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Comparative Evaluation of Antibiotic Susceptibility Testing on Vitek-2 Compact and Direct Sensitivity Test from Blood Cultures from a Tertiary Care Centre in South India

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Bloodstream infections (BSIs), recognized to be a major cause of morbidity and mortality globally, are increasing in incidence. India currently tackles an estimated 7,50,000 cases of BSI every year which includes 2% of hospitalized patients and 70% of patients admitted in the Intensive Care Unit. The associated crude mortality rate is 14-57%. Blood culture samples were subjected simultaneously to susceptibility testing by Direct Sensitivity Test (DST) by disk diffusion method and Antibiotic Sensitivity Test (AST) by Vitek-2 Compact (BioMerieux) a reference method from positive blood cultures flagged by BacT/ALERT 3D System, with the culture bottles FA and PA was used for blood culture. All the blood cultures flagged positive by BacT ALERT 3D system were included in the study. A total of 102 positive blood cultures showing monomicrobial gram-positive cocci or gram-negative bacilli identified after doing a Gram's stain, were taken for further testing. A total of 102 blood cultures yielding mono-microbial bacterial growth were evaluated in this study. Organisms belonging to the family Enterobacteriaceae accounted for 41.2% of the isolates (42/102) followed by Staphylococcus spp. giving 40.2% of the isolates (41/102). E. coli and Klebsiella spp. were the commonest Gram negative isolates. These data suggest that VITEK 2 cards inoculated with samples taken directly from positive Bact/ALERT blood culture bottles would provide acceptable antimicrobial susceptibility testing results for Gram-negative bacilli, but not for Gram-positive cocci. Compared to the reference method, the direct method would reduce turnaround time by at least 24 h.

Keywords: Bloodstream infections, Blood culture, Direct Sensitivity Test.

Bloodstream infections (BSIs), recognized to be a major cause of morbidity and mortality globally, are increasing in incidence. India currently tackles an estimated 7,50,000 cases of BSI every year which includes 2% of hospitalized patients and 70% of patients admitted in the Intensive Care Unit. The associated crude mortality rate is 14-57%.[1] The standard method to detect infectious agents in positive blood cultures involves overnight agar medium subcultures from positive blood culture bottles in order to recover the amount of cells needed for species identification and antimicrobial susceptibility profiling in automated systems. In order to decrease the time needed to obtain identification and susceptibility results, several studies have performed direct inoculation from positive blood cultures into a variety of automated systems.[2-6] With these methods, results could be obtained the same day that the bottles become positive, about 24 h earlier than those obtained with the standard procedure. Some studies suggest that automated systems (cards/panels) inoculated with samples taken directly from positive blood culture bottles provide acceptable identification of Gram negative bacilli but not for Gram positive cocci, whereas susceptibility testing by disk

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diffusion method was applicable for both types.[5-8] Antibiotic treatment for moderate to severe bacterial infections is started early and empirically, before the pathogen and its susceptibilities to antibiotics are known. Approximately one-third of patients with bacterial infections are given inappropriate empirical antibiotic treatment. Although some have demonstrated that inappropriate antibiotic treatment is an independent risk factor for mortality, others did not find appropriate antibiotic treatment to be associated with a significant benefit in terms of survival.[9,10]

MATERIAL AND METHODS

The study was conducted at the Department of Microbiology of a tertiary health care centre between June and July 2016. Blood culture samples which were provided to our hospital Microbiology Laboratory as part of the routine culture and sensitivity testing work were taken and the study was approved by the Institutional Ethics Committee. These blood samples were subjected simultaneously to susceptibility testing by Direct Sensitivity Test (DST) by disk diffusion method and Antibiotic Sensitivity Test (AST) by Vitek-2 Compact (BioMerieux) a reference method from positive blood cultures flagged by BacT/ALERT 3D System, with the culture bottles FA (Adult) and PA (Pediatric) was used for blood culture. Bacterial growth in the bottles was detected through continuous monitoring of the carbon dioxide level. All the blood cultures flagged positive by BacT ALERT 3D system were included in the study. All poly-microbial positive blood cultures were excluded. A total of 102 positive blood cultures showing monomicrobial gram-positive cocci or gram-negative bacilli identified after doing a Gram's stain, were taken for further testing.

Direct Sensitivity Test (DST) by disk diffusion method

Blood culture broth was added to sterile saline, to make the suspension equivalent to a 0.5 McFarland standard, this dilution showed a semi-confluent growth on Mueller-Hinton agar plates used for disk diffusion testing after incubation at 37°C for 18-20 hrs. The following discs were used for Gram positive cocci: Penicillin, Cefoxitin, Erythromycin, Clindamycin, Vancomycin, Gentamicin, Ciprofloxacin, Cotrimoxazole, Tetracycline and Linezolid. For gram negative bacilli, the following discs were tested: Ampicillin, Gentamicin, Amikacin, Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime, Ciprofloxacin, Cotrimoxazole, Imipenem, Meropenem, Ertapenem and Nalidixic acid. Zone inhibition diameters was interpreted as Sensitive (S), Intermediate (I) and Resistant (R) as per CLSI guidelines 2016.[11] DST reports were available in 18-20 hrs. Control: Quality control strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were tested weekly by the AST by disk diffusion method.

Antibiotic Sensitivity Test (AST) by Vitek-2 Compact

This method was our reference method for comparison. One drop of the broth was routinely subcultured on 5% sheep blood agar and MacConkey agar. Both plates were incubated at 35°C for 18-20 hours to obtain isolated colonies. An isolated colony was picked and added to sterile saline solution provided by the manufacturer BioMerieux to make a suspension equivalent to a 0.5 McFarland standard, adjusted by using a DensiCHEK Plus (BioMerieux) and further processed as per the manufacturer's instruction. AST panel AST-P628 was used for gram positive organisms and AST-N280 for gram negative organisms. Result was given as Sensitive (S), Intermediate (I) and Resistant (R).

RESULTS

A total of 102 samples both gram-negative bacilli and gram-positive cocci isolates were compared for DST and AST on VITEK-2. The list of organisms and their number with percentages is shown in table 1. Majority of the isolates were from Enterobacteriaceae family with 42 (41.18%) with split of different Enterobacteriaceae given followed by *Staphylococcus* spp. 41 (40.20%), *Acinetobacter* spp. 10 (9.80%), *Pseudomonas* spp. 7 (6.86%) and *Enterococcus* spp. 2 (1.96%).

A total of 2424 microorganism antimicrobial combinations were analyzed. Antibiotic susceptibility testing by Direct Sensitivity Test and Vitek-2 Compact showed agreement for 2060 (84.98%), Very major error

for 232 (9.57%), Major error for 72 (2.97%) and Minor error for 60 (2.47%).

In Enterobacteriaceae (n=42), maximum very major errors were found in Gentamicin 14 (33.3%), Imipenem 7 (16.7%), Piperacillin/ Tazobactam & Ceftazidime 5 (11.9%), other antibiotics showed less than 10%. Major error was seen maximum in Meropenem 13 (31%) and Maximum minor error was seen in Piperacillin/ Tazobactam 6 (14.3%) table 2.

In *Pseudomonas* spp. (n=7), very major error was found in one antibiotic Levofloxacin 1(14.3%), major error was seen in Meropenem & Ceftazidime 1(14.3%) and minor error was seen in Meropenem 1(14.3%) table 3.

In *Acinetobacter* spp. (n=10), very major error was found maximum in Cefepime & Gentamicin 3(30%), Imipenem & Amikacin 2(20%) and Ciprofloxacin 1(10%), major error was seen in Ceftazidime 1(10%) and minor error was seen in Ciprofloxacin 2(20%) table 4.

In *Staphylococcus* spp. (n=41), very major error were found maximum in Ciprofloxacin 15(36.6%), Penicillin 14(34.1%), Erythromycin & Gentamicin 8(19.5%) and Clindamycin 7(17.1%) whereas other antibiotics showed less than 10%. Major error was seen maximum in Cotrimoxazole 5(12.2%). Other antibiotics showed less than 10% and maximum minor error was seen in Erythromycin 5(12.2%) table 5.

In *Enterococcus* spp. (n=2), no error was seen table 6.

Table 1. Organisms isolated (*n* =102)

Organisms	Number of Isolates	%
Enterobacteriaceae family	42	41.18
-*Escherichia coli*	18	42.36
-*Klebsiella spp.*	13	30.95
-*Citrobacter spp.*	01	02.38
-*Salmonella spp.*	05	11.90
-*Enterobacter spp.*	04	09.52
-*Edwardsiella spp.*	01	02.38
Pseudomonas spp.	07	06.86
Acinetobacter spp.	10	09.80
Staphylococcus spp.	41	40.20
Enterococcus spp.	02	01.96

DISCUSSION

Antibiotics are the greatest gift of the 20th century. A lot of natural, modified or synthetic chemicals have been developed over the last seven to eight decades for the treatment of infections. However, antibiotics are traditionally the secretory products of micro-organisms that are active in high dilutions against other micro-organisms. Therapeutic use of antibiotics began during World War II. A plethora of new antibiotics were

Table 2. Organisms with antibiotics (Enterobacteriaceae)

Enterobacteriaceae (n=42)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Ampicillin	42 (100)	0 (0)	0 (0)	0 (0)
Gentamicin	26 (61.9)	2 (4.8)	0 (0)	14 (33.3)
Amikacin	37 (88.1)	1 (2.4)	0 (0)	4 (9.5)
Amoxicillin/Clavulanic acid	39 (92.9)	2 (4.8)	0 (0)	1 (2.4)
Piperacillin/Tazobactam	31 (73.8)	6 (14.3)	0 (0)	5 (11.9)
Ceftazidime	37 (88.1)	0 (0)	0 (0)	5 (11.9)
Cefotaxime	39 (92.9)	1 (2.4)	0 (0)	2 (4.8)
Ceftriaxone	40 (95.2)	1 (2.4)	0 (0)	1 (2.4)
Cefepime	41 (97.6)	1 (2.4)	0 (0)	0 (0)
Ciprofloxacin	37 (88.1)	1 (2.4)	0 (0)	4 (9.5)
Cotrimoxazole	39 (92.9)	0 (0)	3 (7.1)	0 (0)
Imipenem	33 (78.6)	2 (4.8)	0 (0)	7 (16.7)
Meropenem	26 (61.9)	1 (2.4)	13 (31)	2 (4.8)
Ertapenem	36 (85.7)	1 (2.4)	2 (4.8)	3 (7.1)
Nalidixic acid	40 (95.2)	0 (0)	0 (0)	2 (4.8)

discovered in a short time and it was considered to be the end of the problem of infections. In reality, the response of microbes to the usage of antibiotics was completely unexpected. The organisms quickly started developing resistance to the antibiotics. We are now facing a challenge from drug-resistant bacteria. Antibiotic Sensitivity testing (AST), therefore, has become imperative in the treatment of infections.

Disk diffusion technique of sensitivity testing has been in use for a long time. With the developments in biomedical engineering, automated sensitivity testing is gaining popularity. This has increased the accuracy and speed of sensitivity testing. In the treatment of resistant infections, specific antibiotics need to be started and loss of time to initiate well-directed antibiotic therapy is directly proportional to morbidity and mortality.

This department has developed and tested a modified protocol for early identification and sensitivity testing of blood cultures. It is shown that the identification and sensitivity report can be generated with conventional identification methods within 24 hours. This study has further aimed at reducing the time to report sensitivity by using automated sensitivity testing directly on culture broths.

Most blood stream infections are mono-microbial. We use BacT/ALERT 3D system for incubating blood cultures. As soon as a bottle flags positive the Gram stained films made from the broth provide clue to the mono-microbial or poly-microbial nature of infections. The blood culture sets showing mono-microbial growth can directly be subjected to sensitivity testing by the protocols developed in this laboratory. We here have compared the DST results by Disk Diffusion

Table 3. Organisms with antibiotics (*Pseudomonas spp.)*

Pseudomonas spp. (n=7)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Piperacillin/Tazobactam	7 (100)	0 (0)	0 (0)	0 (0)
Ceftazidime	6 (85.7)	0 (0)	1 (14.3)	0 (0)
Cefepime	7 (100)	0 (0)	0 (0)	0 (0)
Imipenem	7 (100)	0 (0)	0 (0)	0 (0)
Meropenem	5 (71.4)	1 (14.3)	1 (14.3)	0 (0)
Gentamicin	7 (100)	0 (0)	0 (0)	0 (0)
Amikacin	7 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	7 (100)	0 (0)	0 (0)	0 (0)
Levofloxacin	6 (85.7)	0 (0)	0 (0)	1 (14.3)
Cotrimoxazole	7 (100)	0 (0)	0 (0)	0 (0)

Table 4. Organisms with antibiotics (*Acinetobacter* spp.)

Acinetobacter spp. (n=10)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Piperacillin/Tazobactam	10 (100)	0 (0)	0 (0)	0 (0)
Ceftazidime	9 (90)	0 (0)	1 (10)	0 (0)
Cefepime	7 (70)	0 (0)	0 (0)	3 (30)
Imipenem	8 (80)	0 (0)	0 (0)	2 (20)
Gentamicin	7 (70)	0 (0)	0 (0)	3 (30)
Amikacin	8 (80)	0 (0)	0 (0)	2 (20)
Ciprofloxacin	7 (70)	2 (20)	0 (0)	1 (10)
Levofloxacin	10 (100)	0 (0)	0 (0)	0 (0)
Cotrimoxazole	10 (100)	0 (0)	0 (0)	0 (0)

method and routine sensitivity reports on the same sample by Vitek-2 automated sensitivity testing system.

A total of 102 blood cultures yielding mono-microbial bacterial growth were evaluated in this study. Organisms belonging to the family Enterobacteriaceae accounted for 41.2% of the isolates (42/102) followed by Staphylococcus spp. giving 40.2% of the isolates (41/102). *E. coli* and *Klebsiella spp.* were the commonest Gram negative isolates.

Using the permutations and combinations of antibiotics tested against 102 isolates, we could generate a total of 2424 combinations. Whenever there was discrepancy in the sensitivity report by Disk diffusion and the automated methods, it was counted as an error. For counting the errors, the automated sensitivity was considered as the reference method. The errors were subdivided into Very Major error, Major error and Minor error. The reports showing no error were considered as in agreement. Whenever an organism showed Resistance to an antibiotic by Vitek-2 but Sensitive to that antibiotic by Disk diffusion method, it was considered as a Very Major Error. The reverse situation, i.e., Sensitive by Vitek-2 and Resistance by Disk diffusion was counted as Major Error. Minor error was considered as Intermediate sensitivity by either Disk diffusion or Vitek-2 and Sensitive or Resistant shown by the other.

Most of the common bacterial pathogens isolated in hospital setting are showing drug resistance. Multi-Drug resistance and Carbapenem-resistance are daunting problems in the treatment of infections. Escalation in the morbidity, mortality and cost of treatment are frequently seen as consequences of bacterial drug resistance. Automated sensitivity systems are getting wide acceptance and are considered as highly sensitive and reliable methods of sensitivity testing. Because of automation, the personal errors are minimized, improving the reliability. The Disk diffusion method is also reliable, highly standardized and time tested. Both the techniques of sensitivity testing are accepted by various academias like CLSI, EUCAST etc.

Table 5. Organisms with antibiotics (*Staphylococcus* spp.)

Staphylococcus spp. (n=41)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Penicillin	25 (61)	0 (0)	2 (4.9)	14 (34.1)
Cefoxitin	41 (100)	0 (0)	0 (0)	0 (0)
Erythromycin	24 (58.5)	5 (12.2)	4 (9.8)	8 (19.5)
Clindamycin	32 (78)	0 (0)	2 (4.9)	7 (17.1)
Vancomycin	41 (100)	0 (0)	0 (0)	0 (0)
Gentamicin	32 (78)	1 (2.4)	0 (0)	8 (19.5)
Ciprofloxacin	25 (61)	1 (2.4)	0 (0)	15 (36.6)
Cotrimoxazole	36 (87.8)	0 (0)	5 (12.2)	0 (0)
Tetracycline	37 (90.2)	0 (0)	2 (4.9)	2 (4.9)
Linezolid	41 (100)	0 (0)	0 (0)	0 (0)

Table 6. Organisms with antibiotics (*Enterococcus* spp.)

Enterococcus spp. (n=2)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Penicillin	2 (100)	0 (0)	0 (0)	0 (0)
Linezolid	2 (100)	0 (0)	0 (0)	0 (0)
Vancomycin	2 (100)	0 (0)	0 (0)	0 (0)
Erythromycin	2 (100)	0 (0)	0 (0)	0 (0)
Clindamycin	2 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	2 (100)	0 (0)	0 (0)	0 (0)

Considering the reliability and reproducibility of both the above techniques, we evaluated antibiotic sensitivity testing of common pathogens by Vitek-2 and DST by disk diffusion method. Direct Sensitivity by Disk diffusion method has been evaluated in this department and was found to be in excellent agreement with standard culture and sensitivity results. The very major errors, major errors and minor errors, as defined above, were assessed. When a very major error is encountered, the laboratory physician is put in dilemma. Antibiotics like Imipenem and Gentamicin are highly popular in the clinical fraternity. In the present study, we found that members of the family Enterobacteriaceae gave 33.3% very major error for Gentamicin and 16.7% for Imipenem. Major error of 31% was seen for Imipenem. All these antibiotics for which very major or major errors were observed are the mainstay of antibiotic therapy in infections caused by Enterobacteriaceae. Members of this family like *E.coli* are a pathogen having the ability to affect any organ in the body causing localized or systemic infections. Choice of antibiotic on the basis of sensitivity report is the key to prevent mortality and morbidity in infections caused by this potentially dangerous pathogen.

The second major group of organisms evaluated in this work was *Staphylococcus sps*. Penicillin has been the workhorse to treat staphylococcal infections traditionally. The use of this antibiotic has drastically come down because of high resistance, parenteral use and hypersensitivity reactions to this antibiotic. In spite of low use of this antibiotic, 14/41 isolates (34.1%) shows very major errors in this sensitivity report. Ciprofloxacin is another popular antibiotic for the treatment of Staphylococcal infections and it encountered 36.6% very major error. Erythromycin and Gentamicin also showed very major error in 19.5% of the isolates.

The other organisms like *Pseudomonas sps.* and *Acinetobacter sps.* were low in number. In 7 Pseudomonas isolates, only one very major error was encountered, which was for Levofloxacin. For *Acinetobacter spp* (10 isolates), very major error was seen for Cefepime, Imipenem, Gentamicin, Amikacin and Ciprofloxacin while a major error was encountered for Ceftazidime.

Fortunately, no very major, major or minor errors were encountered with *Enterococcus sps.*

Goel *et al* has reported zero very major errors for Enterobacteriaceae.[11] In their work also, other organism like Acinetobacter or other gram negative bacilli were less than or equal to ten in number.

Fraser *et al*. (2006) studied the relationship between inappropriate use of antibiotics and 30 day mortality. They found increased mortality, prolonged hospital stay and increased cost of treatment because of inappropriate use of antibiotics.[9] Similar observations have been expressed by Doern *et al*. (1994) on evaluation of 273 patients in a controlled, prospective, randomized study.[12]

Gabrino *et al*. has also found the negative effect of inappropriate initial antimicrobial treatment on the survival of patients.[13]

Severe sepsis is not uncommon. The treatment is expensive and in spite of using the best treatment modalities, the death rate is high. In the recovered patients, sequelae like organ dysfunction may follow. Precision and reliability of sensitivity test is the only resort for better clinical outcome. An erroneous sensitivity report will be potentially fatal in these conditions.[14]

Barenfanger *et al*. (1999) has reported the clinical and financial benefits of rapid reporting of bacterial identification and AST. A statistically significant decrease in the length of time and hospital costs were shown to be the results of rapid reporting. They have also reported the utility of Vitek-2 in obtaining early sensitivity reports.[2]

Use of multiple antibiotics empirically is detrimental for the outcome of infections. Scaling down the antibiotic therapy to a minimum, preferably monodrug therapy, is essential for better patient outcome.[15]

Discrepancies in the sensitivity results by automated and manual methods are of multifactorial etiology. Inoculum effect, i.e., too heavy or too low inoculums leads to wrong test results. Heavy growth of micro-organisms on culture plate will decrease the zone size and vice versa.

The filter paper discs used for Disk diffusion method often show variation in the potency. Not only batch-to-batch variation but variation in the disk potencies among the disks from the same vial also can be seen. This, again, will result in erroneous results.

Usually, Mueller-Hinton agar is used for sensitivity testing by Disk diffusion method. Quality of the medium, for example, the pH, electrolyte concentration etc. can seriously affect the growth of micro-organisms, leading to inaccurate test results. Batch-to-batch variation in the quality of culture media and manufacture-to-manufacturer variations are frequently seen. Same is true for incubation conditions where the critical maintenance of temperature, humidity and uninterrupted power supply are essential for accurate results.[15]

We have considered Vitek-2 as the reference method for sensitivity testing. However, it must be noted that Disk diffusion method is also an accepted and well-established sensitivity testing method. There is a need to decide if the automated systems have inherent fallacies causing false sensitivity or resistance reports. A feasible approach can be repeating the test by both the methods. Assessment of the clinical outcome and correlating it with the sensitivity result will also be highly useful for this assessment. Highly sophisticated laboratory techniques like detection of antibiotic inhibiting enzymes or assessment of other drug resistance mechanisms, including molecular tests can be used to provide concrete information. Rather than settling the dispute, it is important to establish the reliability of either of the test methods on the basis of firm evidence. Future larger studies should be conducted including more isolates to confirm our results.

CONCLUSION

These data suggest that VITEK 2 cards inoculated with samples taken directly from positive Bact/ALERT blood culture bottles would provide acceptable antimicrobial susceptibility testing results for Gram-negative bacilli, but not for Gram-positive cocci. Compared to the reference method, the direct method would reduce turnaround time by at least 24 h.

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