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IMPROVING DIAGNOSTIC TURNAROUND TIME IN BLOODSTREAM INFECTIONS: A STEP TOWARD TARGETED THERAPY IN LINE WITH THE SURVIVING SEPSIS CAMPAIGN

Clinical Microbiology

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ABSTRACT

Background: Bloodstream infections (BSIs) are time-critical medical emergencies associated with significant morbidity and mortality. Despite advances in diagnostic technologies, delays in pathogen identification and antimicrobial susceptibility testing (AST) continue to impede the initiation of timely and appropriate antimicrobial therapy. This study evaluates the performance of Direct Antimicrobial Susceptibility Testing (DAST) using the VITEK 2 system, in comparison with the conventional method, to determine its potential in reducing diagnostic turnaround time in accordance with the 2021 Surviving Sepsis Campaign guidelines.**Methods:** A prospective study was conducted over one year at a 255-bedded tertiary care hospital in North India. All monomicrobial positive blood cultures detected by the BACT/ALERT® system were included. Samples were processed for both DAST and conventional AST after subculture. DAST employed a standardized in-house centrifugation protocol, and testing was performed on the VITEK 2 platform. Results were compared for categorical agreement, essential agreement, and error rates (major, very major, and minor).**Results:** Out of 2,108 blood cultures, 322 were positive, of which 235 underwent DAST. Identification TAT was reduced to 6–8 hours, and AST results were available within 8–12 hours. High categorical (>98%) and essential agreement (>98%) were observed. Minor and major errors were limited (n=4 and n=2, respectively), with no very major errors. **Conclusion:** DAST significantly reduces the TAT for AST without compromising accuracy, thereby facilitating early targeted therapy in BSIs. It serves as a viable option in settings lacking rapid molecular diagnostic tools, particularly in resource-limited environments.

KEYWORDS

Bloodstream Infections, Direct Antimicrobial Susceptibility Testing, Turnaround Time, Sepsis, Rapid Diagnostics

INTRODUCTION:

Bloodstream infections (BSIs) are a major cause of sepsis and multi-organ dysfunction, contributing significantly to patient morbidity and mortality worldwide. Rapid identification of the causative pathogen and timely antimicrobial susceptibility testing (AST) are critical for effective clinical management. The 2021 *Surviving Sepsis Campaign* guidelines emphasize the importance of early diagnosis and prompt initiation of pathogen-targeted antimicrobial therapy to improve clinical outcomes in patients with sepsis and septic shock [1].

Automated blood culture systems have markedly reduced the time required to detect positive blood cultures. A further reduction in turnaround time can be achieved by directly inoculating automated identification and antimicrobial susceptibility testing (AST) systems from positive blood culture bottles, thereby bypassing the need for overnight subculture. Traditionally, subculturing onto solid media followed by preparation of a standardized inoculum, as per manufacturer recommendations, is required before testing. Among the pathogens causing bloodstream infections, rapidly growing *Enterobacteriaceae* are most likely to yield the fastest and most reliable results with direct inoculation compared to the conventional method. In this study, we evaluated the performance of the direct method versus the standard method using the VITEK 2 system for identification as well as AST across all organisms, including yeasts (2,3).

Traditionally, BSI diagnostics rely on blood cultures followed by organism identification and AST. While effective, these conventional methods are time-consuming. Blood cultures typically take 24–48 hours to indicate microbial growth. Subsequent AST involves subculturing, incubation, and result interpretation, often requiring an additional 24–48 hours. Such delays can result in empirical treatments that may not be optimal, potentially worsening clinical outcomes [2,3] Although automated blood culture systems have reduced time-to-positivity, the AST process remains a critical bottleneck. Direct Antimicrobial Susceptibility Testing (DAST) offers a promising approach for accelerating therapeutic decision-making by enabling susceptibility testing directly from blood culture bottles which have flagged as positive. The present study aims to evaluate the clinical performance of DAST in comparison with the conventional colony suspension method using the VITEK 2 system and evaluates its potential to expedite targeted therapy and align with sepsis care guidelines.

METHODS:

Study Design and Setting

This prospective study was conducted at a newly commissioned 255 bedded super speciality private sector hospital in North India. The study was carried out over a period of one year, from July 2024 to January 2025. Ethical approval was obtained from the Institutional Ethics Committee

(BHR/MSSH/MHPL/DWARKA/MHEC/MICRO/25-02).

Sample Collection and Processing

All positive blood culture samples flagged by the BacT/ALERT system (BioMérieux, Marcy-l'Etoile, France), including both aerobic and anaerobic bottles, from patients with suspected bloodstream infections (BSIs) were included. Gram staining was performed on all positive samples, and only those with a single type of bacterial morphology were included in the study.

Method for Direct Inoculation from Positive Blood Culture Bottle into VITEK 2

An in-house standardized protocol was employed [3], which included a three-step centrifugation method to obtain a bacterial pellet. This approach enables rapid identification (ID) and AST directly from positively flagged blood culture bottles, thereby eliminating the need for subculturing and reducing the time to result by approximately 18–24 hours. Once a positive signal was generated by the automated blood culture system, the bottle was processed immediately for direct testing using the VITEK 2 system; we recorded the **time of positivity** and **clinical indication** for urgency. This was followed by **Gram stain** on the positive bottle to confirm the presence of organisms, type of bacteria for preliminary identification (e.g., Gram-positive cocci, Gram-negative rods or polymicrobial). In case of **polymicrobial infections** (where multiple morphotypes were observed, **we did not proceed with direct method**). Based on the Gram stain morphology, appropriate cards were selected for direct identification and DAST using the VITEK 2 system.

Sample was prepared by centrifugation to separate bacteria from blood components **& washing** the pellet with **saline or sterile water** to reduce interference from blood cells. This pellet was subjected to species identification using VITEK 2 (BioMérieux, Marcy-l'Etoile, France), as per the manufacturer's instructions [4] and the interpretation was done according to the CLSI [5]. For this, **inoculum**

standardization was done, prepared a suspension from the positive bottle (or pellet) and adjusted to a **McFarland standard** (usually 0.5–0.63) using a **densitometer** to ensure accuracy in the inoculum standardization as this is **crucial for AST reliability.** Following this, **VITEK 2 card was inoculated** based on Gram stain: (**ID cards:** GN(Gram-negative) fermenters, GP (Gram-positive), or YST (yeast) and **AST cards.** Based on organism type and clinical need (e.g., AST-N405 for Gram-negatives, in addition P-235 where Enteric fever was suspected clinically, and AST-P628 for Gram-positives). Then we **inoculated the ID and AST cards**, placed the standardized suspension into the inoculation tube, attached the appropriate **VITEK 2 ID and AST cards**. The system automatically **filled and sealed** the cards and began analysis. During the process of **incubation and analysis** cards were loaded into the **VITEK 2 instrument**, which incubated them at 35–37°C, monitored biochemical reactions for identification and growth patterns under antibiotics for AST. **Time to result** for identification was 6–8 hours, while for AST, it was 8–12 hours (varied by organism and card type). We also used the same standard inoculum to perform the disc diffusion testing for AST by the modified Kirby-Bauer method as per CLSI [5] for particular antibiotics which were not included in the AST cards, namely, ampicillin, cefixime, chloramphenicol and azithromycin for the *Salmonella* isolates. The plates were read at 16-18 hours of incubation.

Reference Testing

Positively flagged blood culture broth was sub-cultured onto blood agar and MacConkey agar, which were incubated at 37 degrees C, under aerobic conditions. After 18 hours of incubation, the resulting colonies were identified using VITEK 2 and subjected to AST with the VITEK 2 system as per the manufacturer's instructions [4]. This was considered as the reference and all results of DAST were compared to these. For the disc diffusion technique, the controls strains were used as per the CLSI [5]. The disc diffusion performed on the colonies of the isolates of Salmonella which grew on culture, was used as the reference for the direct disc diffusion AST.

Definitions of the agreements and error categorization used in the study

DAST performance was compared to the results of the reference method. Direct disc diffusion method was compared to the reference method form the colonies. Agreement between the test and the respective reference methods was defined as follows:

Essential agreement: When the Minimum inhibitory concentrations (MICs) for DAST were within ±2-fold dilution of those obtained by the reference method, essential agreement will be fulfilled.

Categorical agreement for both MIC and disc diffusion method:
1)Very Major Error (VME) corresponded to those where DAST reported susceptible while reference method reported resistant, indicating False susceptible results

2)Major Error (ME) corresponded to those where DAST reported a resistant result while reference method reported susceptible, indicating False resistant results

3)Minor Error (mE) corresponded to those where DAST reported an intermediate category while reference method reported either susceptible or resistant indicating intermediate or other discrepancies.

Data Management and Statistical Analysis

All data from DAST and the reference method were entered into Microsoft Excel. Statistical analysis was performed using SPSS software, version 22 (IBM Corp., NY, USA).

RESULTS:

During the study period a total of 2,108 blood cultures were processed, with 322 (15.3%) yielding microbial growth. AST was performed using the VITEK 2 system employing the standard colony suspension technique. DAST was applied directly to positive blood culture broths for 235 of the 322 positive samples (72.98%). The rest were not included in the study.

Table1: Performance of DAST Compared with Reference AST

Organism	Antibiotic Combinations (n × Ab = N)	Categorical Agreement (%)	Minor Error	Major Error	Very Major Error	Total Errors	Essential Agreement (%)
Salmonella Typhi	(124 × 6) = 744	100.0	0	0	0	0	100
Salmo nellaParaty phi A	(45 × 6) = 270	99.63	0	1	0	1	99.6
Escherichia coli	(27 × 17) = 459	98.47	2	0	0	2	98.69
Klebsiella pneumoniae	(23 × 17) =391	98.04	2	1	0	3	98
Proteus mirabilis	(3 x 17)=51	100	0	0	0	0	100
Enterococc us spp	(5 x 19)=95	100	0	0	0	0	100
Streptococc us pneumoniae	(2 × 19) = 38	100.0	0	0	0	0	100
Staphyloco ccus epidermidis	(1 × 19) = 19	100	0	0	0	0	100
Staphyloco ccus aureus	(3 x 19)=57	100	0	0	0	0	100
Candida tropicalis	(1 x 6)= 6	100	0	0	0	0	100
Candida parapsilosis	(1 × 6) = 6	100	0	0	0	0	100

n -Total number of bacterial isolates
Ab - Total Antibiotics
N- Total number of isolates being tested for antibiotic resistance

Note: More extensive studies are necessary to validate DAST across all organism-antimicrobial combination

Turnaround Time (TAT) as determined on the VITEK 2- For Gram-negative bacteria the identification results were available within 6 hours, while, the DAST was available in 10 hours. For Gram-positive bacteria, the TAT for identification was approximately 8 hours, while the DAST was available in 12 hours. For yeasts (e.g., *Candida* spp.), the TAT for identification was 10 hours and the DAST results were available in 22 hours.

DISCUSSION:

This study underscores the critical need to reduce diagnostic turnaround time in bloodstream infections. DAST significantly shortened the time to AST results compared to conventional methods, particularly for Gram-negative and Gram-positive organisms. Notably, *Candida tropicalis* and *Candida parapsilosis* showed perfect categorical and essential agreement, suggesting DAST may have a role in fungal diagnostics.

Any method of AST is considered acceptable when the selection criteria are met, defined as VME $\leq$ 1.5%, ME $\leq$ 3%, categorical agreement $\geq$90%, and essential agreement $\geq$90% as described by the CLSI compared with the reference AST method. These are well within the limits of agreements for AST [6].

Direct inoculation of blood cultures containing Gram-negative bacilli, Gram positive cocci as well as yeasts were performed in this study. When compared to the conventional method, all the isolates were correctly identified by the direct method.

For the selection and validation of a new system for identification, a minimum of 95% accuracy for commonly isolated organisms, such as members of the *Enterobacteriaceae* family and an overall agreement of at least 90% with the reference method, are recommended [7]. Our results are consistent with this and to reference method [4]. The three most frequently isolated organisms were *Salmonella*, *E. coli* and *K.pneumoniae*, followed by *Proteus.* Among the Gram positives which were fewer in number, *Streptococcus* and *Staphylococcus* were the commonest ones detected.

For *Enterobacterales*, antimicrobial susceptibility testing revealed essential agreement for the acceptable threshold (>95%) for piperacillin–tazobactam, and amoxicillin–clavulanic acid, with the occurrence of four minor errors (mEs), one major error (ME) in *Klebsiella pneumoniae* for a carbapenem, and one ME in *Salmonella* Paratyphi A for ampicillin, in categorical interpretation. One of the possible reasons could be due to lack of proper homogenization during inoculum preparation causing the inoculum to stick to the well of the

antibiotic card. Other studies have reported similar discrepancies with these antibiotics [8-12]. We did not observe any discrepancy with the Gram positive cocci and yeasts, though more number of such isolates need to be studied. We did not observe any discrepancy in disc diffusion testing between the test method and the reference method.

The current study also evaluated drug–organism combinations using DAST for Gram-positive cocci. We observed high levels of agreement in both categorical and essential metrics, unlike earlier studies [13-15], but we need more isolates to understand this better. We observed good essential and categorical agreements with cephalosporins and carbapenems unlike previous reports [14, 15].

With the help of Direct ID and DAST as we could provide the complete report of ID and AST within 10 hours for Gram negatives, within 12 hours for Gram positives, and 22 hours for yeasts, improving the TAT tremendously. When compared to the reference method, identification was found to be concordant (100%) with no discrepancy among the Gram-positive, Gram-negative bacteria and yeast. Nevertheless, DAST has limitations. Its performance must be validated for each organism-antibiotic pair to ensure accuracy and reproducibility. Its applicability to slow-growing organisms, and resistant strains also warrants further research. Although a disc diffusion method of AST directly from positively flagged blood culture bottles is recommended by the CLSI [5], our method of DAST is superior or equivalent to it, as it offers MIC values, covers more antibiotics, uses a standard inoculum as recommended for AST (0.5 Mc Farland), allows identification and offers a better TAT. The disc diffusion method of AST directly from positively flagged blood culture bottles also had a TAT of 8-10 hours extendible to 16-18 hours, as per the drug-bug combination CLSI [5]. Moreover, the DAST is much cost effective when compared to molecular methods.

While automated blood culture systems expedite the detection of microbial growth, AST remains a rate-limiting step. Integrating DAST with rapid identification technologies such as MALDI-TOF MS could bridge the gap between pathogen detection and effective therapeutic intervention and in those circumstances where MALDI-TOF MS is not available such as in the resource poor settings, VITEK 2 can be employed for rapid identification and DAST as described here.

LIMITATIONS: The number of Gram-positive bacteria as well as yeast were less and we need to study more numbers for better reliability and reproducibility. We also did not include any non-fermenters in this study as we did not isolate any during the study period.

CONCLUSION:

BSIs pose a significant challenge to timely diagnosis and treatment. Traditional workflows, while dependable, often fall short of the rapid timelines advocated by the Surviving Sepsis Campaign. DAST offers a pragmatic interim solution to accelerate susceptibility testing and enable early implementation of targeted antimicrobial therapy. This method is easily adaptable and reproducible in healthcare settings with limited resources. This may be studied in detail to minimize the existing few errors so as to have a robust, rapid, cost-effective ID and AST method. Future efforts should focus on integrating molecular diagnostics and fully automated AST platforms to optimize patient outcomes in sepsis care.

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CONFLICTS OF INTEREST: None

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