

ORIGINAL RESEARCH

Evaluation Of Direct Antimicrobial Susceptibility Testing From Positive Bactec Blood Culture Bottles

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ABSTRACT

Background: Conventional methods for isolation and antimicrobial susceptibility testing of bacterial isolates from positive blood culture bottles takes at least 48 hours. Direct antimicrobial susceptibility testing (AST) from positively flagged blood culture bottles helps to reduce the turnaround time (TAT) by 24 hours which will be useful in early initiation of appropriate antimicrobial therapy to reduce morbidity and mortality. **Aims and Objectives:** To evaluate the performance of direct AST from positively flagged BACTEC blood culture bottles and its comparison to conventional AST. **Methods:** A total of 356 blood culture bottles that were positively flagged on BACTEC 9120 were processed. Direct AST was performed from positive blood culture bottles. Bacterial isolates were identified using standard microbiological methods and tested against a wide spectrum of antimicrobial agents using the Kirby Bauer's disc diffusion method following the Clinical & Laboratory Standards Institute (CLSI) guidelines. Direct and conventional AST results were compared and Categorical agreement (CA) with various errors was evaluated. **Results:** On direct Gram staining, 107 samples showed Gram negative bacilli and 105 showed Gram positive cocci and 144 samples showed growth of contaminants. A total of 1486 organism-antibiotic combinations were evaluated, out of which 1438 (96.76%) combinations showed CA which was extremely satisfactory. The categorical disagreement was found only in 48 (3.23%) of organism-antibiotic combinations; out of which major error was 23 (1.54%) followed by minor error 17 (1.14%) and very major error 8 (0.53%). **Conclusions:** The present study showed good concordance between the two methods and suggests the use of direct AST to reduce TAT by 24 hours for early initiation of therapy in patients with blood stream infections.

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INTRODUCTION

Sepsis is a life threatening condition and it is a major cause of morbidity and mortality among hospitalized patients. There is an increase in mortality by about 7.6% with every hour of delay in the initiation of appropriate antimicrobial therapy (1). Early diagnosis plays a crucial role in managing sepsis, and hence, prompt detection of such infections is a critical function of clinical microbiology laboratories. Blood culture is a gold standard method for diagnosis of sepsis, and it is included among the early investigations to be sent for sepsis. Even with advancement in molecular diagnostics, for all practical purposes, blood culture still remains the most important microbiological investigation in the management of sepsis (2). The conventional methods of blood culture include inoculation of blood sample into blood culture bottles, followed by incubation and

daily subculturing on solid media for 7 days before declaring negative. The conventional blood culture techniques are labour intensive and time consuming. The automated blood culture systems like BACTEC are superior to conventional method in terms of speed and sensitivity as the bottles are flagged positive once there is any growth. As per the standard practice in most of the microbiology laboratories the turnaround time for AST results from positive flagged blood culture bottle by automated system is about 48 hours as it involves Gram staining and subculture onto blood agar (BA) and MacConkey's agar (MA) of the culture broth and overnight incubation which yield isolated colonies which are then subjected to identification and AST by manual or automated methods (3). On other hand by performing direct AST from positive BACTEC blood culture bottles, the turnaround time (TAT) to generate AST report can

reduce to 24 hours. Rapid TAT of blood culture reports should be the main motive for a clinical microbiologist for optimal patient care (4). Clinicians can get adequate information to tailor the empirical treatment towards targeted antibiotic therapy about 24h earlier than conventional susceptibility testing. This in turn can lead to substantial reduction in mortality and morbidity of the patient. The present study was conducted to evaluate the performance of direct AST from positively flagged BACTEC blood culture bottles and its comparison with conventional AST.

MATERIALS AND METHODS

The study was conducted after taking ethical approval from institutions ethical committee (BFUHS/2K21p-TH/6477). The Study population included patients admitted in different wards with clinical suspicion of bloodstream infection. Blood culture bottles flagged positive by BACTEC 9120 were subjected to gram staining. The specimens which grew more than one type of isolate and showed budding yeast cells on gram staining were excluded from the study. Bottles giving no signal were reported negative after 5 days of incubation. Direct AST was performed according from the flagged bottles, for both Gram negative and Gram positive bacteria. Four drops of blood culture broth from positively flagged BACTEC bottles were inoculated on the Muller Hinton agar (MHA) plate. After 15-20 minutes antibiotic discs, were applied on the surface of agar using sterile forceps (as per the direct gram staining interpretation). The plates were incubated at 37°C for 18 to 24 hrs, and then zone diameters were interpreted as per CLSI guidelines. Simultaneously subculture was performed on Blood agar and MacConkey agar from positive flagged blood culture bottles. On next day conventional AST was performed from isolate grown on subculture plates (according to CLSI guidelines)(7). Antibiotic panels for testing were chosen as per to CLSI guidelines depending on whether the organism was gram positive or gram negative on gram staining. In case of gram negative organisms, antibiotic panel

covering both *Enterobacteriaceae* and non-fermenters, such as Ampicillin (AMP) (10µg), Cefotaxime (CTX) (30µg), Ceftriaxone (CTR) (30µg), Ceftazidime (CAZ) (10µg), Cefepime (CPM) (30µg) Ciprofloxacin (CIP) (5µg), Amikacin (AK) (30µg), Meropenem (MRP) (10µg), Piperacillin/Tazobactam (PIT) (100µg/10µg), Colistin (CL) (10µg). The antibiotic panel used for gram positive organisms covered both *Staphylococcus* species and *Enterococcus* species such as Ampicillin (AMP) (10µg), Erythromycin (E) (15µg), Cefoxitin (CX) (30µg), Ciprofloxacin (CIP) (5µg), Amikacin (AK) (30µg), Vancomycin (VA) (30µg), Linezolid (LZ) (30µg), High level Gentamicin (HLG) (120µg) and Vancomycin screen agar (6µg/ml).

The results of direct disk diffusion were compared with conventional disk diffusion method and results were expressed in terms of Categorical agreement (CA) and Categorical disagreement. The categorical disagreement was further characterized into minor error (mE), major error (ME), and veryME (VME) (6).

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 conventional 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 conventional AST method.
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 conventional AST system.

The data pertaining to socio demographic and other clinical variables was entered in the form of data matrix in Microsoft "Excel" and analysed using IBM SPSS v20.0.0. The descriptive statistics for categorical variables was represented in the form of frequencies and percentage and as means and standard deviations for continuous variables.

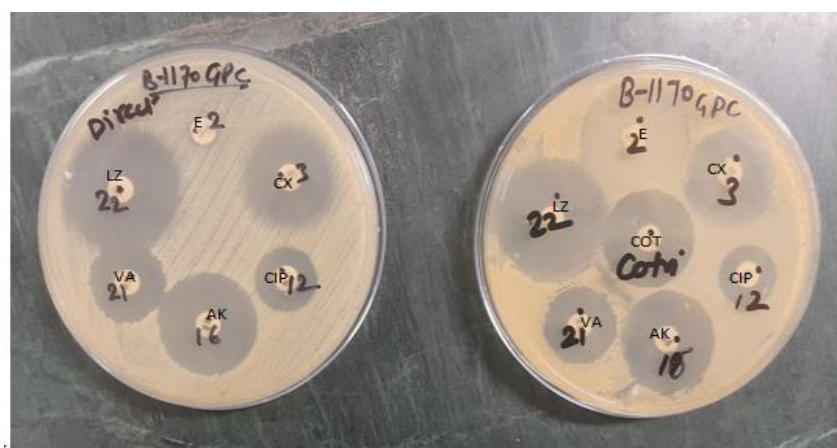


Figure: 1 Direct Disk Diffusion test compared to Conventional Disk Diffusion test for Gram positive cocci.



Figure 2: Direct Disk Diffusion test compared to Conventional Disk Diffusion test for Gram negative bacilli.

RESULTS

Of the total 1564 blood culture analysed, 356 blood culture bottles which flagged positive on BACTEC 9120, were processed. On direct Gram staining, 107 samples showed Gram negative bacilli and 105 showed Gram positive cocci and 144 samples showed contaminants. Table 1 shows the distributions of bacteria isolated from positive flagged blood cultures for which both direct AST and conventional AST were performed. Enterobacteriaceae accounts for

22.16% (47) of total isolates; Non-fermenters 28.3% (60) and Gram positive cocci 49.52% (105). Among Gram negative bacterial isolates, *A. baumannii* complex 37 (17.45%) was the most frequently isolates followed by *K. pneumoniae* 29 (13.67%), *P. aeruginosa* 16 (7.54%) and *E. coli* 15 (7.07%) respectively. Among Gram positive bacterial isolates, *S. aureus* 61 (28.77) was the most common followed by CONS 35 (16.50%) and *Enterococcus* spp. 9 (4.24%).

S. No	Organisms	Number	Percentage (%)
A	Gram negative	107	50.47
I.	Enterobacteriaceae	47	22.16
1)	<i>Klebsiella pneumoniae</i>	29	13.67
2)	<i>Escherichia coli</i>	15	7.07
3)	Citrobacter species	3	1.41
II.	Nonfermenters	60	28.30
1)	<i>Acinetobacter baumannii</i>	37	17.45
2)	complex	16	7.54
3)	<i>Pseudomonas aeruginosa</i> <i>Burkholderia</i> species	7	3.30
B	Gram positive	105	49.52
1)	<i>Staphylococcus aureus</i>	61	28.77
2)	Coagulase negative <i>Staphylococci</i> (CONS)	35	16.50
3)	<i>Enterococcus</i> species	9	4.24

Table 1: Distribution of various organisms isolated from positive blood cultures (n=212)

Table 2 shows a total of 1486 organism-antibiotic combinations were evaluated to perform direct AST from positive blood culture bottles. Out of which 1438 (96.76%) combinations showed categorical agreement whereas categorical disagreement was found only in

48 (3.23%) combinations, of which majority were ME 23 (1.54%) followed by mE 17 (1.14%) and VME 8 (0.53%). Percentage of errors (mE, ME, VME) was overall much lower than the acceptable performance criteria of International Standard (ISO 20776-2) (ME \leq 3%; VME \leq 3%).

Organisms and antibiotics tested (n×Ab=N)	Categorical agreement (CA)	Categorical disagreement			Total
		Minor Error	Major Error	Very Major Error	
Enterobacteriaceae (47×8=376)	359 (95.47%)	8 (2.12%)	7 (1.86%)	2 (0.53%)	17 (4.52%)
Non fermenters	464 (96.66%)	5 (1.04%)	7 (1.45%)	4 (0.83%)	16 (3.33%)

(60×8=480)					
Staphylococcus spp. (96×6=576)	563 (97.74%)	3 (0.52%)	9 (1.56%)	1 (0.17%)	13 (2.25%)
Enterococcus spp. (9×6=54)	52 (96.29%)	1 (1.85%)	0 (0%)	1 (1.85%)	2 (3.70%)
Total (1486)	1438 (96.76%)	17 (1.14%)	23 (1.54%)	8 (0.53%)	48 (3.23%)

Table 2: Performance of direct AST compared to conventional AST for various organisms isolated.

Among Enterobacteriaceae (Table 3), the CA for majority of the antibiotics was above 95% except for CIP (93.61%), MRP (91.48%) and PIT (87.23%) while higher ME was observed for MRP (4.25%) and PIT (6.38%) and higher mE for CTR (4.25%), CIP (4.25%) and PIT (4.25%).

Antimicrobial agents	Categorical agreement (CA)	Categorical disagreement			
		Minor Error	Major Error	Very Major Error	Total
AMP	47 (100%)	NIL	NIL	NIL	NIL
CTX	47 (100%)	NIL	NIL	NIL	NIL
CTR	45 (95.74%)	2 (4.25%)	NIL	NIL	2 (4.25%)
CIP	44 (93.61%)	2 (4.25%)	1 (2.12%)	NIL	3 (6.38%)
AK	45 (95.74%)	1 (2.12%)	1 (2.12%)	NIL	2 (4.25%)
PIT	41 (87.23%)	2 (4.25%)	3 (6.38%)	1 (2.12%)	6 (12.76%)
MRP	43 (91.48%)	1 (2.12%)	2 (4.25%)	1 (2.12%)	4 (8.51%)
CL	47 (100%)	NIL	NIL	NIL	NIL

Table 3: Performance of direct AST compared to conventional AST for Enterobacteriaceae (n=47)

Among Non-fermenters (Table 4), the CA was above 95% for majority of the antibiotics except for CIP (91.66%) and PIT (93.33%). VME for CIP was found to be 3.33%. Higher ME was observed for CIP (3.33%), PIT (3.33%), and MRP (3.33%) and high mE was for CPM (6.25%) and AK (3.33%).

Antimicrobial agents	Categorical agreement (CA)	Categorical disagreement			
		Minor Error	Major Error	Very Major Error	Total
AMP	60 (100%)	NIL	NIL	NIL	NIL
CTX	44 (100%)	NIL	NIL	NIL	NIL
CTR	44 (100%)	NIL	NIL	NIL	NIL
*CAZ	16 (100%)	NIL	NIL	NIL	NIL
*CPM	15 (93.75%)	1 (6.25%)	NIL	NIL	1 (6.25%)
CIP	55 (91.66%)	1 (1.66%)	2 (3.33%)	2 (3.33%)	5 (8.33)
AK	57 (95%)	2 (3.33%)	1 (1.66%)	NIL	3 (5%)
PIT	56 (93.33%)	1 (1.66%)	2 (3.33%)	1 (1.%)	4 (6.66%)
MRP	57 (95%)	NIL	2 (3.33%)	1 (1.66%)	3 (5%)
CL	60 (100%)	NIL	NIL	NIL	NIL

Table 4: Performance of direct AST compared to conventional AST for Non fermenters(n=60).

*CAZ & CPM are tested only for *P. aeruginosa*

For Staphylococcus species (Table 5), the CA for all the antibiotics tested was above 95% with ME was observed for CX (3.12%) and AK (3.12%).

Antimicrobial agents	Categorical agreement (CA)	Categorical disagreement			
		Minor Error	Major Error	Very Major Error	Total
AMP	96 (100%)	NIL	NIL	NIL	NIL
CX	92 (95.83%)	NIL	3 (3.12%)	1 (1.04%)	4 (4.16%)
E	93 (96.87%)	1 (1.04%)	2 (2.08%)	NIL	3 (3.12%)
CIP	94 (97.91%)	1 (1.04%)	1 (1.04%)	NIL	2 (2.08%)
AK	92 (95.83%)	1 (1.04%)	3 (3.12%)	NIL	4 (4.16%)
LZ	96 (100%)	NIL	NIL	NIL	NIL

Table 5: Performance of direct AST compared to conventional AST for Staphylococcus species (n=96).

For *Enterococcus* species (Table 6), the CA was above 95% for all the antibiotics except for E (88.88%) and CIP (88.88%). Higher VME was observed for E (11.11%) and higher mE was for CIP (11.11%). (n=9)

Antimicrobial agents	Categorical agreement (CA)	Categorical disagreement			
		Minor Error	Major Error	Very Major Error	Total
AMP	9 (100%)	NIL	NIL	NIL	NIL
E	8 (88.88%)	NIL	NIL	1 (11.11%)	1 (11.11%)
CIP	8 (88.88%)	1 (11.11%)	NIL	NIL	1 (11.11%)
HLG	9 (100%)	NIL	NIL	NIL	NIL
VA	9 (100%)	NIL	NIL	NIL	NIL
LZ	9 (100%)	NIL	NIL	NIL	NIL

Table 6: Performance of direct AST compared to conventional AST for *Enterococcus* species.

DISCUSSION

Blood stream infections are an important cause of morbidity and mortality in critically ill patients resulting in prolonged hospitalisation, frequent diagnostic testing, greater prescription of antibiotics and increased health care expenses. Exposure to heavy antibiotics in ICU patients plays a critical role in the development of antibiotic-resistant bacteria. Effective treatment of blood stream infections depends on early diagnosis and appropriate and possibly targeted antimicrobial therapy. In the present study, a total of 1486 organism-antibiotic combinations were evaluated. Overall, 1438 (96.76%) combinations showed categorical agreement whereas categorical disagreement was found only in 48 (3.23%) combinations of which majority were ME 23 (1.54%) followed by mE 17 (1.14%) and VME 8 (0.53%) (Table 2). Percentage of errors (mE, ME, VME) were overall much lower than the acceptable performance criteria of International Standard ISO 20776-2 (ME \leq 3%; VME \leq 3%) (8). Similar findings were obtained by Rajshekar et al., who reported the overall CA of 96% between direct and conventional disk diffusion test with 4% combinations showed disagreement of which ME (2.1%) followed by VME (1%) and mE (0.9%) (9). In another studies, Desai et al. found the overall CA of 90.4% between direct and conventional disk diffusion test with 1.8% VME, 1.9% ME, and 5.8% mE and Chandrasekaran et al., showed a CA of 87.9% between direct and 74 conventional disk diffusion test with VME (0.5%), ME (3.5%), and mE (10%) which was lower to that observed in our study (10, 6). Goel et al., in another study on gram negative bacteria, reported CA of 83.7% between direct disk diffusion compared with AST from bacterial colonies by Vitek-2 (5). The present study showed good performance of Direct disk diffusion with Conventional disk diffusion among *Enterobacteriaceae* with 359 (95.47%) combinations showed categorical agreement whereas 17 (4.52%) combinations showed disagreement of which VME 2 (0.53%), ME 7 (1.86%) and mE 8 (2.12%). The categorical agreement for majority of the antibiotics were above 95% except for CIP (93.61%), MRP (91.48%) and PIT (87.23%) with higher ME was observed for MRP (4.25%) and PIT

(6.38%) and higher mE for CTR (4.25%), CIP (4.25%) and PIT (4.25%) (Table 3). Similar findings were observed by Chandrasekaran et al., reported a CA of 83.3% for PIT and Desai et al. who reported CA of 71.7% for ampicillin-sulbactam (6,10). Among Nonfermenters, 464 (96.66%) combinations showed categorical agreement while 16 (3.33%) combinations showed disagreement of which VME 4 (0.83%), ME 7 (1.45%), and mE 5 (1.04%). The categorical agreement was above 95% for majority of the antibiotics except for CIP (91.66%) and PIT (93.33%). VME for CIP was found to be 3.33%. Higher ME was observed for CIP (3.33%), PIT (3.33%), and MRP (3.33%) and high mE was for CPM (6.25%) and AK (3.33%) (Table 4). Similar findings were obtained by Rajshekar et al. who reported CA above 90% for all organism-antimicrobial combinations in *Pseudomonas* species with VME was above the acceptable range in AK (6.2%), G (5.2%), and CF (4.1%) (9). In contrast to our study, Goel et al. reported a low CA for CAZ (76.1%) and this variation was attributed to VME (5). For *Staphylococcus* species, 563 (97.74%) combinations showed complete agreement with a total of 13 (2.25%) combinations showed disagreement which included VME 1 (0.17%), ME 9 (1.56%) and mE 3 (0.52%). The CA for all the antibiotics tested was above 95% with ME was observed for CX (3.12%) and AK (3.12%) (Table 5). Similar findings were obtained by Rajshekar et al. who reported unsatisfactory CA for CX with ME of 4.9%. and Bennet et al. reported CA of 88% for cefoxitin direct with conventional disk diffusion (9,11).

For *Enterococcus* species, 52 (96.29%) combinations showed complete agreement with only 2 (3.70%) combinations showed errors which included VME 1 (1.85%) and mE 1 (1.85%). No ME was noted. The CA was above 95% for all the antibiotics except for E (88.88%) and CIP (88.88%). Higher VME was observed for E (11.11%) and higher mE was for CIP (11.11%) (Table 6). Rajshekar et al. reported higher ME of 4.4% for HLG in *Enterococcus* species (9). The time required for processing AST by conventional method is 48 hrs which requires preparation of media, inoculation of specimen and further incubation. Thus, the proposed study shows

that direct AST could help clinicians to initiate appropriate antibiotic therapy earlier than a conventional method by 24 hrs, and early initiation of infection control measures in the case of multi-drug resistant pathogens.

CONCLUSION

This study showed good concordance between two methods and suggests the use of direct antimicrobial susceptibility testing before the results of conventional antimicrobial susceptibility testing are available; as these results can be useful to the clinician in deciding or modifying the specific antimicrobial therapy at the earliest saving as much as 24 critical hours, thus reducing the mortality and morbidity in patients with blood stream infections

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