ORIGINAL RESEARCH

Comparison of Direct and Standard Susceptibility testing methods for Gram negative blood isolates

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ABSTRACT

Introduction: Blood stream infections are the major cause of morbidity and mortality hence early availability of direct susceptibility reports can be lifesaving Initiation of appropriate antimicrobial at right time can improve the outcome in terms of reduced patient distress in addition to reduced health-care costs. Early preliminary Antimicrobial Susceptibility Test (AST) report will be useful in directing antimicrobial therapy. **Objective**: The objective of the study was to correlate DST (Direct susceptibility testing) by disc diffusion method, directly from positively flagged blood culture bottles, with the AST of bacterial isolates by automated method. **Materials and Methods**: This prospective study was carried in a tertiary care centre of north India. A total of 300 isolates from patients with blood stream infections were included in the study. Gram staining, bacterial identification, direct susceptibility, antimicrobial susceptibility of all the isolates were documented. **Results**: *Escherichia coli* was the commonest isolate (27%), followed by *Klebsiella pneumoniae* (22%). Overall antimicrobial susceptibility pattern among Gram negative bacterial isolates (n=300) detected by DST and automated AST showed maximum susceptibility to cotrimoxazole 54.3% and 53.8% respectively. Comparison of interpretative results with DST and automated AST showed minimum concordance for piperacillin/tazobactam (92.6% and maximum concordance for ceftriaxone (99%). **Conclusion**: DST is an important tool for early initiation of targeted therapy and can be considered as one of the step towards antibiotic stewardship intervention.

Keywords: Blood stream infections, Direct susceptibility testing, Vitek 2, Antimicrobial agents.

INTRODUCTION

Availability of culture and sensitivity results is of importance to the clinicians in guiding them to select the most appropriate antimicrobial for treatment in patients with infections, thereby increasing the chances of maximal therapeutic effect. To this end, it is necessary for the microbiology laboratory to provide such information in a timely manner, especially with reference to cases of blood stream infections. Timely initiation of appropriate antimicrobial therapy along with supportive management may improve the outcome in terms of reduced morbidity and mortality in addition to reduced health-care costs.[1,2] With the advent of automated blood culture methods, the time to detection of the organism decreased from 3 to 4 days to 2 to 3 days. However, even with the automation in place, a subculture is required to obtain a pure growth, so that Antimicrobial Susceptibility Testing (AST) can be carried out either by the Kirby Bauer method or an automated method. Owing to this inherent delay, the empirical therapy started initially with broad spectrum antimicrobials continues till the sensitivity results are made available. However, it is to be emphasized that about 20-50% of all the prescribed antimicrobials are inappropriate.[3]Patients getting these inappropriate antimicrobials get no extra clinical benefits while being at risk of suffering from adverse effects.[4]The most serious and ever-increasing public health problem is emergence of antimicrobial resistance due to the misuse and abuse of antimicrobials.[5] These drug-resistant pathogens pose a threat to health of patients in a health-care setup. Various reports from around the world indicate that there is an increase in the incidence of infections with multidrug resistant organisms (MDRO's) along with

increased mortality being seen in both developing and developed countries.[6-8] One of the useful inputs in implementation of antimicrobial stewardship is early availability of AST, which can help the clinician to de-escalate the antimicrobial, thereby reducing the chances of emergence of resistant organisms. The disc diffusion method for AST takes 48 h for the result to be generated. This includes the 24 hr time taken for subculture from the positively flagged culture bottle onto solid culture media to obtain a pure growth, in addition to AST, which takes another day to complete. Even the automated methods for AST take half to one day for the AST results to be available.

In this study, we have carried out AST by disc diffusion method, directly from the positively flagged blood culture bottles, and correlated it with the AST of isolated bacteria by automated method.

MATERIAL AND METHODS

This was a prospective study. Three hundred consecutive positive blood cultures with Gram negative organism in smear was included in study. All positive blood cultures from admitted patients with clinically suspected blood stream infections (BSIs) were subjected to Gram staining and report was informed to the clinician telephonically. The blood culture bottle flagged positive by the system was taken out, and after gentle shaking, 1.5 mL of the broth was drawn using a sterile syringe. This was centrifuged in a 1.5-mL microcentrifuge tube at 600_g for 10 min to pellet the resin and the red blood cells(MiniSpin centrifuge; Eppendorf, Germany). The supernatant was taken into another 1.5-mL microcentrifuge tube and centrifuged at 3000 _g for 10 min (MiniSpincentrifuge;Eppendorf, Germany) to pellet the bacteria.[9] A smear for Gram stain was prepared from the deposit. The rest of the sediment was processed for preparation of inoculum for direct AST. Samples showing Gram negative organism on Gram staining were further processed for direct AST Kirby Bauer disc diffusion method

Direct susceptibility test by Kirby Bauer disc diffusion method

This pellet was resuspended in sterile saline to make the turbidity equivalent to 0.5 McFarland. This suspension was used for making the lawn culture for AST on Mueller-Hinton Agar (HiMedia, India). Antimicrobial panels for testing were chosen based on the Clinical & Laboratory Standards Institute (CLSI) guidelines 2022 depending on whether the organism was Gram negative on staining.[10] The panel chosen included antimicrobials covering both *Enterobacteriaceae* and non fermenters. The antimicrobial discs were procured from HiMediaLabs, India. The antibiotic disks tested were: amoxicillin–clavulanate (20/10 µg), gentamicin(10 µg) , amikacin (30 µg) , ciprofloxacin (5 µg) ,ceftriaxone (30 µg) , cefuroxime (30 µg), ceftazidime (30 µg) , cefaperazone+sulbactum (75/10 µg), piperacillin–tazobactam (100/10 µg), imipenem (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg) and cefepime (30 µg).After overnight incubation at 37 ^{0} C, the results were interpreted as per CLSI guidelines. Simultaneously, subcultures from the positive-flagged bottle broth were performed on blood and MacConkey agar (HiMedia, India).

Automated identification and AST

The growth on the solid media was further used for bacterial identification and AST using an automated identification (ID) and AST system (VITEK-2 Compact; bioMerieux, France) using appropriate ID and AST cards (N280/N281).

After matching the results of the two methods, four interpretations were given.[11]

1. Categorical agreement: when the results of AST by the two methods were in concordance.
2. Very major errors (VMEs) (false susceptibility): when the isolate was sensitive to a drug by direct AST but turned out to be resistant by the standard automated AST method.
3. Major errors (MEs) (false resistance): when the isolate was resistant to a drug by direct AST but turned out to be sensitive in the standard automated AST.
4. Minor errors (mE): when the isolate was intermediate to a drug by direct AST but turned out to be either sensitive or resistant by the automated AST system.

Methods of statistical analysis

For finding agreement between DST and AST Kappa test was used.

RESULTS

Out of 300 clinically suspected patients with bacteremia, maximum patients were in the age group of 40-60 years (28.3%).Majority of patients were male (71%) as compared to females (29%). Among the Gram negative bacteria, *Escherichia coli* was the commonest isolate (27%), followed by *Klebsiella pneumoniae* (22%), *Acinetobacter spp* (18%), *Salmonella* Typhi (16 %), *Pseudomonas aeruginosa* (7%), *Salmonella* Paratyphi A (3%) and *Aeromonas hydrophila*, *Elizabethkingia meningoseptica*, *Stenotrophomonas maltophila* (1% each) and others.

Overall antimicrobial susceptibility pattern among Gram negative bacterial isolates (n=300) detected by direct susceptibility testing (DST) and automated antimicrobial susceptibility testing method (AST) method showed maximum susceptibility to cotrimoxazole (54.3% & 53.8%) followed by amikacin (54% & 52.1%) and least susceptibility to cefuroxime (13% & 8%) respectively. (Table 1)

While comparing interpretative results with DST and automated AST , we found a total of 47 very major errors (1.3%), 45 minor errors (1.2 %) and 37 major errors (1.0 %) for 300 Gram negative bacterial isolates against 12 antimicrobial agents tested (i.e., total of

3600 isolate/antibiotic combinations). Maximum errors were recorded for piperacillin/tazobactam with 12 (4%) minor errors, 6 (2%) very major errors and 4 (1.3 %) major error. Overall agreement between susceptibility report by DST and automated AST ranged from 92.6% to 99.3%. Maximum agreement of 99.3% was observed for ceftriaxone followed by cefuroxime (98.6%) and least agreement (92.6%) was observed for piperacillin/tazobactam with kappa value of 0.789. (Table 2)

While comparing interpretative results with DST and automated AST for *Enterobacteriaceae* (n=150), we found a total of 27 minor errors (1.6%), 23 major errors (1.3%) and 22 very major errors (1.4%) for 150 *Enterobacteriaceae* isolates against 11 antimicrobial agents tested (i.e., total of 1650 isolate/antibiotic combinations). Maximum errors were recorded for piperacillin/tazobactam with 9 (2%) minor errors, 3 (3.6%) major error 6 (2%) and 3 very major errors (6%) with kappa value of 0.899. (Table 3)

Among non fermenters (n=92), while comparing interpretative results with DST and automated AST, we found a total of 20 very major errors (2.7%), 14 minor errors (1.9%) and 13 major errors (1.7 %) against 8 antimicrobial agents tested (i.e., total of 736 isolate/antibiotic combinations).Maximum errors were recorded for amikacin and ceftazidime with 5 (5.4%), 2(2.1%) major error, minor errors 2 (2.1%), 3 (3.2%), very major errors 2 (2.1%), 4(4.3%) respectively with kappa value of 0.819 for amikacin and 0.687 for ceftazidime. (Table 4).

While comparing interpretative results with DST and automated AST for *Salmonella* (n=58), we found a total of 5 very major errors (2.8%), 4 minor errors (2.2%) and 1 major errors (0.5%) against 3 antimicrobial agents tested (i.e., total of 174 isolate/antibiotic combinations).Maximum errors were recorded for ciprofloxacin with 5 (8.6 %) very major errors and minor errors 3 (5.1%) (Table 5).

Table 1: Antimicrobial agents tested for Gram Negative isolates by DST and AST method and their susceptibility pattern (n=300).

Antibiotics	DST (%)	AST (%)	P- value
Amikacin (30µg)	54	52.1	0.663
Gentamicin (10 µg)	44.8	43.4	0.760
Ciprofloxacin (5 µg)	18	15.3	0.381
Ceftriaxone (30 µg)	36.5	36.5	1.000
Cefuroxime (30 µg)	13	8	0.192
Ceftazidime (30 µg)	37.4	36.6	0.907
Cefepime (30 µg)	22.9	23	0.961
Amoxicillin/Clavulanic Acid (10µg)	29.3	31.3	0.698
Cefoperazone/Sulbactum (5 µg)	49.4	44.1	0.244
Piperacillin/Tazobactum (10 µg)	37.9	38.3	0.933
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	54.3	53.8	0.922
Imipenem (10 µg)	49	44.9	0.368

Table 2: Direct antibiotic susceptibility correlation for Gram Negative Bacilli (n=300)

Antibiotics used	Direct susceptibility method								
	Very major error		Major error		Minor error		Concordance		Kappa value of agreement
	NO.	%	NO.	%	NO.	%	NO.	%	
Amikacin (30µg)	5	1.6	7	2.3	4	1.3	284	94.6	0.867
Gentamicin (10 µg)	3	1	3	1	3	1	291	97	0.899
Ciprofloxacin (5 µg)	8	2.6	2	0.6	6	2	284	94.6	0.808
Ceftriaxone (30 µg)	0	0	1	0.3	2	0.6	297	99.3	0.938
Cefuroxime (30 µg)	3	1	0	0	1	0.3	295	98.6	0.764
Ceftazidime (30 µg)	4	1.3	2	0.6	3	1	290	96.6	0.93
Cefepime (30 µg)	3	1	6	2	4	1.3	287	95.6	0.846
Amoxicillin/Clavulanic Acid (10µg)	2	0.6	7	2.3	3	1	288	96	0.822
Cefoperazone/Sulbactum (5 µg)	3	1	3	1	3	1	291	97	0.901
Piperacillin/Tazobactum (10 µg)	6	2	4	1.3	12	4	278	92.6	0.789
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	4	1.3	2	0.6	0	0	294	98	0.932
Imipenem (10 µg)	6	2	0	0	4	1.3	290	96.6	0.876
Total	47	1.3	37	1.0	45	1.2	171	4.7	

Table 3: Direct antibiotic susceptibility correlation for *Enterobacteriaceae* (n=150)

Antibiotics used	Direct susceptibility method								
	Very major error		Major error		Minor error		Concordance		Kappa value of agreement
	NO.	%	NO.	%	NO.	%	NO.	%	
Amikacin (30µg)	3	2	2	1.3	2	1.3	143	95.3	0.889
Gentamicin (10 µg)	2	1.3	3	2	3	2	142	94.6	0.88
Ciprofloxacin (5 µg)	0	0	1	0.6	2	1.6	147	98	0.898
Ceftriaxone (30 µg)	0	0	1	0.6	1	1.3	148	98.6	0.912
Cefuroxime (30 µg)	3	2	0	0	1	0.6	146	97.3	0.764
Cefepime (30 µg)	2	1.3	5	3.3	2	1.3	141	94	0.784
Cefoperazone/Sulbactum (5 µg)	1	0.6	0	0	0	0	149	99.3	0.933
Amoxicillin/Clavulanic Acid (10µg)	2	1.3	6	4	3	2	139	92.6	0.822
Piperacillin/Tazobactum (10 µg)	3	6	3	2	9	2	135	90	0.806
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	4	2.6	2	1.3	0	0	144	96	0.899
Imipenem (10 µg)	2	1.3	0	0	4	2.6	144	96	0.893
Total	22	1.4	23	1.3	27	1.6	78	4.7	

Table 4: Direct antibiotic susceptibility correlation for non fermenters (n=92)

Antibiotics used	Direct susceptibility method								
	Very major error		Major error		Minor error		Concordance		Kappa value of agreement
	NO.	%	NO.	%	NO.	%	NO.	%	
Amikacin (30µg)	2	2.1	5	5.4	2	2.1	83	90.2	0.819
Gentamicin (10 µg)	1	1.0	0	0	0	0	91	98.9	0.924
Ciprofloxacin (5 µg)	3	3.2	1	1.0	1	1.0	87	94.5	0.852
Ceftazidime (30 µg)	4	4.3	2	2.1	3	3.2	83	90.2	0.687
Cefepime (30 µg)	1	1.0	1	1.0	2	2.1	88	95.6	0.94
Cefoperazone/Sulbactum (5 µg)	2	2.1	3	3.2	3	3.2	84	91.3	0.844
Piperacillin/Tazobactum (10 µg)	3	3.2	1	1.0	3	3.2	85	92.3	0.754
Imipenem (10 µg)	4	4.3	0	0	0	0	88	95.6	0.837
Total	20	2.7	13	1.7	14	1.9	45	6.1	

Table 5: Direct antibiotic susceptibility correlation for *Salmonella* (n=58)

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Ciprofloxacin (5 µg)	5	8.6	0	0	3	5.1	50	86.2
Ceftriaxone (30 µg)	0	0	0	0	1	1.7	57	98.2
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	0	0	0	0	0	0	58	100
Total	5	2.8	0	0	4	2.2	49	28.1

DISCUSSION

Bacterial sepsis remains as one of the leading cause of morbidity and mortality, particularly among neonates and elderly patients in developing countries inspite of advances in diagnosis and treatment in the medical care. The etiological agents causing sepsis and their antimicrobial susceptibility are constantly evolving. Conventional approach requires isolation of the organism followed by AST, while direct susceptibility testing from positively flagged bottles can reduce the turn around time. Hence direct antibiotic susceptibility testing can play an important role in successful early management of blood stream infection cases.[12]

Of the 300 Gram negative bacterial isolates, *Escherichia coli* was the commonest isolate (27%), similar to studies conducted by Rajshekar D et al.

(18.9%), and Annamallaei et al (24.3%) and Lokeshwari (51.4%) [13,14,15]

Comparison of interpretative results with DST and automated AST for Gram negative isolates, agreement ranges from 92.6-99.3 % whereas Rahila and and Gopalkrishna[16] in their study observed an agreement ranging from 75.9-100 %.

Maximum errors were found for Piperacillin tazobactum with concordance of 92.6 % which is similar to study at Puducherry [15] (75%) and but in contrast to study conducted at Mangalore, [16] in which maximum errors were observed for amikacin with concordance of 75.9 %.

For *Enterobacteriaceae* isolates (n=150), categorical agreement between DST result with automated AST method ranges from 90-98.6 % .These findings are similar to study done by Rajshekhar D etal[13](91.8-98.4%) whereas it is in contrast to study by Lokeshwari[15] (75-100 %). We found total of 27 minor errors (1.6%) and 23 major errors (1.3%).Maximum errors were recorded for piperacillin/tazobactam with 9 (2%) minor errors, major error 3 (2%) and 3 very major errors (6%), findings are in contrast to study at South India [13] in which they found maximum errors were for cefoperazone and sulbactum in which 11 (2.5%) minor errors, 24 (5.5 %) major errors and 1 (0.2 %) very major errors were seen and in study by Mahadevan [17], maximum errors observer were for gentamicin with 2 minor errors and 1 very major error.

Among non fermenters ,categorical Agreement between DST result with automated AST method ranges from 90.2-98.9 % whereas in study by Rajshekhar D etal[13] it ranged from 91.2-97.4 % and in study done by Lokeshwari [15], agreement ranges from 80-100%.

Maximum errors were recorded for amikacin with concordance of 90.2%, similar observations were made by study conducted at South India[13] in which maximum errors observed were

for Amikacin with concordance of 91.2% .Findings of our study are in contrast to study conducted at Puducherry[15] in which they found only 2 minor errors for gentamicin and imipenem.

As compared to conventional susceptibility testing method, direct susceptibility report may reduce the turnaround time and promote early initiation of antimicrobial therapy agent; however, there should be higher level of agreement between the susceptibility reports of DST when compared to the automated AST reports. [12,18, 19]

These findings suggest that DST can be used as an alternative/feasible method for susceptibility testing in case of critically ill patients, in whom early institution of antimicrobial therapy can alter the outcome.

Now with the availability of reference documents (CLSA M 47-A and EUCAST RAST method v 1.1), recommending the standard procedures for performing direct susceptibility from the positive blood culture broth and also keeping in mind the advantage of early reporting in management of critically ill patients with sepsis, we should consider DST as effective step towards antimicrobial stewardship to control the unjustifiable use of broad spectrum antibiotics.

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REFERENCES

1. Gaieski DF, Mikkelsen ME, Band RA, Pines JM, Massone R, Furia FF et al. Impact of time to antibiotics on survival in patients with severe sepsis or septic shock in whom early goal-directed therapy was initiated in the emergency department. Crit Care Med.2010;38(4):1045-53.
2. Sterling SA, Miller WR, Pryor J, Puskarich MA, Jones AE. The impact of timing of antibiotics on outcomes in severe sepsis and septic shock: a systematic review and meta-analysis. CritCare Med. 2015;43(9):1907-15.
3. US Department of Health and Human Services. Core elements of hospital antibiotic stewardship programs. US Dep Health Hum Serv CDC. 2014:1-25.
4. Hensgens MPM, Goorhuis A, Dekkers OM, Kuijper EJ. Time interval of increased risk for Clostridium difficile infection after exposure to antibiotics. J Antimicrob Chemother.2011;67(3):742-8.
5. Huttner A, Harbarth S, Carlet J, Cosgrove S, Goossens H, Holmes A et al. Antimicrobial resistance: a global view from the 2013 world health care associated infections forum. Antimicrob Resist Infect Control.2013;2(1):31.
6. Paramythiotou E, Routsi C. Association between infections caused by multidrug-resistant gram-negative bacteria and mortality in critically ill patients. World J Crit Care Med.2016;5(2):111.
7. Colomb-Cotinat M, Lacoste J, Brun-Buisson C, Jarlier V, Coignard B, Vaux S. Estimating the morbidity and mortality associated with infections due to multidrug-resistant bacteria(MDRB), France, 2012. Antimicrob Resist Infect Control. 2016;5:56.
8. Lim C, Takahashi E, Hongsuwan M, Wuthiekanun V, Thamlikitkul V, Hinjoy S et al. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. Elife. 2016;5:1-18.
9. Gherardi G, Angeletti S, Panitti M, Pompilio A, Di Bonaventura G, Crea F et al. Comparative evaluation of the Vitek-2 compact and phoenix systems for rapid identification and antibiotic susceptibility testing directly from blood cultures of Gram-negative and Grampositive isolates. Diagn Microbiol Infect Dis. 2012;72(1):20-31.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing Supplement M100S. 26th ed. 2016. Wayne, Pennsylvania, USA. 252 p
11. Machen A, Drake T, Wang YFW. Same day identification and full panel antimicrobial susceptibility testing of bacteria from positive blood culture bottles made possible by a combined lysis-filtration method

with MALDI-TOF VITEK mass spectrometry and the VITEK2 system. PLoS One. 2014;9(2), e87870
12. Lahanas S, Lee A, Menon V, Janto C. Utility of direct susceptibility testing on blood cultures: is it still worthwhile. J Med Microbiol.2016;65(6):501–9.
13. Rajshekar D, Chaudhari KV, Bhat P, Prakash SS, Raghvan R, Vasanth S, et al. Evaluation of performance of direct disk diffusion test from positively flagged blood culture broth: A large scale study from South India. J Lab Physicians. 2019;11(2):154–60.
14. Annamallaei S, Bhat KS. Correlation of empiric antibiotic use with susceptibility pattern of blood isolates in septicemic patients in an Intensive Care Unit. J Curr Res Sci Med. 2017;3(1):29–35.
15. Lokeshwari, Bhat K and Devi / IP International Journal of Medical Microbiology and Tropical Diseases 2021;7(3):179–185
16. Rahila Yakoob and Gopalkrishna Bhat K., Comparision of Direct Antibiotic Susceptibility Testing with Standard Testing in Blood Culture, *J Pure* Appl Microbiol., 2018; 12(4):2289-96.
17. K Mahadevan , S.P.S. Shergill, T Kundan , S Kavita , R.M. Gupta. Direct antimicrobial susceptibility testing from positive blood culture bottles in laboratories lacking automated antimicrobial susceptibility testing systems, Medical journal Armed forced India 2019;75 :450-457.
18. Coorevits L, Boelens J, Claeys G. Direct susceptibility testing by disk diffusion on clinical samples: a rapid and accurate tool for antibiotic stewardship. *Eur J Clin Microbiol Infect Dis*. 2015;34(6):1207–12.
19. Ceballos E, Martinez-Martinez L, Perea EJ, PascualA. Use of Positive Blood Cultures for Direct Identification and Susceptibility Testing with the Vitek 2 System. J Clin Microbiol. 2004;42(8):3734–8