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Direct testing by VITEK® 2: A dependable method to reduce turnaround time in Gram-negative bloodstream infections

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Abstract:

CONTEXT: Bloodstream infections pose a major health-care burden worldwide. Routine microbiological methods are time-consuming, thereby delaying appropriate treatment.

AIMS: The aim of this study is to evaluate the method of direct testing of identification (ID) and antimicrobial susceptibility testing (AST) of positive blood culture bottles by VITEK®2.

SETTINGS AND DESIGN: This was a prospective study at a tertiary level hospital.

SUBJECTS AND METHODS: One hundred positive BACTEC blood culture bottles with monomicrobial Gram-negative organisms on microscopy were tested in parallel by direct ID/AST as well as conventional method. Results obtained by two methods were compared in terms of ID/AST and turnaround time (TAT).

RESULTS: Of the 100 isolates tested, only one was misidentified by the direct method and there was no unidentified isolate. The AST results demonstrated 99.74% categorical and 99.65% essential agreement. Of 1144 organism-antibiotic combinations, there were 0.44% major error, no very major error, or minor error observed.

CONCLUSIONS: While conventional method is the gold standard, the direct ID/AST methods have demonstrated that it can be successfully utilized to decrease TAT to the final results by 18–24 h, without sacrificing test accuracy. This technique will help to tailor antimicrobial therapy, thereby reducing patient morbidity, mortality, and antibiotic resistance, as well.

Key words:

Blood culture, direct identification/antimicrobial susceptibility testing, turnaround time

Introduction

Bloodstream infections (BSIs) pose a major health-care burden worldwide. There are limited population-based data available on the epidemiology and outcomes of BSIs. A population-based survey in Finland observed an average annualized 30-day mortality rate of 20.8 deaths per 100,000 population.[1] A significant rate of mortality arises in patients who develop nosocomial BSIs (38%) and especially in patients with underlying malignancies (18%–42%).[2,3] Clinically, bacteremia may be transient and self-limiting or may result in life-threatening septicemia.[4] In the USA, septicemia is the tenth leading cause of death.[5] Furthermore, in developing countries, variations in epidemiology, nonstandardized local antimicrobial guidelines, antimicrobial resistance (AMR), and rudimentary diagnostic facilities are some of the key factors responsible for BSI-associated morbidity and mortality.[6] If not diagnosed and treated promptly and adequately, a treatable BSI may progress

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into sepsis and septic shock. Early BSI mortality is associated with delay in seeking care and delayed and/or inappropriate treatment.[7] Consequently, rapid organism identification (ID) and antimicrobial susceptibility testing (AST) are crucial in the management of patients with BSI.

Optimal laboratory diagnosis of BSI is dependent on number of variables such as volume of blood, time of sampling, prior antibiotic usage, and the monitoring system used. There are many commercial systems available in the market nowadays. However, conventional subculture method for carrying out ID and AST from positive blood culture bottles can take 48–72 h (or longer) to generate a final report as the minimum incubation time required by current automated systems is 2–12 h.[8]

Given the potential that decreasing the time to BSI pathogen ID and AST results could significantly improve outcomes for patients with bacteremia or sepsis, methods have been evaluated for determining ID and AST directly from positive blood culture bottles.[9-12] This study examined the performance characteristics of rapid ID and AST testing from positive blood culture bottles using the VITEK® 2 Compact (bioMérieux, Marcy l'Etoile, France) in combination with its associated turnaround time (TAT), compared to that of conventional ID and AST methods.

Subjects and Methods

This prospective study was conducted between May 2016 and September 2016 in a tertiary care hospital in New Delhi, India. During the study period, blood culture specimens were inoculated in BD BACTEC Plus Aerobic culture bottles and then incubated in the BACTEC™ 9120 (Becton Dickinson, Sparks, Maryland, USA), an automated continuous-monitoring blood culture system. When blood culture bottle(s) signaled positive for growth, the respective bottle(s) were processed for Gram staining. Aliquots from 100 consecutive monomicrobial samples with a Gram-negative pathogen were processed for ID/AST directly from positive blood culture broth on VITEK® 2 (direct method). In the conventional method, traditional subculture with inoculation onto blood and MacConkey agars and incubation at 37°C for 18–24 h was done. The colonies so obtained were processed next day for ID/AST on VITEK® 2 as per manufacturer's instructions and Clinical and Laboratory Standards Institute guidelines.[13,14] Gram-positive and polymicrobial cultures, as determined by Gram stain, were excluded from the study.

Protocol for processing positive blood cultures

Three milliliters of blood culture broth was transferred from the positive blood culture bottle into a serum separator tube (SST, Becton Dickinson) and centrifuged at 3000 rpm (1157 ×*g*) for 5 min. The supernatant was discarded, and normal saline was added to the pellet to make it up to 3 ml in SST. It is mixed by inverting 2–3 times. The tube was then recentrifuged at 3000 rpm for 15 min. Supernatant was again discarded and the pellet now transferred to a VITEK® tube with a sterile swab. Add 3 ml normal saline to it followed by brief vortexing. The turbidity of this inoculum was adjusted using normal saline to 0.5–0.6 McFarland. Turbidity of 0.5–0.6 is recommended for inoculum preparation in VITEK (bioMérieux). The McFarland turbidity was checked by an instrument, DensiCHEK Plus, as per manufacturer's (bioMérieux) guidelines.[13] Then, 145 µl of this inoculum was transferred to another VITEK® tube containing 3 ml normal saline. This second inoculum was used for AST. The inoculum was poured onto VITEK® panels, sealed, and was then loaded in the VITEK® 2 analyzer within 30 min of inoculation. A loop of the suspension was also inoculated onto blood agar for purity check.

Data analysis

Direct ID and AST results were compared with the results obtained from the reference method. The reference method results were used as gold standard for ID as well as AST testing. After comparison, direct ID results were classified into three categories: (i) correctly identified (the organism was correctly identified till species level); (ii) misidentified (organism was incorrectly identified either at genus or species level); and (iii) unidentified (no ID given at all).

The direct AST results of the isolates were also evaluated against that of the conventional AST results in terms of categorical and essential agreements. If results of the direct method were concordant with the reference method, such concordance was recorded as "agreement." Discrepancies in terms of interpretation of the AST results were categorized as: (i) very major error (vmj) if the reference result was resistant (R) and the direct method result was susceptible (S); (ii) major error (maj) if reference result was S and the direct method result was R; and (iii) minor error (min) if reference result was R or S and the new method result was intermediate (I) or vice versa. The essential or minimum inhibitory concentration (MIC) agreement stated that direct method MIC should lie within one 2-fold dilution of the standard method without any discrepancies made in the interpretation for each antibiotic.[15]

Results

Of 100 positive blood cultures that met the inclusion criteria of the study, 83 isolates belonged to the family *Enterobacteriaceae*. The isolates identified are enlisted

in Table 1. Of the 100 isolates tested, only one was misidentified by the direct method and there was no unidentified isolate. The single discrepant ID was an *Achromobacter xylosoxidans* which was misidentified as *Pseudomonas pseudoalcaligenes* by the direct method.

The AST results of all the 100 isolates were compared by the two methods. Overall, the AST results demonstrated 99.74% categorical and 99.65% essential agreement. Of 1144 organism-antibiotic combinations, there were three discrepant categorical results showing a maj of 0.44%. There was no vmj or min observed. In one case, we did not have essential agreement although the AST interpretation was the same for both the methods [Table 2].

The time to ID by VITEK 2 for both the methods was comparable. For the direct method, the average ID time was 5 h 41 min, whereas by the standard method, it was 5 h 21 min. A significant difference was observed between the overall times required for the release of final report. The mean time to final release of report(s) by the direct method was 10 h 36 min, while by standard method, it was 9 h 42 min plus 18–24 h of incubation [Table 3].

Table 1: Results of direct identification compared with standard method

Isolates (standard method)	Tested	Correctly identified	Misidentified
Escherichia coli	33	33	None
Klebsiella pneumoniae	23	23	None
Klebsiella oxytoca	1	1	None
Enterobacter cloacae	1	1	None
Salmonella typhi	20	20	None
Salmonella paratyphi A	5	5	None
Pseudomonas aeruginosa	4	4	None
Pseudomonas putida	1	1	None
Stenotrophomonas maltophilia	1	1	None
Acinetobacter baumannii	6	6	None
Acinetobacter lwoffii	2	2	None
Achromobacter xylosoxidans	1	None	1 (*Pseudomonas pseudoalcaligenes*)
Aeromonas spp.	1	1	None
Sphingomonas paucimobilus	1	1	None

Table 2: Discordant antimicrobial susceptibility results

Antimicrobial agent	Organism	Standard method	Direct method	Type of error
Colistin	*Acinetobacter baumannii*	≤0.5 (S)	4 (R)	maj
Imipenem	*Klebsiella pneumoniae*	0.5 (S)	≥16 (R)	maj
Gentamicin	*Klebsiella pneumoniae*	4 (S)	≥16 (R)	maj
Ceftriaxone	*Escherichia coli*	8 (R)	≥64 (R)	MIC discrepant

MIC = Minimum inhibitory concentration, maj = Major error, S = Susceptible, R = Resistant

However, a small percentage of isolates (4%) took >10 h for ID by the direct method. The longest time taken by the direct method was 14.25 h for a strain of *Klebsiella pneumoniae* compared to 5 h with the reference method [Table 3]. However, it did not affect the total time taken for the final release of result.

From cost standpoint, the direct method was negligibly costlier than the reference method and included the incremental cost of two SSTs or an additional Rs. 10.00 per isolate. However, the hand on procedural time per sample is higher (58 min) in the direct method in comparison to the standard method (30 min).

Selecting and administering an appropriate antibiotic therapy as early as possible can have a major impact for a patient with BSI. By performing the direct method, we could have tailored the antibiotics for patients earlier than what could have been achieved using conventional methodologies. Of 100 patients with Gram-negative infections, 32 (32%) were susceptible to carbapenems. Forty-three (43%) needed escalation to colistin which would have been possible at least 18–24 h earlier by the direct method. No alteration of therapy was needed for the 25 cases of *Salmonella* infections as they were already managed as cases of enteric fever according to the clinical features.

Discussion

Over the years, managing patients with BSIs has become increasingly more difficult for health-care professionals, primarily due to the emerging threat of AMR.[16] The Center for Disease Dynamics, Economics and Policy has reported carbapenem resistance rates in *Klebsiella* spp. as high as 57%–60% in India.[17] Consequently, a timely etiological diagnosis and AST profile are paramount, especially in the context of life-threatening illnesses such as BSI, and continue to be a cornerstone for curtailing AMR by allowing more rapid and precise targeted therapy.[17] Therefore, the provision of rapid and reliable diagnostic information in the context of BSI is not only vital for improving patient outcomes in limiting the mortality but also documents a significant reduction in TAT.[18]

While conventional ID and AST method serves as the gold standard, we have demonstrated that the direct method can successfully be utilized to decrease TAT to the final results by 18–24 h, without sacrificing test accuracy. We have correctly identified 99% of the pathogens by direct ID from the blood culture bottle, as compared to the reference method. Only one isolate was misidentified by the direct method; however, it did not affect the choice of antibiotic therapy. Our reported ID rate compares favorably with other studies that have investigated the

Table 3: Generation time to identification by direct and standard methods

	Direct ID from bottle	Total time taken to release final report	Standard method (ID from growth)*	Total time taken to release final report
	5 h 41 min	10 h 36 min	5 h 21 min	9 h 42 min + additional 18-24 h of incubation
Isolates which took longer time to ID by direct method				
Salmonella typhi	10 h 25 min	10 h 25 min	5 h	9.25 h + additional 18-24 h of incubation
Acinetobacter lwoffii	10 h 25 min	15 h 50 min	8 h	17.75 h + additional 18-24 h of incubation
Achromobacter xylosoxidans	10 h 25 min	17 h	6 h 45 min	18 h + additional 18-24 h of incubation
Klebsiella pneumoniae	14 h 25 min	14 h 25 min	5 h	12 h + additional 18-24 h of incubation

*Generation time to ID by standard method includes the subculture incubation period (i.e., approximately 18-24 h). ID = Identification

direct ID and AST approach from positive blood culture bottles using VITEK® 2 Compact.[9-11]

With regard to AST performance, 99.74% showed complete categorical agreement while we reported 0.44% maj with no min or vmj. In addition, we observed a single MIC discrepancy (essential nonagreement); however, the categorical interpretation was consistent between the two methods. Two of the three maj were derived from a single clinical isolate of *K. pneumoniae* with false resistance reported by the direct method for both imipenem and gentamicin. The other maj was observed with an isolate of *Acinetobacter baumannii* which tested falsely resistant to colistin. These could be due to random error or technical deviation; the isolate was not retested by the direct method, and the results of standard method were accepted as final.

In a study conducted by de Cueto *et al*. utilizing VITEK® 2 for direct ID and AST for Gram-negative bacilli from positive blood cultures, an overall 6.6% error rate with 2.4% vmj, 0.6% maj, and 3.6% min was observed.[11] In a similar study, an overall 99.2% MIC agreement was reported with 0.02% maj and 0.8% vmj.[10] In selecting a test system for ID, it has been recommended that overall agreement with reference test system should be at least 90%, and with respect to common isolates (e.g., *Enterobacteriaceae*), at least 95% accuracy should be obtained. In addition, error rates of less than 10% should be obtained for an acceptable AST performance, including <1.5% vmj and <3% maj.[8] With an error rate of 1% and 0.44% for ID and AST, respectively, our results are well within acceptable limits.

Our protocol for the direct method is distinct from other published studies using the VITEK® system.[9-11] While most of the authors have used the supernatant for testing, our approach was to utilize the pellet, washed with sterile saline, and recentrifuged to prepare the 0.5–0.6 McFarland suspension.[9,11] This additional step may have improved the quality and standardization of the inoculum and clean the pellet of any cellular elements which may hamper the test technique. Thus, this study has recorded better and accurate results than most of the published reports, so far.[9-11]

While the direct method does incur minimal additional expense and increased procedural time, it has clearly indicated the potential benefits for patient care. Furthermore, this practice would enhance stewardship efforts related to combating AMR at our hospital and the larger community. Despite this, cost savings are potentially procured from the de-escalation of empirical broad-spectrum antimicrobial therapy, thereby reducing the pharmacological costs and duration of hospital stay. Moreover, it would also help in bringing down the emerging AMR.

The average time taken from blood culture positivity to final release of AST in the direct method is 10 h 36 min (range 9–18 h), whereas it requires additional 18–24 h more by the standard method, i.e., around 35 h. Hence, it takes 18–24 h lesser than the standard method as it eliminates the need of subculturing followed by overnight incubation on a solid media. TAT is the unique selling proposition of the direct method.

The current study did not include Gram-positive or yeast isolates; hence, we do not recommend the direct ID and AST method for the same purpose as additional method validation is needed before adapting the technique. Another limitation of this approach is that it cannot be used for mixed isolates.

Many emerging technologies and platforms such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or multiplexing molecular techniques have recently become available for obtaining rapid and accurate ID and AST information for BSI etiologies. While these methods have the advantage of quicker TAT and improved sensitivity, these platforms are more expensive and may not be economically viable for some laboratories.[19,20] The direct ID and AST method by VITEK® 2, on the other hand, is economical, has reduced TAT, and has proven to be a reproducible and reliable alternative for our laboratory. Finally, adoption of this technique will continue to see opportunities to tailor antimicrobial therapy for BSI and thus reduce patient morbidity and mortality and antibiotic resistance, as well.

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Conflicts of interest
There are no conflicts of interest.

References

1. Skogberg K, Lyytikäinen O, Ollgren J, Nuorti JP, Ruutu P. Population-based burden of bloodstream infections in Finland. Clin Microbiol Infect 2012;18:E170-6.
2. Girão E, Levin AS, Basso M, Gobara S, Gomes LB, Medeiros EA, *et al*. Trends and outcome of 1121 nosocomial bloodstream infections in Intensive Care Units in a Brazilian hospital, 1999-2003. Int J Infect Dis 2008;12:e145-6.
3. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis 2001;33:947-53.
4. Munford RS, Suffredini AF. Sepsis, severe sepsis, and septic shock. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principle and Practice of Infectious Diseases. 7th ed. Philadelphia: Churchill Livingstone-Elsevier; 2010. p. 987-1010.
5. Yu J, Kochanek KD, Murphy SL, Tejada-Vera B. Deaths: Final data for 2006. Natl Vital Stat Rep 2009;57:1-134.
6. Obeng-Nkrumah N, Labi AK, Addison NO, Labi JE, Awuah-Mensah G. Trends in paediatric and adult blood-stream infections at Ghanaian referral hospital: A retrospective study. Ann Clin Microbiol Antimicrob 2016;15:49.
7. 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.
8. Ferraro MJ, Jorgensen JH. Susceptibility testing instrumentation and computerized expert systems for data analysis and interpretation. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of Clinical Microbiology. Washington, DC: American Society for Microbiology; 1999. p. 1593-600.
9. 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;41:4705-7.
10. Bruins MJ, Bloembergen P, Ruijs GJ, Wolfhagen MJ. Identification and susceptibility testing of *Enterobacteriaceae* and *Pseudomonas aeruginosa* by direct inoculation from positive BACTEC blood culture bottles into vitek 2. J Clin Microbiol 2004;42:7-11.
11. de Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascual A. Use of positive blood cultures for direct identification and susceptibility testing with the vitek 2 system. J Clin Microbiol 2004;42:3734-8.
12. Hansen DS, Jensen AG, Nørskov-Lauritsen N, Skov R, Bruun B. Direct identification and susceptibility testing of enteric bacilli from positive blood cultures using VITEK (GNI+/GNS-GA). Clin Microbiol Infect 2002;8:38-44.
13. VITEK® 2 Systems Product Information Manual. Durham (NC): bioMérieux, Inc; 2016.
14. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard, Twenty-Sixth CLSI Document M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
15. Food and Drug Administration. Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA. U.S. Department of Health and Human Services; 2009. Available from: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm. [Last accessed on 2017 Jun 02].
16. Penno EC, Baird SJ, Crump JA. Cost-effectiveness of surveillance for bloodstream infections for sepsis management in low-resource settings. Am J Trop Med Hyg 2015;93:850-60.
17. Gelband H, Miller-Petrie M, Pant S, Gandra S, Levinson J, Barter D, *et al*. The State of the World's Antibiotics 2015. Center for Disease Dynamics, Economics and Policy. Washington, DC: CDDEP; 2015.
18. Kerremans JJ, Verboom P, Stijnen T, Hakkaart-van Roijen L, Goessens W, Verbrugh HA, *et al*. Rapid identification and antimicrobial susceptibility testing reduce antibiotic use and accelerate pathogen-directed antibiotic use. J Antimicrob Chemother 2008;61:428-35.
19. Huang AM, Newton D, Kunapuli A, Gandhi TN, Washer LL, Isip J, *et al*. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis 2013;57:1237-45.
20. Rubach MP, Hanson KE. ID learning unit-diagnostics update: Current laboratory methods for rapid pathogen identification in patients with bloodstream infections. Open Forum Infect Dis 2015;2:ofv174.