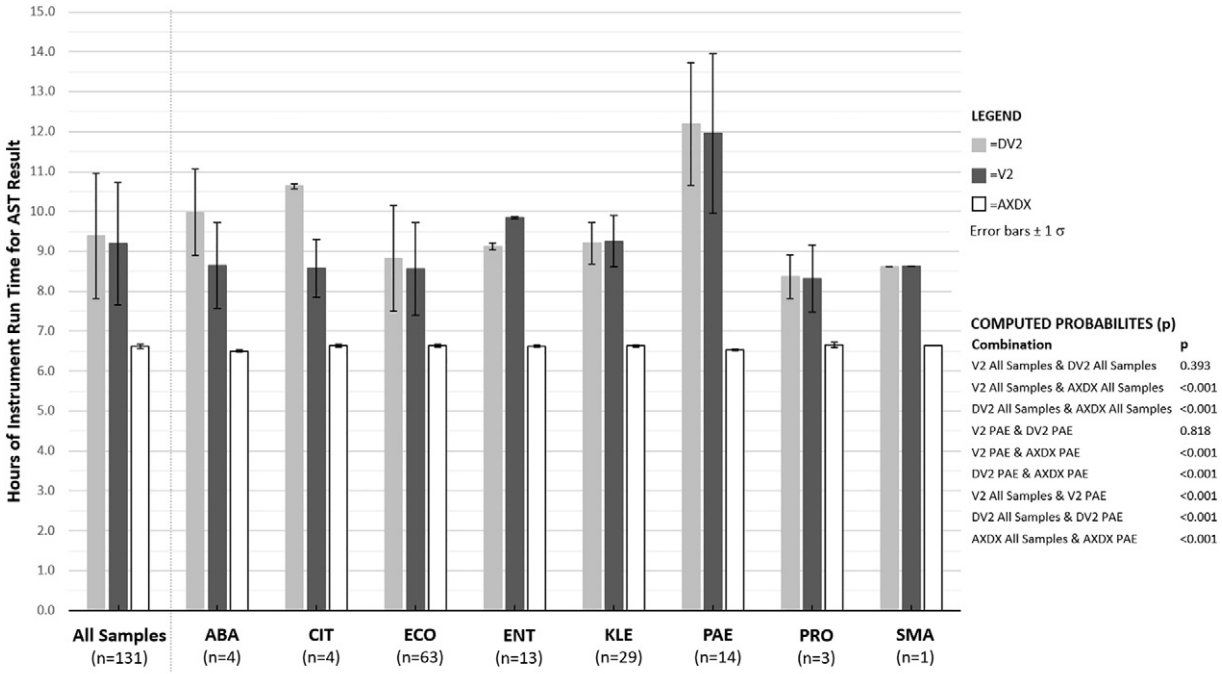
Overall, improper use of broad-spectrum antimicrobial therapy not only leads to an increase in antimicrobial resistant organisms (Kang et al. 2005; Battle et al. 2017; Tamma et al. 2017) but can also increase hospital length of stay, invite secondary infections, and result in additional costs to patients and health systems (Baggs et al. 2018). With the advent of faster phenotypic AST technologies, including the AXDX system, there is potential to significantly improve time to diagnosis and initiation of appropriate antibiotics, including de-escalation, which has been associated with improved clinical outcomes (Raman et al. 2013; Cremers et al. 2014). Similar to other studies (Brazelton de Cárdenas et al. 2017; Marschal et al. 2017; Charnot-Katsikas et al. 2018; Descours et al. 2018; Giordano et al. 2018; Lutgring et al. 2018; Pancholi et al. 2018; Pantel et al. 2018; Sofjan et al. 2018), we found that AXDX provided reliable results for AST, which is comparable to other standard testing such as molecular, proteomics-based, and conventional phenotypic methods (i.e., VITEK® 2 system). This is the first study, however, comparing AXDX AST results directly to the less studied and non–FDA-cleared direct inoculation VITEK® 2 system workflow (DV2), as well as to directly compare AXDX AST performance to both



Computed probability (p) using Mann-Whitney U Test only shown on pair-wise combinations of AST methods in the 'All Samples' category and in 'PAE' category, not computed on other combinations.

Abbreviations: ABA=*A. baumannii*; AST=antimicrobial susceptibility testing; CIT=*Citrobacter* spp.; ECO=*E. coli*; ENT=*Enterobacter* spp.; KLE=*Klebsiella* spp.; PAE=*P. aeruginosa*; PRO=*Proteus* spp.; SMA=*S. marcescens*; σ=standard deviation.

Fig. 4. Mean time to complete AST from blood culture positivity by method.



Computed probability (p) using Mann-Whitney U Test only shown on pair-wise combinations of AST methods in the 'All Samples' category and in 'PAE' category, not computed on other combinations.

Abbreviations: ABA=*A. baumannii*; AST=antimicrobial susceptibility testing; CIT=*Citrobacter* spp.; ECO=*E. coli*; ENT=*Enterobacter* spp.; KLE=*Klebsiella* spp.; PAE=*P. aeruginosa*; PRO=*Proteus* spp.; SMA=*S. marcescens*; σ=standard deviation.

Fig. 5. Mean instrument run time to complete AST by method.

DV2 and V2 workflows on the VITEK® 2 system against reference methods (i.e., BMD).

Although DV2 remains understudied and is not currently FDA-cleared, it has been utilized and validated for clinical testing in medical laboratories in the U.S. and Europe (De Angelis et al. 2019). Given the significant decrease in time to result for AST with AXDX and DV2, we felt it prudent to explore these 2 methods further. When comparing AXDX directly to DV2 for GNR bacteremia, AXDX was observed to have a higher incidence of errors (1.3 times the number of DV2 errors). Despite these errors, however, AXDX was more likely to overcall resistance compared to DV2 (including ESBL isolates), which tended to overcall susceptibility. These overall AST tendencies also applied to testing directly between V2 and AXDX, although not as pronounced. Based on our dataset, both DV2 and V2 workflows had a tendency to overcall susceptibility, specifically with cefepime and ceftazidime (7 and 5 VMEs, respectively for DV2; 2 VMEs each for V2), while AXDX tended to overcall susceptibility for piperacillin-tazobactam (2 VMEs) and notably had 3 MEs for ampicillin-sulbactam. As outlined in the results section, it is important to note that despite AXDX having 12 MEs and 21 MEs compared to V2 and DV2, respectively, these MEs decreased to 4 when compared to BMD adjudicated results. Furthermore, the DV2 workflow only had 7 VMEs when directly compared to V2 but nearly doubled that number postadjudication (13 VMEs). In light of these notable AST patterns, future studies comparing clinical outcomes between AXDX and V2 are needed and planned.

Interestingly, despite DV2 having the capability of decreasing average time to result compared to V2 (12.9 h to 35.5 h), *P. aeruginosa* isolates tended to have a noticeably longer time to result for both VITEK® 2 methods (DV2: 15.1 h; V2: 40.4 h), including instrument run times (DV2: 12.2 h; V2: 12.0 h), compared to 6.6 h for AXDX. The true clinical significance of these time differences is unknown and will need to be studied further. It is important to mention, however, instances in which alternative comparator methods for complete AST were recommended by AXDX on the 175 enrolled samples. These instances include polymicrobial specimens producing at most AST for 1 organism on AXDX (12 samples, 6.9%) and AST nonreportable results on AXDX ($n = 11$, 6.3%). Additionally, 9 samples were 'off-panel' organisms not identified by AXDX (5.1%) and DV2/V2 results terminated on another 4 enrolled samples (2.3%), requiring alternative AST comparator methods (Etests) to be performed in the laboratory. Consequently, like other fast diagnostics, AXDX and DV2 will not be able to replace traditional modalities for all AST combinations, particularly for off-panel or polymicrobial cultures (Bard and Lee 2018; Lutgring et al. 2018; Pancholi et al. 2018; Özenci and Rossolini 2019).

As with any novel diagnostic technology, cost-effectiveness and impact on clinical care must be considered. Though AXDX has shown itself to be a fast and reliable test for ID and AST, cost is a major limitation (Giordano et al. 2018). To that end, AXDX, as with any new, fast diagnostic technology, may prove of greatest benefit to institutions possessing laboratories with multiple shifts and established antimicrobial stewardship teams capable of implementing clinical changes in a timely manner. Additionally, AXDX currently has a lower testing capacity compared to VITEK® 2. Our study utilized 3 AXDX modules, allowing for simultaneous testing of up to 3 samples, without any delays in setup times during our study period, while the VITEK® 2 XL system used in this study could test up to 120 samples simultaneously.

From a laboratory workflow standpoint, AXDX did substantially reduce overall "hands-on" time for laboratory technologists, with the potential to decrease setup and processing time from nearly 45 min when combining DV2 and V2 workflows to approximately 3 min for AXDX. However, other techniques such as VITEK® 2 inoculation after short-term incubation on solid medium from positive blood cultures (SIV2), which was not tested in our study, also provide reliable AST with minimal preparation and cost expenditure (Idelevich et al. 2014; Ha et al. 2018). Interestingly, Idelevich et al. reported SIV2 time to AST result from positive culture of 11.2 h (Idelevich et al. 2014), fitting between AXDX and DV2 times (8.9 h and 12.9 h, respectively) in our study. SIV2 is another method that may be a valuable alternative to traditional methods. As novel, fast diagnostic technologies continue to evolve, further investigation into the impact on laboratory workflows, clinical decision making, and ultimately patient outcomes will be required across a variety of clinical settings to fully understand the utility of these tests.

This study has several limitations. First, our sample size and prevalence of resistance phenotypes are relatively low as it only includes GNR isolates sourced predominantly from our patient population. While this does limit the power and generalizability of the study, these isolates represent the clinically relevant pathogens in the diverse population at our institution. In addition to sourcing samples of Gram-positive bacteremia, future studies may seek a subset of resistant samples, sourced from isolate banks, to improve understanding of performance in more resistant populations. Furthermore, the increasing complexity of antimicrobial resistance (Kang et al. 2005; Peleg and Hooper 2010; Perez et al. 2014; Barlam et al. 2016; Bard and Lee 2018; Özenci and Rossolini 2019), along with the pressing, unmet needs in pathogen diagnostics (Blaschke et al. 2015) for GNRs, emphasizes the importance of these pathogens. Second, polymicrobial samples as indicated by Gram stain (~10% of all PBCs) were excluded due to limitations on AXDX producing AST for all pathogens in these samples and DV2 results being invalid (Marschal et al. 2017; Lutgring et al. 2018). Furthermore, the lack of polymicrobial infections being tested (in addition to excluding Gram-positive BSIs) may have increased the performance of AXDX, DV2, and V2 with respect to other studies. Third, our study site is a quaternary referral center with 24/7 testing capabilities, including a stewardship team with the capability of acting on results during all shifts of the day. Therefore, the potential clinical benefits of our findings are less generalizable to healthcare settings who do not have 24-h a day availability for testing. These findings will have the greatest impact in locales with similar practices and resources to our study site. Finally, the antibiotic panels used for comparison studies among all three modalities are in no way complete or specific for each organism or clinical condition. As a result, it is difficult to deduce whether trends to overcall susceptibility or resistance would translate to better or worse clinical outcomes.

In conclusion, our findings showed that ASTs by AXDX and DV2 from PBCs with monomicrobial GNRs are fast and reliable, which may have significant implications for patient outcomes and antimicrobial stewardship. GNR bacteremia treatment continues to be costly, especially for high-risk patients, and infection can sometimes prove fatal. Consequently, the continued research into how AST results from current technologies are utilized, and developing innovative new diagnostic modalities to acquire even faster AST TTR provide optimism for future success in combating further development of antimicrobial resistance. To evaluate the true clinical impact, studies directly comparing these 3 methods and how each one affects clinical decision making and patient outcomes are needed.

Acknowledgments

Special thanks to the following:

-Medical Technologist Mike Rayl and all other medical laboratory scientists at the Indiana University Health Pathology laboratory who assisted with set up and data acquisition.

-Vera Winn, Director of Clinical Microbiology and Molecular Pathology at Indiana University Health Pathology Laboratory, for technical support and clinical bench space.

-John Prichard and Christina Chantell from Accelerate Diagnostics, Inc., for technical support and manuscript preparation, respectively.

Funding

This study was supported by internal funding by the Department of Pathology and Laboratory Medicine through the Indiana University

School of Medicine. Accelerate Diagnostics, Inc., provided the Accelerate Pheno™ system modules and half of the kits used in the study. Accelerate Diagnostics, Inc., was not involved in study design, data collection, or data interpretation.

Transparency declarations

N.W.S has stock options and is an employee of Accelerate Diagnostics, Inc. N.W.S was involved in data management, figure design, and manuscript preparation. J.G.S has received research grant support from Accelerate Diagnostics, Inc. B.H.S. is a principal investigator in research studies for Accelerate Diagnostics, Inc., which do not include the assay compared in this study. All other authors: none to declare.

BMD adjudication studies were performed by the Discrepancy Laboratory at Accelerate Diagnostics, Inc. Discrepant results were identified by the Indiana University Health Clinical Microbiology Laboratory. Technologists performing confirmatory testing were blinded to all results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2019.05.013.

References

Baggs J, Jernigan JA, Halpin AL, Epstein L, Hatfield KM, McDonald LC. Risk of subsequent sepsis within 90 days after a hospital stay by type of antibiotic exposure. Clin Infect Dis 2018;66(7):1004–12.

Bard JD, Lee F. Why can't we just use PCR? The role of genotypic versus phenotypic testing for antimicrobial resistance testing. Clin Microbiol Newsl 2018;40(11):87–95.

Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ, et al. Implementing an antibiotic stewardship program: guidelines by the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America. Clin Infect Dis 2016;62(10):e51–77.

Barnini S, Brucculeri V, Morici P, Ghelardi E, Florio W, Lupetti A. A new rapid method for direct antimicrobial susceptibility testing of bacteria from positive blood cultures. BMC Microbiol 2016;16(1):185.

Battle SE, Bookstaver PB, Justo JA, Kohn J, Albrecht H, Al-Hasan MN. Association between inappropriate empirical antimicrobial therapy and hospital length of stay in gram-negative bloodstream infections: stratification by prognosis. J Antimicrob Chemother 2017;72(1):299–304.

Bazzi AM, Rabaan AA, Fawarah MM, Al-Tawfig JA. Direct identification and susceptibility testing of positive blood cultures using high speed cold centrifugation and Vitek II system. J Infect Public Health 2017;10(3):299–307.

Blaschke AJ, Hersh AL, Beekmann SE, Ince D, Polgreen PM, Hanson KE. Unmet diagnostic needs in infectious disease. Diagn Microbiol Infect Dis 2015;81(1):57–9.

Brazelton de Cárdenas JB, Su Y, Rodriguez A, Hewitt C, Tang L, Garner CD, et al. Evaluation of rapid phenotypic identification and antmicrobial susceptibility testing in a pediatric oncology center. Diagn Micr Infec Dis 2017;89(1):52–7.

Charnot-Katsikas A, Tesic V, Love N, Hill B, Bethel C, Boonlayangoor S, et al. Use of the Accelerate Pheno system for identification and antimicrobial susceptibility testing of pathogens in positive blood cultures and impact on time to results and workflow. J Clin Microbiol 2018;56(1):e01166-17.

Chen JR, Lee SY, Yang BH, Lu JJ. Rapid identification and susceptibility testing using the VITEK 2 system using culture fluids from positive BacT/ALERT blood cultures. J Microbiol Immunol Infect 2008;41(3):259–64.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement. M100-S26. Wayne. PA; 2016.

Cremers AJ, Sprong T, Schouten JA, Walraven G, Hermans PW, Meis JF, et al. Effect of antibiotic streamlining on patient outcome in pneumococcal bacteraemia. J Antimicrob Chemother 2014;69(8):2258–64.

De Angelis G, Posteraro B, Menchinelli G, Liotti FM, Spanu T, Sanguinetti M. Antimicrobial susceptibility testing of pathogens isolated from blood culture: a performance comparison of accelerate Pheno™ and VITEK® 2 systems with the broth microdilution method. J Antimicrob Chemother 2019;74(Suppl_1):i24–31.

Descours G, Desmurs L, Hoang TLT, Ibranosyan M, Baume M, Ranc AG, et al. Evaluation of the Accelerate Pheno™ system for rapid identification and antimicrobial susceptibility testing of gram-negative bacteria in bloodstream infections. Eur J Clin Microbiol Infect Dis 2018;37(8):1573–83.

Accelerate Diagnostics Inc.. Accelerate PhenoTest™ BC kit, instructions for use. Tucson. AZ; 2018https://www.online-ifu.com/ADX000005/4311/EN-US.

Food and Drug Administration. Guidance for industry and FDA class II Special controls guidance document: antimicrobial susceptibility test (AST) systems. Rockville, MD: Food and Drug Administration; 2009.

Funke G, Monnet D, deBernardis C, von Graevenitz A, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol 1998;36(7):1948–52.

Giordano C, Piccoli E, Brucculeri V, Barnini S. A prospective evaluation of two rapid phenotypical antimicrobial susceptibility technologies for the diagnostic stewardship of sepsis. BioMed Res Int 2018;2018:Article ID 6976923.

Ha J, Hong SK, Han GH, Kim M, Yong D, Lee K. Same-day identification and antimicrobial susceptibility testing of bacteria in positive blood culture broths using short-term incubation on solid medium with the MicroFlex LT, Vitek-MS, and Vitek2 systems. Ann Lab Med 2018;38(3):235–41.

Idelevich EA, Schüle I, Grünastel B, Wüllenweber J, Peters G, Becker K. Acceleration of antimicrobial susceptibility testing of positive blood cultures by inoculation of Vitek 2 cards with briefly incubated solid medium cultures. J Clin Microbiol 2014;52(11):4058–62.

Jo SJ, Park KG, Han K, Park DJ, Park YJ. Direct identification and antimicrobial susceptibility testing of bacteria from positive blood culture bottles by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and the VITEK 2 system. Ann Lab Med 2016;36(2):117–23.

Jones E, Oliphant E, Peterson P, et al. SciPy: open source scientific tools for Python. http://www.scipy.org/, 2001 (accessed 13 May 2019).

Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother 2005;49:760–6.

Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589–96.

Ling TK, Liu ZK, Cheng AF. Evaluation of the VITEK 2 system for rapid direct identification and susceptibility testing of gram-negative bacilli from positive blood cultures. J Clin Microbiol 2003;41(10):4705–7.

Lutgring JD, Bittencourt C, McElvania TeKippe E, Cavuoti D, Hollaway R, Burd EM. Evaluation of the Accelerate Pheno system: results from two academic medical centers. J Clin Microbiol 2018;56(4):e01672-17.

Machen A, Drake T, Wang YF. Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. PloS One 2014;9(2), e87870.

Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat 1947;18(1):50–60.

Marschal M, Bachmaier J, Autenrieth I, Oberhettinger P, Eillmann M, Peter S. Evaluation of the Accelerate Pheno system for fast identification and antimicrobial susceptibility testing from positive blood cultures in bloodstream infections caused by gram-negative pathogens. J Clin Microbiol 2017;55(7):2116–26.

Meropol SB, Haupt AA, Debanne SM. Incidence and outcomes of infections caused by multidrug-resistant Enterobacteriaceae in children, 2007–2015. J Pediatric Infect Dis Soc 2018;7(1):36–45.

Oliphant TE. Python for scientific computing. Comput Sci Eng 2007;9(3):10–20.

Özenci V, Rossolini GM. Rapid microbial identification and antimicrobial susceptibility testing to drive better patient care: an evolving scenario. J Antimicrob Chemother 2019;74(Suppl_1):i2–5.

Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, et al. Multicenter evaluation of the Accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. J Clin Microbiol 2018;56(4):e01329-17.

Pantel A, Monier J, Lavigne JP. Performance of the Accelerate Pheno™ system for identification and antimicrobial susceptibility testing of a panel of multidrug-resistant gram-negative bacilli directly from positive blood cultures. J Antimicrob Chemother 2018;73(6):1546–52.

Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. N Engl J Med 2010;362:1804–13.

Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, et al. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant gram-negative bacteremia. J Infect 2014;69(3):216–25.

Raman K, Nailor MD, Nicolau DP, Aslanzadeh J, Nadeau M, Kuti JL. Early antibiotic discontinuation in patients with clinically suspected ventilator-associated pneumonia and negative quantitative bronchoscopy cultures. Crit Care Med 2013;41(7):1656–63.

Romero-Gómez MP, Gómez-Gil R, Paño-Pardo JR, Mingorance J. Identification and susceptibility testing of microorganism by direct inoculation from positive blood culture bottles by combining MALDI-TOF and VITEK-2 Compact is rapid and effective. J Infect 2012;65(6):513–20.

Schneiderhan W, Grundt A, Wörner S, Findeisen P, Neumaier M. Workflow analysis of around-the-clock processing of blood culture samples and integrated MALDI-TOF mass spectrometry analysis for the diagnosis of bloodstream infections. Clin Chem 2013;59(11):1649–56.

Sofjan AK, Casey BO, Xu BA, Amadio J, Restrepo A, Alam MJ, et al. Accelerate PhenoTest™ BC kit versus conventional methods for identification and antimicrobial susceptibility testing of gram-positive bloodstream isolates: potential implications for antimicrobial stewardship. Ann Pharmacother 2018;52(8):754–62.

Tamma PD, Avdic E, Li DX, Dzintars K, Cosgrove SE. Association of adverse events with antibiotic use in hospitalized patients. JAMA Intern Med 2017;177(9):1308–15.

van Belkum A, Dunn WM. Next-generation antimicrobial susceptibility testing. J Clin Microbiol 2013;51(7):2018–24.