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A study on direct antimicrobial susceptibility testing of positive blood culture broth using BacT/Alert 3D microbial identification system in a tertiary care hospital

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ABSTRACT

Background: Sepsis is one of the major causes of mortality and morbidity in hospitals. Bloodstream infections (BSI) affect approximately 2% of all hospitalized patients and 70% of patients admitted to the Intensive Care Units. Detection of BSI is one of the most important tasks performed in the microbiology laboratory. **Aims and Objectives:** This study aims to evaluate direct antimicrobial susceptibility tests (DAST) from positive blood culture broth in suspected bacteremia and compare the DAST and conventional antimicrobial susceptibility testing (CAST) of the isolates. **Materials and Methods:** A descriptive study involving a total of 112 aerobic blood culture bottles flagged positively by the automated Identification System (Bac T/Alert3D) was processed. The bacteria were pelleted by two-step centrifugation of the broth from the bottle and used to make a smear for Gram stain as well as an inoculum for antimicrobial sensitivity testing by Kirby–Bauer disk diffusion method. **Results:** Out of 842 blood culture samples, 112 flagged positive cultures were subjected to direct gram film, 28 were found to be polymicrobial, 24 Gram-positive cocci, and 60 were Gram-negative bacilli. There was a complete match between the direct Gram stain result from the positive bottle and the Gram stain from subcultures from the bottles obtained after overnight culture on solid media. **Conclusion:** This study has demonstrated good concordance between the DAST and CAST results. Hence, direct AST can be implemented in a routine diagnostic laboratory. Direct AST will be helpful to decrease the turnaround time and to start early antimicrobial therapy in critically ill patients.

Key words: DAST; Conventional antimicrobial susceptibility testing; Blood culture; Agreement

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INTRODUCTION

Sepsis is one of the major causes of mortality and morbidity in hospitalized patients. Blood culture is the gold standard method for the diagnosis of sepsis, and it is included in the early investigation to be sent for sepsis according to the Surviving Sepsis Campaign guidelines.[1]

The availability of culture and sensitivity results in patients with infections is important for clinicians in guiding them to select the most appropriate antimicrobial for treatment, thereby increasing the chances of maximal therapeutic effect.[1] Microbiology laboratory provides such information promptly, especially regarding cases of bloodstream infections (BSI).[1] With the advent of automated blood culture methods, the time taken for detection of the micro-organism has been reduced from 3–4 days to 2–3 days.[1] However, the advent of automation in place of subculture is required to obtain pure growth so that antimicrobial susceptibility testing (AST) can

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be carried out either by the Kirby–Bauer method or an automated method.[1,2]

The empirical therapy started initially with broad-spectrum antimicrobials perforce continues until the sensitivity results are made available.[3] However, it is to be emphasized that about 20–50% of all the prescribed antimicrobials are inappropriate. Patients getting these inappropriate antimicrobials get no extra clinical benefits while being at risk of suffering from adverse effects.[3] The most serious and ever-increasing public health problem is the emergence of antimicrobial resistance due to the misuse of antimicrobials.[4] These drug-resistant pathogens pose a threat to the health of patients in a health-care setup.[5]

One of the useful inputs in the implementation of antimicrobial stewardship is the early availability of AST, which can help the clinician to de-escalate the antimicrobial, thereby reducing the chances of the emergence of resistant organisms. The disk diffusion method for AST takes 48 h for the result to be generated. This includes the 24-h time taken for subculture from the positively flagged culture bottle onto solid culture media to obtain pure growth, in addition to AST, which takes another day to complete. Even the automated methods for AST take another half to 1 day for the results to be available.[6]

Sepsis is one of the major causes of mortality and morbidity in hospitals. BSIs affect approximately 2% of all hospitalized patients and 70% of patients admitted to the Intensive Care Units. Detection of BSI is one of the most important tasks performed in the microbiology laboratory. Rapid identification of isolates and their antimicrobial susceptibility is essential for patients with BSIs. Timely initiation of appropriate antimicrobials can improve the outcome of the patients. The availability of an early preliminary AST report will be useful in direct antimicrobial therapy. This study aims to evaluate the usefulness of the direct AST method from a positive blood cultures broth, thereby helping to reduce the turnaround time (TAT) and early initiation of antibiotics in critically ill patients.[6]

Aims and objectives

This study aims to evaluate the usefulness of direct antimicrobial sensitivity testing from positive blood culture broth in suspected bacteremia and also to compare the direct AST with the conventional disc diffusion method.

MATERIALS AND METHODS

This is a descriptive cross-sectional study conducted in a tertiary care hospital, in Chennai, Tamil Nadu, for 1 year. With the approval from the Institutional Ethical Committee and informed consent, patients were included in the study by simple random sampling method. A total of 842 blood culture samples were collected from patients with clinical suspicion of sepsis.

Inclusion criteria

The following criteria were included in the study:

- Blood culture samples were received in the laboratory for culture and sensitivity from adult patients with suspected sepsis
- Blood cultures with positive signal flagging off from the automated blood culture system show only one type of organism (Gram positive or Gram negative) by direct Gram-film.

Exclusion criteria

The following criteria were excluded from the study:

Positive blood cultures with more than one type of bacteria or skin commensals seen in direct Gram film, also negative alert signaling blood culture bottles were excluded.

Collection of blood samples for blood culture

Under utmost sterile precautions, 10 mL blood samples were collected by doing venipuncture and injected immediately into blood culture bottles. The bottles were loaded into the BACT/ALERT 3D automated system. The blood culture bottles were monitored for flag off signal from the automated system.[7]

Processing of the positive blood culture broth

Direct Gram film was done for positively flagged blood culture bottles from the BACT/ALERT 3D system within 8 h and then all positive blood culture broths were subcultured on blood agar and MacConkey's agar. Those bottles with a single type of organism under Gram film were subjected to direct antimicrobial susceptibility test (DAST) and were performed by disk diffusion method as per CLSI guidelines for Enterobacterales and *Pseudomonas* and the European Committee on AST (EUCAST) rapid AST (RAST) guidelines for Gram-positive organisms and read as per the breakpoints.[8-10]

Procedure for RAST

The inoculum was mixed thoroughly by inverting the blood culture bottle 5–10 times, the 20-gauge venting needle was injected into the blood culture bottle after an alcohol wipe, and blood culture broth was withdrawn, four drops were dispensed on Mueller–Hinton agar (MHA) plate. Then, using a sterile cotton swab blood culture broth was spread across the entire surface of the MHA plate, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. After that leave the lid ajar for 3–5 min, antimicrobial disks were placed and pressed onto

the surface of the inoculated MHA plate. The plates were inverted and placed in the incubator at 37°C for 16–18 h for Gram-negative bacilli. Gram-positive cocci readings were taken after incubation for 8 h. Another blood agar plate was inoculated with the same inoculum to check the purity of the inoculum.[10]

Results were interpreted as follows; before starting interpretation, the blood agar plates were examined to ensure pure growth, and preliminary identification was done to confirm Gram-negative bacillus tested was Enterobacterales or *Pseudomonas aeruginosa* and Gram-positive cocci. Then, the test plates were examined to ensure confluent lawn of growth which was present. The zone diameters were measured and reported using the interpretive categories and zone diameter breakpoints.[8,9]

Conventional identification and susceptibility testing[11]

The bacterial pathogens were identified with colony characters and biochemical parameters from the subcultured media and proceeded with AST.

Conventional AST (CAST)

AST was performed by the Kirby–Bauer disk diffusion method for the isolated organisms.

Procedure

The test organisms grown on culture media were inoculated into peptone water and incubated at 37°C for 2–4 h. The turbidity is matched with 0.5 McFarland. A lawn culture was made and the antibiotic disk was placed on MHA, according to the growth (Gram-positive or Gram-negative organisms) Fig. 1. The plates were incubated for 18–24 h at 37°C. The zone diameters were recorded and interpreted as sensitive, intermediate, or resistant according to CLSI standards 2022.[8]

Statistical analysis

The results were tabulated into Microsoft Excel format and means and percentages were calculated for the susceptibility of pathogens for the given antimicrobials as per CLSI/EUKAST guidelines and analyzed in SPSS software. Based on the categorization of the strains into different susceptibility classes for the different tests, very major error (VME), major error (ME), and minor errors (mE) were calculated using proportions (percent). VME, ME, and mE are defined as a false susceptible result, a false resistant result, and a result involving an intermediate category, respectively. The AST by direct and conventional methods were compared using Cohen's kappa correlation coefficient statistics. The Kappa coefficient values were calculated and based on the kappa value that the results were graded as no agreement, slight agreement, fair agreement, moderate agreement, substantial agreement, near-perfect agreement, and perfect agreement.

RESULTS

Direct AST was performed as per CLSI guidelines for Gram-negative pathogens and Gram-positive cocci as per EUCAST RAST guidelines. Then, the AST results were compared with the conventional disk diffusion. Out of 842 blood culture samples received from patients with suspected BSIs, 112 were flagged for positive culture in the automated system as shown in Fig. 2. All the positive culture bottles were subjected to direct Gram film, and 28 were found to be polymicrobial, skin commensals and in the remaining, 24 were Gram-positive cocci and 60 were Gram-negative bacilli.

Among the 60 pathogens detected Gram-negative bacilli such as *Klebsiella pneumoniae* were 28 (46.6%), *Klebsiella oxytoca* 16 (26.6%), *Escherichia coli* 12 (20%), and *P. aeruginosa* 4 (6.6%) as shown in Fig. 3. Of the 24 Gram-positive cocci *Enterococcus faecalis*, *Enterococcus faecium*, Methicillin sensitive *Staphylococcus aureus*, and Coagulase negative Staphylococci contributed to 8 (33.3%). 2 (8.3%),6 (25%) and 8 (33.3%) respectively

Among the 60 Gram-negative bacilli belonging to Enterobacterales (*Klebsiella* species and *E. coli*), AST results were analyzed as per antimicrobial agents for Enterobacterales, out of 392 antimicrobial agent

Table 1: Correlation agreement between direct AST and conventional AST among Enterobacterales

Antimicrobial agent	Number of isolates tested (n=56) (%)				
	Agreement	VME	ME	mE	Total
Ampicillin	56 (100)				56
Meropenem	55 (98.2)	1			56
Ceftazidime	53 (94.6)		1	2	56
Ciprofloxacin	53 (94.6)	1		2	56
Cotrimoxazole	52 (92.8)	1	1	2	56
Aztreonem	55 (98.2)			1	56
Tobramycin	53 (94.6)	1		2	56
Overall agreement	377 (96.1)	4 (1)	2 (0.5)	9 (2)	392

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

combinations, 377 (96.1%) combinations showed categorical agreement, whereas 15 combinations showed disagreement of which 4 (1%) were VME. Two (0.5%) were ME and 9 (2.2%) were mEs and 100% categorical agreement to antimicrobials such as ampicillin, meropenem, and aztreonam with conventional disk diffusion method. However, fair agreement was found to ceftazidime (63.7%), ciprofloxacin (77%), and cotrimoxazole (53.8%). The antimicrobial tobramycin alone showed 53.8% agreement with the conventional method (Tables 1 and 2).

For *P. aeruginosa,* out of 14 (87.5%) combinations showed categorical agreement whereas two combinations showed disagreement of which 1 (6%) were ME and 1 (6%) mE. The categorical agreement for tobramycin, ceftazidime, and meropenem showed 100%, and fair agreement was found for ciprofloxacin (70%) with the conventional method, as shown in Tables 3 and 4.

Among 24 Gram-positive organisms, *Staphylococcus* species 51 (91%) combinations showed categorical agreement whereas five combinations showed disagreement of which 3 (5.3%) were ME, 2 (3.5%) mE, and fair agreement to cefoxitin (72%), slight agreement to norfloxacin (42%), clindamycin (53.5%), and gentamicin (53%) and no VME for all antibiotic combinations (Tables 5 and 6).

For *Enterococcus* species, 36 (90%) combinations showed categorical agreement whereas four combinations showed disagreement of which 2 (5%) were ME, 2 (5%) mE, and 100% agreement was found to vancomycin and linezolid, a fair agreement was found to ampicillin (43%) high-level gentamicin (66.6%), as shown in Tables 6 and 7.

DISCUSSION

Blood cultures remain the central component to determining the etiology of BSI as they are highly sensitive and easy to perform. An expeditious and appropriate diagnosis of the etiological agents along with their antimicrobial sensitivity pattern is of utmost essential. In this present era with practically a limited number of antimicrobials in the development pipeline, optimum use of the existing antimicrobials is crucial.[12] This misuse or abuse of antimicrobials has a direct relationship with the emergence and dissemination of resistant strains in health-care setups.[13] The appropriate antibiotic treatment within the shortest time can be initiated in BSIs with accurate and timely bacterial identification, and determination of antibiotic susceptibility in the microbiology laboratory and thus, accelerates the time of selection of appropriate antibiotics, shortens the time of stay in the hospital/intensive care unit, and reduces mortality.[13,14] This study proposes to compare the direct

Table 2: Agreement between direct AST and conventional disk diffusion method AST among Enterobacterales

Drug	Direct (%)									Conventional (%)									Agreement (%)	Cohen's K score
	Klebsiella pneumoniae (28)			*Klebsiella oxytoca* (16)			*Escherichia coli* (12)			*Klebsiella pneumoniae* (28)			*Klebsiella oxytoca* (16)			*Escherichia coli* (12)				
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R		
Ampicillin	0		28 100	0		16 100	0		12 100	0		28 100	0		16 100	0		12 100	100	1
Tobramycin	15 53.5		13 46.4	9 56.2		7 43.7	8 66.6	1 8.3	3 25	14 50		14 50	9 56.2	1 6.2	6 37.5	9 75		3 25	53.8 Slight agreement	0.068
Ceftazidime	5 17.8	1 3.5	22 78.5	2 12.5	1 6.25	13 81.2	2 16.6		10 83.3	5 17.8		23 82.1	3 18.7		13 81.2	3 25		9 75	63.7 Fair agreement	0.266
Ciprofloxacin	4 14.2		24 85.7	2 12.5		14 87.5	2 16.6		10 83.3	5 17.8		23 82.1	3 18.7		13 81.2	1 8.3		11 91.6	77 Fair agreement	0.364
Meropenem	20 71.4		8 28.5	14 87.5		2 12.5	10 83.3		2 16.6	20 71.4		8 28.5	14 87.5		2 12.5	10 83.3		2 16.6	100	1
Aztreonam	5 17.8		23 82.1	2 12.5	1 6.2	13 81.2	12 100		0	5 17.8		23 82.1	3 18.7		13 81.2	12 100		0	100	1
Cotrimoxazole	11 39.2	2 7.1	15 53.5	3 18.7		13 81.2	2 16.6		10 83.3	11 39.2		17 60.7	4 25		12 75	1 8.3		11 91.6	63.04 Fair agreement	0.21643

AST: Antimicrobial susceptibility test

Table 3: Agreement between direct AST and conventional AST for *Pseudomonas* spp.

Antimicrobial agent	Number of isolates tested (n=4)				
	Agreement (%)	VME	ME	mE	Total
Tobramycin	3 (75)			1	4
Ceftazidime	4 (100)				4
Ciprofloxacin	3 (75)		1		4
Meropenem	4 (100)				4
Overall agreement	14 (87.5)		1 (6%)	1 (6%)	16

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

Table 4: Agreement between direct AST and conventional disk diffusion method AST among non-fermenters

Drug	Direct (%)			Conventional (%)			Agreement (%)	Cohen's K score
	S	I	R	S	I	R		
Tobramycin	4	0	0	3	1	0	100	1
	100			75	25			
Ceftazidime	2	0	2	2	0	2	100	1
	50		50	50		50		
Ciprofloxacin	1	0	3	2	0	2	70	0.3478
	25		75	50		50	Fair agreement	
Meropenem	2	0	2	2	0	2	100	1
	50		50	50		50		

AST: Antimicrobial susceptibility test

Table 5: Agreement between direct AST and conventional AST among *Staphylococcus* species

Antimicrobial agent	Number of isolates tested (n=14)				
	Agreement	VME	ME	mE	Total
Cefoxitin	12		2		14
Norfloxacin	13			1	14
Gentamicin	13		1		14
Clindamycin	13			1	14
Overall agreement	51 (91%)		3 (5.3%)	2 (3.5%)	56

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

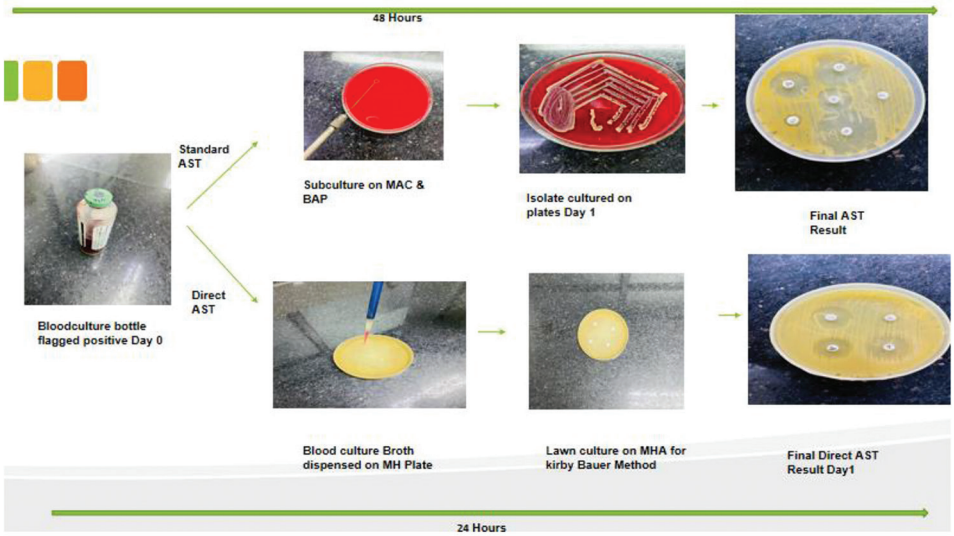

Figure 1: Flow chart showing standard AST and direct AST. BAP: Blood agar, MAC: MacConkey agar, MHA: Mueller-Hinton agar, AST: Antimicrobial susceptibility test. Images were photographed by the authors

Table 6: Agreement between direct AST and conventional disc diffusion method AST among Gram-positive cocci

Drug	Direct (%)								Conventional (%)						Agreement (%)	Cohen's K score
	Staphylococcus aureus (14)			*Enterococcus faecalis* (8)			*Enterococcus faecium* (2)		*Staphylococcus aureus* (14)			*Enterococcus faecalis* (8)			*Enterococcus faecium* (2)			
	S	ATU	R	S	ATU	R	S	R	S	ATU	R	S	R		S	R		
Cefoxitin	10	0	4	-	-	-	-	-	12	0	2	-	-		-	-	72	0.377
	71.4		28.5						85.7		14.2						Fair agreement	
Norfloxacin	9	1	4	-	-	-	-	-	10	0	4	-	-		-	-	100	1
	64.2	7.1	28.5						71.4		28.5							
Gentamicin	7	0	7	-	-	-	-	-	8	0	6	-	-		-	-	53	0.0714
	50		50						57.1		42.8						Slight agreement	
Clindamycin	6	1	7	-	-	-	-	-	6	0	8	-	-		-	-	53.5	0.0718
	42.8	7.4	50						42.8		57.1						Slight agreement	
Ampicillin	-	-	-	6	0	2	2	0	-	-	-	7	1		2	0	43	0.2264
				75		25	100					87.5	12.5		100		Fair agreement	
High Level Gentamicin	-	-	-	5	2	1	1	1	-	-	-	6	2		1	1	66.6	0.3478
				62.5	25	12.5	50	50				75	25		50	50	Fair agreement	
Vancomycin	-	-	-	7	1	0	2	0	-	-	-	8	0		2	0	100	1
				87.5	12.5		100					100			100			
Linezolid	-	-	-	8	0	0	2	0	-	-	-	8	0		2	0	100	1
				100			100					100			100	100		

AST: Antimicrobial susceptibility test

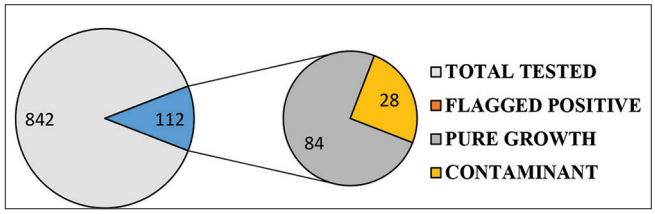

Figure 2: Distribution of blood culture samples

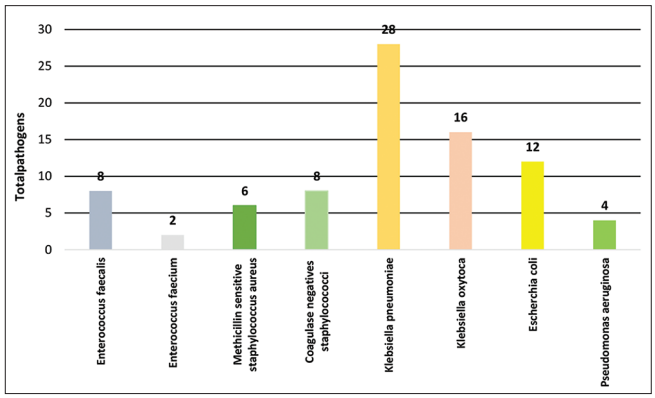

Figure 3: Distribution of organisms from positive blood cultures

antimicrobial sensitivity testing from the positive blood culture broth with the conventional disk diffusion testing for the blood culture bottle flagged off from the automated Bact/Alert3D blood culture system.

Growth of pathogenic microorganisms was found to be 9.8% in our study and this is by a study by Sarode et al., which showed 10.6%. However, in our study, blood culture contamination rate was 3.3% compared to the study by Sarode et al.,[15] where the contamination rate was in 3.5 %. The pathogens detected by the direct Gram stain result from the positive bottles were similar to those by Gram stain from the subsequent subcultures from the bottles obtained after overnight culture. Hence, the direct Gram film is very helpful to start empirical antibiotics in BSIs in the Golden hour.[16]

Early detection of pathogens along with analysis of their antibiotic susceptibility patterns is always the main goals of any diagnostic microbiology laboratory. Compared to CAST, performing DAST on positive blood culture broth provides a clinical team with information on the identity of the pathogen and its antibiotic susceptibility 24 h earlier, which can accelerate switching from empirical therapy to definitive treatment of the disease. Some of the studies have also proposed methods for DAST on clinical specimens.[17] Blood culture tests are critical investigations for any microbiology department, and a delay in reporting the results can significantly affect morbidity and mortality in patients.

In our study, the DAST showed 96.1% categorical agreement for Enterobacterales and 87.5% categorical agreement for *Pseudomonas* species with CAST. Similar

Table 7: Agreement between direct AST and conventional AST among *Enterococcus* spp.

Antimicrobial agent	Number of isolates tested (n=10)				
	Agreement	VME	ME	mE	Total
Ampicillin	9		1		10
High-level gentamicin	8			2	10
Vancomycin	9		1		10
Linezolid	10				10
Overall agreement	36 (90%)		2 (5%)	2 (5%)	50

AST: Antimicrobial susceptibility test, VME: Very major error, ME: Major error, mE: Minor error

findings were reported by Desai et al.,[18] and Rajshekar et al.,[19] who found the categorical agreement to be 90.4% and 96%, respectively. Good categorical agreement for gram-negative organisms has been reported by Kumar et al.[20]

In the case of GPC, DAST showed 91% categorical agreement for *Staphylococcus* species and 90% categorical agreement for *Enterococcus* species. *Staphylococcus* species 51 (91%) combinations showed categorical agreement whereas five combinations showed disagreement of which 3 (5.3%) were ME, 2 (3.5%) mE, and fair agreement to Cefoxitin (72%), slight agreement to norfloxacin (42%), clindamycin (53.5%), and gentamicin (53%). The study by Rajshekar et al., reported among Gram-positive cocci, both *Staphylococcus* species and *Enterococcus* species had CA of >95% for all the antibiotics tested and VME was unsatisfactory in *Staphylococcus* species for cefoxitin (4.9%) and for HLG (4.4%) in *Enterococcus* species. ME and mE were satisfactory among both groups. A similar observation was seen in a study conducted by Bennett and Sharp,[21] well correlating with our studies.

Various studies have compared the direct AST with the standard AST from blood culture bottles using different automated culture systems.[22,23] Most of these studies have found very good categorical agreement for the Gram-negative organisms and not so good agreement for Gram-positive organisms.[23-25] Good categorical agreement for Gram-positive organisms has been reported by Lupetti et al.[22] Nevertheless, our study shows very good categorical agreement for most of the Gram-positive organisms and good agreement for Gram-negative organisms. Moreover, as the correlation analysis shows minimal VME, both methods can be considered to have good concordance.

Limitations of the study

Some of the limitations of this study are : 1) There is a lack of definitive identification of the infecting bacteria. 2) The exclusion of yeasts and polymicrobial organisms on Gram stain are not specified.

CONCLUSION

This study has demonstrated good concordance between the direct AST and CAST results. Hence, direct AST can be implemented in a routine diagnostic laboratory. Direct AST will be helpful to decrease the TAT and to start early antimicrobial therapy in critically ill patients. This direct AST and reporting will be helpful for the implementation of the antimicrobial stewardship program. Although the causative organism cannot be definitively identified by our method, it still enables preliminary AST testing, offering a chance for early institution of appropriate antimicrobial therapy.

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