Evaluation of direct drug susceptibility testing of blood culture isolates comparing it with conventional disk diffusion testing

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Abstract

Objective: To evaluate a direct antibiotic susceptibility testing method for blood culture.

Methods: The cross-sectional comparative study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from December 2016 to October 2017. Direct antimicrobial susceptibility testing was performed from positive blood culture bottles. Bacterial identification was done by using API 10S. Different antimicrobial panels were employed for Gram-negative rods (GNRs), gram-positive cocci (like suspected Staphylococci and Enterococci). Results were compared with conventional disk diffusion testing and very major, major and minor errors were calculated. Result agreement and kappa coefficient scores were generated for categorical agreement. SPSS 24 was used for data analysis.

Results: Of the 101 bacterial isolates, 82(81.2%) were Gram negative rods and 19(18.8%) were Gram-positive cocci. Among 781 bacteria-antibiotic comparisons, the number of very major errors was 3(0.4%), major errors were 7(0.9%) and minor errors were 12(1.5%), while, 759(97.2%) comparisons yielded the same results. The kappa coefficient was 0.946, showing almost perfect agreement. Direct identification of Gram negative rods was successful in 53(64.6%) cases.

Conclusion: Direct susceptibility testing of blood culture produced reliable results for majority of the antimicrobials.

Keywords: Direct susceptibility testing, DST, Disk diffusion testing, Blood stream infections, Blood culture, Antibiotic susceptibility testing, Antimicrobial resistance. (JPMA 70: 105; 2020).
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Introduction

Blood stream infections (BSIs) are an important cause of mortality in hospitalised patients. Early antibiotic treatment remains the cornerstone of management in cases of sepsis. The role of a clinical microbiologist is imperative in aiding the physician in laboratory diagnosis and in being a guide to appropriate antibiotic treatment. Early institution of specific antimicrobial therapy not only reduces mortality and morbidity, but also saves the cost of treatment, decreases hospital stay and prevents development of antibiotic resistance.[1,2]

Routine blood culture takes 1-5 days for a positive result, and a further 24-48 hours are required for bacterial identification and antibiotic susceptibility testing. Till the time results are available, clinicians rely on empirical antimicrobial treatment which may not be effective in cases of multi and extensively drug-resistant nosocomial infections.[3] Thus, there is always a need to make the laboratory diagnoses more efficient. Antibiotic susceptibility testing performed directly on positive cultures can provide results 48 hours in advance and, thus, can save critical time in patient management. In an effort to develop methods for rapid antibiotic susceptibility testing various options, including matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) Vitek, Sensititre, QuickFISH, BC-GP assay etc., have been evaluated with good results. All of these use sophisticated systems which are not widely available in a resource-scarce setting.[3-5] There is enough ground rationale to assess the accuracy of direct susceptibility testing (DST) method for blood cultures to reduce time for preliminary reporting of antibiotic susceptibility results. Many clinical laboratories have adopted different methods,

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but there is no validated method. Studies from Pakistan are lacking in this regard. The current study was planned to evaluate a simple, cost-effective method for direct antibiotic sensitivity testing and bacterial identification, done directly from positive blood culture bottles.

Materials and Methods

The cross-sectional comparative study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from December 2016 to October 2017. After approval from the institutional ethics board, samples for blood culture received from two tertiary care hospitals and medical institutions were evaluated, and samples that were positive on direct Gram stain were included through non-probability convenience sampling. Repeat or duplicate samples from the same patients were excluded, and so were samples showing more than one microorganism and/or yeast cells on Gram stain. Sample size was calculated by taking the prevalence of positive blood cultures from a local study by using the sample size calculation software.[6,7]

The media used for blood culture included commercially available Bactec, BacT/ALERT and in-house prepared Brain Heart Infusion (BHI) broth (Oxoid, UK).

For the standard method, in case of automated systems, Bactec and BacT/ALERT 3D, positive growth signal was indicated by the system. Blood samples collected in BHI broth bottles were incubated at 35±2^{0}C. These bottles were examined daily for any turbidity, clot formation and/or gas production for 7 days before terminal subculture. Blind sub-culturing was performed at days 1, 3 and 7.[8] Standard drug susceptibility was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines by Kirby-Bauer disk diffusion method on Muller-Hilton agar (Oxoid, UK).[9]

For DST, the positive blood culture bottles were confirmed by Gram stain for the presence of microorganisms. The inoculum for direct testing was prepared by taking 1ml of broth from positive blood culture bottles with a needle and syringe and mixing with 1ml of BHI. This was incubated at 35±2^{0}C for 2 hours. The turbidity of the broth mixture was adjusted with 0.9% normal saline to visually match 0.5 McFarland barium sulfate ($BaSO_4$) standards. The standardised inoculum was swabbed onto Muller-Hilton (Oxoid, UK) plate in three directions and antibiotic disks were applied. Antibiotic disk panel (Oxoid, UK) applied was selected according to the Gram stain results as follows;

For Gram-negative rods (GNR)/coccobacilli representative antibiotics from β-lactams, β-lactam/β-lactamase inhibitor combinations, aminoglycosides, fluoroquinolones, tetracyclines and folate pathway inhibitors were used. The drugs included ampicillin (10µg), ciprofloxacin (5µg), amikacin (30µg), ceftriaxone (30µg), meropenem (10µg), minocycline (30µg), amoxicillin-clavulanate (20/10µg) and trimethoprim-sulfamethoxazole (1.25/23.75µg).

A different panel was used for GNR (suspected typhoidal Salmonellae from clinical history). The panel included first-line and second-line anti-typhoidal drugs; ampicillin (10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), chloramphenicol (30µg), ciprofloxacin (5µg) and pefloxacin (5µg), ceftriaxone (30µg) and azithromycin (15µg).

For Gram-positive cocci (GPC), the antimicrobial groups used included penicillins, fluoroquinolones, tetracyclines, glycopeptides, aminoglycosides, lincosamides, phenicols and folate pathway inhibitors. The disks used for GPC in clusters (suspected staphylococci) were cefoxitin (30µg) for detection methicillin resistance, penicillin (10u), clindamycin (2µg), teicoplanin (30µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), doxycycline (30µg), ciprofloxacin (5µg) and amikacin (30µg).

GPC (suspected enterococci) were tested against ampicillin (10µg), penicillin (10u), vancomycin (30µg), linezolid (30µg) and doxycycline (30µg).

Salmonella was considered a separate group from enterobacteriaceae because of the different antimicrobial panel used. Clinical history was obtained from the treating physician.

Zones of inhibition were recorded with transmitted light, using a ruler to the nearest 1mm for both direct sensitivity and the standard testing. CLSI[9] interpretative criteria were used. The results of direct testing were compared with the standard method using the following criteria; Same (S): No change in results of both direct and standard method; Minor error (Min): Sensitive or resistant by the conventional method but intermediate by the direct method and vice versa; Major error (Maj): Sensitive by the conventional method but resistant by the direct method; Very major error (VME): Sensitive by the direct method but resistant by conventional method.[10]

For standard bacterial identification API 20E and 20NE (bioMérieux, France) was used for GNRs. Staphylococci were identified with coagulase and DNAse test as Staphylococcus aureus and coagulase-negative

Staphylococci (CoNS). Enterococci were identified on biochemical reactions of API Strept (bioMérieux, France).[8]

For direct identification of GNRs the inoculum prepared for DST was inoculated onto API 10S (bioMérieux, France). Reactions were read after 18-24 hours incubation at 35±2^{0}C and results were compared with the standard method.

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Data was analysed using SPSS 24. Descriptive statistics were calculated. Frequencies and percentages of isolation of the different species and the percentage of errors were calculated.

Table-1: Distribution of blood culture isolates (n=101).

	Enterobacteriaceae n=36 n (%)	Non-Enterobacteriaceae n=25 n (%)	Salmonella n= 21 n (%)	Staphylococci n=17 n(%)	Enterococci n=2 n (%)
Enterococcus faecalis	0	0	0	0	2 (100)
Staphylococcus (Coagulase negative)	0	0	0	3 (17.6)	0
Staphylococcus aureus	0	0	0	14 (82.4)	0
Salmonella Paratyphi A	0	0	3 (14.3)	0	0
Salmonella Typhi	0	0	18 (85.7)	0	0
Acinetobacter baumannii	0	3 (12)	0	0	0
Aeromonas hydrophila	0	2 (8)	0	0	0
Stenotrophomonas maltophila	0	3 (12)	0	0	0
Burkholderia cepacia	0	12 (48)	0	0	0
Pseudomonas aeruginosa	0	5 (20)	0	0	0
Providencia stuartii	1 (2.8)	0	0	0	0
Serratia marcescens	8 (22.2)	0	0	0	0
Enterobacter cloacae	2 (5.6)	0	0	0	0
Klebsiella pneumonia	17 (47.2)	0	0	0	0
Escherichia coli	8 (22.2)	0	0	0	0

Table-2: Comparison of results for Gram-Negative Rods (GNRs) isolated from blood.

Antibiotic classes	Drugs	Enterobacteriaceae (n=36)				Non-Enterobaceriaceae(n=25)			
		VME n (%)	Maj n (%)	Min n (%)	S n (%)	VME n (%)	Maj n (%)	Min n (%)	S n (%)
β-lactam drugs	Ampicillin	-	1(2.8)	-	35(97.2)	1(4.0)	-	-	24(96)
	Meropenem	-	2(5.6)	4(11.1)	30(83.3)	-	-	1(4.0)	24(96)
	Ceftriaxone	-	-	-	36(100)	-	-	-	25(100)
β-lactam/β-lactam inhibitor combinations	Amoxycillin-clavulanate	-	1(2.8)	-	35(97.2)	-	-	-	25(100)
Aminoglycosides	Amikacin	-	2(5.6)	2(5.6)	32(88.9)	-	-	2(8.0)	23(92)
Fluoroquinolones	Ciprofloxacin	-	-	-	36(100)	-	-	-	25(100)
Tetracyclines	Minocycline	-	-	-	36(100)	-	-	-	25(100)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	1(2.8)	1(2.8)	1(2.8)	33(91.7)	1(4.0)	-	-	24(96)

Simple percentage agreement as well as Kappa scores were generated for categorical agreement. The following standards were used for the strength of agreement for the kappa coefficient: 0-0.0099, poor; 0.01-0.20, slight; 0.21-0.40, fair; 0.41-0.60, moderate; 0.61-0.80, substantial; and 0.81-1, almost perfect.

Results

Of the 101 bacterial isolates, 70(69.3%) were positive through automated systems and 31(30.7%) through BHI broth. Among the positive BHI bottles, turbidity alone was the indicator in 22(71%) isolates and turbidity with gas formation (bubbles) was noted in the rest. Distribution frequency of the isolates was noted (Table 1). The majority of blood stream infections were caused by members of Enterobacteriaceae 57(56.4%), with *Salmonella Typhi* 18(17.8%) being the most frequent isolate.

Of the total isolates, 82(81.2%) were GNRs and 19(18.8%)

Table-3: Comparison of results for Gram-Positive Cocci (GPC) (suspected Staphylococci) (n=17).

Antibiotic classes	Drugs	VME n (%)	Maj n (%)	Min n (%)	S n (%)
β-lactam drugs	Cefoxitin	-	-	-	17(100)
	Penicillin G	-	-	-	17(100)
Lincosamides	Clindamycin	-	-	2(11.8)	15(88.2)
Glycopeptides	Teicoplanin	-	-	-	17(100)
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	-	-	-	17(100)
Tetracyclines	Doxycycline	-	-	-	17(100)
Fluoroquinolones	Ciprofloxacin	-	-	-	17(100)
Aminoglycosides	Amikacin	-	-	-	17(100)

Table-4: Overall error type distribution, percentage agreement and the kappa coefficient for the tested drugs.

Antibiotic classes	Antibiotics	No. of tests	VME n (%)	Maj Errors n (%)	Min Errors n (%)	Agreement %	Kappa score (SE)	*p*-value
β-lactam drugs	Penicillin G	19	-	-	-	100	1.00 [a]	<0.0001
	Ampicillin	84	1(1.2)	1(1.2)	-	97.6	0.953[a] (0.033)	<0.0001
	Ceftriaxone	82	-	-	-	100	1.000 [a]	<0.0001
	Meropenem	61	-	2(3.3)	5(8.2)	88.5	0.779[b] (0.070)	<0.0001
	Cefoxitin	17	-	-	-	100	1.00 [a]	<0.0001
β-lactam/ β-lactam inhibitor combinations	Amoxycillin-clavulanate	61	-	1(1.6)	-	98.3	0.966 a (0.033)	<0.0001
Fluoroquinolones	Ciprofloxacin	99	-	-	-	100	1.00 [a]	<0.0001
	Pefloxacin	21	-	-	-	100	1.00 [a]	<0.0001
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	99	2(2.0)	1(1.0)	1(1.0)	95.9	0.926 a (0.036)	<0.0001
Macrolides	Azithromycin	21	-	-	-	100	1.00 [a]	<0.0001
Phenicols	Chloramphenicol	21	-	-	-	100	1.00 [a]	<0.0001
Tetracyclines	Doxycycline	19	-	-	-	100	1.00 [a]	<0.0001
	Minocycline	61	-	-	-	100	1.00 [a]	<0.0001
Aminoglycosides	Amikacin	78	-	2(2.6)	4(5.1)	92.3	0.853[a] (0.054)	<0.0001
Glycopeptides	Vancomycin	19	-	-	-	100	1.00 [a]	<0.0001
	Teicoplanin	2	-	-	-	100	1.00 [a]	<0.0001
Oxazolidinones	Linezolid	17	-	-	-	100	1.00 [a]	<0.0001
Lincosamides	Clindamycin	17	-	-	2(11.8)	88.2	0.779[b] (0.125)	<0.0001

[a] Almost perfect agreement, [b]Substantial agreement

were GPC. A total of 781 bacteria-antibiotic disk tests were carried out for the 101 isolates, and comparisons were made between direct and standard methods. The number of VMEs was 3(0.4%), Maj 7 (0.9%) and Min errors were 12(1.5%), while 759(97.2%) comparisons yielded the same results. The kappa coefficient was 0.946 (standard error [SE]=0.011) which meant almost perfect agreement. GNRs and GPCs were enumerated separately (Tables 2-3). Overall error type distribution along with kappa coefficient was done separately (Table 4).

Of the 82 GNRs, 53(64.6%) were correctly identified by direct inoculation of API 10S. However, for *Enterobacteriaceae*, including *Salmonella*, 53(92.3%) out of 57 isolates were identified correctly.

Discussion

Early identification of the causative agent of blood stream infections not only helps the initiation of appropriate antibiotic therapy, but also earlier institution of infection control measures in case of multi and extensively drug-resistant organisms.

Protocols for direct sensitivity for blood culture have been proposed in various studies with good results. We used a method that was proposed by Mirret et al., with the difference of BHI broth instead of trypticase soy broth with 1% yeast extract, for inoculum standardisation.[11] In our study with the standardised suspension the percentage of VMEs reached 0.4%, Maj were 0.9% and Min were 1.5%. The percentage agreement between the two methods was 97.2% with a kappa coefficient of 0.946, which showed almost perfect agreement. A study from Singapore reported 0.03% Maj and 2.3% Min errors with inoculum standardisation.[12] With a non-standardised inoculum, Noman et al. reported no VMEs, while 1.3% VMEs were reported in a Turkish study.[10,13]

With a standardised inoculum for direct antibiotic testing, Mirret et al. reported an overall 94.6% agreement between the direct and standard methods.[11] In a study conducted in India that used the British Society for Antimicrobial Chemotherapy (BSAC) methods for antimicrobial susceptibility testing 2011-12, 83.7% comparisons between direct and standard method had the same result.[14,15] Further studies are required which simultaneously compare the two methods of direct testing i.e., with and without the standardised inoculum, to demonstrate superiority of any one of these methods. Theoretically, a standardised inoculum should give more reliable results, as dilution of the broth during standardisation should achieve a better semi-confluent growth on the media and also dilute any inhibitors from blood. The CLSI methods development and standardisation working group tested broth directly from blood culture bottles, using 4 drops of blood culture broth to swab Muller-Hilton plates for DST.[16] It reported an overall agreement of 87.4% which is lower compared to

our study. It also compared the effects of bacterial colony counts on the results of DST and reported improved results with dilution of the blood culture broth inoculum.[16]

Edelmann et al. recommend a 0.5ml inoculum for GPCs and 0.2ml for GNRs to obtain a semi-confluent growth.[17] A semi-confluent growth on the sensitivity plates appears to be an important factor for accurate results. Further studes are needed to be sure if volume has a major impact or not. We did not standardise the inoculum volume in our study. We lawned the agar plates by dipping sterile cotton swab into the standardised suspension, removing the excess by rotating against the wall of the tube above the liquid.

We observed that most of the errors were with beta-lactam drugs and aminoglycosides, which was the case in other studies as well.[10-13]

An interesting finding in our study was 100% agreement in the susceptibility testing of *Salmonella Typhi* and Paratyphi with standard disk diffusion method. Similarly, there was 100% agreement in susceptibility results of cefoxitin for detection of methicillin-resistant *Staphylococcus aureus* (MRSA). This is concurrent with another study from Pakistan.[12] These findings have a significant clinical implication in our setting as both salmonella and MRSA are common pathogens; early and accurate susceptibility testing by direct method may help in early initiation of therapy and infection control measures. The recent epidemic of ceftriaxone-resistant *Salmonella* in Hyderabad further highlights the importance of direct testing for the institution of early antibiotic treatment.

API 10S was used for primary identification of GNRs, and it was successful in the identification of *Enterobacteriaceae*, including *Salmonella*, in 53(92.3%) cases. The API 10S was used for its low cost. None of the other studies have used API for direct bacterial identification. Further multi-centre studies with large number of isolates and carefully selected anti-microbial panels are needed to evaluate the usefulness before recommending it for routine use.

Conclusion

Direct susceptibility testing of blood culture had reliable results for majority of the antimicrobials. Preliminary reporting of DST results can help guide the clinician towards the more effective and less expensive antimicrobial treatment, until the results of standard method are available.

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