Evaluation of the Performance of Direct Susceptibility Test by VITEK-2 from Positively Flagged Blood Culture Broth for Gram-Negative Bacilli

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Abstract

Background Timely initiation of antimicrobial therapy in patients with bloodstream infection is absolutely necessary to reduce mortality and morbidity. Most clinical microbiology laboratories use conventional methods for identification and antimicrobial susceptibility testing (AST) that involve biochemical methods for identification followed by AST by disk diffusion. The aim of the current study is to assess the various errors associated with direct susceptibility testing done from blood culture broth using automated AST system-Vitek-2 compact compared with the reference method of AST done from bacterial colonies.

Materials and Methods The study was conducted in a tertiary care public sector 2,200-bedded hospital in South India for a period of 6 months. The study involved positively flagged blood culture bottles that yielded single morphotype of Gram-negative organism by Gram stain. A total of 120 bacterial isolates were collected that consisted of consecutively obtained first 60 isolates of Enterobacteriaceae family (30 Escherichia coli and 30 Klebsiella pneumoniae) and consecutively obtained first 60 nonfermenters (30 Pseudomonas aeruginosa and 30 Acinetobacter baumannii). Vitek-2 AST was done from these 120 blood culture broth, following the protocol by Biomerieux, and results were obtained. Then, Vitek-2 was done from colonies (reference method) using appropriate panel for Enterobacteriaceae and nonfermenters, and results were obtained. Both the results were compared.

Results Nonfermenters showed a better categorical agreement of 97.6%, as compared to Enterobacteriaceae, which showed 97%. Among Enterobacteriaceae, both E. coli and K. pneumoniae showed categorical agreement of 97% each.

Conclusion The procedure of AST directly from blood culture broth represents a simple and effective technique that can reduce the turnaround time by 24 hours, which in turn benefits the clinician in appropriate utilization of antimicrobials for better patient care.

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Introduction

Bloodstream infections (BSIs) are medical emergencies, which can cause serious morbidities as well as mortalities. BSIs are termed as the third leading cause of health care-related infections.1 The gold standard diagnostic method for BSI is culturing the causative organism from blood and providing the antimicrobial susceptibility testing (AST) result for the same.2 This will help in timely initiation of antimicrobial therapy. But, prolonged laboratory turnaround time (TAT) can increase mortality, time of hospital stay as well as cost of therapy. In the currently used conventional methods, empirical therapy can be started based on the Gram staining done from positive blood culture bottles.3,4 And final confirmatory results are based on biochemical identification and ASTs done from colonies grown on subculture and targeted therapy is given accordingly. As the whole process takes 48 hours or even longer, the need for a robust diagnostic method arises. With every hour of delay in initiating appropriate antimicrobial agents for sepsis, there is an increase in mortality by 7.6%.5

Novel molecular methods and rapid phenotypic methods can help in faster identification, but lack the ability to identify broad range of pathogens and providing antibiotic susceptibility. Moreover, the hands-on processing time as well as monetary investments are high. So, still blood culture remains as the gold standard. Doing direct susceptibility testing (DST) on positive blood culture broth can expedite AST, at least by 24 hours. Since 1980s there have been a lot of studies aiming to standardize DST from blood culture using disc diffusion, automated and molecular testing.6-9 But the categorical error varies in different methods. DST methods are shown to be feasible from previous studies as there is good correlation with reference methods. But disc diffusion done directly from broth showed mixed results.9 And final confirmatory results are based on biochemical identification and ASTs done from colonies grown on subculture and targeted therapy is given accordingly. As the whole process takes 48 hours or even longer, the need for a robust diagnostic method arises. With every hour of delay in initiating appropriate antimicrobial agents for sepsis, there is an increase in mortality by 7.6%.

Our study aims to assess the various errors associated with DST done with automated blood culture system compared with the reference method of AST that is done from bacterial colonies.

Methodology

Study Isolates

The study was conducted in a tertiary care public sector 2,200-bedded hospital in South India from March 2018 to August 2018 (6 months). All the positive aerobic blood cultures (BACT/ALERT® VIRTUO® bioMérieux, United States; aerobic) from patients suspected of having BSIs were subjected to Gram staining. Bottles showing Gram-negative organisms on Gram stain were included for analysis. Repeated isolates from the same patient’s blood culture, positive blood specimens with more than one organism in Gram smear, positive blood specimens that yielded more than one isolate after subculture, positive blood specimens whose direct Gram staining do not correlate with the growth on culture media, and positive blood specimens that yielded fastidious bacteria or yeast were excluded from the study.

Procedure

Once the blood culture bottle (BacTalert aerobic) flagged positive, it was taken out and proceeded with Gram staining. If the Gram staining was showing single morphotype of Gram-negative bacilli, the culture broth was subcultured in blood agar and MacConkey agar. After incubation for 8 hours, colonies from 5% sheep blood agar was subjected to matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) for identification. Then the blood culture broth was inoculated into the Vitek-2 system. For inoculation of blood sample into the Vitek-2, the following procedures were performed.

Aspirate 8 to 15 mL blood sample from the blood culture bottle to 15 mL of gel containing tube or falcon tube. Centrifuge the tube 3,500 to 4,000 rpm for 20 minutes, using the supernatant, and make suspension in a sterile saline, McFarland range from 0.5 to 0.63 in one tube. Respective AST card was inserted based on the identification by MALDI-TOF MS (fermenter-card no. 280; nonfermenter-card no. 281) to the other tube and loaded into the Vitek-2 instrument within 20 minutes (ideally, after inoculum prepared in ≤ 30 minutes, it has to be loaded). Result was available after 8 to 16 hours.

Antibiotics for fermenter panel included were (card no. 280) ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, cefuroxime axetil, ceftriaxone, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, nalidixic acid, ciprofloxacin, tigecycline, nitrofurantoin, colistin, and trimethoprim/sulfamethoxazole.

Antibiotics for nonfermenter panel included were (card no. 281) ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, minocycline, tigecycline, colistin, and trimethoprim/sulfamethoxazole.

Based on the reports, comment has been given by the artificial intelligence data present in the system comparing with Clinical and Laboratory Standards Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Results of the DST by Vitek were compared with the AST results obtained by again doing Vitek from colonies grown on subculture plate.

The performance of DST by Vitek as compared with reference (colony) AST by Vitek was expressed in terms of categorical agreement and categorical disagreement according to ISO 20776–2:2007 guidelines.11 Whenever a test method yielded same susceptibility category as that of reference method, it is said to be categorically agreed. The categorical disagreement was further characterized into minor error (mE), major error (ME), and very major error (VME). When result in one method is intermediate and other method is susceptible or resistant, it is said to be mE. When reference method yields resistant category and test method yields susceptible category, this is called as very ME. When reference method result is susceptible and test method result is
resistant, it is said to be ME. Essential agreement for an isolate meant that minimum inhibitory concentrations (MICs) obtained for the test direct blood culture method was within ±1 2-fold dilution of the currently used routine method. All collected data were entered into Microsoft Excel sheet. Analysis of data was performed using SPSS software. Our study was funded by Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER) Intramural fund.

**Results**

One twenty positively flagged blood cultures during the study period were included in the study. For analysis to be valid, a minimum of 30 isolates should be included. So, 60 isolates in Enterobacteriaceae family were included, which comprise 30 *Escherichia coli* and 30 *Klebsiella pneumoniae*. Also, 60 nonfermenters were included, with 30 *Pseudomonas aeruginosa* and 30 *Acinetobacter baumannii*.

As explained in Table 1, nonfermenters showed a better categorical agreement of 97.6% compared with Enterobacteriaceae, which showed 97%. Among Enterobacteriaceae, both *E. coli* and *K. pneumoniae* showed categorical agreement of 97% each. Nonfermenters behaved in a different way. *P. aeruginosa* showed an excellent categorical agreement of 99.4%, whereas *A. baumannii* showed 95.8% which was least among all the four.

Assessment of categorical disagreement was also done. ME was highest in *A. baumannii* (3.8%), ME and VME were highest in *K. pneumoniae* (1.2 and 0.5%, respectively). Essential agreement was 97.1%. *P. aeruginosa* showed an excellent essential agreement of 99.4%, whereas *A. baumannii* showed maximum essential disagreement, (4.2%), among the isolates tested.

For selective reporting of antibiotics, analysis of DST and AST of individual antibiotics should be done. Among Enterobacteriaceae, most of the antibiotics in the Vitek panel showed an excellent categorical agreement > 95% except piperacillin/tazobactam and nitrofurantoin that showed only 93.3 and 85.0%, respectively. Categorical disagreement was more than acceptable level (> 3%) in mE for amoxicillin/clavulanic acid (3.3%), piperacillin/tazobactam (5.0%), imipenem (3.3%), and nitrofurantoin (13.3%). ME and VME were showing acceptable levels of categorical disagreement (< 3%). Essential disagreement of > 3% was seen in piperacillin/tazobactam (6.7%), imipenem (5.0%), meropenem (3.4%), amikacin (3.3%), gentamicin (3.3%), ciprofloxacin (3.4%), and nitrofurantoin (15.0%) as shown in Table 2.

Nonfermenters also showed excellent categorical agreement > 95% except levofloxacin (91.7%) and minocycline (90.0%). ME and VME showed excellent categorical agreement > 97%, but mE showed categorical disagreement > 3% for ticarcillin/clavulanic acid (95.0%), levofloxacin (8.3%), minocycline (10.0%), and tigecycline (3.3%) as shown in Table 3.

**Discussion**

Rapid AST for invasive infections like BSI is the need of the hour. Even though molecular mechanisms of resistance to different antibiotics can be detected by nucleic acid amplification tests, the practical utility is less. On a clinical perspective, rapid identification of antibiotics to which organism is susceptible is more important to modify the empirical antibiotics. Most of the studies were done based on disk diffusion method directly from blood culture broth and other clinical samples such as urine, bile, and respiratory samples. Here comes the importance of an automated AST system with software and disposable reagent cards like Vitek that has shorter TAT and gives MIC values also. There are only few studies, conducted on this idea, which implemented laborious workflow involving bacterial pellets as extra step. We have used supernatant after centrifugation as bacterial inoculums. And also most of the similar studies have used AST-N020, AST-202, AST-N009 Vitek cards. We have used card no. N280 for fermenter organism and card no. N281 for nonfermenter organism.

**Table 1** Performance of direct DST test compared with reference (colony) AST method test by automated VITEK-2 System

| Organisms and antibiotic tested \n\(n \times Ab = N\) | Categorical agreement, \(n (\%)\) | Categorical disagreement, \(n (\%)\) among isolate-antibiotic combinations tested | Essential agreement |
|-----------------------------|---------------------------------|---------------------------------------------------------------------------------|-------------------|
|                             | Minor                          | Major                            | Very major         | Total              | Agreed | Disagreed |
| Enterobacteriaceae \(60 \times 18 = 1080\) | 1048 (97.0%)                  | 16 (1.4%)                        | 12 (1.1%)          | 4 (0.4%)           | 32 (3.0%) | 1,044 (97.7%) | 36 (3.3%) |
| *Escherichia coli* \(30 \times 18 = 540\) | 524 (97.0%)                   | 10 (1.8%)                        | 5 (0.9%)           | 1 (0.1%)           | 16 (3.0%) | 521 (96.4%) | 19 (3.6%) |
| *Klebsiella pneumoniae* \(30 \times 18 = 540\) | 524 (97.0%)                   | 18 (11.1%)                       | 7 (1.2%)           | 3 (0.5%)           | 16 (3.0%) | 523 (96.7%) | 17 (3.3%) |
| Nonfermenters \(60 \times 15 = 900\) | 878 (97.6%)                   | 19 (2.1%)                        | 2 (0.2%)           | 1 (0.1%)           | 22 (2.4%) | 878 (97.6%) | 22 (2.4%) |
| *Pseudomonas aeruginosa* \(30 \times 15 = 450\) | 447 (99.4%)                   | 2 (0.4%)                         | 1 (0.2%)           | 0 (0.0%)           | 3 (0.6%) | 447 (99.4%) | 3 (0.6%) |
| *Acinetobacter baumannii* \(30 \times 15 = 450\) | 431 (95.8%)                   | 17 (3.8%)                        | 1 (0.2%)           | 1 (0.2%)           | 19 (4.2%) | 431 (95.8%) | 19 (4.2%) |

Abbreviations: AST, antimicrobial susceptibility testing; DST, direct susceptibility testing.
organism that were easily available and routinely used card in most of the laboratories.

Barnini et al.\(^{15}\) conducted similar study in Italy in 2014, where they used MALDI-TOF MS for identification and AST by Vitek-2. But their accordance of direct AST result with culture AST was less than our study. Usage of serum separator tubes or short-term subculture in liquid medium may be the reason for that. In Belgium, Maelegheer and Nulens\(^{16}\) did similar study in 2016, where they got a better concordance of 98.9% than our study. They microcentrifuged positive blood sample and MALDI-TOF identification and AST by BD Phoenix was performed. Study by Mauri et al.\(^{17}\) showed an excellent categorical agreement of 98.1% and essential agreement of 97.7%. They used MALDI-TOF mass spectrometry identification and automated AST.

Among the isolates tested, \textit{A. baumannii} showed maximum essential disagreement (4.2%). mE was highest in \textit{A. baumannii}, whereas ME and VME were highest in \textit{K. pneumoniae}, but they were within the acceptable range (< 3%). But in study by Mauri et al, ME and VME were not reported. Pan et al showed less essential agreement (< 95%) for meropenem, imipenem, cefepime, ceftriaxone, and ceftazidime, which was not reflected in our study.\(^{18}\)

In this study we demonstrated that Vitek-2 can be used to provide rapid, highly accurate susceptibility reports directly from positive blood cultures with Enterobacteriaceae and nonfermenters. The advantages of using Vitek include widespread availability in majority of laboratories around the globe, so that the need for additional equipment and facilities can be circumvented. Less hands-on time and low additional cost are the added advantages. Moreover, there is no need of dedicated manpower to perform the test.\(^{19}\) In a study conducted by Höring et al, comparing Vitek-2 and BD Phoenix for direct blood culture inoculation, Vitek-2 demonstrated higher test accuracy.\(^{20}\)

Finally, in addition to the high performance for rapid AST, our method reduced the TAT also considerably.

### Table 2 Performance of direct DST test compared with reference (colony) AST method test for Enterobacteriaceae by VITEK-2 system

| Enterobacteriaceae (60) | Categorical agreement, n (%) | Categorical disagreement n (%) | Essential agreement |
|-------------------------|-----------------------------|--------------------------------|---------------------|
|                         | Minor | Major | Very major | Total | Agreed | Disagreed |
| Ampicillin              | 60 (100.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 60 (100.0%) | 0 (0.0%) |
| Amoxicillin/clavulanic acid | 58 (96.7%) | 2 (3.3%) | 0 (0.0%) | 2 (3.3%) | 58 (96.7%) | 2 (3.3%) |
| Piperacillin/tazobactam | 56 (93.3%) | 3 (5.0%) | 1 (1.7%) | 4 (6.7%) | 56 (93.3%) | 4 (6.7%) |
| Cefuroxime              | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Cefuroxime axetil       | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Ceftriaxone             | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Cefoperazone/sulbactam  | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Cefepime                | 59 (98.3%) | 0 (0.0%) | 0 (0.0%) | 1 (1.7%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Imipenem                | 57 (95.0%) | 2 (3.3%) | 1 (1.7%) | 3 (5.0%) | 57 (95.0%) | 3 (5.0%) |
| Meropenem               | 58 (96.6%) | 0 (0.0%) | 1 (1.7%) | 2 (3.4%) | 58 (96.6%) | 2 (3.4%) |
| Amikacin                | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 1 (1.7%) | 58 (96.7%) | 2 (3.3%) |
| Gentamicin              | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 58 (96.7%) | 2 (3.3%) |
| Nalidixic acid          | 60 (100.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 60 (100.0%) | 0 (0.0%) |
| Ciprofloxacin           | 58 (96.6%) | 1 (1.7%) | 1 (1.7%) | 0 (0.0%) | 2 (3.4%) | 58 (96.6%) | 2 (3.4%) |
| Tigecycline             | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Nitrofurantoin          | 51 (85.0%) | 8 (13.3%) | 0 (0.0%) | 1 (1.6%) | 9 (15.0%) | 51 (85.0%) | 9 (15.0%) |
| Colistin                | 59 (98.3%) | 0 (0.0%) | 1 (1.7%) | 0 (0.0%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |
| Trimethoprim/sulfamethoxazole | 59 (98.3%) | 0 (0.0%) | 0 (0.0%) | 1 (1.7%) | 1 (1.7%) | 59 (98.3%) | 1 (1.7%) |

Abbreviations: AST, antimicrobial susceptibility testing; DST, direct susceptibility testing.
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Table 3 Performance of direct test compared with reference (colony) test for nonfermenters by VITEK-2 system

| Nonfermenter (60) | Categorical agreement (%) | Categorical disagreement (%) | Essential agreement |
|-------------------|---------------------------|-----------------------------|---------------------|
|                    | Minor         | Major         | Very major       | Total         |
| Tigecycline        | 57 (95.0%)    | 1 (1.7%)      | 0 (0.0)          | 59 (98.3%)    |
| Ciprofloxacin      | 59 (98.3%)    | 0 (0.0)       | 1 (1.7%)         | 59 (98.3%)    |
| Ceftazidime        | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Cefoperazone/sublactam | 59 (98.3%) | 1 (1.7%)      | 0 (0.0)          | 59 (98.3%)    |
| Ticarcillin/clavulanic acid | 57 (95.0%) | 0 (0.0)       | 1 (1.7%)         | 59 (98.3%)    |
| Piperacillin/tazobactam | 59 (98.3%) | 0 (0.0)       | 0 (0.0)          | 59 (98.3%)    |
| Doripenem         | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Amikacin          | 59 (98.3%)    | 0 (0.0)       | 1 (1.7%)         | 59 (98.3%)    |
| Gentamicin        | 59 (98.3%)    | 1 (1.7%)      | 0 (0.0)          | 59 (98.3%)    |
| Colistin          | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Imipenem          | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Meropenem         | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Imipenem          | 60 (100.0%)   | 0 (0.0)       | 0 (0.0)          | 60 (100.0%)   |
| Ciprofloxacin      | 59 (98.3%)    | 1 (1.7%)      | 0 (0.0)          | 59 (98.3%)    |

Conclusion
The new procedure of doing direct AST from blood culture represents a simple, effective technique that can reduce the TAT by 24 hours, which in turn benefits the patient outcome by reducing the mortality, time to de-escalation, length of stay, and antibiotic-related side effects like Clostridium difficile infection.

Conflict of Interest
None.

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