Comparison of Accelerate PhenoTest™ BC Kit vs. MALDI-TOF MS/VITEK®2 System for the Rapid Identification and Antimicrobial Susceptibility Testing of Gram-negative Bacilli Causing Bloodstream Infections

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DECLARATION OF INTEREST

The authors have nothing to disclose.

ETHICS

This study received ethical approval from the Conjoint Health Regional Ethics Board of the University of Calgary (#REB17-1705).

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

CONFLICTS OF INTEREST AND FUNDING

The use of the Accelerate Pheno™ system and its kits were provided by Accelerate Diagnostics, Inc. The authors acted independently with respect to study design, data collection, data analysis and the preparation and contents of this publication.

AUTHORS' CONTRIBUTIONS

WS helped design the study, collect and analyze the data and prepared the manuscript. LC helped design the study and collect the data. JC helped design the study and was a major contributor in writing the manuscript. JP helped design the study, provided the multi-drug resistant isolates, and was a major contributor in writing the manuscript. DC helped design the study and was a major contributor in writing the manuscript. DG helped design the study, was a

major contributor in writing the manuscript, and provided guidance to WS in all aspects of the study.

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ABSTRACT

Introduction: Our laboratory uses MALDI-TOF MS (MALDI) and the VITEK® 2 system (DV2) directly from positive blood cultures (BC) for organism identification (ID) and antimicrobial susceptibility testing (AST). Our objective was to compare direct MALDI/DV2 to a commercial BC ID/AST platform, the Accelerate Pheno™ system, in the ID/AST of clinical and seeded BC positive for Gram-negative bacilli (GNB).

Methods: BC positive for GNB were collected over a three month period and tested using AXDX, direct MALDI/DV2, and compared to conventional methods. A subset of sterile BC were seeded with multi-drug resistant GNB. Discrepancies in very major errors (VME) and major errors (ME) were confirmed with broth microdilution.

Results: A total of 29 clinical samples and 35 seeded samples were analyzed. Direct MALDI had a higher ID failure rate (31.0%) compared to AXDX (3.4%) [$p<0.001$]. Time to ID/AST was 1.5/6.9 hours, 5.8/16.5 hours and 21.6/33.0 hours for AXDX, direct MALDI/DV2 and conventional methods, respectively ($p<0.001$). For clinical samples, AXDX and DV2 had essential agreement (EA)/categorical agreement (CA) >96%. For seeded samples, AXDX had EA, CA, VME, ME and mE of 93.2%, 89.0%, 2.2%, 0%, and 9.2%, respectively. AXDX had a large number of non-reports (6.1%), stemming from meropenem testing. DV2 had EA, CA, VME, ME and mE of 97.5%, 94.7%, 1.3%, 0%, and 4.4%, respectively.

Conclusions: AXDX had fewer ID failures and more rapid ID/AST results. Direct MALDI/DV2 and AXDX both had high agreement for clinical samples but direct MALDI/DV2 had higher agreement when challenged with MDR GNB.

INTRODUCTION

Time to antimicrobial identification (ID) and antibiotic susceptibility testing (AST) is a critical factor for patients with bloodstream infections (BSI) due to Gram-negative enteric bacilli, especially in this era of ever-increasing antimicrobial resistance. Conventional microbiologic evaluation using phenotypic methods takes approximately 36-48 hours from the time a blood culture flags positive to the reporting of final antimicrobial ID and AST results.

Different methods and commercial platforms are available to help reduce the turnaround time in ID and AST for positive blood cultures. For instance, using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS, abbreviated henceforth as MALDI) and the VITEK[®] 2 system (VITEK[®] 2) directly from positive blood culture isolates has decreased the organism ID and AST turnaround time in our laboratory to 18-24h (i.e. a full 1 – 1.5 days faster than the conventional pipeline). The Accelerate PhenoTest[™] BC kit (AXDX) (Accelerate Diagnostics Inc., Tucson, AZ, United States), on the other hand, has been shown to reduce the turnaround time for final organism ID/AST from positive blood cultures to approximately 7 hours.¹

AXDX has a reported sensitivity of 97.4% and specificity 99.3% for pathogen identification, and 96.3% essential and categorical agreement for AST compared to broth microdilution.² Several studies also found that the Accelerate PhenoTest[™] BC kit was more rapid and more accurate for ID/AST of Gram-negative bloodstream infections than MALDI/automated susceptibility testing from short-incubation cultures.^{3,4} In addition, AXDX can be run with minimum sample

preparation, hands on assay time or training, making it feasible to run on nighttime shifts in our high-volume operations when there may be only one highly skilled technologist present. The use of direct MALDI and VITEK[®] 2 analyses of positive blood cultures to report a final ID and AST, respectively, has been shown to be non-inferior to conventional methods.^{5,6} This approach has also been documented to reduce mortality, decrease hospital length of stay, and decrease the time to prescription of optimal antibiotic therapy.⁷⁻¹⁰ Our laboratory recently implemented direct MALDI-TOF MS and VITEK[®] 2 using a lysing procedure with ammonium chloride for all positive blood cultures. For Gram-negative bacilli, we demonstrated sensitivity (86%) and specificity (100%) for pathogen ID using direct MALDI and an essential agreement (EA) (96.4%) and categorical agreement (CA) (99.0%) for direct VITEK[®] 2 as compared to our previous conventional protocol.¹¹

This study compares the performance of the AXDX platform with direct MALDI/VITEK[®] 2 for the reporting of a final ID and AST on clinical and seeded blood culture samples positive for Gram-negative enteric bacilli. The potential impact of more rapidly reporting final ID/AST using the AXDX system was estimated for patients with BSI due to Gram-negative enteric organisms.

METHODS

Calgary Laboratory Services (CLS) provides centralized laboratory services for the city of Calgary and surrounding areas, for a catchment population of over 1.6 million individuals. Positive blood cultures identified as Gram-negative bacilli based on Gram stain was included if reported from 0800 - 1600 Monday to Friday in our laboratory. Pellets from positive blood culture bottles, incubated using the BACT/ALERT[®] VIRTUO[®] system (bioMérieux, Laval,

QC, Canada), were prepared using the protocol outlined in Prod'hom et al.,¹² with several modifications as outlined: i) 1.5mL of positive blood culture medium was transferred into a microcentrifuge tube and the medium sat for at least 30 seconds before 1.25mL of medium was transferred into a 15mL tube containing 11.25mL of sterile water. The solution was then centrifuged at 1000 x g for 10 minutes followed by removal of the supernatant followed by the addition of 1 mL ammonium chloride. The pellet was subsequently re-suspended using a pipette, transferred into a microcentrifuge tube, and centrifuged at 200 x g for 10min. The supernatant was again removed and the pellet re-suspended by adding 1 mL of sterile water and then vortexed. The solution was again centrifuged at 200 x g for 10min, the supernatant discarded, the pellet re-suspended in 200 uL of sterile water, and then vortexed. For direct MALDI analyses, 1 µL of the final solution was spotted onto the slide. 1 µL of 70% formic acid was then added to the slide, followed by 1 µL of matrix after drying. Proteomics analyses using MALDI was then performed using standard procedures as outlined by the manufacturer. For direct susceptibility testing, a 0.5 McFarland standard was created from the final pellet solution and antimicrobial susceptibility testing using VITEK® 2 was then performed using standard procedures outlined by the manufacturer.

Medium from each positive blood culture was also simultaneously inoculated onto sheep's blood agar, chocolate agar and MacConkey agar as required to perform conventional ID methods using MALDI-TOF MS and VITEK 2 on the recovered isolate using standard methods according to the manufacturer's instructions. The Accelerate Pheno™ system (software version 1.3.1.15) using Accelerate PhenoTest™ BC kit was also inoculated and run according to the manufacturer's instructions within 8 h from the time the blood culture flagged positive. Serial

blood cultures from the same patient were excluded. Stored isolates were also recovered from the CLS biorepository of multi-drug resistant Gram-negative enteric organisms and seeded into sterile blood culture bottles, and processed as outlined above. A total of 35 seeded samples of carbapenemase-resistant *E. coli*, *K. pneumoniae*, *K. oxytoca* or *E. cloacae* were included in the study. Types of carbapenemase resistance included 5 NDMs, 4 VIMs, 4 IMPs, 9 OXAs, 6 KPC, 1 GES, 1 OXA/NDM and 5 non-carbapenemase isolates with impermeability to carbapenems due to porin mutations. The enzymes were previously characterized with whole genome sequencing as part of several ongoing global molecular epidemiology studies using procedures described before.¹³

Time to ID and AST for direct and conventional MALDI/VITEK[®] 2 was determined by comparing the time from Gram stain result to time of reporting of the final ID and AST results. Time to ID and AST for AXDX was based on the system run time but doesn't include other parameters such as time to instrument loading. Overall ID agreement was calculated by comparing ID result to our reference standard (conventional MALDI).

AST results were interpreted as per the 2018 Clinical and Laboratory Standards Institute (CLSI) guidelines.¹⁴ Essential agreement, categorical agreement, very major error, major error and minor error were calculated by comparing AST results to our reference standard (conventional VITEK[®] 2 system). Amoxicillin/clavulanate, ampicillin, cefoxitin and trimethoprim/sulfamethoxazole (TMP-SMX) were not compared, as the antibiotics were not available on the tested AXDX panel currently approved by the United States Food and Drug Administration. The VITEK[®] 2 system is unable to differentiate cefazolin intermediate from

susceptible due to changes in recommended MIC breakpoints for cefazolin.¹⁴ For the purposes of this study, a VITEK[®] 2 cefazolin minimum inhibitory concentration (MIC) reading of <4 was considered intermediate. Discrepancies in antimicrobial susceptibility testing between VITEK[®] 2 and the Accelerate PhenoTest[™] BC kit were resolved with broth microdilution on bacterial colonies that grew on blood agar using CLSI reference methods.¹⁴

Patient data including demographics; antibiotic usage; changes in antibiotic management at time of AST results, and clinical outcomes were collected through electronic chart review.

Our sample size was 65 (30 clinical isolates, 35 seeded isolates) and was based on the number of Accelerate PhenoTest[™] BC kits supplied by Accelerated Diagnostics, Inc. Statistical analysis was conducted using STATA (Version 14.1). Categorical variables were compared using Pearson chi-squared or Fisher's exact test as appropriate. Continuous variables were compared using Wilcoxon rank-sum test.

This protocol was approved by the University of Calgary Conjoint Health Research Ethics Board (#REB17-1705).

RESULTS

Clinical results

A total of 30 clinical samples were tested in the study. One isolate was excluded (N=29) from identification agreement, time comparison and AST comparison because it was identified as an

anaerobe (*Bacteroides fragilis*). Another isolate was excluded (N=28) from AST comparison because it failed to provide an AST on AXDX.

The identification agreement and number of failures is provided in Table 1. The number of failures was high in the direct MALDI (31.0%) compared to AXDX (3.4%). AST failures were similar between AXDX and direct VITEK (p=0.32). Compared to conventional MALDI/VITEK[®] 2, direct MALDI/VITEK[®] 2 was more rapid for both ID (mean time 5.8 hours vs 21.6 hours, p<0.001) and AST (mean time 16.5 hours vs 33.0 hours, p<0.001). AXDX obtained the fastest ID (mean time 1.5 hours) and AST (mean time 6.9 hours) results, even when compared to direct MALDI/VITEK[®] 2 (p<0.001).

For the clinical samples, AXDX had EA, CA and minor error (mE) of 97.0%, 96.3%, and 3.7%, respectively. Direct VITEK[®] 2 had EA, CA, and mE of 99.3%, 99.3%, and 0.7%, respectively. AXDX and direct VITEK[®] 2 had no very major errors (VME) or major errors (ME). Further details are provided in Table 2.

Details of patient characteristics from clinical samples are provided in Table 3. The mean age was 68 years with 54% being male gender. Eleven percent of patients had nosocomial bacteremia with urosepsis being the main diagnosis (50%). Shorter time to ID and AST may have had major impact in 4/28 (14%) clinical cases in which the organism isolated was resistant to the empiric antibiotics used. Empiric antibiotics were escalated based on the organism ID (2/4, 50% of cases) and AST (2/4, 50% of cases). Once AST was known, 57% of patients had their antibiotics narrowed.

Seeded Samples

A total of 35 seeded isolates were tested in the study. Two isolates (N=33) were excluded from ID comparison and six isolates were excluded (N=29) from AST comparison. Two failed to ID on AXDX and therefore could not provide ID and AST results (*E. coli* NDM-6, *E. coli* OXA-48), 2 failed to provide AST with AXDX (*K. pneumoniae* OXA-48, *K. oxytoca* OXA-48) and 2 failed to provide AST with direct VITEK®2 (*K. pneumoniae* OXA-232, *K. pneumoniae* NDM-1).

Identification agreement was similar between direct MALDI and AXDX ($p=0.65$) but overall had lower agreement compared to that found for the clinical samples (Table 1). Seeded samples again showed the high number of non-reports (NR) (40.0%) when using direct MALDI compared to AXDX (5.7%) ($p < 0.001$).

In the seeded samples, AXDX had EA, CA, VME, mE and NR of 93.2%, 89.0%, 2.2%, 9.2%, and 6.1%, respectively. Direct VITEK® 2 had EA, CA, VME, mE and NR of 97.5%, 94.7%, 1.3%, 4.4%, and 0.3%, respectively (Table 2). The AXDX and direct VITEK® 2 had no major errors (ME). The majority of NRs for AXDX (15/19) were due to meropenem. More than 50% of meropenem AST for AXDX gave a non-report.

Combined results

Combined results per organism are displayed in Table 2 (N=57). AXDX had EA, CA, VME, mE and NR of 95.1%, 92.7%, 2.3%, 6.4%, and 3.3%, respectively. Direct VITEK® 2 had EA, CA, VME, mE and NR of 98.2%, 97.0%, 1.3%, 2.6%, and 0.5%, respectively. There were statistically significant differences between EA ($p = 0.003$), CA ($p < 0.001$), mE ($p = 0.001$), and

NR ($p < 0.001$) for the AXDX compared to direct VITEK[®] 2. There were no major differences in results seen between organisms.

DISCUSSION

Time to blood culture isolate identification and susceptibility testing are critical factors in patient care. For Gram-negative bacteremia, determining the identification of the Gram-negative bacteria can lead to improved patient management, such as broadening antibiotic therapy to a carbapenem when organisms known to be resistant to cephalosporins are identified (e.g. organisms with inducible chromosomal AmpC beta-lactamases).¹⁵

In our clinical isolates, organisms with inducible chromosomal AmpC beta-lactamases organisms were identified in 7% of isolates. Due to increases in antibiotic resistance, particularly among Gram-negatives harboring extended-spectrum beta-lactamases (ESBL), organism identification alone is not sufficient to gain reassurance that the organism is sensitive to first-line empiric antimicrobial therapies such as third generation cephalosporins.¹⁶⁻¹⁸ We detected a 3/29 (10%) incidence of ESBL producing organisms among our clinical isolates, 2 of which were resistant to the empiric antibiotic therapy the patient was given (ceftriaxone). Ten percent ESBL prevalence is lower than that seen in our center which has an 18% prevalence of ESBL-producing *E. coli* among community patients and emergency room patients with *E. coli* bacteremia.¹⁹ Therefore, the clinical impact of rapid ID/AST results may be even higher. In addition, 57% of patients had their therapy narrowed shortly after the results of AST, suggesting that narrowing of therapy can occur sooner with more rapid AST results. Narrowing of antibiotic

therapy not only reduces antimicrobial resistance but can also improve patient outcomes such as reduced mortality.²⁰

In our study, time to ID and AST was, on average, 5.8 hours and 16.5 hours after Gram-stain result, respectively. This corresponds to a reduction of 15.8 hours for direct MALDI (73% reduction) and 16.5 hours (50% reduction) for direct VITEK[®] 2 in comparison to conventional methods. AXDX was the fastest, reducing time to identification to 1.5 hours (93% reduction) and time to AST to 6.9 hours (79% reduction) compared to conventional methods. Compared to direct MALDI/VITEK[®] 2, AXDX reduced time to ID and time to AST by 74% and 58%, respectively. Moreover, AXDX had the most convenient workflow compared to direct MALDI/VITEK[®] 2 as the processing of the blood culture pellet for MALDI/VITEK[®] 2 is labour intensive.¹ With that being said, AXDX is more expensive per test, generates large amounts of plastic waste, and each system module can process only one blood culture isolate approximately every seven hours (maximum 8 modules per system). Due to these limitations, AXDX is difficult to consider implementing in our high-volume laboratory. It may have a niche in other settings, such as in lower volume laboratories, laboratories without access to MALDI/VITEK[®] 2, or during times when personnel is limited (e.g. overnight).

In the overall results, direct VITEK[®] 2 had higher EA and CA and lower mE and NR (all p <0.05) than AXDX. When broken down, AST results were similar between AXDX and direct VITEK[®] 2 in the clinical samples but direct VITEK[®] 2 had higher agreement than AXDX when challenged with multi-drug resistant organisms. For clinical samples, both the AXDX and direct VITEK[®] 2 achieved >96% EA/CA with no VME or ME and only low numbers of mE (<4%). In

the seeded samples, AXDX had a higher number of VMEs compared to direct VITEK[®] 2, though these differences were not statistically significant and were still less than 3%. Interestingly, Schneider et al., found higher rates of VME using direct VITEK[®] 2 (3.2%) compared to AXDX (1.3%) when compared to conventional VITEK.²¹ This may be related to differences in their pellet making procedure from positive blood cultures.

AXDX also had a high number of non-reportable results that occurred with meropenem (over 50% of the time). A study testing AXDX with carbapenemase producing organisms also found major issues with meropenem AST results, with VMEs occurring in 25% of meropenem tested.⁴ These results are problematic since meropenem is a commonly used carbapenem and using more conventional methods, such as Kirby-Bauer disk diffusion, to clarify meropenem sensitivities will take away AXDX's speed advantage. However, AXDX plans to address this issue in a future software release (already released in Europe).

Strengths of our study included direct MALDI/VITEK[®] 2 being an established protocol within our laboratory and, therefore, the time to ID/AST for direct MALDI/VITEK[®] 2 were obtained through retrospective data from normal laboratory workflow. Another strength of our study was the ability to challenge AXDX and direct MALDI/VITEK[®] 2 with a wide variety of rare carbapenem resistant isolates that were collected worldwide.

There are several study limitations worth mentioning. Our sample size was small and limited by the number of kits that were provided by Accelerate Diagnostics, Inc. Furthermore, our reference for AST was not a true gold standard (VITEK[®] 2 from bacterial colonies grown on blood agar).

Broth microdilution was done only for discrepant results between AXDX and direct VITEK[®] 2 for ME and VMEs. By using VITEK[®] 2 as our reference standard, the results of direct VITEK[®] 2 may appear better than they actually are.

There are many different methods available in reducing time to ID and/or AST for positive blood cultures, all with their differing advantages and disadvantages. Short incubation MALDI and AST (using an automated AST platform such as VITEK[®] 2), direct MALDI and AST, AXDX, peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH), polymerase chain reaction (PCR)-based assays, and the T2Bacteria[®] panel (T2 Biosystems) are the most notable.^{22,23} The T2Bacteria[®] panel has the advantage of not requiring blood culture incubation prior to identification, but does not provide susceptibility information. The main limitation with most of these panels is their high cost. Given that many major laboratories already have a MALDI and/or VITEK[®] 2, short incubation MALDI/VITEK[®] 2 and direct MALDI/VITEK[®] 2 are attractive options. The main limitation identified with using direct MALDI was in its high failure rate which, in this study, was as high as 40%. However, our lab has since improved the blood culture pellet extraction process to improve failure rates down to approximately (20%) [unpublished data], which is more in keeping with other published in-house extraction methods for direct MALDI.²⁴⁻²⁷ Also, commercial kits such as the MALDI Sepsityper[®] (Bruker) are now available for purifying and extracting bacteria from positive blood cultures for the use of performing direct MALDI. However, one head to head study of an in-house extraction method vs the MALDI Sepsityper[®] did not find any statistically significant differences in direct MALDI failure rates.²⁴

Short incubation MALDI is a similar alternative to direct MALDI but has many of the same challenges associated with it. Failure rates for identification using short incubation MALDI is comparable to that of direct MALDI at approximately 20%.^{28,29} The downside to short incubation MALDI and VITEK[®] 2 compared to direct MALDI is the number of hours required to incubate before undergoing MALDI and VITEK[®] 2. As such, studies examining short-incubation MALDI/VITEK[®] 2 had increased time to ID/AST compared to our direct method.^{30,31} However, the extraction process required for direct MALDI/VITEK[®] 2 has its own limitations, such as the hands-on personnel time required (our extraction process takes approximately 45 minutes to complete).

CONCLUSIONS

AXDX and MALDI/VITEK[®] 2 directly from blood cultures positive for Gram-negative bacilli is an effective means to obtain ID and AST results compared to conventional methods. Faster results may improve patient care by speeding the time to antibiotic de-escalation and reducing the time patients are on less effective empiric antibiotic therapy due to bacterial drug resistance. While direct MALDI suffered from high failure rates for ID (up to 40%), direct VITEK[®] 2 had higher agreement, and higher reportability for meropenem, than AXDX in the AST for multi-drug resistant organisms. Direct MALDI/VITEK[®] 2 and AXDX each have their own advantages and disadvantages with respect to costs, turn-around time, workflow, performance and environmental impact and all these factors must be taken into account when deciding on the best diagnostic test for blood culture ID/AST for a laboratory.

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521 **LIST OF ABBREVIATIONS**

522

523 ID Identification

524 AST Antibiotic susceptibility testing

525 BSI Bloodstream infection

526 MALDI Matrix-assisted laser desorption/ionization time-of-flight mass
527 spectrometry

528 VITEK® 2 VITEK® system

529 AXDX Accelerate PhenoTest™ BC kit

530 EA Essential agreement

531 CA Categorical agreement

532 CLS Calgary laboratory services

533 CLSI Clinical and Laboratory Standards Institute

534 TMP-SMX Trimethoprim/sulfamethoxazole

535 MIC Minimum inhibitory concentration

536 VME Very major error

537 ME Major error

538 mE Minor error

539 NR Non-reports

540 ESBL Extended-spectrum beta-lactamases

541 PNA FISH Peptide nucleic acid fluorescence *in situ* hybridization

542 PCR Polymerase chain reaction

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544 **Table 1:** Identification agreement and number of failures for AXDX and direct MALDI-TOF

545 MS (DM) compared to conventional MALDI-TOF MS ($p < 0.001$ for all comparisons).

Sample Type	Method	Agreement	Non-report [95% CI]
Clinical (N=29)	AXDX	28 (100%)	1 (3.4%) [-3.6% – 10.5%]
	Direct MALDI	20 (100%)	9 (31.0%) [13.1% - 48.9%]
Seeded (N=35)	AXDX	31 (93.9%) [79.8% - 99.3%]	2 (5.7%) [-2.4% - 13.8%]
	Direct MALDI	19 (90.5%) [76.8% - 104.2%]	14 (40.0%) [22.9% - 57.1%]

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549 **Table 2:** AXDX and direct VITEK® 2 system AST results^a for clinical samples, seeded samples

550 and combined samples per organism.

Clinical Samples (N=28)									
Antibiotic	EA	CA	VME	ME	mE	NR	S	I	R
AXDX									
Amikacin	27	28	0	0	0	0	28	0	0
Cefazolin ^b	23	21	0	0	2	0	0	19	4
Cefepime	26	26	0	0	2	0	27	1	0
Ceftazidime	26	26	0	0	2	0	27	0	1
Ceftriaxone	27	27	0	0	0	0	24	0	3
Ciprofloxacin	26	27	0	0	1	0	24	0	4
Ertapenem	27	27	0	0	0	1	27	0	0
Gentamicin	28	28	0	0	0	0	25	0	3
Meropenem	27	28	0	0	0	0	28	0	0
Piperacillin/Tazobactam	27	27	0	0	1	0	27	0	1
Tobramycin	28	25	0	0	3	0	24	3	1
Total (%) [95% CI]	292 97.0% [94.3% - 98.5%]	290 96.3% [93.5 – 98.0]	0 0%	0 0%	11 3.7% [2.0% – 6.6%]	1 0.3%	261 86.7%	23 7.6%	17 5.6%
Direct VITEK® 2 system									
Antibiotic	EA	CA	VME	ME	mE	NR	S	I	R
Amikacin	28	28	0	0	0	0	28	0	0

Cefazolin	28	28	0	0	0	0	0 ^c	22	6
Cefepime	27	27	0	0	1	0	27	0	1
Ceftazidime	28	28	0	0	0	0	27	0	1
Ceftriaxone	27	27	0	0	0	1	24	0	3
Ciprofloxacin	27	27	0	0	1	0	23	1	4
Ertapenem	27	27	0	0	0	1	27	0	0
Gentamicin	28	28	0	0	0	0	25	0	3
Meropenem	28	28	0	0	0	0	28	0	0
Piperacillin/Tazobactam	28	28	0	0	0	0	27	0	1
Tobramycin	28	28	0	0	0	0	24	3	1
Total (%)	304 99.3% [97.5% - 99.98%]	304 99.3% [97.5% - 99.98%]	0 0%	0 0%	2 0.7% [0.02% - 2.7%]	2 0.7%	282 92.2%	4 1.3%	20 6.5%
Seeded Samples (N=29)									
Antibiotic	EA	CA	VME	ME	mE	NR	S	I	R
AXDX									
Amikacin	25	25	2	0	2	0	24	1	4
Cefazolin ^b	20	20	0	0	0	1	0	0	20
Cefepime	24	22	1	0	6	0	7	3	19
Ceftazidime	28	26	0	0	3	0	2	2	25
Ceftriaxone	27	26	0	0	2	1	3	1	24
Ciprofloxacin	29	27	0	0	2	0	3	0	26
Ertapenem	24	23	2	0	2	2	5	1	21
Gentamicin	27	26	0	0	3	0	15	2	12
Meropenem	13	14	0	0	0	15	1	0	13
Piperacillin/Tazobactam	27	25	0	0	4	0	4	0	25
Tobramycin	28	26	0	0	3	0	7	7	15
Total (%)	272 93.2% [89.6% - 95.6%]	260 89.0% [84.9% - 92.2%]	5 2.2% [0.8% - 5.0%]	0 0%	27 9.2% [6.4% - 13.1%]	19 6.1% [3.9% - 9.4%]	71 24.3%	17 5.8%	204 69.9%
Direct VITEK® 2 system									
Antibiotic	EA	CA	VME	ME	mE	NR	S	I	R
Amikacin	28	27	1	0	1	0	22	2	5
Cefazolin	29	29	0	0	0	0	0 ^c	0	29
Cefepime	28	27	0	0	2	0	12	3	14
Ceftazidime	29	29	0	0	0	0	3	0	26
Ceftriaxone	28	28	0	0	1	0	2	0	27
Ciprofloxacin	29	29	0	0	0	0	3	2	24

Ertapenem	28	28	1	0	0	0	3	1	25
Gentamicin	29	27	0	0	3	0	15	3	11
Meropenem	25	23	1	0	4	1	6	3	19
Piperacillin/Tazobactam	28	27	0	0	2	0	3	2	24
Tobramycin	29	28	0	0	1	0	7	7	15
Total (%)	310 97.5% [95.0% - 98.8%]	302 94.7% [91.6% - 96.7%]	3 1.3% [0.3% - 3.9%]	0 0%	14 4.4% [2.6% - 7.3%]	1 0.3%	76 23.9%	23 7.2%	219 68.9%
Combined samples per organism (N=57)									
AXDX^b									
Organism	EA	CA	VME	ME	mE	NR	S	I	R
<i>E. coli</i> (N=23)	245	241	0	0	10	2	182	25	44
<i>Klebsiella</i> spp. (N=21)	213	210	2	0	11	8	74	13	136
<i>Enterobacter</i> spp. (N=8)	65	58	2	0	12	8	27	9	36
<i>Citrobacter</i> spp. (N=2)	16	16	1	0	2	1	13	0	6
<i>P. mirabilis</i> (N=2)	20	20	0	0	0	0	20	0	0
<i>P. aeruginosa</i> (N=1)	5	5	0	0	3	1	8	0	0
TOTAL	564 95.1% [93.1% - 96.7%]	550 92.7% [90.4% - 94.7%]	5 2.3% [0.07% - 5.2%]	0 0%	38 6.4% [4.6% - 8.7%]	20 3.3% [2.1% - 5.2%]	324 54.6%	47 7.9%	222 37.4%
Direct VITEK[®] 2 system									
Organism	EA	CA	VME	ME	mE	NR	S	I	R
<i>E. coli</i> (N=23)	248	247	0	0	6	0	195	11	47
<i>Klebsiella</i> spp. (N=21)	230	224	1	0	6	0	87	9	135
<i>Enterobacter</i> spp. (N=8)	84	82	2	0	4	1	33	7	47
<i>Citrobacter</i> spp. (N=2)	21	22	0	0	0	0	13	0	9
<i>P. mirabilis</i> (N=2)	22	22	0	0	0	0	22	0	0
<i>P. aeruginosa</i> (N=1)	9	9	0	0	0	2	8	0	1
TOTAL 625	614 98.2% [96.9% - 99.1%]	606 97.0% [95.3% - 98.2%]	3 1.3% [0.3 - 3.6%]	0 0%	16 2.6% [1.5% - 4.1%]	3 0.5% [0.1% - 1.4%]	358 57.4%	27 4.3%	239 38.3%

551

552 Abbreviations: CA=categorical agreement; EA=essential agreement; I=intermediate; mE=minor

553 error; ME=major error; NR=non-report; R=resistant; S=susceptible; VME=very major error.

554 ^aMICs were truncated to overlapping reportable range prior to analysis.

555 ^bAXDX only reports cefazolin for *E. coli* and *Klebsiella* spp.

556 ^cVITEK® 2 system unable to differentiate cefazolin I from S. For the purposes of this study, a

557 VITEK® 2 system cefazolin minimum inhibitory concentration (MIC) reading of <4 was

558 considered intermediate.

559 **Table 3:** Clinical samples - baseline characteristics and changes to antibiotics made after AST
560 results (N=28).

Mean age (SD) [95% CI]	68 (3) [61 – 74]
Male gender [95% CI]	54% [34% – 73%]
Diagnosis	Urosepsis (50%)
	Fever NYD (21%)
	Liver/biliary sepsis (11%)
	Other (18%)
Nosocomial bacteremia [95% CI]	11% [-1% - 23%]
Death within 3 months [95% CI]	11% [-1% - 23%]
Organism identified	<i>E. coli</i> (61%)
	<i>K. pneumoniae</i> (14%)
	<i>K. oxytoca</i> (7%)
	<i>P. mirabilis</i> (7%)
	<i>P. aeruginosa</i> (4%)
	<i>E. cloacae</i> (4%)
	<i>C. freundii</i> (4%)
Empiric antibiotics used	Ceftriaxone (62%)
	Pip/Tazo (31%)
	Ertapenem (4%)
	Meropenem (4%)
Organism resistant to empiric antibiotics [95% CI]	14% [0.5% - 28%]
Antibiotics narrowed after AST results [95% CI]	57% [38% - 77%]

561 Nosocomial bacteremia: Positive blood cultures (for Gram-negative bacilli) that were drawn >48
562 hours after hospital admission. Clinical data was unknown for one patient.