International Journal of Medicine
pISSN 1468-3814 | eISSN 2667-7008
https://theinternationalmedicine.com/

Original Researcher Article *DOI: 10.61336/im/26-04-95*

Direct versus automated antimicrobial susceptibility testing in positive blood cultures: a comparative study in a cancer care setting

[1*]Dr. Nagma Rafi, [2]Dr. Yaseera S, [3]Sujith A
[1]*Consultant Microbiology, MVRCCRI, Kozhikode, Kerala*
[2]*Assistant Professor, Govt. Medical College, Kasargod, Kerala*
[3]*Technical Manager & Lab Safety Officer, MVRCCRI, Kozhikode, Kerala*

Correspondence:
Dr. Nagma Rafi.
Consultant Microbiology, MVRCCRI, Kozhikode, Kerala.

Received: 18-02-2026
Revised: 18-03-2026
Accepted: 10-04-2026
Published: 29-04-2026

Abstract

Background: Bloodstream infections (BSIs) are a major cause of morbidity and mortality among cancer patients. Although automated antimicrobial susceptibility testing (AAST) is the reference method, its prolonged turnaround time (TAT) may delay appropriate antimicrobial therapy. Direct antimicrobial susceptibility testing (DAST) offers a rapid alternative. Objectives: To compare the accuracy and reliability of DAST with AAST, evaluate their turnaround time, and assess the potential clinical impact of earlier susceptibility reporting. ***Materials and Methods***: This retrospective comparative study included 181 clinically significant bacterial isolates recovered from positive blood cultures processed between January and December 2024. DAST was performed directly from positively flagged blood culture bottles using the disk diffusion method, while identification and AAST were performed using the VITEK® 2 Compact system. Agreement, diagnostic performance, turnaround time, and potential clinical impact were analyzed. ***Results***: Gram-negative bacilli accounted for 79.6% of isolates, with Escherichia coli (35.9%) being the predominant pathogen. Overall categorical agreement between DAST and AAST was 90.1%, with sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of 98.5%, 93.6%, 89.6%, 99.1%, and 95.4%, respectively (κ=0.814). Mean turnaround time was significantly shorter with DAST than AAST (17.4 ± 2.1 vs. 42.8 ± 5.7 hours; p<0.001), providing susceptibility results approximately 25 hours earlier. Earlier availability of DAST facilitated timely optimization of antimicrobial therapy. ***Conclusion***: DAST demonstrated excellent agreement with automated AST while significantly reducing turnaround time. It is a reliable adjunct to automated susceptibility testing and may facilitate earlier targeted antimicrobial therapy and improved antimicrobial stewardship in cancer-care settings.

Keywords: Bloodstream infection; Cancer patients; Direct antimicrobial susceptibility testing; Automated antimicrobial susceptibility testing; Antimicrobial resistance; Turnaround time.

INTRODUCTION

Bloodstream infections (BSIs) are a major cause of morbidity and mortality among patients with cancer. Underlying malignancy, chemotherapy-induced neutropenia, impaired immune function, mucosal barrier disruption, prolonged hospitalization, invasive procedures, and indwelling vascular devices increase susceptibility to serious infections[1]. Repeated healthcare exposure and frequent use of broad-spectrum antimicrobial agents further increase the risk of infection with multidrug-resistant organisms. Consequently, BSI in cancer patients may rapidly progress to sepsis and septic shock, making early initiation of effective antimicrobial therapy crucial for improving clinical outcomes [2].The increasing burden of antimicrobial resistance further complicates the management of BSI in oncology patients. Gram-negative bacilli, including Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii, are important bloodstream pathogens and may exhibit resistance to multiple antimicrobial classes [2,3]. In the absence of definitive microbiological results, broad-spectrum empirical antimicrobial therapy is frequently initiated. However, inappropriate empirical therapy may result in treatment failure and adverse outcomes, whereas unnecessary continuation of broad-spectrum antibiotics contributes to antimicrobial resistance, drug-related toxicity, and increased healthcare costs. Studies among cancer patients have demonstrated that inappropriate antimicrobial treatment of BSI is associated with poorer clinical outcomes, emphasizing the importance of early administration of active therapy [4,5].Blood culture remains the reference standard for the microbiological diagnosis of BSI. Following detection of a positive blood culture, conventional laboratory processing generally involves Gram staining, subculture onto solid media, incubation to obtain isolated colonies, organism identification, and antimicrobial susceptibility testing (AST). Although automated AST systems have improved the accuracy, standardization, and reproducibility of susceptibility testing, conventional testing generally requires isolated

colonies and a standardized inoculum, thereby adding an overnight subculture step before definitive susceptibility results become available [6,7]. This delay is particularly relevant in immunocompromised and neutropenic patients, in whom timely administration of appropriate antimicrobial therapy may substantially influence clinical outcomes [4,5].

Direct antimicrobial susceptibility testing (DAST) from positively flagged blood-culture bottles provides a practical approach to shortening this diagnostic interval. In DAST, material obtained directly from positive blood-culture broth is subjected to susceptibility testing without waiting for overnight isolation of colonies. By bypassing this step, direct AST can provide susceptibility information substantially earlier than conventional testing and may facilitate earlier transition from empirical to targeted antimicrobial therapy [6-8].Previous studies have demonstrated encouraging agreement between direct and conventional or automated susceptibility methods. Kumar et al. reported high categorical agreement between direct disk-diffusion testing and automated AST from positive blood cultures [8]. Similarly, direct susceptibility testing using automated systems has demonstrated good agreement with conventional testing while substantially reducing the time to susceptibility results [6,7,9]. More recently, a study conducted in a tertiary cancer-care hospital also demonstrated the feasibility of direct AST as a rapid approach for susceptibility testing of bloodstream isolates [10].Despite its shorter turnaround time, the clinical usefulness of direct AST depends on its analytical reliability. Discrepancies between direct and automated methods, particularly false-susceptible results, may result in inappropriate antimicrobial selection. Therefore, assessment of categorical agreement and clinically significant discrepancies is essential before direct AST can be incorporated into routine diagnostic workflows.The potential utility of DAST is particularly relevant in cancer-care settings, where immunosuppression, frequent antimicrobial exposure, multidrug-resistant pathogens, and rapid clinical deterioration create a need for both timely and reliable microbiological information. However, evidence regarding its performance and clinical utility specifically in oncology populations remains limited. Therefore, the present study was designed to compare direct AST with automated AST in positive blood cultures from cancer patients, assess their accuracy and reliability in detecting antimicrobial resistance.

MATERIALS AND METHODS

This retrospective, observational, comparative study was conducted in the Department of Microbiology, MVR Cancer Centre and Research Institute, Kozhikode, Kerala, India, over a one-year period from January 2024 to December 2024. The study was designed to compare the performance of direct antimicrobial susceptibility testing (DAST) with automated antimicrobial susceptibility testing (AAST) in positive blood cultures obtained from patients with malignancy.

Study population

The study included all clinically significant bacterial isolates recovered from positive blood cultures processed in the Department of Microbiology during the study period. A total of 181 bacterial isolates were included for analysis.

Inclusion criteria

- Positive blood culture bottles yielding clinically significant bacterial isolates.
- Isolates for which both direct antimicrobial susceptibility testing (DAST) and automated antimicrobial susceptibility testing (AAST) results were available.

Exclusion criteria

- Blood cultures yielding fungal or other non-bacterial pathogens.
- Blood cultures considered contaminants according to standard microbiological criteria.
- Duplicate isolates recovered from the same patient during the same infectious episode.

Blood culture processing

Blood culture specimens were processed using the BacT/ALERT® 3D automated blood culture system (bioMérieux, France). Bottles flagged positive were subjected to Gram staining, and the Gram-stain morphology was used to guide the selection of antimicrobial agents for direct susceptibility testing.

Direct antimicrobial susceptibility testing

Direct antimicrobial susceptibility testing was performed using broth aspirated aseptically from the positively flagged blood culture bottle. Approximately four drops of broth were inoculated onto Mueller–Hinton agar (MHA) and spread uniformly using a sterile cotton swab to obtain a confluent lawn culture. After allowing the surface to dry for 3–5 minutes, appropriate antimicrobial discs were placed on the agar. Plates were incubated at 35 ± 2°C in ambient air for 16–18 hours, following which inhibition zone diameters were measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Conventional culture and automated antimicrobial susceptibility testing

Simultaneously, broth from each positive blood culture bottle was subcultured onto Blood agar, MacConkey agar, and Chocolate agar plates and incubated at 37°C for 18–24 hours. Following isolation of pure colonies, bacterial identification and antimicrobial susceptibility testing were performed using the VITEK® 2 Compact automated system (bioMérieux, France) according to the manufacturer's instructions. The automated AST results were considered the reference standard for comparison.

Outcome measures

The primary outcome was the agreement between DAST and automated AST in determining antimicrobial susceptibility. The secondary outcomes were:

- Comparison of turnaround time (TAT) between DAST and AAST.
- Assessment of the potential clinical impact of earlier availability of DAST results on antimicrobial therapy.

Agreement between the two methods was evaluated by calculating categorical agreement (CA). Discordant results were classified as very major errors (VME), major errors (ME), and minor errors (mE) according to CLSI recommendations.

Data collection

Laboratory data were retrieved retrospectively from the microbiology records for the study period. The variables collected included demographic details, bacterial isolates, antimicrobial susceptibility results obtained by DAST and automated AST, turnaround time of both methods, and antimicrobial therapy modifications wherever available.

Statistical analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics software 26. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution.

The diagnostic performance of DAST was assessed by calculating categorical agreement, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy, and Cohen's kappa coefficient (κ), using automated AST as the reference method. Error rates were reported as very major errors (VME), major errors (ME), and minor errors (mE).Turnaround times between DAST and AAST were compared using the paired *t*-test or Wilcoxon signed-rank test, as appropriate. Comparisons of categorical variables were performed using the Chi-square test or Fisher's exact test. A two-sided *p*-value <0.05 was considered statistically significant.

RESULTS

A total of 181 clinically significant bacterial isolates recovered from positive blood cultures were included in the study. Of these, 144 (79.6%) were Gram-negative bacilli (GNB) and 37 (20.4%) were Gram-positive cocci (GPC). Among the GNB isolates, *Escherichia coli* was the predominant pathogen (65; 35.9%), followed by *Klebsiella pneumoniae* (52; 28.7%), *Pseudomonas aeruginosa* (21; 11.6%), and *Acinetobacter baumannii* (6; 3.3%). Among GPC isolates, *Staphylococcus aureus* was the most frequently isolated organism (17; 9.4%), followed by *Streptococcus* spp. (10; 5.5%) and *Enterococcus* spp. (10; 5.5%) (Table 1 and Figure 1).Overall, 1,662 organism–antimicrobial comparisons were evaluated to compare direct antimicrobial susceptibility testing (DAST) with automated antimicrobial susceptibility testing (AAST). DAST demonstrated an overall categorical agreement of 90.1% (1,497/1,662), with 165 (9.9%) discrepant results. Minor errors accounted for 126 (7.6%), major errors for 36 (2.2%), and very major errors for 3 (0.2%) of all comparisons. Categorical agreement was 89.9% among Gram-negative bacilli and 91.0% among Gram-positive cocci (Table 2).Using automated AST as the reference method, DAST demonstrated an overall sensitivity of 98.5%, specificity of 93.6%, positive predictive value of 89.6%, negative predictive value of 99.1%, and an overall diagnostic accuracy of 95.4%. Agreement between the two methods was strong, with an overall Cohen's kappa coefficient (κ) of 0.814. Performance was comparable between Gram-negative and Gram-positive isolates, with categorical agreement of 89.9% and 91.0%, respectively (Table 3 and Figure 2).Among Gram-negative bacilli, antibiotic-wise categorical agreement ranged from 86.1% to 95.1%. The highest agreement was observed for meropenem (95.1%; κ=0.863), followed by gentamicin (93.1%; κ=0.826) and ceftriaxone (91.0%; κ=0.827). The lowest agreement was observed for amikacin (86.1%; κ=0.630). Overall categorical agreement for Gram-negative isolates was 89.9%, with an overall κ value of 0.816 (Table 4).

Among Gram-positive cocci, 100% categorical agreement was observed for both penicillin G and ampicillin. Agreement for the remaining antibiotics ranged from 83.8% to 89.2%. Overall categorical agreement among Gram-positive isolates was 91.0%, with an overall κ value of 0.766, indicating substantial agreement between DAST and AAST (Table 5 and Figure 3).

The mean turnaround time (TAT) for DAST was 17.4 ± 2.1 hours, compared with 42.8 ± 5.7 hours for automated AST, representing a mean reduction of 25.4 hours ($p < 0.001$). Similar reductions were observed for both Gram-negative (25.2 hours) and Gram-positive (26.1 hours) isolates. DAST provided susceptibility results within 24 hours for 169 (93.4%) isolates compared with only 4 (2.2%) using automated AST ($p < 0.001$). All DAST results were available within 48 hours,

whereas only 84.5% of automated AST reports were available during the same period. Overall, DAST reduced the mean turnaround time by 59.3% (Table 6).

Evaluation of the clinical impact demonstrated that DAST enabled availability of appropriate definitive antimicrobial therapy within 24 hours in 151 (83.4%) patients compared with 7 (3.9%) using automated AST ($p < 0.001$). Within 48 hours, appropriate therapy was possible in 174 (96.1%) patients using DAST compared with 146 (80.7%) using automated AST ($p < 0.001$). Earlier DAST reporting also facilitated earlier escalation to an active antimicrobial agent in 35 (19.3%) patients, earlier de-escalation in 46 (25.4%), and an earlier switch to another appropriate antimicrobial in 29 (16.0%) patients. In 71 (39.2%) patients, no modification of antimicrobial therapy was required because the initial treatment was already appropriate (Table 7).

Table 1. Distribution of bacterial isolates recovered from positive blood cultures (N=181)

Organism	n	%
Gram-negative bacilli (GNB)	**144**	**79.6**
Escherichia coli	65	35.9
Klebsiella pneumoniae	52	28.7
Pseudomonas aeruginosa	21	11.6
Acinetobacter baumannii	6	3.3
Gram-positive cocci (GPC)	**37**	**20.4**
Staphylococcus aureus	17	9.4
Streptococcus spp.	10	5.5
Enterococcus spp.	4	2.2
Enterococcus faecalis	3	1.7
Streptococcus pneumoniae	2	1.1
Enterococcus faecium	1	0.6
Total	**181**	**100.0**

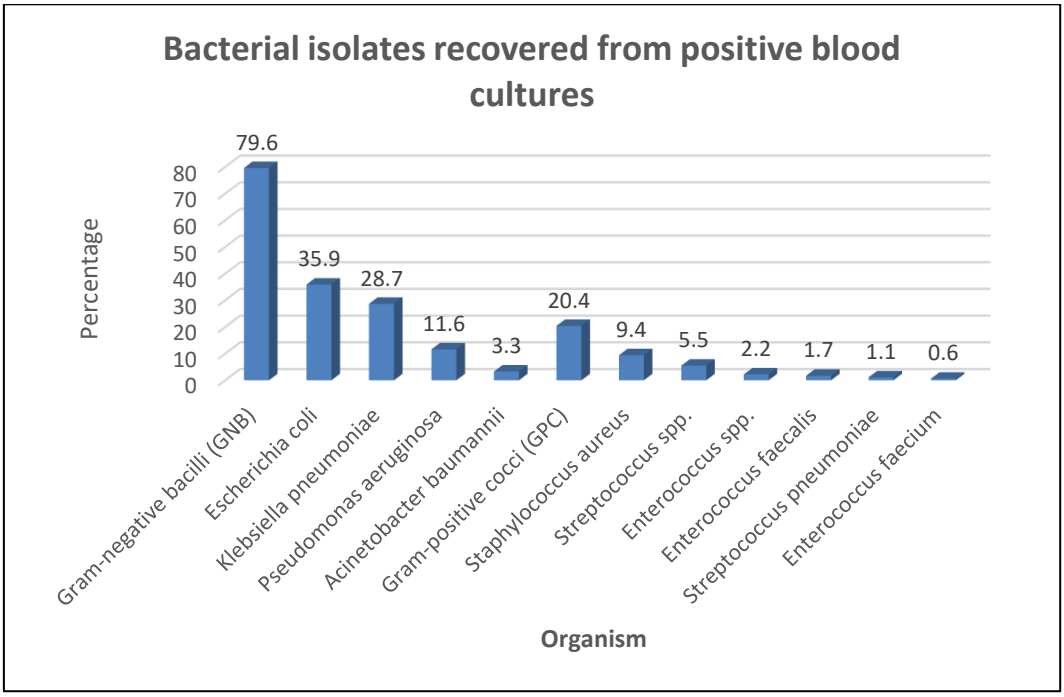

Figure 1. Distribution of bacterial isolates recovered from positive blood cultures (N=181)

Table 2. Overall categorical agreement and disagreement between DAST and AAST

Organism group	Categorical agreement, n (%)	Minor error, n (%)	Major error, n (%)	Very major error, n (%)	Total disagreement, n (%)
GNB	1,295 (89.9)	113 (7.8)	31 (2.2)	1 (0.1)	145 (10.1)
GPC	202 (91.0)	13 (5.9)	5 (2.3)	2 (0.9)	20 (9.0)

Table 3. Accuracy and reliability of DAST for detection of antimicrobial resistance using AAST as the comparator

Performance parameter	GNB	GPC	Overall
Categorical agreement (%)	89.9	91.0	**90.1**
Cohen's κ	0.816	0.766	**0.814**
Sensitivity (%)	98.9	93.6	**98.5**
Specificity (%)	93.0	97.1	**93.6**
Positive predictive value (%)	89.6	89.8	**89.6**
Negative predictive value (%)	99.3	98.3	**99.1**
Overall accuracy (%)	95.2	96.4	**95.4**

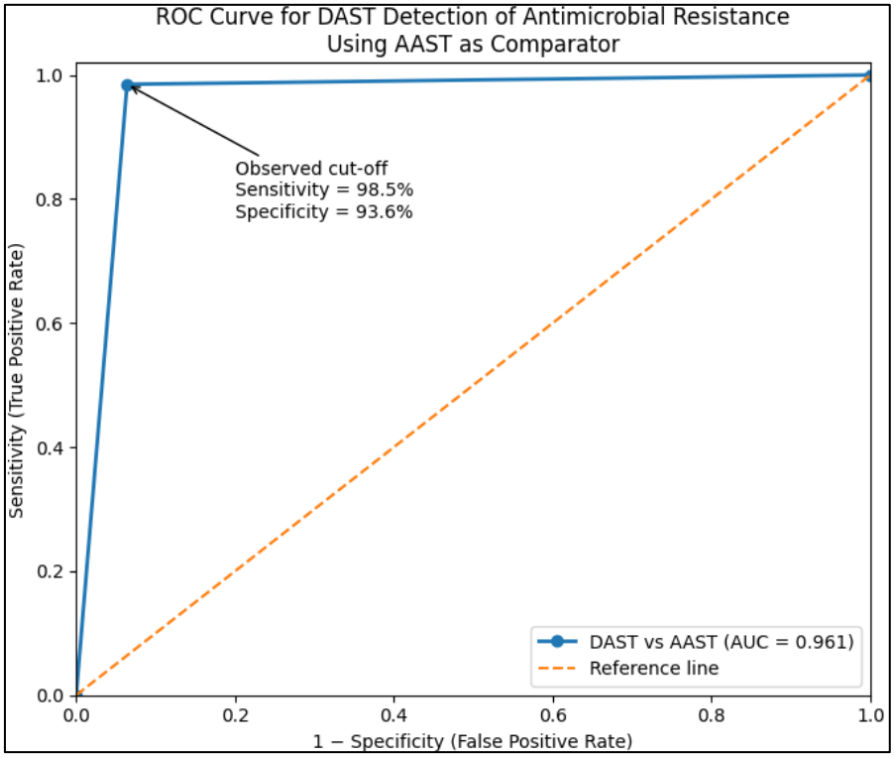

Figure 2. Accuracy and reliability of DAST for detection of antimicrobial resistance using AAST as the comparator

Table 4. Antibiotic-wise categorical agreement between DAST and AAST among Gram-negative bacilli

Antibiotic	Agreement, n (%)	Disagreement, n (%)	Cohen's κ
Gentamicin	134 (93.1)	10 (6.9)	0.826
Amikacin	124 (86.1)	20 (13.9)	0.630
Amoxicillin–clavulanate	129 (89.6)	15 (10.4)	0.813
Piperacillin–tazobactam	125 (86.8)	19 (13.2)	0.748
Meropenem	137 (95.1)	7 (4.9)	0.863
Ceftriaxone	131 (91.0)	13 (9.0)	0.827
Cefepime	129 (89.6)	15 (10.4)	0.813
Ceftazidime	127 (88.2)	17 (11.8)	0.789
Cotrimoxazole	129 (89.6)	15 (10.4)	0.804
Ciprofloxacin	130 (90.3)	14 (9.7)	0.821

Table 5. Antibiotic-wise categorical agreement between DAST and AAST among Gram-positive cocci

Antibiotic	Agreement, n (%)	Disagreement, n (%)
Penicillin G	**37 (100.0)**	0 (0.0)
Ampicillin	**37 (100.0)**	0 (0.0)
Vancomycin	31 (83.8)	6 (16.2)
Teicoplanin	32 (86.5)	5 (13.5)
Linezolid	33 (89.2)	4 (10.8)
Clindamycin	32 (86.5)	5 (13.5)

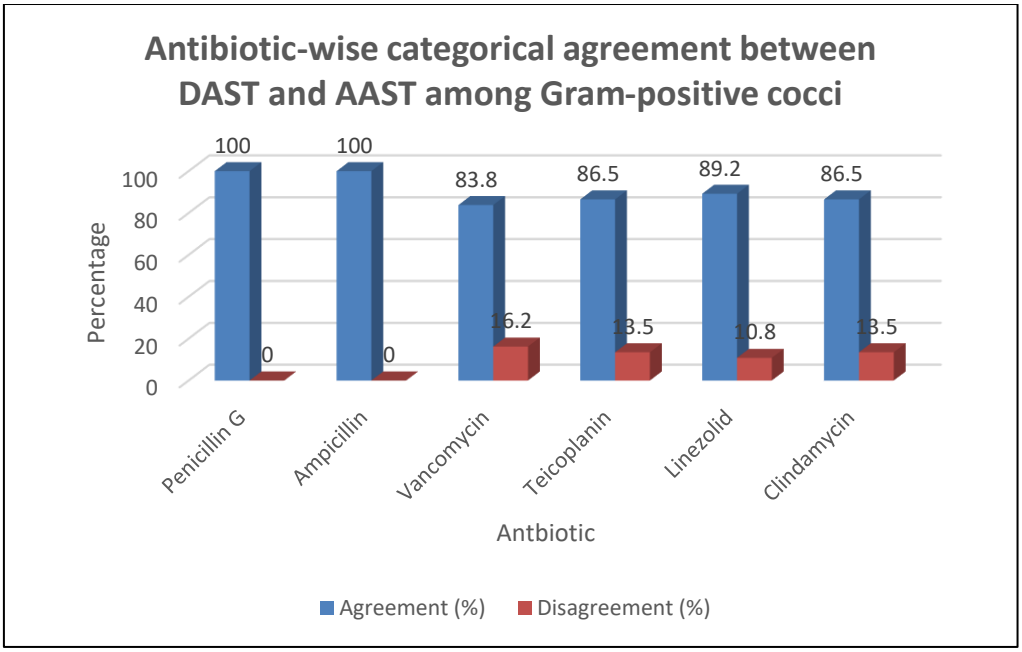

Figure 3. Antibiotic-wise categorical agreement between DAST and AAST among Gram-positive cocci

Table 6. Comparison of turnaround time between DAST and AAST (N=181)

TAT parameter	DAST	AAST	Difference	p-value
Overall TAT, h, mean ± SD	**17.4 ± 2.1**	**42.8 ± 5.7**	**25.4 h**	**<0.001**
Median TAT, h (IQR)	17.2 (16.1–18.5)	42.1 (39.0–46.0)	24.9 h	**<0.001**
GNB TAT, h, mean ± SD (n=144)	17.3 ± 2.0	42.5 ± 5.5	25.2 h	**<0.001**
GPC TAT, h, mean ± SD (n=37)	17.8 ± 2.3	43.9 ± 6.2	26.1 h	**<0.001**
Result available within 24 h, n (%)	169 (93.4)	4 (2.2)	+91.2	**<0.001**
Result available within 48 h, n (%)	181 (100.0)	153 (84.5)	+15.5	**<0.001**

Table 7. Impact of earlier DAST availability on timely initiation of appropriate antimicrobial therapy (N=181)

Clinical parameter	DAST-based availability, n (%)	AAST-based availability, n (%)	p-value
Appropriate definitive therapy possible within 24 h	**151 (83.4)**	**7 (3.9)**	**<0.001**
Appropriate definitive therapy possible within 48 h	**174 (96.1)**	**146 (80.7)**	**<0.001**
Earlier escalation to an active antimicrobial	35 (19.3)	12 (6.6)	**<0.001**
Earlier de-escalation possible	46 (25.4)	18 (9.9)	**<0.001**
Earlier switch to another active antimicrobial	29 (16.0)	11 (6.1)	**<0.001**
No modification required/current therapy appropriate	71 (39.2)	71 (39.2)	—

DISCUSSION

In the present study, Gram-negative bacilli (GNB) constituted 79.6% of bloodstream isolates, while Gram-positive cocci (GPC) accounted for 20.4%. Escherichia coli (35.9%) was the predominant pathogen, followed by Klebsiella pneumoniae (28.7%) and Pseudomonas aeruginosa (11.6%). Similar predominance of Gram-negative organisms has been reported by Parajuli et al. [11]and Sahoo et al. [12], who identified Gram-negative bacilli, particularly E. coli and Klebsiella spp., as the leading causes of bloodstream infections in tertiary-care hospitals, reflecting the increasing burden of multidrug-resistant Gram-negative pathogens in hospitalized patients.

The present study demonstrated an overall categorical agreement (CA) of 90.1%, with 7.6% minor errors, 2.2% major errors, and only 0.2% very major errors, indicating good concordance between DAST and AAST. These findings are comparable to Rajshekar et al. [13], who reported an overall 96% CA with 2.1% major, 1.0% very major, and 0.9% minor errors. Similarly, Desai et al. [14] reported an overall CA of 90.4%, whereas Choi et al. [15] demonstrated agreement exceeding 90% using a rapid microscopic AST platform. Together, these studies support the reliability of direct AST for preliminary susceptibility reporting.

Using automated AST as the comparator, DAST achieved an overall sensitivity of 98.5%, specificity of 93.6%, PPV of 89.6%, NPV of 99.1%, accuracy of 95.4%, and Cohen's κ of 0.814, indicating excellent diagnostic performance. Similarly high levels of agreement have been reported by Rajshekar et al. [13], Kumar et al., and Choi et al. [15], all of whom concluded that direct susceptibility testing provides reliable results while considerably shortening reporting time.

Among Gram-negative isolates, agreement ranged from 86.1% for amikacin to 95.1% for meropenem, with the highest κ value for meropenem (0.863). Overall agreement among Gram-positive cocci was 91.0%, with 100% agreement for penicillin G and ampicillin. Comparable observations were made by Rajshekar et al. [13], who reported excellent agreement (>95%) for most antimicrobial agents but relatively lower agreement for β-lactam/β-lactamase inhibitor combinations, aminoglycosides against Pseudomonas spp., and cefoxitin against Staphylococcus spp. Similar findings have also been reported by Kumar et al. [8] and Choi et al. [15], emphasizing that caution is required while interpreting selected organism–antibiotic combinations despite overall good agreement.

The mean turnaround time was significantly shorter with DAST (17.4 ± 2.1 hours) than with AAST (42.8 ± 5.7 hours), resulting in an average reduction of 25.4 hours ($p<0.001$). Furthermore, 93.4% of DAST reports were available within 24 hours compared with only 2.2% of automated reports. Similar reductions of approximately 24 hours have been reported by Desai et al. [14], Rajshekar et al. [13], and the CLSI working group, who highlighted that direct susceptibility testing substantially shortens the time to clinically actionable AST results without compromising reliability.

Earlier availability of DAST enabled appropriate definitive antimicrobial therapy within 24 hours in 83.4% of patients compared with 3.9% using AAST. Earlier escalation (19.3%), de-escalation (25.4%), and modification of antimicrobial therapy (16.0%) were also possible with DAST. Although Desai et al. [14] primarily evaluated laboratory performance rather than clinical outcomes, they concluded that earlier direct AST reporting facilitates earlier targeted therapy. Likewise, Choi et al. [15] demonstrated that rapid AST significantly reduces the interval between blood culture positivity and susceptibility reporting, thereby supporting earlier optimization of antimicrobial therapy and antimicrobial stewardship.

CONCLUSION

Direct antimicrobial susceptibility testing demonstrated high agreement with automated antimicrobial susceptibility testing, with an overall categorical agreement of 90.1% and diagnostic accuracy of 95.4%. DAST significantly reduced the turnaround time by approximately 25 hours, enabling earlier availability of susceptibility results and timely optimization of antimicrobial therapy. These findings support the use of DAST as a reliable adjunct to automated AST for the early management of bloodstream infections in cancer patients.

Limitation

This was a single-center retrospective study with a relatively limited sample size, which may affect the generalizability of the findings. Clinical outcome assessment was limited, and larger prospective multicenter studies are required to further validate the impact of DAST on patient outcomes and antimicrobial stewardship.

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