DOI: 10.7860/JCDR/2025/79981.21497

Original Article

Microbiology Section

Comparative Analysis of Conventional and Rapid Antimicrobial Susceptibility Testing in Patients with Gram-negative Bacteraemia Directly from Blood Culture in a Tertiary Care Hospital: A Cross-sectional Study

S SHANMUGAPRIYA[1], S ALAGAMMAI[2], B APPALARAJU[3]

ABSTRACT

Introduction: Early administration of appropriate antimicrobial therapy in Gram-negative bacteraemia would influence the patient's prognosis. Conventional Antibiotic Susceptibility Testing (AST)- disk diffusion and VITEK-2 rely on bacterial isolates obtained from subculturing positive blood cultures. Direct AST done from positive blood culture fluid would reduce subculturing time.

Aim: To compare rapid AST directly from the positive blood culture with the conventional method of performing in isolated colonies obtained from positive blood cultures.

Materials and Methods: The present cross-sectional study was done on 350 blood culture samples received at the diagnostic Microbiological laboratory of PSG hospital, Coimbatore, Tamil Nadu, India from August 2024 to October 2024. Consecutive positive blood cultures received during the study period showing monomicrobial (gram negative bacilli identified by doing gram stain of culture fluid) was included in the study. Blood culture samples were subjected simultaneously to susceptibility testing by Direct Sensitivity Test (DST) by Kirby bauer disk diffusion method (CLSI recommended) and Antibiotic Sensitivity Test (AST) by Vitek-2 Compact which is an automated (BioMerieux) reference method from positive blood cultures flagged by BacT/ALER3D System. AST was done directly from the positive blood culture fluids and on sub cultures by disc diffusion and VITEK 2 method. Data analysed using Statistical Package for Social Sciences (SPSS) v.28.0 and p-value less than 0.05 was considered statistically significant.

Results: Antibiotic susceptibility test results of *Klebsiella pneumoniae* and *E.Coli* between direct and conventional disc diffusion method showed complete agreement in 98% of the samples in total. More than 95% samples showed complete agreement for all the antibiotics. On comparison between direct and conventional VITEK 2 method, 99% of the samples showed complete agreement between two tests in total. A 100% of samples showed complete agreement for the antibiotics like ceftazidime, ceftazidime+clavulanic acid, cefoxitin, cotrimoxazole, meropenem, ertapenem, piperacillin-tazobactam, cefaperazone-sulbactam, colistin. 91.25 and 90.7% samples showed complete agreement for amikacin and Ciprofloxacin.

Conclusion: The therapeutic value of this approach is underlined by the excellent agreement rates obtained for antibiotics of critical importance. This may contribute to improved outcomes through earlier directed therapy.

Keywords: Antimicrobial therapy, Disk diffusion, *Escherichia coli*, *Klebsiella pneumoniae*

INTRODUCTION

Bloodstream Infections (BSIs) were identified as one of the major cause of morbidity and according to a global surveillance system named Sentry antimicrobial surveillance program proportion of gram negative bacteria causing blood stream infection was 43.4% by 2016 [1]. It has the significant impact on the life of the affected individuals with the mortality rate of 12 to 38%. Various factors like causative organisms, host factors like sociodemographic factors and antimicrobial therapy affects the patient's outcome [2,3]. In India annually 7,50,000 blood stream infections are reported and it constitutes 2% of hospitalised patients and 70 % of Intensive Care Unit (ICU) admissions. Crude mortality rate is estimated to be 14 to 57% [4].

Antimicrobial resistance among Gram-negative bacteria has also raised due to extended spectrum β-lactamase production and carbapenem-resistant *Enterobacterales*. Antimicrobial resistance has complicated the management, which in turn has raised the mortality rate to 40% [5].

Early administration of appropriate antimicrobial therapy influences the patient's prognosis. Study has proved that delay in administration of effective antibiotic has resulted in higher risk of hospital mortality among the sepsis patient (OR -1.04 per hour at 95% CI) [6]. But it takes 48-72 hours after blood culture to get the final results of antibiotic susceptibility from conventional AST methods, leading to the delay in initiating the effective antibiotic therapy [7]. The patients receive empirical broad-spectrum antimicrobial therapy before the antibiotic test results are got, this may in turn lead to the development of resistance.

Conventional AST methodologies, including disk diffusion, broth microdilution, and automated systems such as VITEK-2 and Phoenix, rely on pure bacterial isolates obtained from subculturing positive blood cultures. While these methods are well-standardised and provide reliable results, their time-to-result significantly delays targeted therapy adjustments [8]. In contrast, rapid AST techniques have emerged as promising alternatives, offering potential time saving of 24-48 hours. These include molecular-based methods Polymerase

Chain Reaction (PCR) Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry (MALDI-TOF MS), Fluorescence In Situ Hybridisation (FISH), microfluidic techniques, and direct inoculation methods [9,10]. However, these rapid methods vary considerably in their accuracy, cost, technical complexity, and range of antimicrobials that can be tested.

Conventional Antibiotic susceptibility test is performed by subculturing positive blood cultures on to blood agar and MacConkey agar and will be incubated at 37ºC for 18 hours. The colonies of organisms will be identified and AST will be done. AST done from positive blood culture fluid reduces the time of subculturing. Hence it can provide AST results 18-24 hours earlier than conventional methods [11]. Studies have proved that direct AST may give result which is 90-95% concordant with the conventional methods for gram-negative bacteraemia when properly optimised [12].

Despite the short turn over time of rapid AST and its concordance with conventional AST in end results, significant lacunae still exists in studying the impact of rapid AST when compared to the conventional techniques in the tertiary care hospital setting. By comparing the efficacy of rapid AST methods with conventional methods standard AST protocols can be framed and it leads to early initiation of the appropriate antibiotics which will in turn prevent development of antibiotic resistance and thereby improving the patient's outcome. Hence, the present study was done to compare rapid AST directly from the positive blood culture with conventional method of performing in isolated colonies obtained from positive blood culture.

The primary objectives of the study were to compare AST directly from the positive blood cultures with that performed in isolated colonies obtained from positive blood cultures by Kirby Bauer's disc diffusion method and to compare AST directly from the positive blood cultures with that performed in isolated colonies obtained from positive blood cultures by vitek 2 compact system. The secondary objective of the study was to estimate the level of agreement between test results of rapid AST directly from the positive blood culture with the conventional method of performing isolated colonies obtained from positive blood cultures.

MATERIALS AND METHODS

The present cross-sectional study was done on all the blood culture samples received at the Diagnostic Microbiological laboratory from all the wards of PSG hospital, a tertiary care hospital in Coimbatore, Tamil Nadu, India from August 2024 to October 2024, for the period of four months. Institutional ethical clearance was obtained (IEC NO 24/365) and the confidentiality of the patients was maintained throughout the study.

Sample size calculation: Total number of samples with Gram negative bacilli received during the study period was 407 with an average of 135 samples each month. By the pilot study minimal expected kappa value got was (level of agreement) 70%, with absolute precision 8% and 95% confidence interval, sample size calculated was 307, but however 350 samples out of 407 samples were included which were received during the study period after applying the exclusion criteria. No specific sampling technique was followed as all the samples got during the study period were included.

Inclusion criteria: Consecutive positive blood cultures received at the microbiology laboratory of PSG hospitals during the study period showing monomicrobial (gram negative bacilli identified by doing Gram stain of culture fluid) was included in the study.

Exclusion criteria:

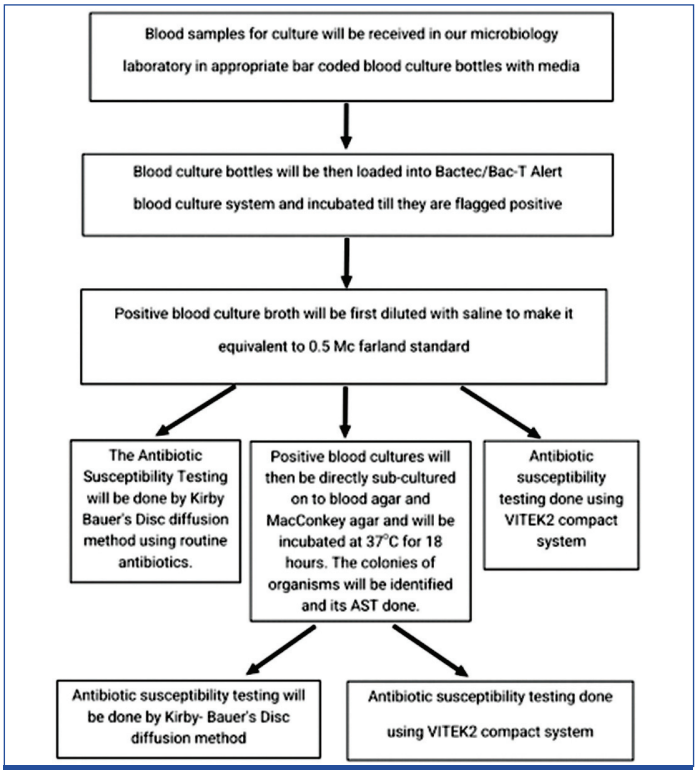
- Cultures showing gram positive cocci, gram negative cocci, gram positive bacilli anaerobic organisms and yeast cells were excluded from the study.
- Positive blood cultures that contain more than one type of bacteria will also be excluded from the study.

Study Procedure

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, the bottle was removed and an aliquot of sample was used for smear preparation and Gram staining. Samples which showed single type of bacteria were used for the study.

AST from sub culture colonies: Positive blood culture broth was first diluted with saline to make it equivalent to 0.5 Mc farland standard [13] and directly sub cultured on to blood agar and Mac Conkey agar and incubated at 37°C for 18 hours. The colonies of organisms were identified and its AST was done by Kirby Bauer's Disc diffusion method [14] and by VITEK 2 COMPACT system [15].

AST from direct blood culture broth: Positive blood culture broth was diluted with saline to make it equivalent to 0.5 Mc farland standards [13] and AST was done by Kirby Bauer's Disc diffusion method [13] and by VITEK 2 COMPACT system from direct blood culture fluid [15]. The study's methodology is displayed in [Table/Fig-1].



[Table/Fig-1]: Methodology of the study.

Direct from blood culture disc diffusion: Four drops of blood culture broth from the venting needle is placed on Muller-Hinton agar and swabbed in three directions to get lawn culture. Disc appropriate for gram negative bacteria were placed as per CLSI [14] and incubated overnight at 35°C.

Direct from blood culture VITEK 2 susceptibility testing: Five mL positive blood culture sample was centrifuged at 160 × g for five minutes to pellet RBC. Supernatant was centrifuged at 650 × g for 10 minutes to pellet bacteria. Turbidity of bacterial suspension was matched to Mc Farland 0.5% standard using 0.45% sodium chloride [13]. Then the suspension was loaded manually into VITEX 2 system.

Conventional disc diffusion from subcultures: Muller Hinton Agar (MHA) was the media used for performing disc diffusion from subculture growth. Sterile petri dishes were taken and it was poured to a depth of 4 mm. Disc diffusion was performed by Kirby bauer method on MHA [14]. A pure growth from subculture plate was picked with a loop, inoculated in peptone water and incubated. Peptone water growth was matched for 0.5 McFarland turbidity standards [13]. This inoculum was cultured onto MHA using a sterile cotton swab. After almost 15 minutes, appropriate antibiotic discs

were placed. A maximum of five discs can be placed in a single plate. Now plates were incubated for 18-24 hours at 37°C. Antibiotic zone sizes were interpretated as per CLSI guidelines [14].

Conventional VITEK 2 susceptibility testing from subcultures [15]: A pure isolated colony from subculture plate was picked with a loop, inoculated in peptone water and incubated. Peptone water growth was matched for 0.5 Mcfarland turbidity standards [13]. Appropriate gram-negative AST cards were inoculated following the manufacturer's instruction.

STATISTICAL ANALYSIS

The collected data were entered into excel sheets and analysed using SPSS v. 28.0. Descriptive statistics was used to find frequencies, percentages, mean, and standard deviation. Kappa statistics was done to find the level of agreement between two tests whose test results were categorical (sensitive, resistant and intermittent)

Interpretation on level of agreement based on Kappa value was as follows [16]:

Kappa	Interpretation
<0	No agreement
0.0-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-1.00	Almost perfect agreement

The susceptibility data from the rapid AST method were compared with those obtained from the conventional test. The comparisons were as follows [17]:

Very Major Error (VMJ): A susceptible result using the direct method and a resistant result using the standard method.

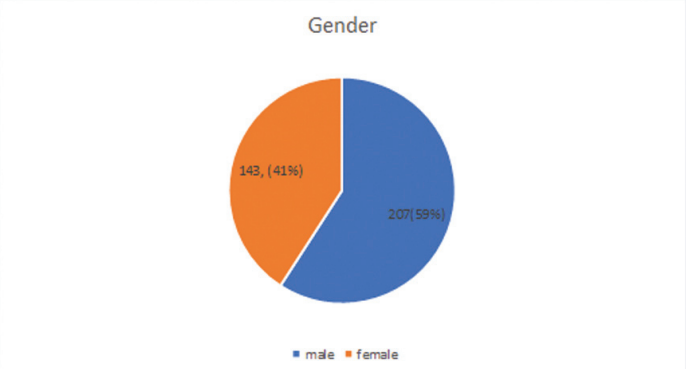
Major Error (MJ): Resistant result by the direct method and susceptible result by the standard method.

Minor Error (MN): Any discrepancy involving intermediate susceptibility by one method and susceptibility or resistance by the other.

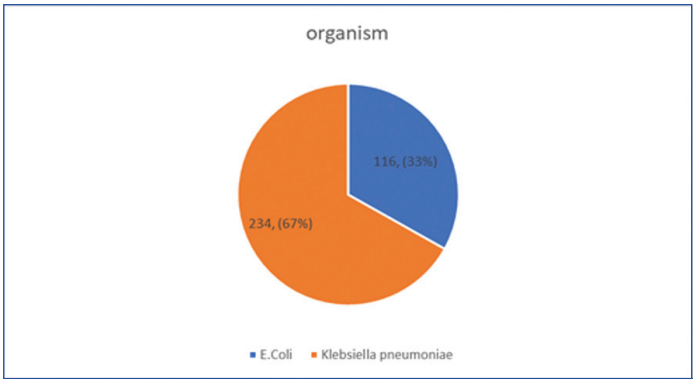
Agreement (A): Agreement or "no error" when both methods' results agree with the respective criteria.

RESULTS

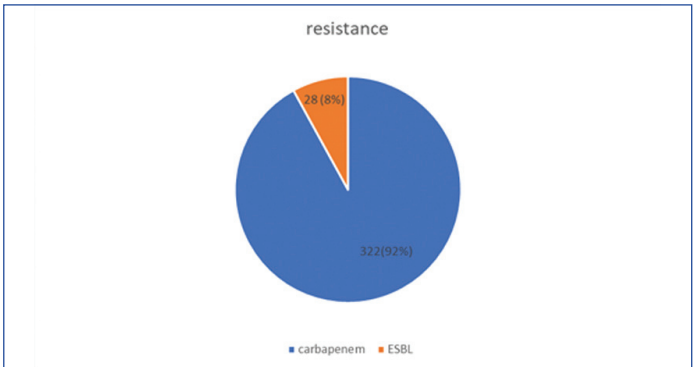
In the present study, done among 350 study subjects mean age was 49.6 with standard deviation 15.4. Maximum number of study participants 102 (29%) belonged to the age group 41 to 50 years and minimum number of study participants (8.3%) was within 30 years of age. [Table/Fig-2] shows the gender distribution among the respondents. Majority of them were males. [Table/Fig-3] shows the organism distribution in the sample tested and most commonly detected organism was Klebsiella pneumoniae and [Table/Fig-4] shows the resistance pattern among the isolates studied. 92% of them showed carbapenem resistance.



[Table/Fig-2]: Gender distribution among the respondents (N=350).

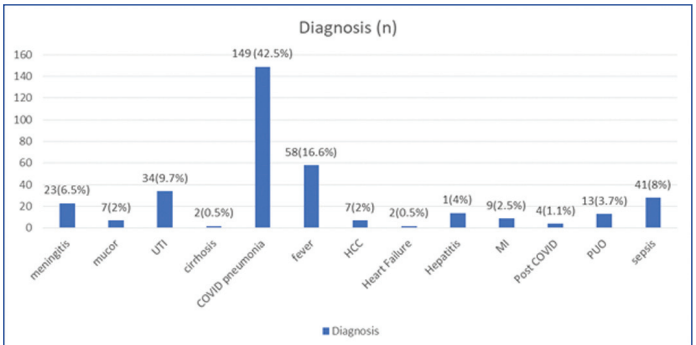


[Table/Fig-3]: Distribution of organism among the study participants (N=350).



[Table/Fig-4]: Resistance pattern among the isolates studied (N=350).

[Table/Fig-5] shows the diagnosis among the patients from whom samples were collected. First three common causes were COVID pneumonia followed by fever followed by UTI. Least common cause for bacteraemia was cirrhosis, Heart failure and post COVID.



[Table/Fig-5]: Diagnosis among the study subjects (N=350).

[Table/Fig-6] shows the agreement of antibiotic susceptibility test results between direct and conventional disc diffusion method, 98% of the samples showed complete agreement between two-test in total. All the samples showed complete agreement for the antibiotics like ceftazidime, ceftazidime+clavulanic acid. Cefoxitin, cotrimoxazole, meropenem, ertapenem, piperacillin-tazobactam, cefaperazone-sulbactum, colistin. All most 95% of the sample showed complete agreement for all other antibiotics.

Antibiotics	Categorical agreement in %	Minor Error (MN) in %	Major Error (MJ) in %	Very Major Error (VMJ) in %
Amikacin	96	4	0	0
Gentamycin	96	2.5	0.5	1
Ciprofloxacin	96	4	0	0
Ceftazidime	100	0	0	0
Ceftazidime+ Clavulanic acid	100	0	0	0
Cefoxitin	100	0	0	0
Cefepime	99.5	0	0.5	0
Cotrimoxazole	100	0	0	0

Imipenem	97.5	0.5	1	1
Meropenem	100	0	0	0
Ertapenem	100	0	0	0
Piperacillin-tazobactum	100	0	0	0
Cefaperazone-sulbactam	100	0	0	0
Colistin	100	0	0	0
Tigecycline	92	8	0	0
Ampicillin	98	2	0	0
Total	98	1	0.1	0.9

[Table/Fig-6]: Agreement of antibiotic susceptibility test results of rapid and conventional disc diffusion method (N=350).

[Table/Fig-7-9] shows the kappa statistics of level of agreement for various antibiotics when tested by conventional and rapid disc diffusion method. All antibiotics showed perfect or all most perfect agreement (kappa value from 0.81 to 1.000) except gentamycin, ampicillin, cefepime and tigecycline which showed substantial agreement (kappa value 0.61 tp 0.80).

Antibiotic		Conventional		Kappa value	p-value
Amikacin	Rapid	Sensitive	Resistant	0.812	<0.001
	Sensitive	252	0		
	Resistant	40	58		
Gentamycin	Rapid	Sensitive	Resistant	0.799	<0.001
	Sensitive	173	13		
	Resistant	22	142		
Ciprofloxacin	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	134	0		
	Resistant	0	216		
Ceftazidime	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	275	0		
	Resistant	0	75		
Ceftazidime+ Clavulanic acid	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	300	0		
	Resistant	0	50		
Cefoxitin	Direct	Sensitive	Resistant	1.000	<0.001
	Sensitive	226	0		
	Resistant	0	124		

[Table/Fig-7]: Comparison between test results of conventional and rapid Method of disc diffusion (n =350).

Antibiotic		Conventional		Kappa value	p-value
Cefepime	Rapid	Sensitive	Resistant	0.741	<0.001
	Sensitive	273	18		
	Resistant	9	50		
Tigecycline	Rapid	Sensitive	Resistant	0.632	<0.001
	Sensitive	295	18		
	Resistant	9	28		
Ampicillin	Rapid	Sensitive	Resistant	0.795	<0.001
	Sensitive	243	8		
	Resistant	20	79		
Cotrimoxazole	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	253	0		
	Resistant	0	97		
Imipenem	Rapid	Sensitive	Resistant	0.939	<0.001
	Sensitive	322	1		
	Resistant	2	25		

[Table/Fig-8]: Antibiotic sensitivity on comparing conventional and direct Method of disc diffusion (N=350).

Antibiotic		Conventional		Kappa value	p-value
Meropenem	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	333	0		
	Resistant	0	26		
Ertapenem	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	335	0		
	Resistant	0	23		
Piperacillin-tazobactam	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	307	0		
	Resistant	0	54		
Cefaperazone-sulbactum	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	314	0		
	Resistant	0	36		
Colistin	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	346	0		
	Resistant	0	4		

[Table/Fig-9]: Agreement of test results of conventional and rapid Method of disc diffusion (N=350).

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.

[Table/Fig-10] shows the agreement of antibiotic susceptibility test results between direct and conventional VITEK method, 99% of the samples showed complete aggreement between two test in total. All the samples showed complete agreement for the antibiotics like ceftazidime, ceftazidime+clavulanic acid, cefoxitin, cotrimoxazole, meropenem, ertapenem, piperacillin-tazobactam, cefaperazone-sulbactam, colistin. All most 95% of the sample showed complete agreement for all other antibiotics except amikacin (91.2%) and ciprofloxacin (90.7%).

Antibiotics	Categorical agreement in %	Minor Error (MN) in %	Major Error (MJ) in %	Very Major Error (VMJ) in %
Amikacin	91.2	5	0.5	3.3
Gentamycin	97	2	1	0
Ciprofloxacin	90.7	5	0	4.3
Ceftazidime	100	0	0	0
Ceftazidime+ clavulanic acid	100	0	0	0
Cefoxitin	100	0	0	0
Cefepime	99.5	0	0.5	0
Cotrimoxazole	100	0	0	0
Imipenem	97.4	0	1	1.6
Meropenem	100	0	0	0
Ertapenem	100	0	0	0
Piperacillin-tazobactum	100	0	0	0
Cefaperazone-sulbactum	100	0	0	0
Colistin	100	0	0	0
Tigecyclin	95	5	0	0
Ampicillin	96.4	3.6	0	0
Total	99	0	0.9	0.1

[Table/Fig-10]: Agreement of antibiotic susceptibility test results of rapid and conventional VITEK method (N=350).

[Table/Fig-11-13] Shows the kappa statistics of level of agreement for various antibiotics when tested by conventional and rapid VITEK method .All antibiotics showed perfect or all most perfect agreement (kappa value from 0.81 to 1.000) except amikacin, gentamycin,

ampicillin, cefepime and tigecyclin which showed substantial agreement (kappa value 0.61 tp 0.80).

Antibiotic		Conventional		Kappa value	p-value
Amikacin	Rapid	Sensitive	Resistant	0.615	<0.001
	Sensitive	245	0		
	Resistant	49	56		
Gentamycin	Rapid	Sensitive	Resistant	0.799	<0.001
	Sensitive	172	15		
	Resistant	20	143		
Ciprofloxacin	Rapid	Sensitive	Resistant	0.970	<0.001
	Sensitive	135	2		
	Resistant	3	210		
Ceftazidime	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	277	0		
	Resistant	0	73		
Ceftazidime+ Clavulanic acid	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	302	0		
	Resistant	0	48		
Cefoxitin	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	226	0		
	Resistant	0	124		

[Table/Fig-11]: Comparison between test results of conventional and rapid Method-VITEK (n =350).

Antibiotic		Conventional		Kappa value	p-value
Cefepime	Rapid	Sensitive	Resistant	0.896	<0.001
	Sensitive	269	1		
	Resistant	9	53		
Tigecyclin	Rapid	Sensitive	Resistant	0.623	<0.001
	Sensitive	296	17		
	Resistant	10	27		
Ampicillin	Rapid	Sensitive	Resistant	0.792	<0.001
	Sensitive	245	8		
	Resistant	20	77		
Cotrimoxazole	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	257	0		
	Resistant	0	93		
Imipenem	Rapid	Sensitive	Resistant	0.958	<0.001
	Sensitive	323	0		
	Resistant	2	25		

[Table/Fig-12]: Antibiotic Sensitivity Test (AST) results of conventional and rapid Method -VITEK (N=350).

DISCUSSION

Especially in BSIs caused by Enterobacterales like Escherichia coli and Klebsiella Pneumoniae, early identification of bacterial pathogens and antibiotic sensitivity patterns is critical to guide the appropriate treatment course. In this study, disc diffusion and automated systems such as VITEK 2 were utilised to compare the performance of direct methodologies versus conventional culture-based methods for AST.

Concordance between traditional and direct disc diffusion methods: Total categorical concordance between direct and traditional disc diffusion methods was noted in 98% of isolates in the present study. The effectiveness of the use of direct AST methods for early treatment recommendations is evidenced by this high concordance rate.

Findings of the current study are comparable to Bhattacharya S et al., who reported that direct disc diffusion testing in Gram-negative bacilli, i.e., *KE pneumoniae* and *E.coli*, had a total categorical agreement of 96.4% [18]. The present study showed less categorical agreement for antibiotics like ciprofloxacin, Amikacin, Gentamycin,

Antibiotic		Conventional		Kappa value	p-value
Meropenem	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	337	0		
	Resistant	0	13		
Ertapenem	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	335	0		
	Resistant	0	23		
Piperacillin-tazobactam	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	308	0		
	Resistant	0	53		
Cefaperazone-sulbactum	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	314	0		
	Resistant	0	36		
Colistin	Rapid	Sensitive	Resistant	1.000	<0.001
	Sensitive	346	0		
	Resistant	0	4		

[Table/Fig-13]: Comparison between rapid and conventional test results of VITEK Method (N=350).

Tigecycline between conventional and direct disc diffusion method, this was in concordant with another study done by Menon et al., (Ciprofloxacin 2.7%, Meropenem 1.4%), [19] and KJ R et al., (Ciprofloxacin 6.6%) [20].

Rapid and conventional VITEK 2 methods: A comparison: This research found an exceptional 99% concordance between the rapid and standard VITEK 2 systems for each antibiotic tested. Such a high agreement supports the accuracy of the rapid VITEK 2 method in making clinical decisions. This finding surpass those of Altun O et al., who noted 91.3% categorical concordance between rapid and standard VITEK 2 AST for Enterobacterales from positive blood cultures [21]. Paluch M et al., likewise noted agreement at 98.4% and with Very major discrepancies were for amoxicillin-clavulanate (4.9%), piperacillin-tazobactam (7.5%) and meropenem (33%) for Enterobacterales and gentamicin for Staphylococci (4.6%) [22]. In another study done by C.A. Hogan et al agreement rate was 97.7% for gram negative bacilli which was lower when compared to the present study [23]. Enhanced internal quality control methods, increased automation, and stringent sample processing protocols can also be a possible explanation for the higher agreement in our study.

A closer look at aminoglycoside and Ciprofloxacin: Relative to the other antibiotics, ciprofloxacin and aminoglycoside demonstrated relatively less concordance in this study. In study done by agreement for P. aeruginosa and A. baumannii were, 98.6 and 100% (ciprofloxacin), 88.4 and 100% (gentamicin)[24]. In direct testing,differences in inoculum concentration and protein-binding effects can produce differences in fluoroquinolone and aminoglycoside performance. These mismatches show the importance of carefully interpreting these agents in rapid workflows. The susceptibility agreement was high with Extended spectrum of beta lactamase , resistance to ceftazidime, carbapenems and cefepime according to study done by Munoz-Dávila et al which was similar to the present study. [25]

Colistin intolerance: Technical challenges: As colistin's MICs are variable and have poor diffusion in agar-based systems, testing its susceptibility is still technically challenging. Despite these limitations, the current study surpassed several previous studies in having high rates of agreement. Matuschek E et al., for example, highlighted the inefficiency of diffusion-based methods to use with colistin and proposed broth microdilution, which is a resource-intensive method [26].

The use of enhanced interpretation criteria and compliance with CLSI/EUCAST guidelines could be the cause of the elevated degree of colistin agreement in our study, underpinning the value of meticulous standardisation. Turnaround time for susceptibility results is significantly shortened by 24 to 48 hours when utilising direct AST

and rapid automated methods. Better patient outcomes, reduced antibiotic resistance, and an earlier de-escalation of empirical therapy are just a few of the important impacts this has on patient care. Combined antibiotic stewardship and rapid diagnoses lowered mortality and hospitalisation in BSIs, as indicated by Banerjee R et al., [27]. This was corroborated by our data, which indicate that rapid identification and AST derived directly from blood cultures can greatly improve patient outcomes and resource utilisation. The present study excluded polymicrobial bacteraemia which accounts for 10 to 20% of infections in tertiary care hospital settings which is one of the limitations of the present study [28].

Limitation(s)

Despite of these advantages there were some limitations in the study. Firstly, the study analysis was mainly on *E. coli* and *K. pneumoniae* which were predominant Gram-negative pathogens in study setting. AST for other Gram-negative organisms (e.g., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) was not done making broad implementation questionable. The present study also excluded polymicrobial bacteraemia which accounts for 10 to 20% of infections in tertiary care hospital settings. Secondly, our study was done in a single tertiary care centre which has its out antibiotic resistance pattern. However, this study forms the base for future large multi-centric studies with large sample size. Thirdly we did not assess patient's outcome by diagnosis rapidly through direct methods.

CONCLUSION(S)

Unlike conventional methods, our study demonstrates that direct AST from blood culture fluid provides highly precise susceptibility results for E. coli and K. pneumoniae bacteraemia, with the potential to have a significant reduction in time to results. The therapeutic value of this approach is underlined by the excellent agreement rates obtained for antibiotics of critical importance. Direct AST is a promising tool to accelerate antibiotic optimisation in Gram-negative bacteraemia patients, even though there are some limitations. This may contribute to improved outcomes through earlier directed therapy.

REFERENCES

[1] Diekema DJ, Hsueh PR, Mendes RE, Pfaller MA, Rolston KV, Sader HS, et al. The microbiology of bloodstream infection: 20-year trends from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother. 2019;63(7):e00355-19.

[2] Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: International guidelines for management of sepsis and septic shock: 2016. Intensive Care Med. 2017;43(3):304-77.

[3] Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med. 2019;47(4):486-95.

[4] Bearman GM, Wenzel RP. Bacteremias: A leading cause of death. Arch Med Res. 2005;36:646-59.

[5] World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: 2023. Geneva: WHO; 2023.

[6] Seymour CW, Gesten F, Prescott HC, Friedrich ME, Iwashyna TJ, Phillips GS, et al. Time to treatment and mortality during mandated emergency care for sepsis. N Engl J Med. 2017;376(23):2235-44.

[7] Kahlmeter G, Dahlager JI. Antimicrobial susceptibility testing of bacteria that grow aerobically—EUCAST standardized disk diffusion method. Clin Microbiol Infect. 2021;27(8):1119-28.

[8] Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. Front Pediatr 2018;6:285. Doi: 10.3389/fped.2018.00285.

[9] Idelevich EA, Becker K. How to accelerate antimicrobial susceptibility testing. Clin Microbiol Infect. 2019;25(11):1347-55.

[10] Pancholi P, Carroll KC, Buchan BW, Chan RC, Dhiman N, Ford B, et al. Multicenter evaluation of the Accelerate PhenoTest BC kit for rapid identification and phenotypic antimicrobial susceptibility testing using morphokinetic cellular analysis. J Clin Microbiol. 2018;56(4):e01329-e01317.

[11] Chandrasekaran S, Abbott A, Campeau S, Zimmer BL, Weinstein M, Thrupp L, et al. Direct-from-blood-culture disk diffusion to determine antimicrobial susceptibility of Gram-negative bacteria: Preliminary report from the Clinical and Laboratory Standards Institute methods development and standardization working group. J Clin Microbiol. 2018;56(3):e01678-17.

[12] Delport JA, Strikwerda A, Armstrong A, Schaus D, John M. MALDI-ToF short incubation identification from blood cultures is associated with reduced length of hospitalization and a decrease in bacteremia-associated mortality. Eur J Clin Microbiol Infect Dis. 2021;40(11):2373-79.

[13] Collee JG, Mackie TJ, McCartney JE. Mackie & McCartney Practical Medical Microbiology. New York: Churchill Livingstone; 1996. p. 121-24.

[14] Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100. Wayne (PA): CLSI; 2025.

[15] Ling TKW, Tam PC, Liu ZK, Cheng AFB. Evaluation of VITEK 2 rapid identification and susceptibility testing system against gram-negative clinical isolates. J Clin Microbiol. 2001;39(8):2964-66.

[16] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74.

[17] Yakoob R, Bhat GK. Comparison of direct antibiotic susceptibility testing with standard testing in blood culture. J Pure Appl Microbiol. 2018;12(4):2289-96.

[18] Bhattacharya S, Srinivasan R, Ayyagari A. Direct susceptibility testing from blood culture bottles for Gram-negative bacilli. Indian J Med Microbiol. 2019;37(1):38-42.

[19] Menon VS, Lahanas C, Janto, Lee A, Utility of direct susceptibility testing on blood cultures: Is it still worthwhile? J Med Microbiol. 2016;65:501-509.

[20] KJ R, Harisree S, Baijayanti M, Evaluation of Direct Susceptibility Testing for Monomicrobial Growth from Bactec Blood Culture Bottles-A Pilot Study. 2017. 10.18535/jmscr/v5i8.10.

[21] Altun O, Almuhayawi M, Ullberg M, Özenci V. Clinical evaluation of the FilmArray Blood Culture Identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol. 2013;51(12):4130-36.

[22] Paluch M, Lleres-Vadeboin M, Poupet H, Chanard E, Wilhelm N, Nadji S, et al. Multicenter evaluation of rapid antimicrobial susceptibility testing by VITEK®2 directly from positive blood culture. Diagnostic Microbiology and Infectious Disease. 2023;106(3):115950.

[23] Hogan CA, Watz N, Budvytiene N. Banaei Rapid antimicrobial susceptibility testing by VITEK®2 directly from blood cultures in patients with Gram-negative rod bacteremia.

[24] Poyanes P, del Carmen Conejo M, Martínez-Martínez L, Perea EJ. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. J Clin Microbiol. 2001;39(9):3247-53.

[25] Munoz-Dávila MJ, Yagüe G, Albert M. et al. Comparative evaluation of Vitek 2 identification and susceptibility testing of Gram-negative rods directly and isolated from BacT/ALERT-positive blood culture bottles. Eur J Clin Microbiol Infect Dis 31. 2012;663-69. Available from: https://doi.org/10.1007/s10096-011-1356-1.

[26] Matuschek E, Åhman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter spp. Clin Microbiol Infect. 2018;24(8):865-70.

[27] Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, et al. Randomized trial of rapid multiplex polymerase chain reaction–based blood culture identification and susceptibility testing. Clin Infect Dis. 2020;70(1):132-40.

[28] Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, et al. The clinical significance of positive blood cultures in the 1990s: A prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis. 1997;24(4):584-602.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, PSG IMS and R, Coimbatore, Tamil Nadu, India.
2. Undergraduate Student, Department of Microbiology, PSG IMS and R, Coimbatore, Tamil Nadu, India.
3. Professor, Department of Microbiology, PSG IMS and R, Coimbatore, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
S Shanmugapriya,
PSG Hospital, Peelamedu, Coimbatore, Tamil Nadu, India.
E-mail: drshanmamicro@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Apr 20, 2025
- Manual Googling: Jul 17, 2025
- iThenticate Software: Jul 19, 2025 (8%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 17, 2025**
Date of Peer Review: **May 18, 2025**
Date of Acceptance: **Jul 21, 2025**
Date of Publishing: **Sep 01, 2025**