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Original Research Article

Assessing the effectiveness of direct susceptibility testing from positive blood culture broth using the VITEK-2 system

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ABSTRACT

Background: Bloodstream infections (BSIs) are prevalent in intensive care units, often requiring timely diagnosis and treatment. Traditional methods of diagnosing bacteraemia, including Gram staining, subculture, and antibiotic susceptibility testing (AST), can yield results in 12-48 hours. The advent of MALDI-TOF MS has expedited bacterial identification from colonies. Recently, methods to directly identify bacteria from positive blood cultures using MALDI-TOF MS have emerged.
Aim and Objective: This study compared the findings of traditional colony-based AST with the VITEK-2 automated testing technology for direct AST from the blood culture broth.
Materials and Methods: Blood cultures were processed using BacT/Alert Virtuo. Aerobic-positive cultures showing single organism types were subjected to direct identification and susceptibility with VITEK MS and VITEK 2, respectively. The methodology involved lysis centrifugation and on-plate extraction for MALDI-TOF MS identification, followed by AST using VITEK 2.
Results: A total of 60 isolates, comprising 44 Gram-negative and 16 Gram-positive bacteria, were included. This study tested 902 drug-bug combinations, revealing better categorical agreement for Gram-negative (93.7%) than Gram-positive organisms, which showed higher error rates, particularly with Vancomycin, Linezolid, and Oxacillin for *Staphylococcus aureus,* and Cefepime for *E. coli*.
Conclusion: The results highlight that VITEK 2 and MALDI-TOF MS allow identification on the same day and whole panel AST from positive blood cultures, cutting the turnaround time by a day compared to traditional methods. This rapid approach facilitates timely and targeted antimicrobial therapy, potentially improving patient outcomes and antimicrobial stewardship. However, larger studies are needed to validate these findings, especially for Gram-positive isolates.

1. Introduction

Bloodstream infections (BSIs) are a common reason for admission to intensive care units (ICUs), which affect as much as 2% of patients.[1] Sepsis can be caused by a variety of bacteria. The widely followed procedure for diagnosing bacteraemia from positively flagged blood cultures like BacT/ALERT or Bactec involves using Gram stain, followed by subculturing blood culture broth onto a solid medium and incubating overnight for colonies. These colonies are subsequently employed as a standardized inoculum for traditional antibiotic susceptibility testing (AST). All these procedures require a minimum 12-48 hours to provide organism identification and AST report to the clinician for effective targeted

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therapy.[1–3] The development of protein fingerprinting-based Matrix Assisted Laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has successfully shortened time required to identify organisms from colonies in microbiology laboratories worldwide. MALDI-TOF MS is desirable alternative to directly identify bacteria from clinical specimens due to its excellent diagnostic accuracy. In recent years, methods have been developed using MALDI-TOF MS, bacteria from positive blood cultures may be identified immediately without having to wait for a laborious colony to form in a subculture plate.[4]

Previously, disc diffusion testing with a restricted number of drugs was used in many studies to assess direct susceptibility of isolates from positive blood culture.[2] Although direct susceptibility testing (DST) by MIC-based methods was not explicitly mentioned in the guidelines of Clinical and Laboratory Standards Institute (CLSI), these methods are also found to be valuable.[5] Minimum Inhibitory Concentration (MIC) testing provides quantitative data on lowest concentration of antimicrobial that inhibits growth of bacteria, offering precise information on the degree of susceptibility or resistance. This study aimed to evaluate the VITEK-2 automated testing system for direct susceptibility from blood culture broth using an algorithm. It was discovered that AST could be accelerated more consistently by combining the lysis filtration method for identification by MALDI TOF MS with AST utilising VITEK 2 from positive blood culture broth.[4] This study compared the antibiotic susceptibility test (AST) results from colonies generated by VITEK-2 with those from blood culture broth (DST).

2. Materials and Methods

This prospective observational study was conducted at the Department of Microbiology blood culture section, JIPMER, Puducherry from January 2024 to March 2024. All the blood culture bottles that are collected from patients suspected of bacteraemia and sent by clinicians are routinely loaded into BacT/Alert Virtuo (bioMe´rieux). This automated blood culture system continuously monitors microbial growth and incubates blood culture bottles.

2.1. Inclusion and exclusion criteria

Only aerobic-positive blood culture bottles upon microscopy showing a single type of organism were included for direct identification by VITEK MS and AST with the VITEK 2. Every blood culture exhibiting yeast or polymicrobial growth was excluded.

2.2. Methodology

Positive blood cultures were subjected to identification and AST for comparison by following standard protocols. Based on a published methodology, an in-house method was developed to employ saponin to separate bacteria from the blood culture broth.[6] The pellet was picked up for MALDI TOF MS by using the On-plate extraction method with formic acid and α-Cyano-4-hydroxycinnamic acid (CHCA). Using VITEK Densichek (bioMérieux), the pellet was adjusted to match the McFarland 0.5 standard in 0.45% saline. The bacterial suspension and the matching VITEK 2 AST cards (N405, N406, ST03, P628) were manually fed into the VITEK 2 system based on MALDI TOF MS identification. Table 1 lists all of the antibiotics that were tested for each panel. As controls, standard strains of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used.

2.3. Data analysis

The sample size was calculated to be 60 and consecutive samples were collected. A sample size of 60 provides sufficient precision for estimating key parameters, such as means or proportions, with narrow confidence intervals. It has been done as a pilot study in our institute it has been decided to take the first 60 samples. VITEK MS further confirmed the direct identification of organisms from pure isolated colonies. Only when the organism identification was found to be the same, the analysis was proceeded. AST results were interpreted as susceptible, intermediate, susceptible dose-dependent, and resistant by VITEK2. The interpretation of AST by VITEK 2 from the broth was compared with colony VITEK 2. The three categories of susceptibility discrepancies such as categorical, essential and absolute agreements and disagreements were reported. Categorical disagreements include very major errors, major errors and minor errors. When the reference method is susceptible or resistant but the test method gives an intermediate result, it is a minor error. When the reference method is susceptible but the test method gives a resistant result, it is a major error. When the reference method is resistant but the test method gives a susceptible result, it is a major error. Essential agreed is when the MIC of the test method is within plus or minus one two-fold serial dilution of MIC of the reference method. If the MIC of the test method is greater than plus or minus one two-fold serial dilution of MIC of the reference method, it is termed as essential disagreement. The absolute agreement is when the MIC of the test method exactly matches the MIC of the reference method, when the MIC value is different it is absolute disagreement.[7]

3. Results

A total of sixty isolates - 44 Gram-negatives and 16 Gram-positives for which the organism identification corresponded with that of pure isolated colonies were included in the study. 902 drug bug combinations were tested excluding intrinsic resistance for 60 organisms,

Table 1: Antibiotics tested under each panel

Antibiotic classes	N405	N406	ST03	P628
Aminoglycosides	Amikacin Gentamicin	Amikacin Gentamicin	Gentamicin	Gentamicin
Antifolates	Trimethoprim-Sulphamethoxazole	Trimethoprim-Sulphamethoxazole	Trimethoprim-Sulphamethoxazole	Trimethoprim-Sulphamethoxazole
Beta lactams				
Penicillinase labile penicillin			Ampicillin Benzylpenicillin	Benzylpenicillin
Penicillinase stable penicillin				Oxacillin
Cephalosporins	Cefepime Ceftriaxone Cefuroxime	Cefepime Ceftazidime	Cefotaxime Ceftriaxone	
Carbapenems	Ertapenem Imipenem Meropenem	Imipenem Meropenem		
Monobactams		Aztreonam		
Beta lactam – Beta lactamase inhibitors	Amoxicillin-Clavulanic acid Cefoperazone Sulbactam Piperacillin-Tazobactam	Cefoperazone Sulbactam Piperacillin-Tazobactam		
Chloramphenicol			Chloramphenicol	
Fluroquinolones	Ciprofloxacin	Ciprofloxacin Levofloxacin	Levofloxacin Moxifloxacin	Ciprofloxacin Levofloxacin
Fosfomycin	Fosfomycin	Fosfomycin		
Glycopepetides			Vancomycin Teicoplanin	Vancomycin Teicoplanin
Glycylcycline	Tigecycline	Tigecycline	Tigecycline	Tigecycline
Lincosamides			Clindamycin	Clindamycin
Lipopeptides				Daptomycin
Macrolides			Erythromycin	Erythromycin
Nitrofurans				Nitrofurantoin
Oxazolidinones			Linezolid	Linezolid
Polymyxins	Colistin	Colistin		
Rifamycins			Rifampicin	Rifampicin
Tetracycline		Minocycline	Tetracycline	Tetracycline
Other Properties			Inducible Clindamycin Resistance	Inducible Clindamycin Resistance Cefoxitin screen Gentamicin High-level Synergy

including 640 and 262 drug bug combinations for Gram-negative and Gram-positive organisms, respectively, as depicted in Table 2.

Gram-negative organisms showed a better categorical agreement of 93.7%. Gram-positive organisms showed a total error of 14.5% with a major error of 10.7%, more than the acceptable limit. (Table 3) shows the breakup of organisms and their categorical error percentages.

The essential agreement and absolute agreement between Direct VITEK AST and colony VITEK AST are shown in (Table 4).

Of 640 Gram-negative drug bug combinations, 31 had an essential MIC mismatch, resulting in a 4.8% essential disagreement and 95.2% essential agreement. 75 had an absolute MIC mismatch, leading to an 11.7% absolute disagreement and 88.3% absolute agreement. Contrastingly, among 262 Gram-positive drug bug combinations, 33 had an essential MIC mismatch, resulting in a 12.6% essential disagreement and 87.4% essential agreement. 77 had an absolute MIC mismatch, leading to a 29.4% absolute disagreement and 70.6% absolute agreement. The data indicates better agreement and lower mismatch

Table 2: Antibiotics and number of organisms tested

Organism	No of isolates	Antibiotics tested per isolate	Total Antibiotics tested
Gram Negative Organisms: Fermenters			
Escherichia coli	20	16	320
Klebsiella pneumoniae	8	16	128
Enterobacter cloacae	3	15	45
Aeromonas hydrophila	1	13	13
Gram Negative Organisms: Non - Fermenters			
Acinetobacter baumannii	4	16	64
Pseudomonas aeruginosa	4	11	44
Elizabethkingia anophelis	2	8	16
Burkholderia cepacia complex	2	5	10
Gram Positive Organisms			
Enterococcus faecalis	2	12	24
Enterococcus faecium	2	11	22
Staphylococcus aureus	12	18	216
	60		902

Table 3: Categorical errors in drug-bug combination tested

Organism	Minor Error (%)	Major Error (%)	Very Major Error (%)	Total (%)
Acinetobacter baumannii	1.6	4.7	1.6	7.8
Enterobacter cloacae	2.2	0.0	0.0	2.2
Escherichia coli	1.6	1.9	1.9	5.3
Klebsiella pneumoniae	0.8	0.0	0.0	0.8
Pseudomonas aeruginosa	4.5	2.3	2.3	9.1
Elizabethkingia anophelis	43.8	0.0	0.0	43.8
Aeromonas hydrophila	0.0	0.0	7.7	7.7
Burkholderia cepacia Complex	20.0	0.0	20.0	40.0
GNB	3	1.6	1.7	6.3
Enterococcus faecalis	8.3	16.7	0.0	25.0
Enterococcus faecium	4.5	4.5	0.0	9.1
Staphylococcus aureus	0.9	10.6	2.3	13.9
GPC	1.9	10.7	1.9	14.5
Total	2.7	4.2	1.8	8.6

Table 4: Essential agreement and absolute agreement between direct VITEK AST and colony VITEK AST

	Drug bug tested	Essential MIC mismatch (outside -1 to +1 dilution)	Essential disagreement %	Essential agreement%	Absolute MIC mismatch	Absolute MIC disagreement %	Absolute MIC agreement %
Total	902	64	7.1%	93.9%	152	16.9 %	83.1%
GNB	640	31	4.8%	95.2%	75	11.7 %	88.3%
GPC	262	33	12.6%	87.4%	77	29.4 %	70.6%

Table 5: Drug-bug combination showed higher errors anddisagreement

	Minor Error %	Major Error %	Very major error %	Total Error %	Essential disagreement %
Vancomycin-S. aureus	8.3% (1/12)	25% (3/12)	-	33% (4/12)	33% (4/12)
Linezolid- S. aureus	-	25% (3/12)	-	25% (3/12)	42% (5/12)
Oxacillin - S. aureus	-	33% (4/12)	8.3% (1/12)	42% (5/12)	42% (5/12)
Cefepime – E. coli	5% (1/20)	5% (1/20)	25% (5/20)	35% (7/20)	15% (3/20)

rates for Gram-negative bacteria than Gram-positive cocci. Specifically, Gram-positive drug-bug combinations that showed higher errors are Vancomycin, Linezolid, and Oxacillin for *Staphylococcus aureus,* and Gram-negative drug-bug combinations that showed higher errors are Cefepime – *E. coli* (Table 5).

Error percentages were drastically high for *Elizabethkingia anophelis* (43.8)*, Burkholderia cepacia* Complex (40.0), and *Enterococcus faecalis* (25.0), this should be reconfirmed by increasing the sample size.

4. Discussion

Antimicrobial susceptibility testing (AST) is crucial for guiding the appropriate antimicrobial therapy, especially in bloodstream infections (BSIs) and sepsis.[8] High rates of mortality are linked to sepsis and a key factor in lowering morbidity and mortality is the window of opportunity for efficient antibiotic treatment.[9] The desire to expedite the completion of antimicrobial susceptibility testing stems from the realization that prompt initiation of the correct antibiotic regimen is linked to better outcomes in patients with serious infections and prompt de-escalation minimizes exposure to unduly broad antibiotic therapy.[10]

Antimicrobial stewardship and rapid identification and susceptibility procedures together greatly shortened hospital stays, decreased overall expenditures and expedited the time to optimum therapy.[11] VITEK 2 is a new automated instrument for susceptibility testing and bacterial identification. Investigations revealed that when using pure bacterial cultures, this method might produce trustworthy susceptibility data.[12] Turnaround time (TAT) of the susceptibility testing is further reduced greatly when susceptibility testing is performed immediately from positively flagged blood cultures.[2,8–10,13] This in turn facilitates pathogen-directed antimicrobial stewardship, resulting in significant reductions in patient morbidity, mortality, and financial burden.

VITEK 2 System and lysis centrifugation method along with MALDI-TOF VITEK MS allow identification on the same day and whole panel AST from positive blood cultures. A study by Bazzi A. et al. has shown that lysis centrifugation with reagents such as 30% acetic acid and formalin has identified organisms down to species level with 100% accuracy. It was better than MALDI TOF with colonies from 90 to 180-minute subculture plates.[14] Additionally, it should be noted that transferring organisms from the pellet to the mass spectrometry slide is a skill acquired by experience and contributes to inter-laboratory variations.

In tandem with conventional procedures, Romero-Gomez et al. investigated 6156 isolate/antimicrobial agent combinations utilising direct identification by MALDI-TOF MS and AST by VITEK. There was 96.67% agreement between the Enterobacteriaceae group and non-fermentative Gram-negative Bacilli, and 92.30% agreement between the two groups. The total agreement for cocci that are Gram-positive was 97.84%.[15] This observation contradicts our findings, where Gram-positive cocci showed a categorical agreement of less than 90%. The categorical agreement was demonstrated by Machen et al. for 93.5% (946/1012) of the antimicrobial-microorganism combinations. According to their findings, 1.7% (17/1012) of the antibiotics had a major error and 1.3% (13/1012) had a very major error, which is lesser than our observation.[16] Our combined approach reduces the duration from flagging positive blood culture to reported antimicrobial susceptibility with the lowest turnaround time by 18 hours when compared to standard protocol.

A small sample size of this study, Gram positives and the absence of yeast testing are two of its limitations. The exclusion of polymicrobial infections during the research period is another drawback. To validate our findings, larger-scale investigations in the future should include more isolates, especially more Gram-positive isolates. According to this study, findings of antimicrobial susceptibility testing and microbe identification might be obtained as early as the day a blood culture tested positive, providing information for optimal antibiotic therapy and antibiotic stewardship.

5. Conclusion

In most clinical laboratories, AST is mostly performed by disk diffusion or by automated systems from pure colony growth. Using AST straight from blood culture broth using VITEK-2 is a useful method that can cut turnaround time in half, up to 24 hours in turn aids the medical professional in selecting antibiotics for improved patient care. This study demonstrates the efficacy of the lysis centrifugation method for the direct and quick identification of bacteria and AST in a clinical scenario when used in conjunction with VITEK MS and VITEK 2. In particular, the lysis centrifugation process produces a clean, concentrated sample of microorganisms as a pellet in less than 30 minutes. This pellet can be utilised for full panel AST by VITEK 2 in around 12 hours on average, and for direct identification by VITEK MS in approximately an hour. Thus, same-day ID and full panel AST can be completed in a clinical setting using this method.

6. Ethical statement

This study was permitted by the JIPMER Institute Human Ethics Committee (JIP/IEC/2022/0181), JIPMER, Puducherry, India.

7. Source of Funding

None.

8. Conflicts of Interest

There are no conflicts of interest to declare for any authors listed.

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