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Comparative Study on Direct Susceptibility Test And Standardised AST in Positive Blood Cultures of Cancer And Critically Ill Patients

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Abstract
Introduction: *Blood stream infection is one of the major cause of morbidity & mortality in developing countries as of ours. The incidence of sepsis is increasing all over the world particularly in developing countries. Moreover the scenario is complicated in high risk patients particularly neutropenic patients. Standardized methods of blood culture take 48 hours for the Antibiotic susceptibility test result even after being flagged positive by an automated blood culture system and even more by conventional blood culture method. We proceed with this study so as to establish a system so that we can cut short the duration of initial result of AST available to the clinicians for the betterment of these patients.*
Materials & Methods: *The study was a hospital based comparative study conducted in a tertiary care centre Guwahati. Analysis of two methods for Antibiotic sensitivity, Direct Sensitivity test (DST) described in BSAC and standardised AST described in CLSI guidelines against the organisms isolated from critical & FN patients with BSI, admitted from September 2014 to September 2016.*
Result: *A total of 106 GNB & 53 GPC bacterial isolates were compared for standardized AST & DST. DST results were typically available 24 hrs after the Blood culture was flagged positive by the VersaTrek system compared to 48 hrs for standardized AST results. Thus DST results were released and was available 24 hrs before the standardized AST*

I. Introduction

Blood stream infection is one of the major cause of morbidity & mortality in developing countries as of ours. The incidence of sepsis is increasing all over the world particularly in developing countries. Moreover the scenario is complicated in high risk patients particularly neutropenic patients. Detection of bacteremia or fungemia by blood culture is critical in managing patients with infection, and directs the appropriate selection of antimicrobials. Timely administration of appropriate antibiotics plays a crucial role in the morbidity & mortality of these patients, with data showing an overall reduction (11%) in crude mortality rate is associated with adequate early empirical antimicrobial therapy & inappropriate initial antimicrobial therapy was identified as an independent risk factor for mortality [(1)].

Standardized methods of blood culture take 48 hours for the Antibiotic susceptibility test result even after being flagged positive by an automated blood culture system and even more by conventional blood culture method. Direct susceptibility testing (DST) form positive Blood culture broths is a well established method, in inoculums standardisation culture broth for direct sensitivity in British society for Antimicrobial Chemotherapy (BSAC) methods[(2,3)]. Isolates from positive blood cultures has been evaluated with automated systems to get a comparable result[(4,5)] in many studies. Studies have also been done directly on clinical samples to deduce methods like DST to reduce the turn - around time[(6,7)].

The earliest initiation of appropriate antibiotics is directly related to the morbidity and mortality of the patients with BSI. As both early initiation and appropriate antibiotics are essential, as in this era of antibiotic resistant organisms, administration of right antibiotics at the earliest is important in the outcome of the patient. Keeping this in mind we proceed with this study so as to establish a system so that we can cut short the duration of initial result of AST available to the clinicians for the betterment of these patients.

II. Materials & Methods

The study was a hospital based comparative study conducted in a tertiary care centre Guwahati. Analysis of two methods for Antibiotic sensitivity, Direct Sensitivity test (DST) described in BSAC and standardised AST described in CLSI guidelines against the organisms isolated from critical & FN patients with BSI, admitted from September 2014 to September 2016. The Inoculum standardization was done by inoculating five colonies of one of the test organisms (ATCC 25922, ATCC700603 & ATCC 25923) in the BHI bottles, which were incubated at 35°C for 24 h, after which the growth was evenly suspended in the broth.

Various combination of the suspension 1 drop in 2ml, 3ml & 5ml sterile 0.45% saline, 2 drops in 2ml, 3ml & 5ml sterile 0.45% saline etc., were prepared & streaked in duplicate onto the surfaces of 90-mm Mueller-Hinton agar plates. Himedia sterile swabs were used to streak the inoculum in three planes. Antibiotic discs were then applied, and the plates were incubated at 35°C overnight. The zone diameters were measured to the nearest millimeter, and values of the duplicate trials for each antibiotic at each inoculums volume were averaged. The entire experiment was repeated 10 times for each of the three test organisms to get the right inoculum.
All the blood culture bottles which were labelled positive by the VersaTrek of these patients were followed. The blood culture bottles were first processed by doing the gram stain, the blood culture with poly microbial growth were not included in the study. Then one part is processed in the conventional way by sub culturing it on Mac Conkey and Blood Agar and other part was subjected to DST.

Exclusion criteria:
1. Polymicrobial growth
2. Growth of gram positive cocci other than staphylococcus and Enterococcus species.
3. When inoculums of DST is discordant with the standard

Direct Susceptibility Testing By Disk Diffusion Method

For DST of GNB 1 drop of blood culture media (BHI broth) flagged positive by the Versa Trek was added to 5 ml of 0.45% sterile saline by using sterile 2ml Dispo van syringe, which shows semi confluent growth on the Muller- Hinton Media used for disk diffusion testing after incubation at 37^0 c for 18 – 20 hours, simultaneously the flagged positive bottle was processed on Blood and Macconkey agar as done routinely and AST was done by Kirby- Bauer method according to CLSI guidelines on the subsequent day. Result of DST was communicated to the clinician as provisional report & final report was given a day after, the standardised susceptibility test report. Result of the DST was interpreted as Sensitive (S), Intermediate (I), or Resistant (R) as per standards of the BSAC methods for Antimicrobial Susceptibility Testing.

For DST of GPC, Using a 2ml syringe (Dispo Van) three drops of the Positive flagged blood culture is dispensed in 5 ml of sterile 0.45% saline, then a sterile cotton-wool swab was dipped in the suspension and excess removed by turning the swab against the inside of the tube, Using the swab the inoculum is spreaded evenly over the surface of the susceptibility plate. Plates were then incubated within 15 min of disc application. Antibiotic discs strength used for DST was according to the recommendation of BSAC guidelines. Susceptibility results obtained by DST and standardised AST were then compared. The tests were however not repeated on discordant results, DST results were considered as provisional report and the Standardised AST result was considered as the final report. Major error was labelled when test procedure (DST) result was S & reference procedure revealed either I or R and vice-versa. Minor error was labelled when test procedure (DST) result was S/I/R & reference procedure also revealed S/I/R, but the zone sizes were different i.e. difference of more than 2 mm.

III. Result

A total of 106 GNB & 53 GPC bacterial isolates were compared for standardized AST & DST. DST results were typically available 24 hrs after the Blood culture was flagged positive by the VersaTrek system compared to 48 hrs for standardized AST results. Thus DST results were released and was available 24 hrs before the standardized AST because of isolating pure colonies which require overnight incubation on Blood & Macconkey Agar media.

Table 1:

Sl No.	**Antibiotics**	Major Error	Minor Error
1.	Meropenem	0	3
2.	Ertapenem	0	2
3.	Imipenem	0	3
4.	Ampicillin	0	7
5.	Amoxycillin + clavulinic acid	1	15
6.	Piperacillin + Tazobactam	0	12
7.	Cefuroxime	1	11
8.	Ceftriaxone	0	9
9.	Cefoperazone + sulbactam	0	14
10.	Cefepime	0	9
11.	Ceftazidime	1	17
12.	Amikacin	0	11
13.	Gentamicin	0	16
14.	Ciprofloxacin	0	14
15.	Teigecycline	0	3
16.	Colistin	0	2

17.	Cotrimoxazole	2	27
18.	Nalidixic acid	0	9
19.	Penicillin	0	14
20.	Levofloxacin	0	12
21.	Clindamycin	2	12
22.	Erythromycin	2	9
23.	Linezolid	0	7
24.	Daptomycin	0	6
25.	Teicoplanin	0	11
26.	Vancomycin	0	6
27.	Tetracycline	3	15

Table 1 shows there is a high concordance with the DST result.

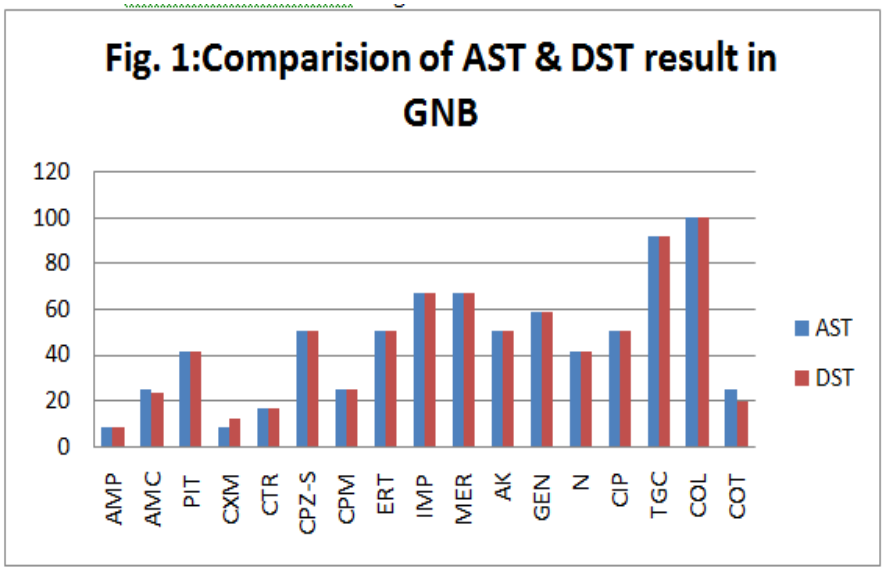
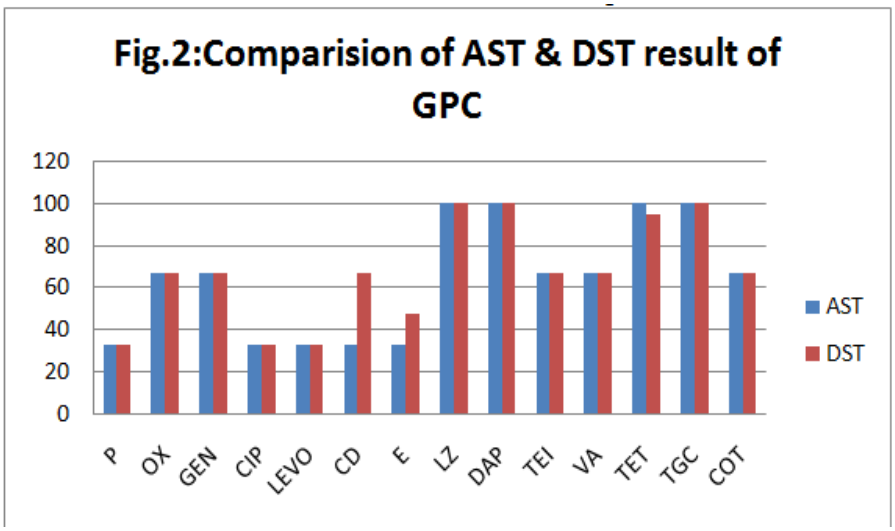


Fig. 1 & 2 shows the comparison of antibiotic susceptibility results of the neutropenic patients by both the methods, which show high concordance.



IV. Discussion

As early initiation and right antibiotic i.e. sensitive antibiotic to the particular causative organism plays a crucial role in the outcome of critically ill and neutropenic patients, in an attempt to shorten reporting time for susceptibility results, several authors have suggested that in urgent situations susceptibility plates may be inoculated directly with clinical material. The results of these direct or preliminary tests are then confirmed the following day by using one of the accepted standardized methods. In our study we observed that the standardisation of the inoculums for GNB & GPC is the most critical step, as described by BSAC the semi confluent growth to be obtained. We have to exclude few plates due to lack of lesser or heavier lawn growth as is described semi-confluent bacterial growth on the disk diffusion plates, as required by BSAC guidelines.

Major problems with DST was the preparation of right semi- confluent growth as described by BSAC, which showed variation from person to person and also type of organisms. Apart from it polymicrobial growth can't be evaluated.

However we get some advantage on doing DST. The heteroresistant organisms were well detected in DST Which many a times got skipped in the Standardised AST, we found 9 heteroresistant organisms. As our study reveals there is a high concordance in the DST & AST results, DST can be utilised for those critical patients suffering from BSI specially the neutropenic patients to cut short the duration of initiation of appropriate antibiotics by at least 24 hours, thus reducing the mortality and morbidity. There is high agreement between the results of DST & Standardised AST susceptibility results, thus it can be incorporated as a preliminary report. However more studies with more number of causative organisms and the antibiotics are warranted to standardize the reporting. An added advantage of DST is Heteroresistant organisms is taken care of thus giving an actual sensitive result in comparision to the Standardised AST where the Heteroresistant organisms may get skipped. Similar results were produced by different authors, Miño de Kaspar H found the two methods yielded identical susceptibility results in 409 (88%) of the 467 tests in 24 endophthalmitis aspirates.

V. Conclusion

Our findings suggest that DST of positive monbacterial BSI specimens could safely used along with standardized susceptibility testing for critically ill patients and to reduce the duration of early initiation of appropriate Antibiotics under certain circumstances. Initial antibiotic therapy can quite reliably be based on the results of DST, followed by confirmation of the result the following day. More research on a larger scale is warranted to extend it to other clinical specimen like CSF, body fluid and vitreous.

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