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RESEARCH ARTICLE

Comparision of Direct Antibiotic Susceptibility Testing with Standard Testing in Blood Culture

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Abstract

To perform Direct Antibiotic Susceptibility Testing (DAST) on positive blood culture and compare with that of standard antibiotic susceptibility testing. Blood cultures performed at Department of Microbiology Kasturba Medical College and Microbiology diagnostic centre (KMC hospital Ambedkar circle), Mangalore. Blood cultures with monobacterial bacteraemia. Cross sectional comparative time bound study of 116 samples. Out of 116 positive blood culture samples. It was seen that over all error rate was 55.17% minor error/categorical error, 15.5% of major error and 0.8% of very major errors were detected. The inoculum size influences the result of DAST and standard AST. Therefore standardization of inoculum is crucial. To conclude DAST using disk diffusion from positive blood culture bottles help to start early antibiotic treatment for bacteraemia/septicaemia.

Keywords: Blood stream infection, Direct antibiotic susceptibility testing, concordance.

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INTRODUCTION

Sepsis is one of the leading causes of morbidity and mortality in hospitalised patients[1]. Accurate information of patients with blood stream infections can be obtained by performing antibiotic susceptibility test and identification of bacteria[2]. Timely and early information regarding the identification and susceptibility pattern of significant bacteria helps the clinicians in rapid diagnosis, determine resistance pattern both in community and institutions and also contribute to the reduction in hospital-care associated costs[3]. Appropriate treatment and early diagnosis of blood stream infection can save the life of people[4].

The conventional phenotypic methods, based on culturing on agar (e.g.: Disk diffusion test) or on micro titration plates (e.g.: broth dilution method) is one of the commonly employed methods[2]. In case of standard AST, the results are available only with a delay of 48-72h after sampling as bacteria needed to be cultured before AST can be executed[2]. Standardised of inoculum is a main problem in DAST, so it has been criticised by American Society for Microbiology (ASM), the British Society for Antimicrobial Chemotherapy (BASC), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[2].

Disk diffusion has many advantages such as it allows the visibility of growth, correct inoculum, mixed cultures and other abnormalities and it is flexible and cheap. And another benefit is the possibility of executing DAST[2.] Rapid and most reliable results for direct and standard inoculation are provided by the non-fastidious *Enterobacteriaceae* family among all the other bacteria[5]. DAST by turbid broth is an accepted method for rapid reporting. Although not mentioned in CLSI, but standardization of broth for direct sensitivity is mentioned in BSAC (British Society for Antimicrobial Chemotherapy) methods[6,7]. Previous studies comparing DAST with conventional method have yielded varying results and these studies were conducted only on gram negative bacilli. The study focuses on performance and clinical significance of DAST in blood culture.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, Kasturba Medical College, Mangalore and blood samples for culture were received from District Wenlock Hospital, Lady Goschen Hospital and Kasturba Medical College Hospital, Ambedkar Circle, Mangalore.

Study duration

The study was conducted from January 2017 to June 2017.

Study design

Cross sectional comparative time bound study.

Sample size

A total of 116 positive blood culture samples were included in the study (time bound).

Inclusion criteria

Blood cultures with monobacterial bacteraemia.

Exclusion criteria

1) Blood cultures with two or more isolates.
2) Blood cultures positive for fungi.

Ethical consideration

The present study had approval of the Institutional Ethics Committee (IEC KMC MLR 11-16 /305,16th November 2016).

Processing of samples

Samples of blood was collected with aseptic precaution from patients with suspected bacteraemia/septicaemia and was inoculated into blood culture bottles and incubated in BACTEC 9050 or BacT/ALERT systems. When the system beeps showing growth of bacteria in blood culture bottles, bottle was removed and an aliquot of sample was used for smear preparation and Gram staining. Samples which show single type of bacteria were used for the study[8].

Direct Antibiotic Susceptibility Testing (DAST) By Disk Diffusion Method

Gram negative bacilli

A drop (20µl) of blood was placed in 5 ml of sterile water using the venting needle. A sterile cotton wool swab was dipped and excess was removed by turning the swab against the walls of the container. The swab was used to spread the inoculum evenly over the surface of susceptibility plate (6).The following panels of antimicrobial disks were used: amikacin (30µg), ampicillin (10µg), amoxicillin/ clavulanic acid (20/10µg),cefuroxime(30µg), cefepime(30µg), ceftriaxone(30µg), ciprofloxacin (5µg),cefoperazone/sulbactum (5µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), ertapenem (10µg), gentamicin (10µg), imipenem

(10µg), meropenem (10µg), piperacillin/ tazobactum (10µg), tigecycline (15µg). The zone inhibition were interpreted as Susceptibile (S), Intermediate (I), Resistant (R) as per CLSI guidelines 2016[9].

Gram positive cocci

Three drops (60µl) of blood was mixed in 5 ml of sterile water using the venting needle. A sterile cotton wool swab was dipped and excess was removed by turning the swab against the walls of the container. The swab was then used to spread the inoculum evenly over the surface of susceptibility plate (1). The following panels of antimicrobial disks were used; For *Staphylococcus aureus* and coagulase negative staphylococci: clindamycin (2µg), cefoxitin (30µg) ciprofloxacin(5µg),erythromycin (5µg) gentamicin (10µg), linezolid (30µg), rifampicin (5µg), penicillin (10units), trimethoprim/sulfamethaxazole (1.25/3.75µg), teicoplanin (30µg).

For enterococcus species: Ampicillin (10 µg), amikacin (30 µg), high level gentamicin (120 µg), high level streptomycin (300 µg) ,imipenem (10 µg),meropenem (10 µg), pencillin (10units), teicoplanin (30 µg) and vancomycin(30 µg). The zone inhibition were interpreted as Susceptibile(S), Intermediate (I), Resistant (R) as per CLSI guidelines 2016[9]. Each bottle positive flagged by the BACTEC or BacT/ALERT instrument was inoculated on Mac Conkey agar, blood agar and chocolate agar and was incubated at 37°C for 24 hours. Standard antibiotic susceptibility testing was done using the culture isolates, by Kirby Bauer disk diffusion method or VITEK 2 system[1,8].

Conventional susceptibility testing

An aliquot of the positive blood culture broth was plated on Mac Conkey Agar, Blood Agar and chocolate agar which were procured from HiMedia Laboratories Limited, Mumbai, India, and incubated at 35°C overnight to obtain isolated colonies. These colonies will be inoculated to Muller-Hinton Broth to make a suspension equivalent to a 0.5 McFarland standard[8]. The above mentioned antibiotics were procured from HiMedia Laboratories Limited, Mumbai, India and interpreted accordingly,(CLSI guidelines 2016)[9].

Quality control

The quality control strains, *E.coli* ATCC 25922, *S.aureus* ATCC 25923, *E.faecalis* 29212 and *P.aeruginosa* ATCC 27853 were tested weekly by the reference method[1].

Interpretation of results and data analysis

Susceptibility results obtained by direct antibiotic susceptibility testing were compared with conventional susceptibility test.

The following definitions were used:

1) Essential agreement or minor errors: standard method is susceptible (S) or resistant (R) and DAST is intermediate (I); alternatively, standard method is intermediate (I) and DAST is susceptible (S) or resistance (R).
2) Major errors; standard method yields susceptible (S) result whereas DAST yields resistance (R)
3) Very major errors: standard method is resistance (R) and DAST yields susceptible (S) [1].

RESULTS

A total of 116 gram negative and gram positive bacterial isolates were obtained. The distribution of gram negative bacilli and gram positive cocci is given in fig 1.

The most frequent isolates among gram negative bacilli and gram positive isolates is given in table 1 and table 2 respectively.

It was observed that out of 29 *E.coli* isolates 26(93.10%) were susceptibile to tigecycline, 24 (86.2%) to ertapenem and meropenem. Among 23 *k.pneumoniae* isolates 23(100%) was susceptibile to tigecycline,19(82.6%) to meropenem and imepenem. All the strains of *S.aureus* (25)isolated were found to be resistant to 25(100%) pencillin.

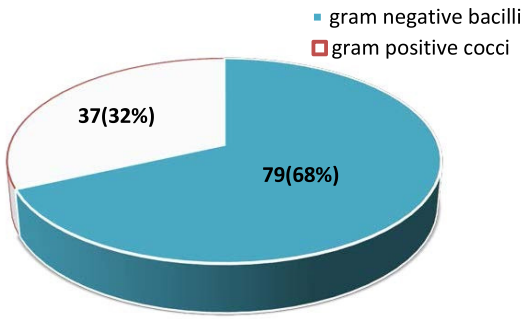

Fig. 1. Isolation of bacteria from blood culture

Table 1. Total number of gram negative isolates from blood culture (n=79)

Gram negative organisms	No. (%)isolated
Escherichia coli	29 (25%)
Klebsiella pneumoniae	23 (19.82%)
Pseudomonas aeruginosa	7 (6.03%)
Salmonella typhi	5 (4.3%)
Acinitobacter baumannii	3 (2.58%)
Klebsiella oxytoca	2 (1.72%)
Enterobacter cloacae	2 (1.72%)
Citrobacter freudii	2 (1.72%)
Aeromonas hydrophila	1 (0.86%)
Proteus mirabilis	1 (0.86%)
Achromabacter xylosoxidans	1 (0.86%)
Ralstonia picketti	1 0.86%)

Table 2. Total number of gram positive isolates from blood culture (n=37)

Gram positive organisms	No. (%) isolated
Staphylococcus aureus	25 (21.5%)
Coagulase negative Staphylococcus	7(6.3%)
Enterococcus faecalis	3 (2.58%)
Enterococcus faecium	2(1.72%)

For all 116 gram negative isolates; we observed 94.3% of no errors. DST yields 55.17% categorical agreement / minor error, 15.5 % major error and 0.8 % very major error. Direct antimicrobial susceptibility correlation for gram negative bacteria and gram positive bacteria is given in table 3,4,5,6 and 7 respectively.

DISCUSSION

Although there are many advances and diagnosis for the treatment of sepsis. Sepsis remains a major cause of death these days. Early detection of pathogen and their susceptibility pattern, plays a vital role in the diagnosis of sepsis. Immediate administration of antibiotics in sepsis is essential so as to reduce the morbidity and mortality rate in the hospitals. Mixed cultures and improper standardisation of the inoculums are the two major issues stated in the previous studies[10]. In the present study 116 positive blood culture samples were evaluated which were suspicious cases of septicaemia. Only unimicrobial culture was included in our study. Polymicrobial cultures were avoided based on gram stain results but few specimen that appeared to be unimicrobial were later found to produce polymicrobial growth in

Table 3. Direct antibiotic susceptibility correlation for gram negative bacilli (n=79)

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error/ essential agreement		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Amikacin(30µg)	1	1.26	6	7.59	12	15.18	60	75.9
Amoxicillin/Clavulanic Acid(10µg)	1	1.2	5	6.3	7	8.8	66	83.5
Ampicillin(10 µg)	0	0	0	0	0	0	79	100
Ceftriaxone(30 µg)	0	0	0	0	0	0	79	100
Cefuroxime(30 µg)	0	0	0	0	0	0	79	100
Cefoperazone/Sulbactum(5 µg)	0	0	0	0	1	1.2	78	98.7
Ciprofloxacin(5 µg)	0	0	1	1.2	1	1.2	77	97.4
Ertapenem(10 µg)	0	0	0	0	2	2.5	77	97.4
Gentamicin(10 µg)	0	0	0	0	7	8.8	72	91.1
Imipenem(10 µg)	0	0	0	0	1	1.2	78	98.7
Meropenem(10 µg)	0	0	0	0	0	0	79	100
Piperacillin/Tazobactum(10 µg)	0	0	0	1.8	7	8.8	72	91.1
Tigecycline(15 µg)	0	0	6	7.5	6	7.5	73	92.4
Cefepime(30 µg)	0	0	0	0	10	12.6	69	87.3
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	1	1.2	0	0	0	0	78	98.7

Table 4. Direct antibiotic susceptibility correlation for *E.coli* (n=29)

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error/ essential agreement		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Amikacin(30µg)	1	3.4	6	20.6	6	20.6	16	55.1
Amoxicillin/Clavulanic Acid(10µg)	0	0	5	17.2	5	17.2	19	65.5
Ampicillin(10 µg)	0	0	0	0	0	0	29	100
Ceftriaxone(30 µg)	0	0	0	0	0	0	29	100
Cefuroxime(30 µg)	0	0	0	0	0	0	29	100
Cefoperazone/Sulbactum(5 µg)	0	0	0	0	0	0	29	100
Ciprofloxacin(5 µg)	0	0	0	0	0	0	29	100
Ertapenem(10 µg)	0	0	0	0	0	0	29	100
Gentamicin(10 µg)	0	0	0	0	4	13.7	25	86.2
Imipenem(10 µg)	0	0	0	0	0	0	29	100
Meropenem(10 µg)	0	0	0	0	0	0	29	100
Piperacillin/Tazobactum(10 µg)	0	0	0	0	4	13.7	25	86.2
Tigecycline(15 µg)	0	0	5	17.2	2	6.8	22	75.8
Cefepime(30 µg)	0	0	0	0	6	20.6	23	79.3
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	0	0	0	0	0	0	29	100

Table 5. Direct antibiotic susceptibility correlation for *K.pneumoniae* (n=23)

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error/ essential agreement		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Amikacin(30µg)	0	0	0	0	4	17.3	19	82.6
Amoxicillin/Clavulanic Acid(10µg)	1	4.3	0	0	2	8.6	20	86.9
Ampicillin(10 µg)	0	0	0	0	0	0	23	100
Ceftriaxone(30 µg)	0	0	0	0	0	0	23	100
Cefuroxime(30 µg)	0	0	0	0	0	0	23	100
Cefoperazone/Sulbactum(5 µg)	0	0	0	0	0	0	23	100
Ciprofloxacin(5 µg)	0	0	0	0	0	0	23	100
Ertapenem(10 µg)	0	0	0	0	2	8.6	21	91.3
Gentamicin(10 µg)	0	0	0	0	2	8.6	21	91.3
Imipenem(10 µg)	0	0	0	0	0	0	23	100
Meropenem(10 µg)	0	0	0	0	2	8.6	21	91.3
Piperacillin/Tazobactum(10 µg)	0	0	0	0	0	0	23	100
Tigecycline(15 µg)	0	0	1	4.3	2	8.6	20	86.9
Cefepime(30 µg)	0	0	0	0	2	8.6	21	91.3
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	1	4.3	0	0	0	0	22	95.6

Table 6. Direct antibiotic susceptibility correletion for *S.aureus* and Coagulase Negative *Staphylococcus* (n=32)

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error/ essential agreement		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Cefoxitin (30 µg)	0	0	0	0	3	8.10	29	90.62
Clindamycin(2 µg)	1	2.7	0	0	0	0	31	96.87
Ciprofloxacin (5 µg)	0	0	0	0	0	0	32	100
Erythromycin(5 µg)	0	0	0	0	0	0	32	100
Linezolid (30 µg)	0	0	0	0	0	0	32	100
Gentamicin (30 µg)	0	0	0	0	2	5.4	30	93.75
Penicillin(10unit)	0	0	0	0	0	0	32	100
Rifampicin(5 µg)	1	2.7	0	0	0	0	31	96.87
Teicoplanin(30 µg)	0	0	4	10.8	4	10.8	24	75

Table 7. Direct antibiotic susceptibility correletion for *Enterococcus faecium* and *Enterococcus faecalis (n=5)*

Antibiotics used	Direct susceptibility method							
	Very major error		Major error		Minor error/ essential agreement		Concordance	
	NO.	%	NO.	%	NO.	%	NO.	%
Amikacin(30 µg)	0	0	0	0	0	0	5	100
Ampicillin(10 µg)	0	0	0	0	0	0	5	100
High Level Gentamicin (120 µg)	0	0	0	0	0	0	5	100
High Level Streptomycin (300 µg)	0	0	0	0	0	0	5	100
Imipenem (10 µg)	0	0	0	0	0	0	5	100
Meropenem (10 µg)	0	0	0	0	0	0	5	100
Penicillin (10 units)	0	0	0	0	0	0	5	100
Teicoplanin (30 µg)	0	0	0	0	0	0	5	100
Vancomycin (30 µg)	0	0	0	0	0	0	5	100

subcultures. Also blood cultures positive for fungus were also excluded from our study. Frequency of isolation in our study for gram negative isolates were found to be higher than gram positive isolates. This was similar to many other studies[1,2]. There was a contradiction in many other studies, that gram positive isolates was higher than gram negative isolates[11,19]. In the study done by fay and Oldfather , method of standardisation of inoculum was directly on the blood samples. Due to this there was a possibility of different inhibitory factors present in the blood to interfere with the results produced[11]. Therefore to avoid the error that can occur due to standardisation of inoculums we followed the guidelines of BSAC[6]. For standardisation we used the method of diluting the blood samples with sterile saline ,which reduced the inhibitory factor.

We used different volumes which was slightly different from the study done by Fay and Oldfather. They used an inoculum of 0.03 ml whereas in the present study we used a volume of 5 ml sterile water for the dilution of blood culture samples, according to BSAC guidelines[6]. Similar

study done from an oncology centre in Eastern India also used the similar method instead of sterile water they used 0.45% saline[1].

Since 1970's many studies have been done based on comparison of Direct susceptibility testing with that of conventional testing. Even though several studies reported an agreement of 90-97%, repeating the sensitivity with the conventional method is proposed. In our study the percentage rate for very major error was found to be low followed by major error and the minor error rate was observed to be highest. Similar findings were reported in the study done by Washington and Johnson were the direct susceptibility testing was found to be both feasible and accurate as compared with standardised susceptibility testing[12]. Studies done by Mirret *et al* and Doern *et al* also observed the error rates to be similar to our study with however they did not report any very major errors[13,14].

A study done in Eastern India also showed that the rate of errors to be less[1]. However, the very major error rate was found to be high in several other studies which contradict the findings in our study[10,15]. Among the antibiotic panels evaluated for the gram negative isolates, it was observed that, very major errors were found in *E.coli* and *Klebsiella* for drugs amikacin , amoxicillin clavulanic acid , and trimethoprim/sulfamethoxazole and major error for amikacin, amoxicillin clavunic acid, tigecycline, and ciprofloxacin. Several other studies have reported different error rates for different antibiotic panels[1,16,17]. In case of gram positive isolates we obtained very major errors for clindamycin and rifampicin and major error for teicoplanin.

The frequent isolates in gram positive bacteria was *S.aureus* which was consistent with earlier studies[18]. Similarly in Gram negative isolates it was *Escherichia coli* followed by *Klebsiella* species. The isolate rate in case of gram negative bacteria was similar to the previously reported studies[10,18,19]. Among gram negative bacteria, imipenem and meropenem showed lesser resistance. *Enterobacteriaceae* family showed high resistance to ampicillin, amoxillin and gentamicin which was also reported in the studies[14,20]. All the strains of *S.aureus* isolated in our study were found to be resistant to penicillin which was comparable to another study done by Garg A *et al.*[21].

Since there was an concordance of 94.7% for Direct susceptibility testing result obtained by gram staining as compared to that of standard susceptibility testing, we can suggest the use of DAST as a part of empirical therapy.

CONCLUSION

We compared DAST and standard AST for blood culture isolates. For gram negative bacilli, the concordance rate between these two methods was 94.14%, whereas for gram positive cocci ,it was 95.26%. Gram negative bacilli showed highest concordance of 100% for the following antibiotics; ampicillin, cefrriaxone, cefuroxime and meropenem. Gram positive cocci showed highest concordance for clindamycin, erythromycin and pencillin.The inoculum size influences the result of DAST and standard AST. Therefore standardization of inoculum is crucial. To conclude DAST can be used to start early antibiotic treatment for bacteraemia/septicaemia.

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