IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 9 Ser. 11 (September. 2024), PP 02-06
www.iosrjournals.org

"Evaluation Of Direct Disk Diffusion Antimicrobial Susceptibility Testing Of Blood Culture In Patients With Gram Negative Bacteremia And Its Usefulness In Patient Outcome"

Komali K, Harika V, Jayaprada R

Abstract
Background: *A reduction in turnaround time may be beneficial to the timely appropriate antimicrobial administration to patients in sepsis, especially with Gram negative bacteria. This study was done to evaluate direct antimicrobial susceptibility testing (DAST) on positive blood cultures with Gram negative bacteremia in comparison to conventional method.*
Materials and method: *This is a prospective observational study where 64 positive blood culture samples with monomorphic Gram negative bacterial growth were processed by conventional and direct blood culture disk diffusion methods in accordance with procedure followed in preliminary report by CLSI. The data was entered and analyzed in a Microsoft excel sheet. Statistical significance was done with SPSS.*
Results: *The agreement of direct method with conventional method was 100% and 94.6% for identification and antimicrobial susceptibility respectively. There were 0.39% very major errors (VME), 0.59% major errors (ME) and 4.42% minor errors (mE), bringing the total disagreement to 5.4%. Pseudomonas spp. (12.9%) had the highest disagreement followed by Acinetobacter spp. (11.4 %) and Enterobacterales (3.7%). Errors due to carbapenems were more in the case of Enterobacterales, while errors with Ciprofloxacin were more with Non-fermenters.*
Conclusion: *The TAT of blood culture and susceptibility report can be reduced by 24 hours with the direct method which can expeditate the delivery of appropriate antibacterial agent, the major advantage of this study. Direct method is to be reported with caution for beta lactam agents and Non-fermenters.*
Keywords: *Direct blood culture, Gram negative bacteremia, Beta lactams.*

Date of Submission: 17-09-2024 Date of Acceptance: 27-09-2024

I. Introduction

Sepsis due to bacterial blood stream infections (BSIs) continues to be the prime contributor of hospital related morbidity and mortality up to 50% **[1].** Majority of BSIs in health care is contributed by ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp*) and SPICE *(Serratia, Pseudomonas,* Indole-positive *Proteus, Citrobacter, Enterobacter)* group of organisms **[2,3].**

Though molecular methods are available for rapid diagnosis, it is rarely affordable or available in Indian health care settings with 11.3 million cases of sepsis **[4]**. Hence, clinicians abide by blood cultures to track down the bacterial etiology of sepsis. Bare identification is only a goal half reached in the era of antimicrobial resistance. It is mandatory to promptly determine antimicrobial susceptibility pattern of the causative bacterial, as every one-hour delay in targeted antibiotic administration may lead to 3-7% increase in the odds of a poor clinical outcome **[5,6]**.

The standard method of processing positive blood cultures done in accordance with Clinical laboratory and Standards institute (CLSI) guidelines has a disadvantage of long turnaround time (TAT) **[7]**. Despite the use of automation in swift picking out of positive blood cultures, it would take 18-24 hours further for isolation of bacterial colonies and additional 18-24 hours for complete identification and Antimicrobial Susceptibility Testing (AST) report. To hasten antimicrobial susceptibility results, few studies have performed Direct disk diffusion susceptibility testing of positive blood cultures of Gram-negative bacteria. This ensued in a reduction of TAT to 18-24 hours from the point of detection **[8-11]**. While TAT is reduced, results of direct method may not be as accurate as that of standard method since the inoculum is not standardized. Report from one study stated that there was 91.6% agreement between direct and standard AST methods among Gram-negative bacilli, with poor agreement observed with certain antibacterial agents **[8]**. This study was undertaken to evaluate the feasibility and accuracy of direct blood culture

disk diffusion method of AST in comparison to standard method.

II. Materials & Methodology:

This was a prospective observational study carried out in a teaching hospital in Andhra Pradesh from April 2021 to June 2021 after obtaining ethical committee clearance.

This study aimed to compare Direct disk diffusion testing (dDDT) with reference disk diffusion testing (rDDT) advised by CLSI for Antimicrobial Susceptibility Testing (AST) for positive blood cultures. Direct method was also used for identification of organisms of the same.

Substituting the prevalence of monomicrobial Gram negative bacteremia from the study area in the formula [n = Zα2 p(1-p)/d2], sample size was 75 **[12]**. Convenient nonprobability sampling was done on blood culture samples received from ICU patients suspected of bacterial blood stream infection (BSI) to a total of the same.

In the study area, BacT/Alert automated blood culture system (bioMeriuex Pvt Ltd, India) is being used for the diagnosis of BSI's. All the blood cultures which flagged positive within 24hrs of loading and demonstrating monomorphic Gram-negative bacilli on Gram stain were processed by both the methods. *Candida*, Gram positive cocci, contaminants like diptheroids, false positives, smear negative (no growth) and polymicrobial growth identified at any time of the study were excluded.

All the culture media (rehydrated or ready to use) and antimicrobial discs used for disk diffusion testing were procured from HiMedia Laboratories, Mumbai. MacConkey agar and 5% Sheep Blood Agar (SBA) were used for aerobic blood subculture while Mueller Hilton Agar (MHA) was used for disk diffusion testing.

For the various antimicrobial discs tested and reported for *Enterobacterales, Pseudomonas spp., Acinetobacter spp.* and *Nonfermenters* kindly refer to supplementary material. Zones of inhibition were recorded with transmitted light using a ruler and to the nearest 1mm for both the methods. Quality controls used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923.

Standard method of AST (rDDT): A 10 µl loopful of the positive culture broth was sub-cultured onto SBA and MacConkey agars, incubated aerobically for 18-24 hours at 37˚C. Isolated colonies from these subcultures were processed onto appropriate biochemical media for identification **[13]**. Colonies were emulsified in sterile peptone water and turbidity adjusted to 0.5 McFarland standard. A lawn culture of this was made on MHA and discs placed within 15 min of pouring for AST by standard method according to CLSI protocols **[7]**. Reporting was done after 18 to 24 hours aerobic incubation, at 9 am every morning and results recorded.

Direct Susceptibility Testing (dDDT): Within 8-10 hours of flagging positive, a sterile disposable syringe was used to take 1ml of blood culture broth from the bottle, four drops of which were poured over MHA, swabbed in three directions at 60^{o} and discs placed. For the purpose of biochemical identification, another four drops were added to 2 ml peptone broth, centrifuged at 2,500 rpm for 5 minutes. The supernatant was used for biochemical characterization. All the plates and tubes were incubated aerobically at 37 ^{o}C for 18-24 hours after which reporting was done and results recorded **[14]**. Based on the following definitions, results were compared **[1]**:

Direct identification

(i) Correctly identified
(ii) Misidentified (organism was incorrectly identified either at genus or species level)
(iii) Unidentified (no identification given at all)

AST

(i) Minor errors (mE): Standard method is susceptible (S) or resistant (R) and DST is intermediate (I); Alternatively, standard method is intermediate (I), and DST is susceptible (S) or resistance (R).
(ii) Major errors (ME): Standard method is susceptible (S), DST is resistant (R)
(iii) Very major errors (VME): Standard method is resistant (R), DST is susceptible (S).

Data was entered in Microsoft Excel sheet and analyzed. The categorical variables were represented as percentages; goodness of fit test was done comparing both methods (Chi square method). The data was analyzed using SPSS version 21.0 (IBM, New York).

III. Results And Interpretation

From loading a blood culture bottle to flagging positive, the mean time to positivity was 18 hr, 40 min (6hr 32 min to 84 hr, 30 min). The TAT from flagging positive to reporting of results for dDDT was earlier than rDDT by 24 hours (shown in **Figure 1**). All positive blood culture reports were immediately communicated to

the clinicians through telephone as well as updated later in the Hospital Information System (HIS).

Among the 75 Gram negative bacterial isolates processed in our study, majority belonged to Enterobacterales - 60% (n=45) and the remaining 40% were constituted by Non-fermenters (n=30), as shown in **Figure 2**. Among the isolated Enterobacterales, *Klebsiella spp.* were 30% (23), *E. coli* were 19% (14), *Enterobacter spp.* were 8% (6), and *Citrobacter spp.* were 2% (2). Among the Non-fermenters, *Pseudomonas spp.* were 10% (7), *Acinetobacter spp.* were 16% (12) and other Non-fermenting Gram-negative bacilli - NFGNB were 15% (11).

All the isolates were correctly identified and there were no misidentifications or un identification in this study. As the current study followed the procedure by Chandrasekharan et al [14] where NFGNB other than *Pseudomonas* and *Acinetobacter* were not included for direct method of AST we excluded them as well. Hence the number of isolates compared were n = 64. Fixed panel of antibiotics were reported in case the isolate is phenotypically identified as Enterobacterales (18) or Non-fermenter (11). The Antibiotic-Isolate Combinations (AIC's) reported for each type of organism is shown in **Figure 3**.

Total AIC's were 1019. Disagreement was calculated amongst AIC for all isolates in each genus. *Pseudomonas spp.* (12.9%) had the highest disagreement followed by *Acinetobacter* spp. (11.4 %) and Enterobacterales (3.7%). In Enterobacterales, Enterobacter (11.1%) had higher disagreement compared to *Klebsiella* spp. (2.8%) and *E. coli* (2.7%). Among 1019 AIC's, there was a total of 55 errors (5.4%) - 6 ME, 45 mE and 4 VME bringing the percentage to 0.59 %, 4.42 % and 0.39 % respectively. The distribution of these errors is *Enterobacterales* (n=30), *Acinetobacter spp.* (n=15) and *Pseudomonas spp.*(n=10). Isolates with error for ≤ 2 antibiotics was 76.7% (23/30), while 23.3% (7/30) isolates had error with ≥2 antibiotics. The categorical agreement was 94.6% while overall disagreement was 5.4%.

Figure 4 depicts the isolate distribution among agents consistently causing errors. Majority of the errors in Carbapenems were contributed by Enterobacterales (92.3%), while *Acinetobacter spp*. contributed most to the errors in Cephalosporins (57%) and Ciprofloxacin (44.4%). Errors with Piperacillin – Tazobactam were contributed consistently by Enterobacterales and Non-fermenters, indicating it is suitable for neither by dDDT.

The distribution of the three categories of errors among the tested antibiotics is shown in **Figure 5.** Maximum errors were observed with Piperacillin-Tazobactum - 15.6% (10/64), Ciprofloxacin - 12% (9/75), Cephalosporins - (7/64) and Carbapenems – 7.5% (13/173), while single error was seen with Cotrimoxazole, Aztreonam and Aminoglycosides.

Between dDDT and rDDT, goodness of fit test was done using Chi-square method **(supplementary material)**. Statistically significant error rate (%) were noted while testing for Ceftazidime (15.7%), Ceftriaxone (33.3%), Ciprofloxacin (7.8%), Imipenem (6.3%), Meropenem (4.7%) and Piperacillin-Tazobactam (15.6%) by dDDT method, proving it is inferior compared to rDDT for these agents. The reader can refer to supplementary material for various antibiotic used for DDT, and errors in Enterobacterales, *Pseudomonas spp.* and *Acinetobacter spp* for direct method.

IV. Discussion

Turnaround time is the unique selling proposition of the direct method of AST of positive blood cultures. As it eliminates the need for subculture and isolated colonies, the average time taken from blood culture positivity to final release of AST report is reduced. For this purpose, since the 1970's multitude of studies have been done to compare direct method of AST with either conventional or automated methods and there was a reported agreement of **90-97% [14-20]**. This resulted in a decreased time to effective antibiotics in 9.3% patients and increase in targeted antibiotics in 14.3 % of cases **[24]**. Despite the advantage, direct method differed in the rates of agreement in these various studies which could be attributed to lack of standardization of inoculum for performing AST of blood cultures**.**

Hence in January 2018, a preliminary report was given by the CLSI group ***Chandrasekaran et al*** **[14]** for direct blood culture disk diffusion testing using a standardized inoculum, followed by official release of guidelines by CLSI in M100 31st edition (August 2021) for bacteremia with Enterobacterales and *Pseudomonas.* They compared the direct method with conventional and automated methods along with time of incubation and inoculum effect. The current study was done before the release of these guidelines, following the same procedure as in the preliminary report for direct blood culture disk diffusion testing, in order to expedite AST report in positive blood cultures with Gram negative bacilli. We tried comparing our study findings with similar studies in and around India **[15-19]** who followed the similar procedures for direct testing and compared with conventional disk diffusion testing. The direct method was used for identification as well and it correctly identified 100% of the isolates. Among these isolates, *Enterobacterales* (n=45, 60%) were more than non-fermenters (n=30, 40%). This finding is similar to the studies by ***Imtiaz et al*** **[15]** and ***Rajshekar et al*** **[16]** where greater number of Enterobacterales were isolated than Non-fermenters.

The categorical agreement between conventional (rDDT) and direct (dDDT) methods was 94.6% in

our study implying there is good correlation between both the methods. This is similar to ***Imtiaz et al, Rajsekhar et al, Yakoob et al,*** and ***Shantharaju et al*** **[15-18]**

where the CA ranged 97.2 %, 96%, 94.3% and 91.5% respectively, while it was between 75 to 100% in study by ***Bhat et al*** **[19]**. ***Imtiaz et al*** **[15]** reported 0.4% VME, 0.9%ME and 1.5% mE. ***Bhat et al*** **[19]** reported no VME, 0.97% ME and 4.8% mE similar to ***Rajsekhar et al*** **[16]** with no VME, ME- 0.9%, mE – 4.7%. In the current study, there were 0.39% very major errors (VME), 0.59% major errors (ME) and 4.42% minor errors (mE), bringing the total disagreement to 5.4%. Our next task was to assess if this disagreement of 5.4% could make use of dDDT in routine testing impractical.

The majority of the errors in our study were due to β lactam antibiotics including Carbapenems followed by Ciprofloxacin. ***Rajsekhar et al*** **[16]** reported similar findings in their study while Carbapenems and Aminoglycosides have contributed in case of study by ***Imtiaz et al*** **[15].** The preliminary report by **CLSI [14]** have reported similar findings in their study, stating the reason for errors could be inhibition of translocation of β lactam agents into bacterial cell due to the blood components present in the inoculum. While β lactam antibiotics are the major empirical and first line choice of antibiotics in case of sepsis, this could be a major setback for using dDDT in case of Gram-negative bacteremia.

Percentages of errors (mE, ME, and VME) were lower than the acceptable criteria of International Standard ISO 20776-2 (ME ≤3%; VME ≤3%) **[14,16,25].** The percentage of isolates with error ≥2 antibiotics was 10.7% in study by ***Rajsekhar et al*** **[16]**, while in our study it was 23.3%. Significant disagreement at ≥2 antibiotics was observed with Pseudomonas (20%) and Acinetobacter (37.5%) than Enterobacterales (17.6%).

An important observation in our study was while errors due to carbapenems were more in the case of Enterobacterales, errors with Ciprofloxacin were more with Non-fermenters. Also, majority of these were minor errors which could be attributed to incubation time of 24 hours, suggesting these agents require not more than 18 hours incubation for good agreement for the respective organisms. In this study we found a decrease in TAT by 24 hours which may help in better patient outcome when coupled with effective antibiotic stewardship measures **[20,21].**

V. Conclusion

The TAT of blood culture and susceptibility report can be reduced from 56 hours to 32 hours approximately by the direct method which is quite remarkable. This helps in expediting the delivery of appropriate antibacterial agent, the major advantage of this study. Being economical and having lesser TAT, the direct identification and antimicrobial susceptibility testing method can be a reliable alternative for our laboratory in case of *Enterobacterales* and *Acinetobacter spp*, The AST of *Acinetobacter spp*. by direct method needs to be reported with caution especially for Piperacillin-Tazobactam, in which case confirmation should be done by standard method. It is prudent to use conventional method of AST for *Pseudomonas spp.* as many antimicrobial agents have shown very major, major, and minor errors.

When paired with timely antimicrobial stewardship interventional measures, direct method of AST may reduce infection related mortality for which further robust studies are required. Exclusion of Gram-positive organisms and *Candida*, lack of comparison with standard MIC methods are few limitations of our study. The dDDT approach is not suitable for mixed BSI's.

Institutional Ethics Committee approval: IEC no. 1129, dated 23-04-2021

References

[1] Gupta S, Kashyap B. Bacteriological Profile And Antibiogram Of Blood Culture Isolates From A Tertiary Care Hospital Of North India. Trop J Med Res. 2016;19(2):94.

[2] Goel G, Das D, Mukherjee S, Bose S, Das K, Bhattacharya S. A Method For Early Detection Of Antibiotic Resistance In Positive Blood Cultures: Experience From An Oncology Centre In Eastern India. Indian J Med Microbiol. 2015; 33:53-8.

[3] Macdougall C. Beyond Susceptible And Resistant, Part I: Treatment Of Infections Due To Gram-Negative Organisms With Inducible B-Lactamases. J Pediatr Pharmacol Ther. 2011;16(1):23-30.

[4] Jeganathan N. Burden Of Sepsis In India. Chest. 2022;161(6):1438-9.

[5] Kumar A, Haery C, Paladugu B, Kumar A, Symeoneides S, Taiberg L Et Al. The Duration Of Hypotension Before The Initiation Of Antibiotic Treatment Is A Critical Determinant Of Survival In A Murine Model Of Escherichia Coli Septic Shock: Association With Serum Lactate And Inflammatory Cytokine Levels. J Infect Dis. 2006; 193:251–8.

[6] Ferrer R, Loeches Mi, Phillips G, Osborn Tm, Townsend S, Dellinger Rp Et Al. Empiric Antibiotic Treatment Reduces Mortality In Severe Sepsis And Septic Shock From The First Hour: Results From A Guideline-Based Performance Improvement Program. Crit Care Med. 2014; 42:1749–55.

[7] Performance Standards For Antimicrobial Susceptibility Testing (Clsi) 30th Ed. Clsi M100. Wayne, Pa: Clinical And Laboratory Standards Institute; 2020. Available From M100ed30 | Performance Standards For Antimicrobial Susceptibility Testing, 30th Edition (Clsi.Org). Accessed On March 23rd, 2021.

[8] Edelmann A, Pietzcker T, Wellinghausen N. Comparison Of Direct Disk Diffusion And Standard Microtitre Broth Dilution Susceptibility Testing Of Blood Culture Isolates. J Med Microbiol 2007; 56:202–7.

[9] Doern Gv, Scott Dr, Rashad Al, Kim Ks. Evaluation Of A Direct Blood Culture Disk Diffusion Antimicrobial Susceptibility Test. Antimicrob Agents Chemother 1981; 20:696 8.

[10] Johnson Je, Washington Ja 2nd. Comparison Of Direct And Standardized Antimicrobial Susceptibility Testing Of Positive Blood Cultures. Antimicrob Agents Chemother. 1976;10(2):211-4.
[11] Mirrett S, Reller Lb. Comparison Of Direct And Standard Antimicrobial Disk Susceptibility Testing For Bacteria Isolated From Blood. J Clin Microbiol. 1979;10(4):482-7.
[12] Daniel Ww And Cross Cl. Biostatistics: A Foundation For Analysis In The Health Sciences. 10th Ed. New Jersey: John Wiley & Sons; 2013.
[13] Mackie, Mccartney. Practical Medical Microbiology 14th Edition; Philadelphia: Elsivier Churchill Livingstone; 2006; P.95-151.
[14] Chandrasekaran S, Abbott A, Campeau S, Zimmer Bl, Weinstein M, Thrupp L, Et Al. Direct-From Blood Culture Disk Diffusion To Determine Antimicrobial Susceptibility Of Gram- Negative Bacteria: Preliminary Report From The Clinical And Laboratory Standards Institute Methods Development And Standardization Working Group. J Clin Microbiol.2018;56: E01678-17.
[15] Imtiaz A, Ikram A, Zaman G, Satti L, Sana F. Evaluation Of Direct Drug Susceptibility Testing Of Blood Culture Isolates Comparing It With Conventional Disk Diffusion Testing. Jpma. 2020; 70:105.
[16] 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.
[17] Yakoob R And Bhat Gk. Comparison Of Direct Antibiotic Susceptibility Testing With Standard Testing In Blood Culture J Pure Appl Microbiol. 2018;12(4):2289-96.
[18] Shanthraju Lr, Devi G. Evaluation Of Direct Sensitivity Testing As A Method For Early Initiation Of Treatment In Gram-Negative Sepsis. Journal Of Academy Of Clin Micro. 2016;18(2):131-4.
[19] Lokeshwari V, Bhat Sk, Devi Ip. Direct Susceptibility Testing By Disk Diffusion On Positive Bact/Alert Blood Cultures: A Rapid And Definite Tool For Antibiotic Stewardship. Int J Of Med Microbiol And Trop Diseases 2021;7(3):179–185.
[20] Kavipriya D, Prakash Ss, Dhandapani S, Rajshekar D, Sastry As. Evaluation Of The Performance Of Direct Susceptibility Test By Vitek-2 From Positively Flagged Blood Culture Broth For Gram-Negative Bacilli. J Lab Physicians. 2021;13(4):374-9.
[21] 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.
[22] Waites Kb, Brookings Es, Moser Sa, Zimmer Bl. Direct Susceptibility Testing With Positive Bact/Alert Blood Cultures By Using Microscan Overnight And Rapid Panels. J Clin Microbiol 1998; 36:2052 6.
[23] Yu Fl, Lin Mh, Lee Jc, Lian Ly, Lin Cw, Chen Ct, Et Al. Comparison Of Antimicrobial Susceptibility Testing Of Isolates From Blood Cultures By Direct Inoculation Method And Phoenix. J Biomed Lab Sci 2011; 23:23-7.
[24] Menon V, Lahanas S, Janto C, Lee A. Utility Of Direct Susceptibility Testing On Blood Cultures: Is It Still Worthwhile? J Med Microbiol. 2016;65(6):501-9.
[25] Guidance For Industry And Fda Class Ii Special Controls Guidance Document: Antimicrobial Susceptibility Test (Ast) Systems [Internet]. 2009. Available From: Https://Www.Fda.Gov/ Downloads/Medicaldevices/Deviceregulationandguidance/Guidancedocuments/Ucm071462.Pdf. Accessed On March 24,2023.