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Original Research Article

Effect of charcoal supplementation on the accuracy of direct antimicrobial susceptibility testing from blood cultures for key Gram-negative bacilli: an *in vitro* evaluation

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ABSTRACT

Background: Gram negative bacilli such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are major causes of bloodstream infection and are associated with high mortality rates in critical care settings. Rapid antimicrobial susceptibility testing (AST) directly from positive blood cultures can reduce diagnostic turnaround time and improve patient management. To evaluate the in vitro performance of direct antimicrobial susceptibility testing from blood culture systems, with and without charcoal supplementation, for *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
Methods: In this study, using clinical isolates from various blood culture systems, Brain Heart Infusion broth (BHI), BHI with charcoal (BHI-C), Tryptic Soy Broth (TSB) and TSB with charcoal (TSB-C). DST was performed directly on the positive broth samples using disk diffusion, and the results from each medium type were compared.
Result: Charcoal supplementation significantly increased mean zone diameters for most antibiotics across all organism. TSB-C demonstrated consistently higher susceptibility reading compared to other media.
Conclusion: Direct AST using TSB supplemented with charcoal enhances susceptibility accuracy and could be adopted in laboratories lacking automated AST systems for rapid bloodstream infection management.

Keywords: Direct susceptibility testing, Bloodstream infections, Charcoal, Tryptic soy broth, Brain heart infusion broth, Gram-negative bacteria

INTRODUCTION

Bloodstream infections (BSI) are defined broadly as the presence of viable microorganisms in the blood, which can lead to inflammation in the host and alter the clinical and hemodynamic properties and lead to morbid consequences.[1] The presence of microorganisms, however, transiently in the circulation poses a threat to most organs. The consequences of bloodstream infections if not treated can lead to shock, disseminated intravascular coagulation (DIC), multiple organ failure, and death.[2] Bloodstream infections are a major public health problem worldwide, and it has been associated with significant morbidity and mortality. There is an emerging trend of BSI caused by Gram-negative organisms such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and an increased incidence of drug-resistant strains.[3,4]

Timely intervention in the treatment of bloodstream infection is of paramount importance to increase the chances of a favourable outcome, since empirical therapy can be modified upon receipt of in vitro antimicrobial susceptibility testing (AST) results. AST results can assist in modifying antimicrobial therapy, and investigators have demonstrated decreased mortality with early treatment. Further data from outcome-based studies assessing the effect of rapid reporting of susceptibility results have

shown a decrease in the number of laboratory tests and procedures ordered, decreased length of stay, and decreased health care costs and quicker modification of antimicrobial therapy.[5]

Resin-based media are known to absorb antibiotics present in blood culture media, which is useful for the evaluation of sepsis patients who have already received antibiotics. Charcoal-based media have the same effect but might hinder the microscopic observation of Gram staining. These bottles are known to enhance the detection of microorganisms, even for sepsis patients who do not receive antibiotics, possibly by inhibiting the activities of antibodies, complement factors, or cytokines.[6] Exploring the reliability and accuracy direct antimicrobial susceptibility testing using clinical isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa*, with and without charcoal, can contribute valuable insights to optimize diagnostic practice in clinical settings. Active charcoal absorbs antimicrobial agent and other substances that may be present in the blood specimens, potentially impacting bacterial growth. In this in vitro study, comparing the zone of inhibition in tests with and without charcoal can help determine the effectiveness of this approach in improving diagnostic accuracy.

METHODS

This study was conducted in Department of Medical Microbiology, Centre for Professional and Advanced Studies (CPAS), SME, Gandhinagar from April 2024 to May 2025. A total of 150 isolates were analysed, comprising 50 isolates each of *E. coli*, *K. pneumoniae* and *P. aeruginosa*, were collected from St. Mary's hospital, Thodupuzha, Kerala.

Blood culture media and antimicrobial agents

A cohort of 150 clinical Gram-negative isolates were collected and tested, each isolate was tested across all four media conditions, established a matched-pair design essential for rigorous statistical comparison. The four media conditions utilized for generating the positive blood culture broths were BHI, TSB, BHI supplemented with activated charcoal BHI-C, and TSB supplemented with activated charcoal TSB-C. These broths served as the source material for the direct testing. AST was performed on Mueller–Hinton agar (MHA) using the Kirby–Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines. Antimicrobial susceptibility disks were selected based on standard clinical usage for Gram-negative bacteremia. The tested agents included Amoxicillin-Clavulanic Acid (AMC,10-20 µg), Aztreonam (AT,30 µg), Ceftazidime (CAZ,30 µg), Cefuroxime (CXM,30 µg), Ciprofloxacin (CIP,5 µg), Gentamicin (HLG,10 µg), Tetracycline (TE,30 µg), and Imipenem (IMP,10 µg). In the case of *E. coli* Ampicillin (AMP,30 µg) was used and in *K. pneumoniae* Piperacillin-Tazobactam (PIT,100-10 µg) was used. For *P. aeruginosa* Cefepime (CPM,30µg), Amikacin (AK,30 µg), ceftazidime/avibactam (CZA,30/20 µg) and also Piperacillin\tazobactam (PIT,100/10 µg) was used.

Measurement of zone

Zone diameters (in millimeters) were measured using a calibrated ruler according to routine disk diffusion recommendation in CLSI M02-A13. The measured values were recorded in Microsoft Excel for systematic analysis and compare the zone differences between each set of plate (BHI, TSB with and without charcoal) Each isolates response was interpreted as Sensitive (S), Intermediate (I), or Resistant (R) based on zone diameter breakpoints provided by the CLSI guidelines.

Statistical analysis

The study was approved by the institutional ethical committee at School of Medical Education. The data was analysed using Paired "t" test to compare the antibiotic susceptibility, employing Statistical Package for the Social Sciences (SPSS Inc.;Chicago,IL) version 29.0.10. A p-value of less than 0.05 is typically considered statistically significant.

RESULTS

In the present study, a total of 150 isolates were obtained from various blood culture systems, comprising *E. coli* (n=50), K. pneumoniae (n=50) and *P. aeruginosa* (n=50). The analysis provides a quantitative comparison of the mean susceptibility values, measured as zone of inhibition (in millimeters) between different media pairs using a paired t-test. This statistical test was applied to determine whether the average susceptibility value is significantly different between two conditions.

Direct susceptibility testing of E. coli

The data in Table 1 consistently showed that the addition of charcoal has a statistically significant effect on susceptibility measurements for 7 out of 9 antibiotics, the comparison between BHI and BHI-C yielded a p value of less than 0.001, indicating a highly significant difference in mean susceptibility. Furthermore, the remaining two antibiotics, the comparison between BHI and BHI-C yielded a p value that vary from 0.215-0.001, indicating variability. Additionally, the mean difference was consistently negative, which signifies that the mean susceptibility measured in the charcoal supplemented medium (BHI-C) was higher than in the un-supplemented medium (BHI).

The same pattern holds true for the TSB vs TSB-C comparative with negative difference, demonstration a uniform and significant increase a measured susceptibility with the addition of charcoal. The analysis also reveals a significant difference between the two-base media. The paired t test result for BHI -TSB also showed statistically significant difference for all antibiotics (p value ranging from 0.001 to 0.510). The mean difference was

consistently negative indicating that the TSB media generally yielded a higher susceptibility value than BHI media. These comparisons between the charcoal supplemented media BHI-C vs TSB-C, also show statistically significant different (p value <0.05) that the inherent difference between the base media persist even with the addition of charcoal.

Direct susceptibility testing of K. pneumoniae

The data in Table 2 showed that, both BHI-C and TSB-C consistently demonstrated significantly higher mean susceptibility compared to their respective without charcoal base media (BHI and TSB). The p-values for these comparisons were overwhelmingly significant, ranging from <0.001 to 0.001. The consistently negative mean differences further confirm that the supplemented media yielded higher susceptibility values. For instance, for AMC, the mean susceptibility in BHI-C (19.94) was significantly higher than in BHI (18.69), with a mean difference of −1.25 and a p-value of 0.001. Similarly, TSB-C (20.59) showed significantly higher susceptibility than TSB (19.16), with a mean difference of −1.43 and a p-value of 0.001. The overwhelming statistical significance (p-values consistently <0.001 or 0.001) for 8 out of 9 antibiotics, however, IPM, there was no statistically significant difference between BHI and BHI-C (p=0.088) or between TSB and TSB-C (p=0.189). This indicates that IPM susceptibility is largely unaffected by the charcoal.

Comparison between BHI and TSB, statistically significant differences were observed where TSB showed higher mean susceptibility than BHI for a subset of antibiotics. For AT, TSB (Mean: 21.14) yielded significantly higher susceptibility than BHI (Mean: 20.10, p<0.001). Similarly, for CXM, TSB (Mean: 19.64) showed significantly higher susceptibility than BHI (Mean: 18.90, p=0.002). CIP and TE also exhibited significantly higher susceptibility in TSB compared to BHI (Mean: 20.39, p=0.017). The comparison between BHI-C and TSB-C, TSB-C generally proved superior, demonstrating statistically significant higher mean susceptibility for a majority of antibiotics. For AMC, TSB-C (Mean: 20.59) was significantly higher than BHI-C (Mean: 19.94, p=0.026). Similarly, for AT, TSB-C (Mean: 22.40) showed significantly higher susceptibility than BHI-C (Mean: 21.68, p=0.014). CAZ (TSB-C Mean: 20.68 vs BHI-C Mean: 20.00, p=0.003), CXM (TSB-C Mean: 21.00 vs BHI-C Mean: 20.06, p<0.001), CIP (TSB-C Mean: 22.57 vs BHI-C Mean: 21.67, p=0.002), and TE (TSB-C Mean: 21.46 vs BHI-C Mean: 20.52, p<0.001) all showed significantly higher susceptibility in TSB-C compared to BHI-C. However, for PIT (p=0.157), HLG (p=0.092), and IPM (p=0.189), no statistically significant differences were observed between BHI-C and TSB-C. Given that TSB-C consistently shows significantly higher mean susceptibility than BHI-C for a majority of antibiotics (6 out of 9), it emerges as the generally preferred medium among the supplemented options for eliciting higher *K. pneumoniae* susceptibility in vitro.

Direct susceptibility testing of P. aeruginosa

The data in Table 3 consistently showed that the addition of charcoal has a statistically significant effect on susceptibility measurements. For all eight antibiotics, the comparison between BHI and BHI-C yielded a p value of less than 0.001, indicating a highly significant difference in mean susceptibility. Furthermore, the mean difference was consistently negative (e.g., -1.36 for AK and -1.54 for PIT), which signifies that the mean susceptibility measured in the charcoal-supplemented medium (BHI-C) was higher than in the un-supplemented medium (BHI). The same pattern holds true for the TSB vs. TSB-C comparison, with all p-values also below 0.001 and negative mean differences, demonstrating a uniform and significant increase in measured susceptibility with the addition of charcoal.

The analysis also reveals significant differences between the two-base media. The paired t-test results for BHI versus TSB also showed statistically significant differences for all antibiotics (p values ranging from 0.001 to 0.034). The mean differences were consistently negative, indicating that TSB media generally yielded higher susceptibility values than BHI media. These comparisons between the charcoal-supplemented media, BHI-C vs. TSB-C, also showed statistically significant differences (p values <0.05), suggesting that the inherent differences between the base media persist even with the addition of charcoal.

Table 1: DST of *E. coli* on BHI and TSB media, with and without charcoal supplementation.

Drugs	Culture emdias		Mean	S.D.	Mean difference	"t"	P value
AMC	BHI vs. BHI-C	BHI	16.19	4.88	-1.98	-7.80	<0.001*
		BHI-C	18.17	4.53			
	TSB vs. TSB-C	TSB	18.22	5.49	-2.02	-8.62	<0.001*
		TSB-C	20.24	5.69			
	BHI vs. TSB	BHI	16.41	5.04	-1.76	-3.40	0.001*
		TSB	18.17	5.44			
	BHI-C vs. TSB-C	BHI-C	18.31	4.58	-1.67	-2.97	0.005*
		TSB-C	19.98	5.55			
AMP	BHI vs. BHI-C	BHI	16.31	5.06	-1.94	-15.48	<0.001*
		BHI-C	18.25	5.25			

Continued.

Drugs	Culture emdias		Mean	S.D.	Mean difference	"t"	P value
	TSB vs. TSB-C	TSB	19.23	5.39	-2.17	-9.75	<0.001*
		TSB-C	21.40	4.97			
	BHI vs. TSB	BHI	16.51	4.97	-2.71	-6.01	<0.001*
		TSB	19.23	5.39			
	BHI-C vs. TSB-C	BHI-C	18.49	5.13	-2.91	-7.32	<0.001*
		TSB-C	21.40	4.97			
AT	BHI vs. BHI-C	BHI	20.20	4.98	-0.98	-1.74	0.089
		BHI-C	21.18	4.97			
	TSB vs. TSB-C	TSB	20.85	4.73	-1.88	-7.91	<0.001*
		TSB-C	22.73	4.56			
	BHI vs. TSB	BHI	20.39	4.74	-0.63	-0.85	0.403
		TSB	21.02	4.60			
	BHI-C vs. TSB-C	BHI-C	21.17	4.90	-1.59	-2.63	0.012*
		TSB-C	22.76	4.53			
CAZ	BHI vs. BHI-C	BHI	19.36	4.75	-0.87	-1.26	0.215
		BHI-C	20.23	4.02			
	TSB vs. TSB-C	TSB	19.76	4.67	-2.44	-7.86	<0.001*
		TSB-C	22.20	4.73			
	BHI vs. TSB	BHI	19.43	4.71	-0.55	-0.67	0.510
		TSB	19.98	4.48			
	BHI-C vs. TSB-C	BHI-C	20.00	4.17	-2.37	-3.93	<0.001*
		TSB-C	22.37	4.40			
CFX	BHI vs. BHI-C	BHI	18.97	4.75	-2.21	-9.49	<0.001*
		BHI-C	21.18	4.74			
	TSB vs. TSB-C	TSB	21.05	4.62	-2.08	-6.11	<0.001*
		TSB-C	23.13	4.34			
	BHI vs. TSB	BHI	18.95	4.73	-2.11	-3.26	0.002*
		TSB	21.05	4.62			
	BHI-C vs. TSB-C	BHI-C	21.11	4.73	-2.03	-3.34	0.002*
		TSB-C	23.13	4.34			
CIP	BHI vs. BHI-C	BHI	21.13	5.34	-1.35	-2.90	0.006*
		BHI-C	22.48	4.87			
	TSB vs. TSB-C	TSB	20.59	4.48	-2.13	-10.89	<0.001*
		TSB-C	22.72	4.65			
	BHI vs. TSB	BHI	21.09	5.39	0.27	0.33	0.744
		TSB	20.82	4.23			
	BHI-C vs. TSB-C	BHI-C	22.17	5.20	-0.54	-0.77	0.447
		TSB-C	22.72	4.65			
HLG	BHI vs. BHI-C	BHI	17.89	4.79	-2.28	-9.61	<0.001*
		BHI-C	20.17	4.53			
	TSB vs. TSB-C	TSB	18.74	4.67	-2.20	-7.73	<0.001*
		TSB-C	20.93	4.50			
	BHI vs. TSB	BHI	17.87	4.84	-0.87	-1.16	0.253
		TSB	18.74	4.67			
	BHI-C vs. TSB-C	BHI-C	20.15	4.58	-0.78	-1.11	0.271
		TSB-C	20.93	4.50			
IPM	BHI vs. BHI-C	BHI	19.10	4.52	-1.10	-2.12	0.040*
		BHI-C	20.20	4.23			
	TSB vs. TSB-C	TSB	19.85	4.86	-2.06	-8.03	< 0.001*
		TSB-C	21.92	4.49			
	BHI vs. TSB	BHI	19.10	4.52	-0.59	-0.88	0.385
		TSB	19.69	4.94			
	BHI-C vs. TSB-C	BHI-C	20.23	4.27	-1.69	-3.46	0.001*
		TSB-C	21.92	4.49			

Continued.

Drugs	Culture emdias		Mean	S.D.	Mean difference	"t"	P value
TE	BHI vs. BHI-C	BHI	19.00	4.64	-1.24	-4.91	<0.001*
		BHI-C	20.24	4.49			
	TSB vs. TSB-C	TSB	20.32	5.00	-1.98	-10.60	<0.001*
		TSB-C	22.29	4.67			
	BHI vs. TSB	BHI	18.98	4.70	-1.34	-2.24	0.031*
		TSB	20.32	5.00			
	BHI-C vs. TSB-C	BHI-C	20.22	4.54	-2.07	-4.31	<0.001*
		TSB-C	22.29	4.67			

*Significant.

Table 2: DST of K. pneumoniae on BHI and TSB media, with and without charcoal supplementation.

Drugs	Culture medias		Mean	S.D.	Mean difference	"t"	P value
AMC	BHI vs. BHI-C	BHI	18.69	2.42	-1.25	-13.16	<0.001*
		BHI-C	19.94	2.24			
	TSB vs. TSB-C	TSB	19.16	3.42	-1.43	-7.22	<0.001*
		TSB-C	20.59	2.87			
	BHI vs. TSB	BHI	18.69	2.42	-0.47	-1.74	0.089
		TSB	19.16	3.42			
	BHI-C vs. TSB-C	BHI-C	19.94	2.24	-0.65	-2.29	0.026*
		TSB-C	20.59	2.87			
PIT	BHI vs. BHI-C	BHI	20.06	2.08	-1.28	-10.01	<0.001*
		BHI-C	21.34	2.06			
	TSB vs. TSB-C	TSB	20.36	2.32	-1.34	-8.48	<0.001*
		TSB-C	21.70	2.23			
	BHI vs. TSB	BHI	20.06	2.08	-0.30	-1.27	0.210
		TSB	20.36	2.32			
	BHI-C vs. TSB-C	BHI-C	21.34	2.06	-0.36	-1.44	0.157
		TSB-C	21.70	2.23			
AT	BHI vs. BHI-C	BHI	20.10	2.14	-1.58	-7.97	<0.001*
		BHI-C	21.68	2.64			
	TSB vs. TSB-C	TSB	21.14	2.17	-1.26	-8.36	<0.001*
		TSB-C	22.40	2.18			
	BHI vs. TSB	BHI	20.10	2.14	-1.04	-3.84	<0.001*
		TSB	21.14	2.17			
	BHI-C vs. TSB-C	BHI-C	21.68	2.64	-0.72	-2.55	0.014*
		TSB-C	22.40	2.18			
	BHI vs. BHI-C	BHI	18.98	1.70	-1.02	-10.10	<0.001*
		BHI-C	20.00	1.76			
	TSB vs. TSB-C	TSB	19.44	2.20	-1.24	-11.00	<0.001*
		TSB-C	20.68	2.07			
	BHI vs. TSB	BHI	18.98	1.70	-0.46	-1.94	0.059
		TSB	19.44	2.20			
	BHI-C vs. TSB-C	BHI-C	20.00	1.76	-0.68	-3.09	0.003*
		TSB-C	20.68	2.07			
CXM	BHI vs. BHI-C	BHI	18.90	2.46	-1.16	-13.27	<0.001*
		BHI-C	20.06	2.52			
	TSB vs. TSB-C	TSB	19.64	2.72	-1.36	-9.77	<0.001*
		TSB-C	21.00	2.31			
	BHI vs. TSB	BHI	18.90	2.46	-0.74	-3.24	0.002*
		TSB	19.64	2.72			
	BHI-C vs. TSB-C	BHI-C	20.06	2.52	-0.94	-4.73	<0.001*
		TSB-C	21.00	2.31			
CIP	BHI vs. BHI-C	BHI	20.39	3.07	-1.29	-10.39	<0.001*
		BHI-C	21.67	3.28			

Continued.

Drugs	Culture medias		Mean	S.D.	Mean difference	"t"	P value
	TSB vs. TSB-C	TSB	21.06	3.29	-1.51	-9.14	<0.001*
		TSB-C	22.57	2.97			
	BHI vs. TSB	BHI	20.39	3.07	-0.67	-2.47	0.017*
		TSB	21.06	3.29			
	BHI-C vs. TSB-C	BHI-C	21.67	3.28	-0.90	-3.22	0.002*
		TSB-C	22.57	2.97			
HLG	BHI vs. BHI-C	BHI	17.92	2.67	-1.20	-9.02	<0.001*
		BHI-C	19.12	2.73			
	TSB vs. TSB-C	TSB	18.33	3.28	-1.16	-9.58	<0.001*
		TSB-C	19.49	3.07			
	BHI vs. TSB	BHI	17.92	2.67	-0.41	-1.53	0.133
		TSB	18.33	3.28			
	BHI-C vs. TSB-C	BHI-C	19.12	2.73	-0.37	-1.72	0.092
		TSB-C	19.49	3.07			
IPM	BHI vs. BHI-C	BHI	16.35	2.64	-0.51	-1.74	0.088
		BHI-C	16.86	2.87			
	TSB vs. TSB-C	TSB	17.82	2.26	-0.29	-1.33	0.189
		TSB-C	18.10	2.37			
	BHI vs. TSB	BHI	16.35	2.64	-0.51	-1.74	0.088
		TSB	16.86	2.87			
	BHI-C vs. TSB-C	BHI-C	17.82	2.26	-0.29	-1.33	0.189
		TSB-C	18.10	2.37			
TE	BHI vs. BHI-C	BHI	19.46	2.04	-1.06	-11.64	<0.001*
		BHI-C	20.52	2.15			
	TSB vs. TSB-C	TSB	20.06	2.79	-1.40	-7.35	<0.001*
		TSB-C	21.46	2.49			
	BHI vs. TSB	BHI	19.46	2.04	-0.60	-2.48	0.017*
		TSB	20.06	2.79			
	BHI-C vs. TSB-C	BHI-C	20.52	2.15	-0.94	-4.62	<0.001*
		TSB-C	21.46	2.49			

*Significant.

Table 3: DST of P. aeruginosa on BHI and TSB media, with and without charcoal supplementation.

Drugs	Culture media		Mean	S.D.	Mean difference	"t"	P value
AK	BHI vs. BHI-C	BHI	19.94	2.17	-1.36	-9.98	0.001*
		BHI-C	21.30	2.10			
	TSB vs. TSB-C	TSB	20.82	2.21	-1.52	-9.67	0.001*
		TSB-C	22.34	2.38			
	BHI vs. TSB	BHI	19.94	2.17	-0.88	-2.73	0.009*
		TSB	20.82	2.21			
	BHI-C vs. TSB-C	BHI-C	21.30	2.10	-1.04	-3.61	0.001*
		TSB-C	22.34	2.38			
PIT	BHI vs. BHI-C	BHI	19.60	1.93	-1.54	-10.33	0.001*
		BHI-C	21.14	2.26			
	TSB vs. TSB-C	TSB	20.70	2.25	-1.38	-9.31	0.001*
		TSB-C	22.08	2.45			
	BHI vs. TSB	BHI	19.60	1.93	-1.10	-4.66	0.001*
		TSB	20.70	2.25			
	BHI-C vs. TSB-C	BHI-C	21.14	2.26	-0.94	-3.67	0.001*
		TSB-C	22.08	2.45			
AT	BHI vs. BHI-C	BHI	19.18	1.92	-1.25	-10.20	0.001*
		BHI-C	20.43	1.66			
	TSB vs. TSB-C	TSB	20.06	2.17	-1.20	-10.67	0.001*
		TSB-C	21.27	1.90			

Continued.

Drugs	Culture media		Mean	S.D.	Mean difference	"t"	P value
	BHI vs. TSB	BHI	19.18	1.92	-0.88	-3.56	0.001*
		TSB	20.06	2.17			
	BHI-C vs. TSB-C	BHI-C	20.43	1.66	-0.84	-4.00	<0.001*
		TSB-C	21.27	1.90			
CAZ	BHI vs. BHI-C	BHI	18.36	2.57	-1.44	-9.33	<0.001*
		BHI-C	19.80	2.44			
	TSB vs. TSB-C	TSB	20.06	2.17	-1.20	-10.67	<0.001*
		TSB-C	21.27	1.90			
	BHI vs. TSB	BHI	18.36	2.57	-0.94	-3.98	<0.001*
		TSB	19.30	2.89			
	BHI-C vs. TSB-C	BHI-C	19.80	2.44	-1.10	-5.23	<0.001*
		TSB-C	20.90	2.79			
CZA	BHI vs. BHI-C	BHI	19.02	2.11	-1.45	-8.39	<0.001*
		BHI-C	20.47	1.88			
	TSB vs. TSB-C	TSB	19.90	2.07	-1.37	-8.33	<0.001*
		TSB-C	21.27	2.18			
	BHI vs. TSB	BHI	19.02	2.11	-0.88	-3.44	0.001*
		TSB	19.90	2.07			
	BHI-C vs. TSB-C	BHI-C	20.47	1.88	-0.80	-3.02	0.004*
		TSB-C	21.27	2.18			
CIP	BHI vs. BHI-C	BHI	22.88	3.04	-1.59	-9.98	<0.001*
		BHI-C	24.47	3.18			
	TSB vs. TSB-C	TSB	23.55	3.14	-1.59	-8.87	<0.001*
		TSB-C	25.14	3.22			
	BHI vs. TSB	BHI	22.88	3.04	-0.67	-2.56	0.014*
		TSB	23.55	3.14			
	BHI-C vs. TSB-C	BHI-C	24.47	3.18	-0.67	-2.97	0.005*
		TSB-C	25.14	3.22			
CPM	BHI vs. BHI-C	BHI	19.08	1.54	-1.25	-10.83	<0.001*
		BHI-C	20.33	1.69			
	TSB vs. TSB-C	TSB	19.59	1.95	-1.43	-10.22	<0.001*
		TSB-C	21.02	1.97			
	BHI vs. TSB	BHI	19.08	1.54	-0.51	-2.19	0.034*
		TSB	19.59	1.95			
	BHI-C vs. TSB-C	BHI-C	20.33	1.69	-0.69	-3.26	0.002*
		TSB-C	21.02	1.97			
IMP	BHI vs. BHI-C	BHI	13.04	2.30	-1.36	-8.12	<0.001*
		BHI-C	14.40	2.53			
	TSB vs. TSB-C	TSB	13.68	2.59	-1.53	-8.79	<0.001*
		TSB-C	15.21	2.66			
	BHI vs. TSB	BHI	13.04	2.30	-0.64	-3.39	0.001*
		TSB	13.68	2.59			
	BHI-C vs. TSB-C	BHI-C	14.40	2.53	-0.81	-4.22	<0.001*
		TSB-C	15.21	2.66			

*Significant.

DISCUSSION

Bacteraemia is a worldwide cause of hospitalization and any kind of delay in appropriate antibiotic therapy could be harmful or even fatal for the patient. A bacteraemia diagnosis with speeded-up identification and AST is mandatory to adjust empirical broad-spectrum antibiotic therapy and avoid the emergence of multi-resistant bacteria. Speeded-up positive blood culture testing is therefore an important challenge for the hospital microbiology laboratory.[7] In this study, performed an in vitro evaluation of direct susceptibility testing (DST) using different blood culture systems-BHI, BHI-C, TSB, and TSB-C-to determine the most reliable and efficient medium for AST directly from positive blood culture bottles of clinical *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates, is recognised as standard procedure by the CLSI. According to Mahadevan et al in which a

high degree of agreement observed between direct antimicrobial testing susceptibility and the conventional AST method suggests that direct AST results are reliable and clinically useful. By comparing present study and Mahadevan et al study this rapid approach allows clinicians to initiate or modify antimicrobial therapy much earlier, potentially saving up to 24 critical hours in the management of bloodstream infections. Similarly, our study also supports the reliability of direct AST however, specifically demonstrated that TSB supplemented with charcoal provided superior results compared to other media as it enhanced bacterial recovery and produced larger susceptibility zones for most antibiotics. Thus, while Mahadevan et al confirmed the general utility of direct AST in clinical practice our study further refines this approach by identifying TSB with charcoal as the most effective medium for accurate and rapid susceptibility testing in *E. coli*, *K. pneumoniae* and *P. aeruginosa*.[8]

Sturm et al conducted DST on urine is a reliable method in which AST results substantially contribute to antimicrobial management of patients with UTI in general practice and DST can further improve antimicrobial prescription in these patients.[9] In the present study direct AST was done from the blood culture bottles which also showed high reliable results. The study demonstrated that TSB-C outperformed other media in terms of higher mean susceptibility zones for most antibiotics, suggesting that this combination provides optimal growth and better representation of bacterial susceptibility. These findings are consistent with Pfaller et al., who observed that TSB-based formulations provided better recovery rates compared to sucrose-supplemented alternatives, although they noted no significant advantage for fungal recovery.[10]

Henrichsen and Brun et al and Ellner et al found that although the use of hypertonic media did not result in an increase in total recovery, significant differences were seen in the recovery rates of individual species and groups of organisms.[11,12] Similarly, our result support the finding of Eng et al found that the use of brain heart infusion broth containing SPS, gelatin, and 20% sucrose like charcoal, resulted in increased numbers and rate of isolation of *Enterobacteriaceae* and *Staphylococcus aureus*.[13] But these results were not supported by Washington et al who found that although the recovery of Bacillus spp. was greater in TSB with 15% sucrose, other organisms, including *Haemophilus* spp., *S. aureus*, and the Bacteroidaceae, were isolated more frequently from TSB without sucrose supplementation, in the present study used TSB and TSB supplemented with charcoal and found greater zone difference in TSB with charcoal supplemented media, charcoal enhance the bacterial recovery by removing inhibitory substances such as residual antibiotic or host derived components, charcoals adsorptive property likely facilitated more accurate susceptibility patterns in the study.[14] As time changes the technological impact on clinical laboratory practices will be increasing so many authors focused on rapid identification by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) directly on positive blood cultures one such study was conducted by Wüppenhorst et al who studied Several protocols from positive blood cultures and doing direct MALDI-TOF MS from the same by using charcoal-containing BacT/ALERT bottles and also charcoal free bottles.[15] Also identification rates are high and results are accurate for the BACTEC™ system and for charcoal-free bottles. In our study conventional methods were followed in which the TSB supplemented with charcoal enhanced the results of AST in a reliable manner. So, for the future generation charcoal can also be used in the detection of AST in MALDI-TOF MS as it is a fast and accurate tool for direct species identification, even from positive charcoal-containing BC bottles.

Automated systems such as VITEK-2 and Phoenix (BD, USA) have also been employed to perform AST directly from positive blood culture bottles, showing a good correlation with conventional methods. In addition, chromogenic media, such as CHROMagar (BioMérieux, France), have been developed for rapid identification of methicillin-resistant Staphylococcus aureus (MRSA) directly from blood cultures, while the CHROMagar Mueller-Hinton Orientation medium has subsequently been used for organism identification in urinary tract infections. These specialized media can also be adapted for direct AST, demonstrating their versatility in rapid diagnostic workflows.[8]

The study has a few limitations as it was carried out on a limited number of an *E. coli*, *K. pneumoniae* and *P. aeruginosa* isolates from a single hospital, which may not reflect wider resistance trends. This work was one organism and few antibiotics, so these results cannot be generalised to other pathogens or drug classes. Moreover, only phenotypic methods were used, and advanced automated identification and susceptibility testing systems such as VITEK and MALDI-TOF were not employed, which may have limited the accuracy and comprehensiveness of the result.

CONCLUSION

The present study highlights the importance of rapid and reliable AST directly from positive blood culture bottles for the effective management of bloodstream infections caused by *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Among the different media tested, TSB-C proved to be the most efficient, showing larger and more consistent susceptibility zones for the majority of antibiotics. These findings indicate that charcoal supplementation enhances bacterial recovery by neutralizing inhibitory substances present in the blood culture media, thereby providing more accurate susceptibility patterns. This study also demonstrated that rapid antimicrobial sensitivity will be helpful for the hospital with laboratories lacking automated system.

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