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Time is Survival: Early Detection of Antimicrobial Susceptibility in Bloodstream Infections

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Research Article

Keywords: Bloodstream infections (BSIs), Antimicrobial resistance (AMR), Direct Susceptibility Testing (DST), Antimicrobial susceptibility testing (AST)

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Title- Time is Survival: Early Detection of Antimicrobial Susceptibility in Bloodstream Infections

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ABSTRACT

Introduction: Bloodstream infections (BSIs) are associated with morbidity and mortality, requiring timely effective antimicrobial therapy. However, conventional antimicrobial susceptibility testing (AST) from positive blood cultures generally requires 24-48 hours after culture positivity confirmation, delaying targeted treatment and sustaining broad-spectrum antibiotic use. Direct susceptibility testing (DST) from positive blood culture bottles is a rapid, cost-effective approach that may shorten turnaround time. This study compared direct and conventional AST.

Materials and Methods: This cross-sectional study was conducted from April 2024 to November 2025 in bacteriology at SMS&R, Sharda Hospital, Greater Noida. Positive blood culture bottles from BacT/ALERT were included; contaminated or mixed-growth samples were excluded. Direct Gram staining and susceptibility testing on flagged bottles were compared with traditional culture methods. Data were analysed using Excel and SPSS.

Results: Of 10,248 blood samples, 302 flagged positive and 256 with single morphotype on Gram stain were included. Gram-negative bacilli predominated (202/256, 78.90%), followed by Gram-positive cocci (54/256, 21.10%). Enterobacterales predominated (57.81%), and categorical agreement between direct susceptibility testing and conventional AST was highest for Enterococcus species (91.23%).

Conclusion: DST from flagged positive blood culture bottles offers rapid and reliable early susceptibility results, with high agreement and no very major errors compared with conventional AST. Its routine use can shorten reporting time by 18-24 hours, support earlier targeted therapy, and strengthen antibiotic stewardship in bloodstream infections.

Keywords: Bloodstream infections (BSIs), Antimicrobial resistance (AMR), Direct Susceptibility Testing (DST), Antimicrobial susceptibility testing (AST)

INTRODUCTION

Bloodstream infections (BSIs) remain a major cause of morbidity and mortality worldwide, partly because they may progress rapidly to sepsis and septic shock if effective therapy is delayed [1]. Sepsis continues to represent a substantial global health burden, with large numbers of deaths attributable to infection each year and persistent challenges in timely recognition and management. Evidence from septic shock cohorts has shown that each hour of delay in administering effective antimicrobial therapy is associated with worse survival, reinforcing the clinical importance of early diagnosis and prompt treatment [2]. In routine clinical practice, clinicians are frequently compelled to initiate empirical antimicrobial therapy before microbiological confirmation is available, especially in severe infections where delays can worsen outcomes. However, this strategy must balance urgency with precision, because empirical regimens may be ineffective against resistant pathogens, and unnecessary broad-spectrum antibiotic use can contribute to toxicity and antimicrobial resistance [3]. Thus, the principal challenge in bloodstream infection (BSI) management lies not only in accurate pathogen detection, but in the rapid determination of antimicrobial susceptibility, which is essential to guide timely and appropriate de-escalation or escalation of therapy [4].

Blood culture remains the diagnostic reference standard for bloodstream infections because it enables recovery of viable organisms for species identification and susceptibility testing. However, the conventional framework is intrinsically slow and lengthy: after a bottle is flagged positive, laboratories still must perform Gram staining, subculture, colony isolation, organism identification, and antimicrobial susceptibility testing, which can delay actionable results by at least 24 to 48 hours or longer depending on the workflow. This delay often forces clinicians to continue broad empirical therapy until laboratory results can guide targeted treatment [5,6].

The clinical consequences of diagnostic delay are magnified by the continuing rise in antimicrobial resistance (AMR), which remains a major global health threat [7]. Recent global analyses have confirmed that bacterial AMR contributes substantially to mortality and disability worldwide, underscoring its growing burden [8]. In bloodstream infections (BSIs), antibiotic-resistant organisms are consistently associated with worse outcomes, prolonged hospitalization, and increased healthcare costs [9]. Gram-negative bacteria are particularly critical in this context due to their high prevalence in BSIs and their remarkable capacity to acquire and disseminate resistance determinants [7]. These limitations have accelerated interest in rapid diagnostic approaches, including molecular and other culture-independent methods, as well as integrated workflows that combine rapid testing with antimicrobial stewardship interventions [8]. Systematic reviews and clinical practice guidelines indicate that rapid diagnostic tests can shorten time to organism identification and improve clinical decision-making, particularly when paired with stewardship support, though outcome benefits vary across healthcare settings [9]. Accordingly, there remains a pressing need for faster and more reliable diagnostic strategies capable of improving early pathogen identification and susceptibility-guided treatment in patients with BSIs [10].

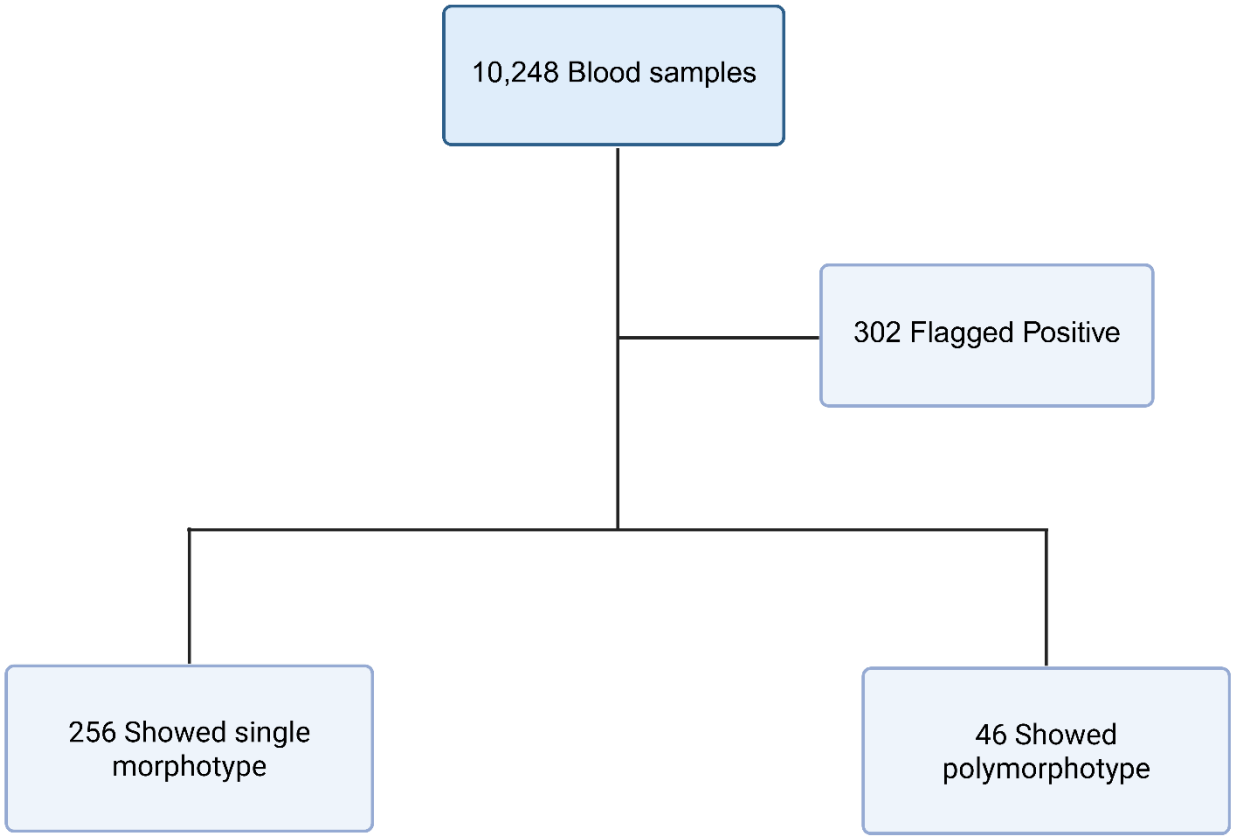
MATERIALS AND METHODS

This cross-sectional and observational study was performed at the bacteriology section of the Microbiology Unit, Central Laboratory, School of Medical Sciences and Research (SMS&R), Sharda Hospital, Greater Noida, over a period of 20 months from April 2024 to November 2025. This study aimed to evaluate and validate rapid diagnostic methods that determine antimicrobial susceptibility directly from flagged positive blood culture bottles, substantially reducing the turnaround time (TAT) for targeted antibiotic therapy compared to conventional subculturing methods. Blood culture bottles [FA (adult) and PF (pediatric)] (bioMerieux, France) that flagged positive in the BacT/ALERT system (bioMerieux, France) were included in the study. Blood samples with more than one type of organism on gram staining and indicative of commensal flora or contamination were excluded.

Procedure: Well-labelled blood culture bottles were subjected to an automated BacT/ALERT system. The bottles that flagged positive in the BacT/ALERT system were cultured on solid media, that is, blood and McConkey agar. Simultaneously, direct Gram staining was performed on blood culture bottles, and Direct Susceptibility Test (DST) was performed according to the Gram staining results. The next day, growth was identified using the conventional method; however, the doubtful strain was confirmed using the Automated VITEK 2 Compact system, and conventional Antimicrobial Susceptibility Test (AST) was performed as per CLSI M100, 35th edition (2025). The results of DST and conventional AST were compared. Data were managed using Microsoft Excel 2019 and SPSS 2022 for the analysis. Concordance between the results of conventional AST and DST was calculated using an appropriate formula.

RESULTS:

A total of 10,248 blood samples were received between April 2024 and November 2025 at the Bacteriology Lab, Department of Microbiology, Central Laboratory, School of Medical Sciences and Research (SMSR), Sharda Hospital, Greater Noida. A total of 302 samples were identified as positive, of which 256 samples were included in the study as they showed a single morphotype on Gram staining. Of these, 202 (78.90%) showed growth of gram-negative bacilli, whereas 54 (21.10%) were gram-positive cocci. All samples were processed for blood culture and sensitivity testing as per CLSI guidelines. All observations were statistically analyzed and presented as bar graphs and histograms.



The majority of the isolates were gram-negative bacilli (GNB), accounting for 79% of the total, while gram-positive cocci (GPC) comprised the remaining 21%, as shown in Figure 1, where sample origins were markedly skewed toward hospitalized patients, with the majority of isolates recovered from in-patient department (IPD) settings (n = 244; 95.32%), while out-patient department (OPD) contributed only a minor share with 12 samples (4.68%) as illustrated in **Figure 2**. The total number of samples collected was divided into five groups according to the age of the patients whose samples were used for the study. The total number of isolates was 256, of which *23 (8.98 %) were Acinetobacter species,* 148

(57.81%) Enterobacterales, *31 (12.10 %) Pseudomonas species*, 38 (14.84%) *Staphylococcus species, and 16 (6.25 %) Enterococcus species*. *Enterobacterales* isolated were *Escherichia coli, Klebsiella species, Salmonella Typhi, Salmonella Paratyphi, Proteus species, Citrobacter species, and Enterobacter species.* The Gram-positive cocci isolated were *Enterococcus species, Staphylococcus aureus, and* coagulase-negative *Staphylococcus species* **(Figure 3)**.

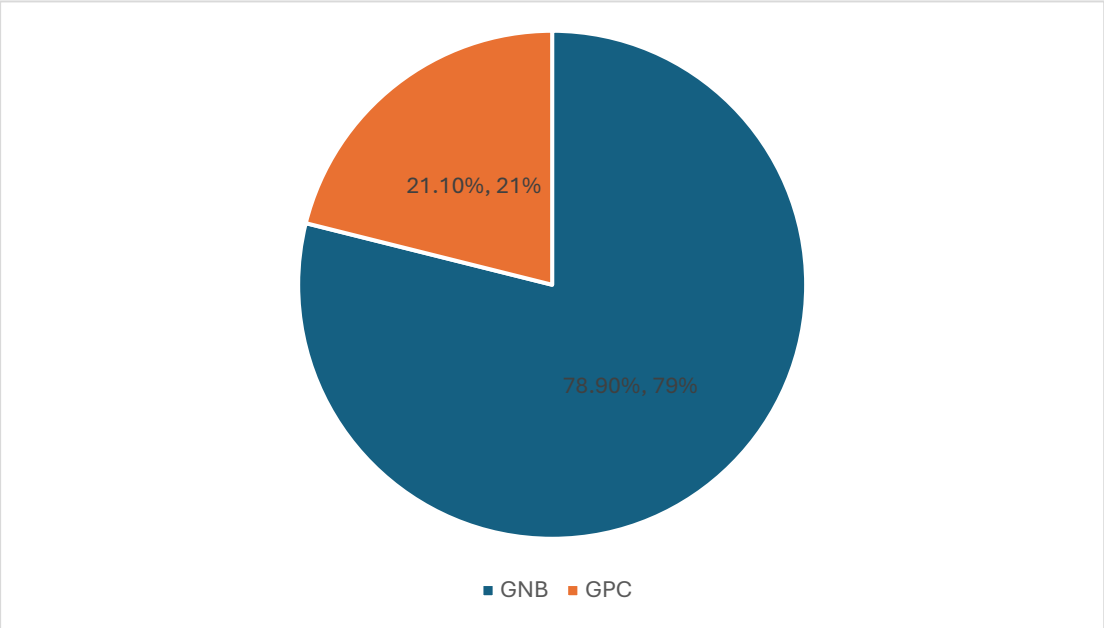


Figure 1: Distribution of organisms based on Gram staining

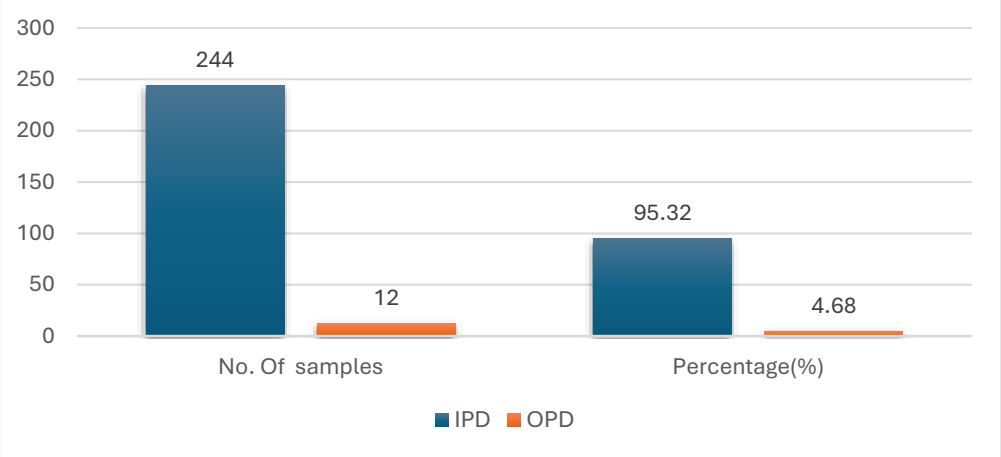


Figure 2: Distribution of the total no. of samples received across the Inpatient and Outpatient departments.

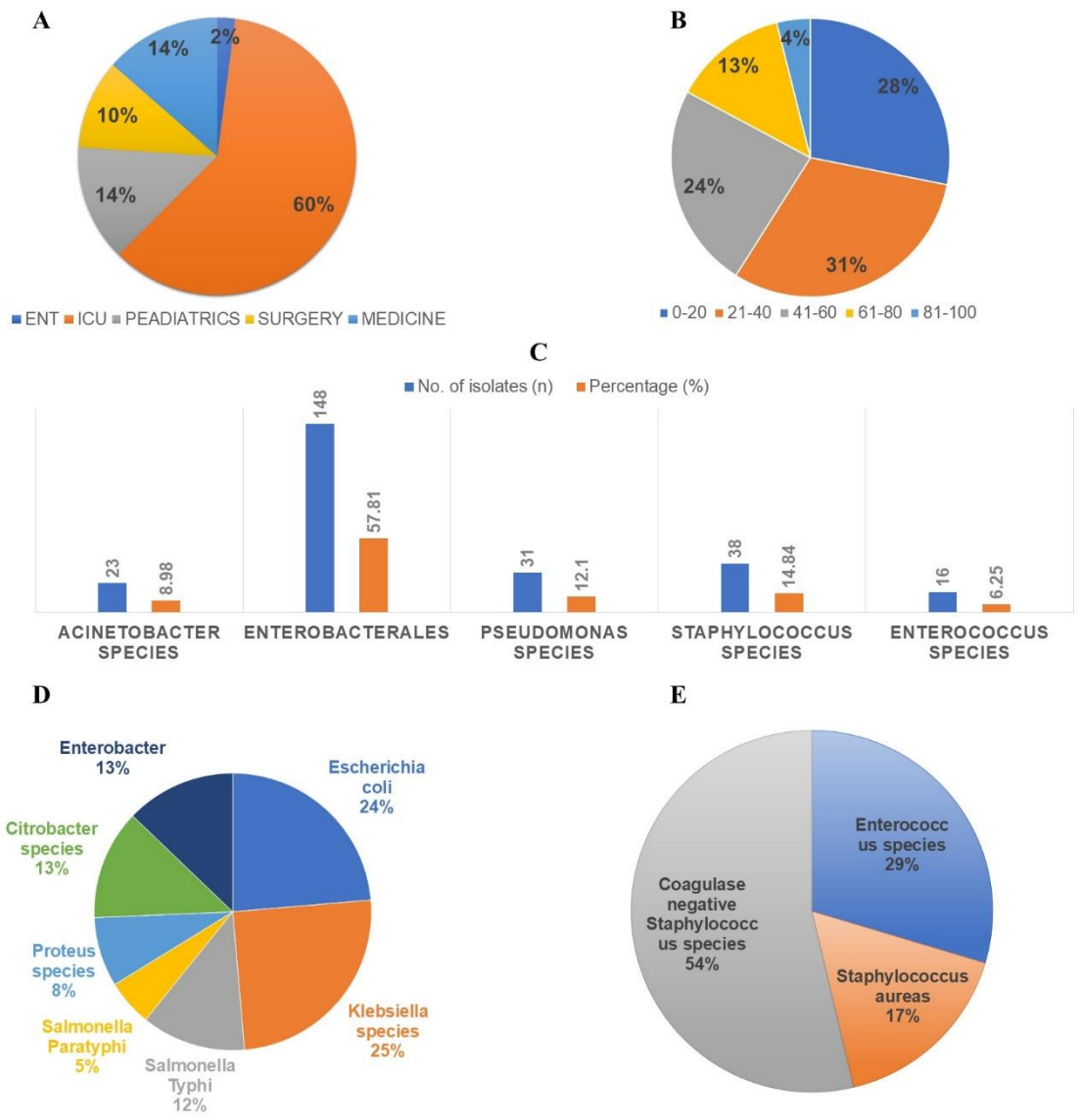


Figure 3: Overview of the sample distribution and bacterial isolate profiling. (A) Distribution of total samples across the inpatient departments. (B) Age-wise breakdown of the blood samples. (C) Distribution of different bacterial isolates among the total number of positive blood samples. (D) Proportion of Enterobacterales isolated. (E) Total percentage of gram-positive cocci isolated.

Conventional AST and DST showed a very high concordance in the case of *Acinetobacter species* (n=23). Antibiotics such as ampicillin, penicillin, ciprofloxacin, teicoplanin, erythromycin, cefoxitin, gentamicin, ceftriaxone, amoxicillin clavulanate, and tobramycin showed 100% concordance. A total of *148 Enterobacterales were isolated.* All were evaluated together. The concordance of ampicillin, levofloxacin, ciprofloxacin, amikacin, pipracillin-tazobactam, imipenem, meropenem, and aztreonam were 100%. The total number of *Staphylococcus s*pecies isolated was 38, including *S. aureus* and coagulase-negative *Staphylococcus species.* The antibiotics that showed 100% concordance were linezolid, ampicillin, levofloxacin, teicoplanin, cefoxitin, vancomycin, and gentamicin. *Enterococcus species (n=16)* showed the highest concordance pattern among all species isolated. Almost all antibiotics showed 100% concordance, except teicoplanin and gentamicin.

The overall performance of the evaluated testing method was assessed by calculating the categorical agreement, major errors, and minor errors against the reference standard for four key groups of clinical isolates: Acinetobacter species, Enterobacterales, Staphylococcus species, and Enterococcus species.

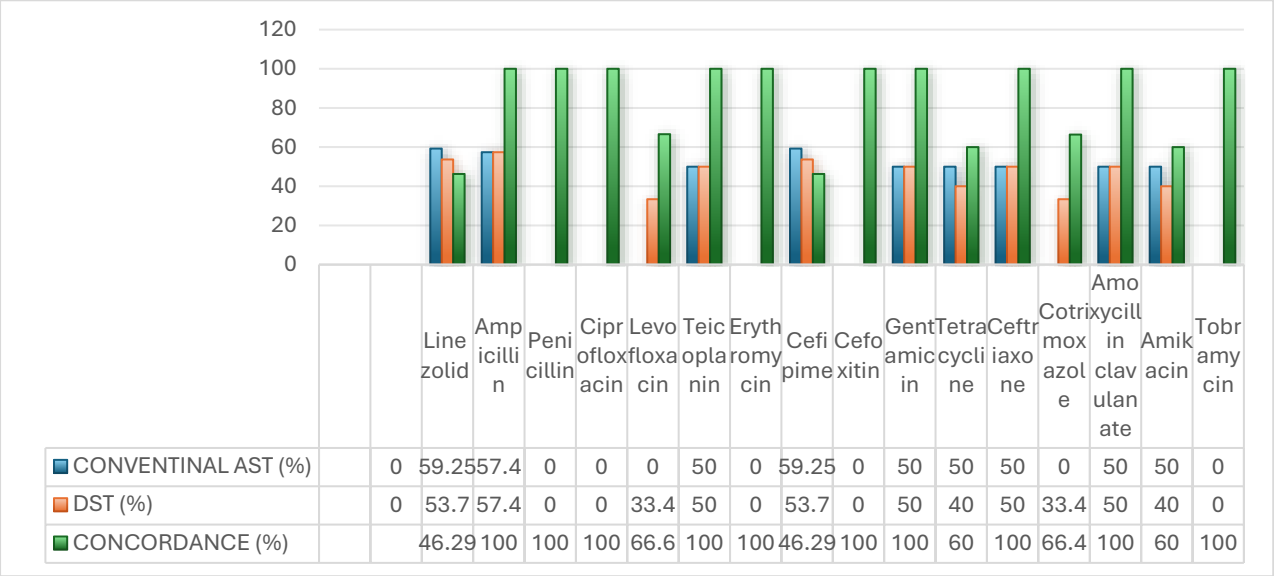
CONCORDANCE EVALUATIONS

Concordance evaluations were performed based on categorical agreement. As illustrated in **Figure 4-8** the categorical agreement varied among the evaluated pathogens. The highest categorical agreement was observed for Enterococcus species at 91.23%, indicating strong diagnostic reliability for this group. Staphylococcus species and Acinetobacter species also demonstrated high concordance rates of 88.67% and 87.26%, respectively. In contrast, Enterobacterales exhibited the lowest categorical agreement among the tested organisms at 82.37%.

Discrepancies between the test and reference methods were further stratified into major and minor errors. The major error rates (Figure 9), representing false-resistant categorizations, were highest among Enterobacterales (4.86%) and Staphylococcus species (3.80%). Acinetobacter species demonstrated a major error rate of 2.30%, while Enterococcus species showed the lowest rate of major discrepancies at just 1.26%.

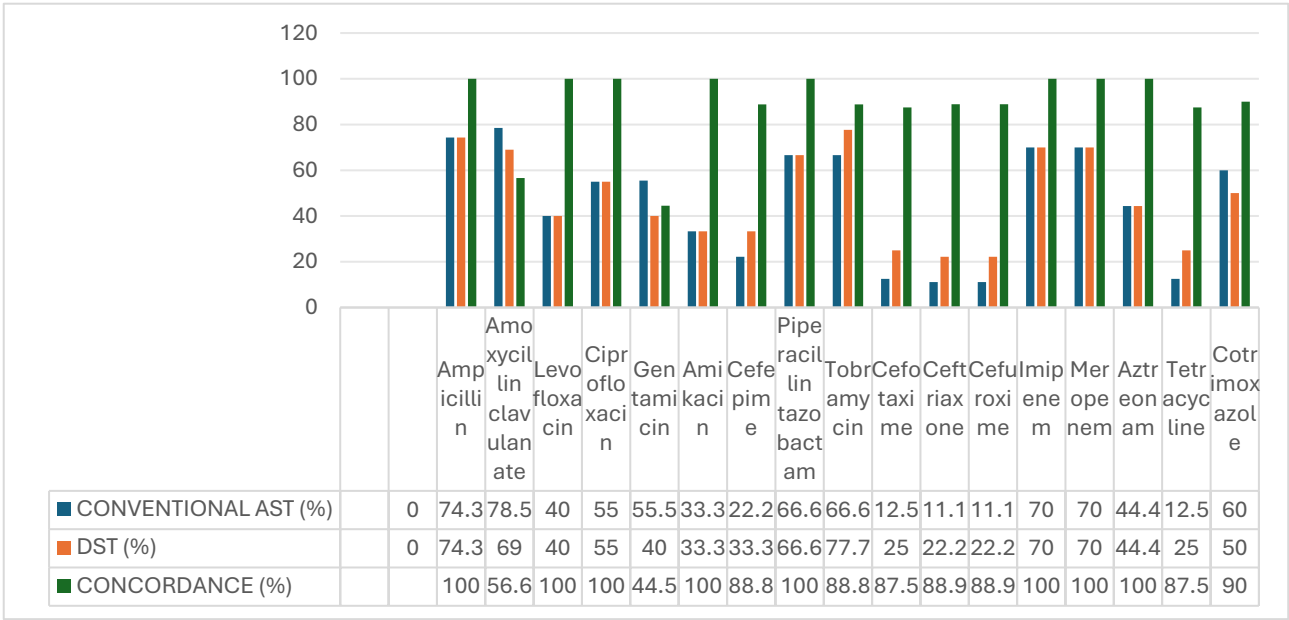
The analysis of minor errors (Figure 10), representing shifts involving the intermediate susceptibility category, revealed a different distribution. Acinetobacter species exhibited a notably higher minor error rate (10.32%) compared to the other groups. Enterococcus species and Enterobacterales recorded minor error rates of 7.00% and 6.00%, respectively, while Staphylococcus species produced the lowest minor error rate at 5.76%.

Overall, the data indicates that while the testing methodology is highly reliable for Gram-positive cocci such as Enterococcus species, further optimization may be required to reduce the error rates observed in Gram-negative isolates, particularly Enterobacterales.



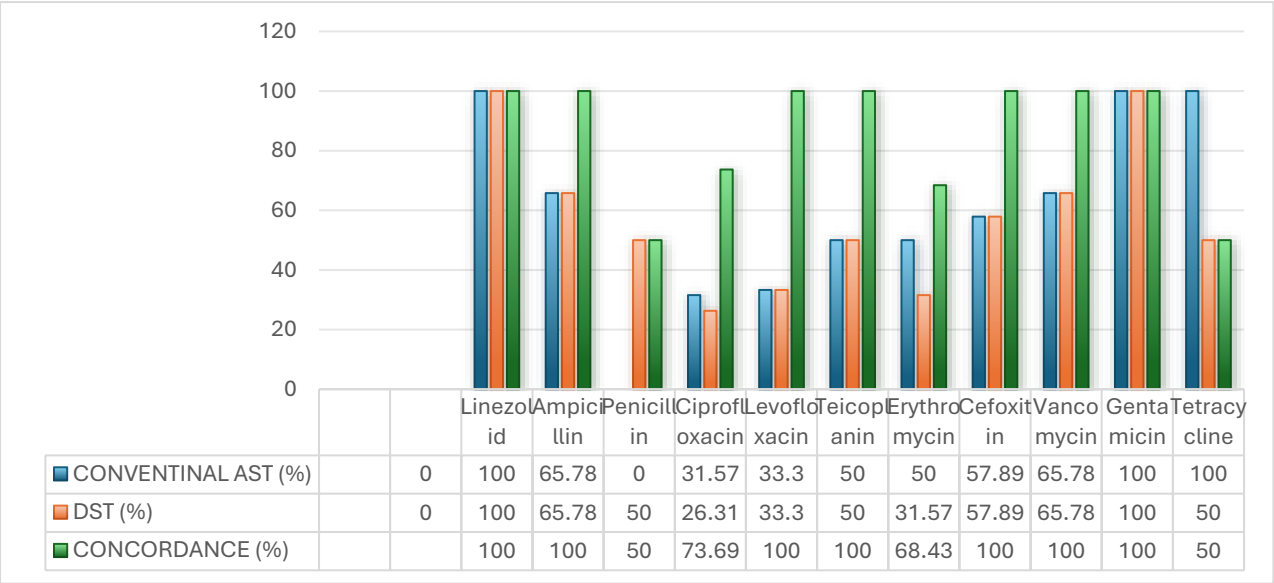
		Linezolid	Ampicillin	Penicillin	Ciprofloxacin	Levofloxacin	Teicoplanin	Erythromycin	Cefipime	Cefoxitin	Gentamicin	Tetracycline	Ceftriaxone	Cotrimoxazole	Amoxycillin clavulanate	Amikacin	Tobramycin
CONVENTINAL AST (%)	0	59.25	57.4	0	0	0	50	0	59.25	0	50	50	50	0	50	50	0
DST (%)	0	53.7	57.4	0	0	33.4	50	0	53.7	0	50	40	50	33.4	50	40	0
CONCORDANCE (%)		46.29	100	100	100	66.6	100	100	46.29	100	100	60	100	66.4	100	60	100

Figure 4: Concordance pattern by *Acinetobacter species.*



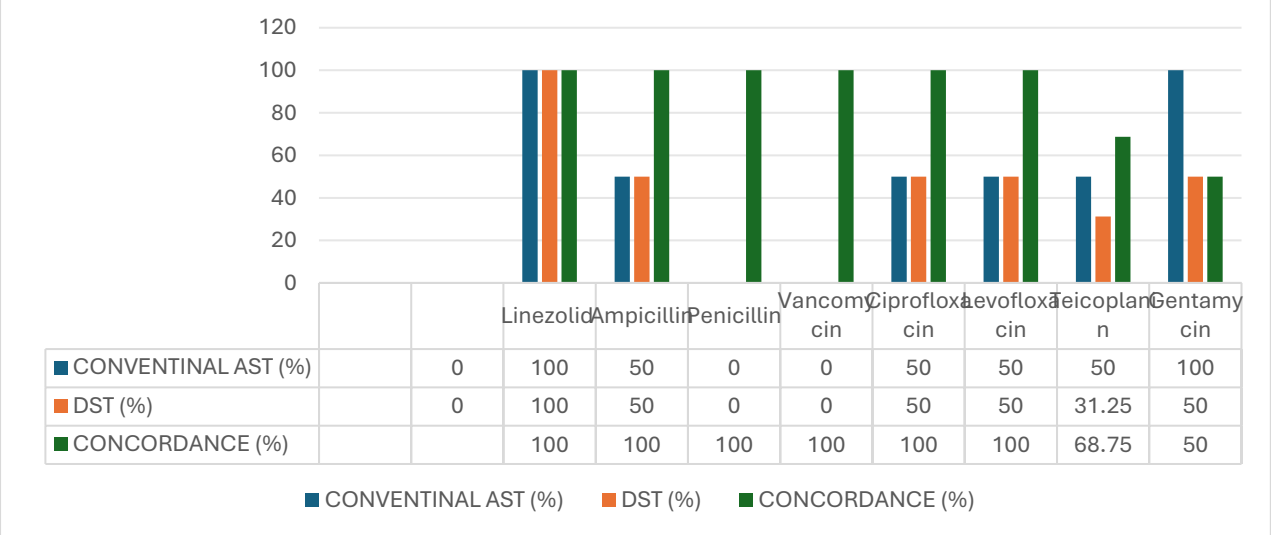
		Ampicillin	Amoxycillin clavulanate	Levofloxacin	Ciprofloxacin	Gentamicin	Amikacin	Cefepime	Piperacillin tazobactam	Tobramycin	Cefotaxime	Ceftriaxone	Cefuroxime	Imipenem	Meropenem	Aztreonam	Tetracycline	Cotrimoxazole
CONVENTIONAL AST (%)	0	74.3	78.5	40	55	55.5	33.3	22.2	66.6	66.6	12.5	11.1	11.1	70	70	44.4	12.5	60
DST (%)	0	74.3	69	40	55	40	33.3	33.3	66.6	77.7	25	22.2	22.2	70	70	44.4	25	50
CONCORDANCE (%)		100	56.6	100	100	44.5	100	88.8	100	88.8	87.5	88.9	88.9	100	100	100	87.5	90

Figure 5: Concordance of *Enterobacterales:*



		Linezolid	Ampicillin	Penicillin	Ciprofloxacin	Levofloxacin	Teicoplanin	Erythromycin	Cefoxitin	Vancomycin	Gentamicin	Tetracycline
CONVENTINAL AST (%)	0	100	65.78	0	31.57	33.3	50	50	57.89	65.78	100	100
DST (%)	0	100	65.78	50	26.31	33.3	50	31.57	57.89	65.78	100	50
CONCORDANCE (%)		100	100	50	73.69	100	100	68.43	100	100	100	50

Figure 6: Concordance pattern of *Staphylococcus species:*



		Linezolid	Ampicillin	Penicillin	Vancomycin	Ciprofloxacin	Levofloxacin	Teicoplanin	Gentamycin
CONVENTINAL AST (%)	0	100	50	0	0	50	50	50	100
DST (%)	0	100	50	0	0	50	50	31.25	50
CONCORDANCE (%)		100	100	100	100	100	100	68.75	50

Figure 7: Concordance pattern of *Enterococcus:*

CONCORDANCE EVALUATIONS

The concordance evaluations were done on the basis of categorical agreement.

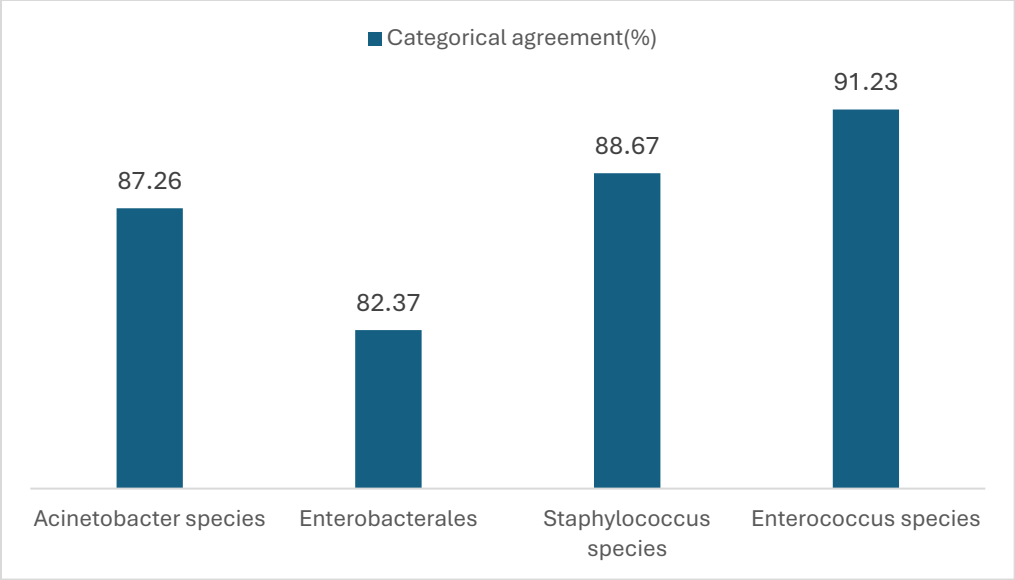


Figure 8: Total categorical agreement shown by all the organisms.

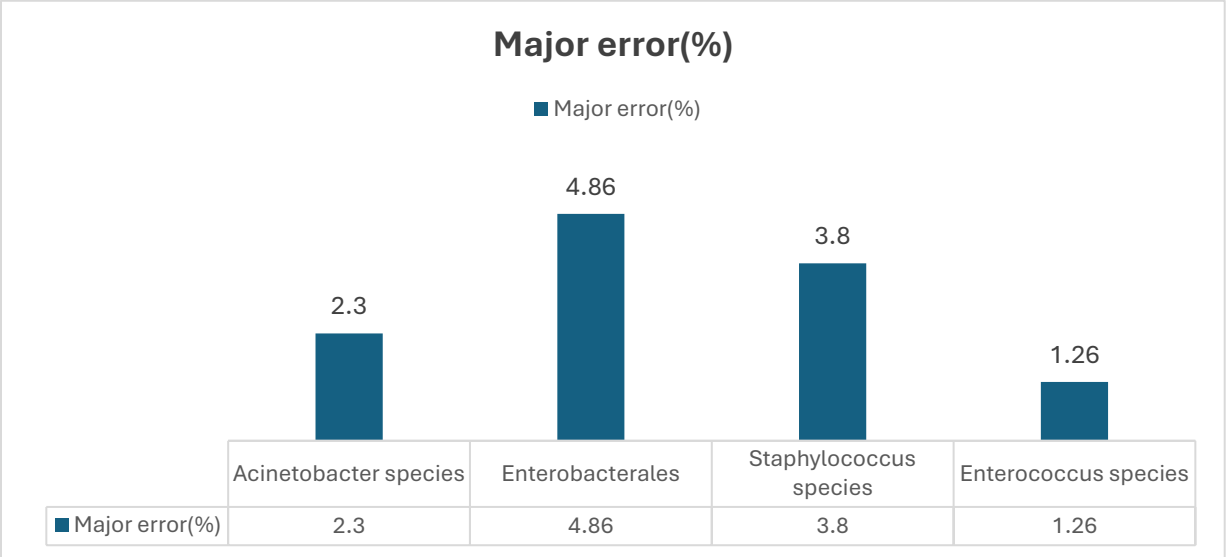


Figure 9: Major errors in the isolated organisms:

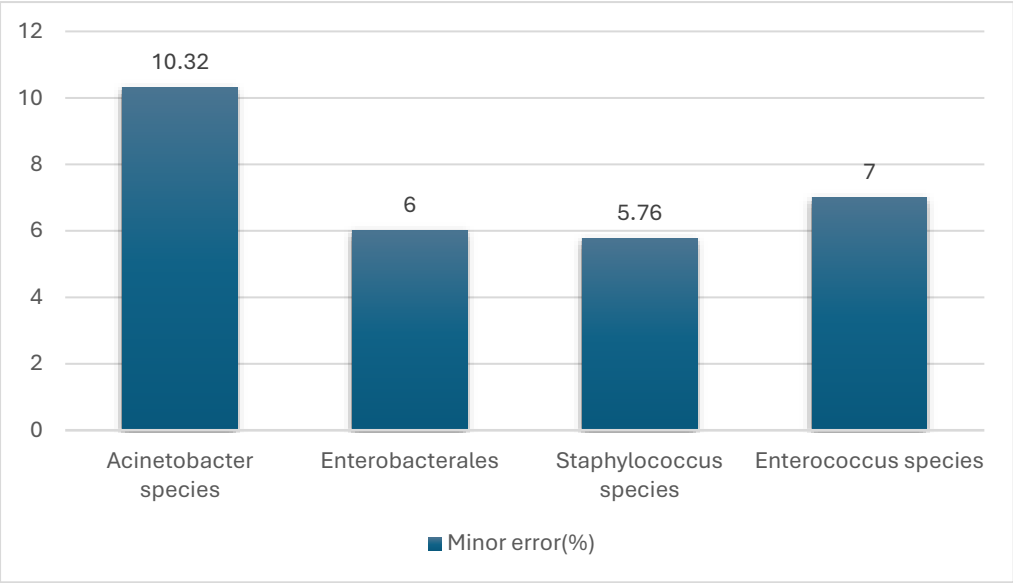


Figure 10: The minor errors by all the isolated organisms:

DISCUSSION

Bloodstream infections remain one of the most serious and life-threatening conditions in clinical medicine. The timely identification of causative organisms and their antibiotic susceptibility patterns plays a crucial role in guiding appropriate treatment and improving patient outcomes. Direct Susceptibility Testing (DST) from positive blood culture broth has emerged as a promising approach to reduce the turnaround time compared to conventional Antibiotic Susceptibility Testing (AST). In this study, we aimed to evaluate the early detection of antibiotic susceptibility in positive blood cultures using DST and compare its findings with conventional AST results, thereby assessing the potential clinical utility of this approach in a tertiary care hospital setting.

Blood Culture Positivity Rate and Sample Distribution

In our study, a total of 10,248 blood samples were received at the Bacteriology Laboratory of the Department of Microbiology, Central Laboratory, School of Medical Sciences and Research (SMSR), Sharda Hospital, Greater Noida, over the period of April 2024 to November 2025. Of these, 302 samples were flagged as positive, and after excluding polymicrobial cultures, 256 samples were finally included in the study. This translates to an overall blood culture positivity rate of approximately 2.95%. The majority of samples were received from inpatient departments (IPD), which accounted for 244 samples (95.32%), while only 12 samples (4.68%) were from outpatient departments (OPD). This overwhelming contribution from IPD is expected, as hospitalized patients, particularly those in ICUs and medical wards that carry a higher risk of developing bloodstream infections due to prolonged hospital stays, invasive procedures, and immunocompromised conditions.

Our positivity rate is consistent with that of several previously published studies from India and other countries. Nazir et al. (2018) conducted a study at a teaching hospital where 25.3% of 2,231 blood culture samples showed culture positivity, with 447 samples (79.1%) showing bacterial growth [11]. In contrast, Li et al. (2023) reported positivity from 3,660 non-repeating strains at Suining Central Hospital, where Gram-negative bacteria constituted 76.7% of all isolates [12]. Kumar et al. (2024) noted that among 669 blood culture samples processed at Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow, India, 213 samples (31.8%) showed bacterial or fungal growth [13]. The difference in positivity rates across studies is largely attributable to differences in patient populations, types of hospitals (teaching versus non-teaching), geographic location, local epidemiology, and the volume of samples processed. Higher positivity rates were observed in hospitals with large ICU populations or immunocompromised patient cohorts. The high representation of IPD in our study is similarly noted in global literature, where hospitalized patients remain the primary population at risk for bacteremia and septicemia.

Distribution of Organisms Based on Gram Staining

In our study, all 256 positive blood culture samples showed a single morphotype on gram staining. Of these, 202 samples (78.90%) demonstrated gram-negative bacilli, while the remaining 54 samples (21.10%) showed gram-positive cocci. This predominance of Gram-negative organisms over Gram-positive organisms in blood culture isolates is a well-recognized finding in the Indian subcontinent and in many developing countries. The high proportion of Gram-negative bacteremia reflects the environmental conditions, sanitation practices, healthcare-associated infection burden, and the widespread use of broad-spectrum antibiotics that can suppress Gram-positive flora while selecting for resistant Gram-negative pathogens.

Our findings are supported by multiple previous studies. Girija et al. in 2021 conducted a prospective cross-sectional study at a tertiary care center involving 708 blood samples, of which bacteria were isolated from 201 samples (28.3%). The majority were Gram-negative in nature, with Coagulase-negative Staphylococci (CoNS) constituting 55.2% of all isolates; however, this was partly influenced by neonatal samples, where CoNS predominates [14]. Similarly, Kumar et al. (2024) highlighted that septicemia was predominantly caused by gram-negative bacteria in their retrospective study[13]. Gupta et al. (2021) reported a slightly different picture from a pediatric-heavy cohort, where 56.1% were Gram-positive and 43.9% were Gram-negative, which can be attributed to the high proportion of neonatal samples where CoNS is a leading pathogen [15]. The predominance of Gram-negative bacilli in our study underscores the need for early identification and targeted therapy to avoid inappropriate empirical treatment.

Distribution of Bacterial Isolates

Among the 256 positive blood cultures in our study, Enterobacterales was the most commonly isolated group, accounting for 148 isolates (57.81%). Within this group, *Klebsiella* species were the most frequent (37 isolates), followed by *Escherichia coli* (35), *Citrobacter* species (19), Enterobacter (19), *Salmonella Typhi* (18), *Proteus* species (12), and *Salmonella Paratyphi* (8). *Staphylococcus* species were the second most common group overall, with 38 isolates (14.84%), which included Coagulase-negative Staphylococci (CoNS) (29) and *Staphylococcus aureus* (9). *Pseudomonas* species accounted for 31 isolates (12.10%), followed by *Acinetobacter* species with 23 isolates (8.98%), and *Enterococcus* species with 16 isolates (6.25%).

The dominance of Enterobacterales; especially *Klebsiella* and *E. coli* in our study reflects the commonly reported pattern from tertiary care hospitals in India. Li et al. (2023) analyzed blood culture-positive isolates from 2018 to 2021 and found *E. coli* (44.8%) and *Klebsiella* spp. (19.2%) as the most prevalent Gram-negative organisms [12]. Datta et al. (2012), in a large retrospective analysis of 77,618 blood culture samples, similarly highlighted *E. coli* and *Klebsiella pneumoniae* as the dominant Gram-negative isolates, with concerning trends of ESBL production and carbapenem resistance over time [16]. Analysis of 135,268 blood cultures by Gandra et al. in 2016 over seven years documented resistant pathogens, among them *Acinetobacter* species and *Pseudomonas aeruginosa* were frequently isolated [17]. Nazir et al. (2018) found *Acinetobacter* species as the most common Gram-negative isolate (63.1%) in their teaching hospital study, while CoNS (67.9%) dominated Gram-positive isolates; a pattern that is slightly different from ours, likely reflecting ICU-heavy patient populations[11]. Gupta et al. (2021) similarly documented Klebsiella pneumoniae as the predominant organism in neonates and CoNS in older age groups[15]. The presence of *Salmonella Typhi* and *Paratyphi* in our study is particularly notable and consistent with the typhoid-endemic nature of North India.

Overall Concordance Between DST and Conventional AST

One of the primary objectives of our study was to evaluate the concordance between Direct Susceptibility Testing (DST) performed directly from positive blood culture broth and the Conventional Antibiotic Susceptibility Testing (AST) performed on isolated colonies. In our study, the overall categorical agreement was high across all organism groups: *Enterococcus* species showed the highest concordance at 91.23%, followed by *Staphylococcus* species at 88.67%, *Acinetobacter* species at 87.26%, and Enterobacterales at 82.37%. These findings indicate that DST is a reliable and reproducible method that can provide susceptibility results approximately 18-24 hours earlier than conventional AST, which is critically important in the management of sepsis and bloodstream infections.

Several studies have corroborated the high concordance rates between DST and conventional AST. Goel et al. (2015) evaluated DST using the disk diffusion method on blood cultures flagged positive between May 2011 and December 2012. They found high agreement between DST and AST, with

minor errors of 12.5%, major errors of 3.33%, and very major errors of only 0.47%, concluding that DST is an efficient tool for early targeted antibiotic therapy [18]. Baba et al. (2023) evaluated DST from BacT/ALERT blood culture vials against VITEK-2 as the reference method and observed 99.2% essential agreement for Gram-positive isolates (minor errors 4.04%, major errors 1.18%) and 98.99% for Gram-negative isolates (minor errors 4%, major errors 2%), with no very major errors in either group [19]. Rishu et al. (2024) reported 97.76% categorical agreement among 1,486 organism-antibiotic combinations, with major errors of only 1.54%. Singh et al. (2024) found 99% categorical agreement for *E. coli* and *K. pneumoniae* among Enterobacterales isolates in a 25-bedded ICU in North India, with *Pseudomonas aeruginosa* showing 99.6% categorical agreement [20]. Mazhar et al. (2025) conducted a cross-sectional study in Bangladesh and demonstrated that categorical agreement between DST and standard AST exceeded 93% for major pathogens including Staphylococcus species, *Salmonella Typhi*, *E. coli*, *Klebsiella*, *Enterobacter*, and *Pseudomonas* species [21]. Wong et al. (2025) reported an overall categorical agreement of 97.9% across diverse organism groups [22]. Collectively, these studies strongly support the high reliability of DST as an early susceptibility testing tool.

Concordance of *Acinetobacter* species

In our study, among the 23 *Acinetobacter* species isolates, the overall categorical agreement between DST and conventional AST was 87.26%. Antibiotic-wise, 100% concordance was achieved for Ampicillin, Penicillin, Ciprofloxacin, Teicoplanin, Erythromycin, Cefoxitin, Gentamicin, Ceftriaxone, Amoxycillin clavulanate, and Tobramycin. However, discrepancies were noted for Linezolid (46.29% concordance), Cefepime (46.29%), Levofloxacin (66.6%), Tetracycline (60%), Cotrimoxazole (66.4%), and Amikacin (60%). These discordances may be related to the inherent difficulties in standardizing inoculum from broth, which can affect zone sizes in disk diffusion testing. The conventional AST showed zero sensitivity for Ciprofloxacin, Penicillin, Erythromycin, Cefoxitin, and Tobramycin, suggesting a multidrug-resistant profile for *Acinetobacter* in our hospital environment represents a critical concern, particularly in light of the limited therapeutic armamentarium.

The high rate of multidrug resistance in *Acinetobacter* spp. is well documented globally. Gandra et al. (2016) reported resistance rates in *Acinetobacter* species of approximately 69.6% in a large retrospective Indian study spanning seven years (2008–2014) [17]. Kumar et al. (2024) noted *Acinetobacter* species as the third most common isolate (17.8%) and highlighted significant resistance to multiple antibiotics among Gram-negative pathogens in their Lucknow-based cohort [13]. Gupta et al. (2021), in a two-year study analyzing 12,173 blood samples, noted increased resistance in *Acinetobacter* spp. to a range of antibiotics over the study period [15]. Singh et al. (2024), studying DST in an ICU setting in North India, reported 95-96% categorical agreement for *Acinetobacter* spp. and exotic Gram-negative bacteria, acknowledging that non-fermenters like *Acinetobacter* can be more challenging to

test directly due to variable growth characteristics in broth [13]. Bianco et al. (2019) also observed that categorical agreement for non-fermenting Gram-negative bacilli, including *Acinetobacter*, ranged from 88.2% to 100% for the rapid E-test from blood culture broth, underscoring that while accuracy is generally good, some antibiotic-organism combinations can be less reliable [23]. The high concordance for most antibiotics in our study, despite the complex resistance background of Acinetobacter, is an encouraging finding.

Concordance of Enterobacterales

Enterobacterales constituted the largest group in our study (n=148, 57.81%), an overall categorical agreement of 82.37%. Perfect concordance (100%) was observed for Ampicillin, Levofloxacin, Ciprofloxacin, Amikacin, Piperacillin-Tazobactam, Imipenem, Meropenem, and Aztreonam; antibiotics that are critical for the management of serious Gram-negative infections. Some discordance was noted for Amoxicillin-Clavulanate (56.58% concordance), Gentamicin (44.5%), and minor discrepancies for Cefepime (88.8%), Tobramycin (88.8%), Cefotaxime (87.5%), Ceftriaxone (88.9%), Cefuroxime (88.9%), Tetracycline (87.5%), and Cotrimoxazole (90%). Notably, sensitivity to Carbapenems (Imipenem and Meropenem) was 70% in both DST and conventional AST, which suggest a significant burden of carbapenem-resistant Enterobacterales in our facility that is a finding of major public health significance.

Datta et al. (2012), in their decade-long retrospective study, documented a dramatic rise in carbapenem resistance in Klebsiella pneumoniae from 2.4% to 52% between 2000 and 2009, and increasing ESBL production in E. coli from 40% to 61%. These alarming trends underscore the rapid changes in the resistance landscape in hospital settings [16]. Lokeshwari et al. (2021) evaluated DST in 37 bacteremic patients and found 15 minor errors (4.8%) and 3 major errors (0.97%) among 28 Gram-negative Enterobacteriaceae isolates, with Piperacillin-Tazobactam showing the most errors, a finding that aligns with the challenges we observed [24]. Bianco et al. (2019), evaluating rapid E-test directly from blood culture bottles, reported categorical agreement between 95.1% and 100% for Enterobacteriaceae, and for carbapenemase-producing Enterobacteriaceae specifically, a categorical agreement of 87.5%, highlighting the challenges in this subgroup [23]. Pan et al. (2018) using a lysis-centrifugation-wash method with VITEK-2 direct testing reported an agreement rate of 96.89% for Gram-negative bacteria, with a minor error rate of 2.63%, producing estimates in agreement with our study observations [25]. Kumaresan et al. (2024) noted better categorical agreement for Gram-negative organisms (93.7%) compared to Gram-positive organisms using VITEK-2 direct testing, reinforcing the general reliability of direct susceptibility methods for Enterobacterales [26].

Concordance for Staphylococcus Species

Among the 38 *Staphylococcus* species isolates in our study, the categorical agreement between DST and conventional AST was 88.67%. Complete concordance (100%) was observed for Linezolid, Ampicillin, Levofloxacin, Teicoplanin, Cefoxitin, Vancomycin, and Gentamicin. Discordances were observed for Penicillin (50% concordance), Ciprofloxacin (73.69%), Erythromycin (68.43%), and Tetracycline (50%). The achievement of 100% concordance for Linezolid and Vancomycin, both cornerstone therapies for MRSA and resistant Gram-positive pathogens, carries considerable clinical importance. The cefoxitin disk screen for MRSA showed 57.89% sensitivity in both DST and conventional AST, indicating a substantial prevalence of methicillin resistance among our Staphylococcal isolates.

The prevalence of MRSA and resistance patterns in staphylococcal isolates have been well characterized in the literature. Gandra et al. (2016) reported persistent MRSA rates of approximately 44.2% from a large Indian private laboratory network [17], and Li et al. (2023) observed MRSA detection rates of 39.3% with methicillin-resistant coagulase-negative staphylococci (MRCNS) at 71.8% in their Chinese cohort, with no resistance detected for vancomycin or linezolid [12], consistent with our 100% concordance for these agents. Gupta et al. (2021) reported maximum sensitivity of Gram-positive organisms to Linezolid and Vancomycin, with significant resistance to Erythromycin and Ciprofloxacin, a finding reflected in our study, where discordance for Erythromycin (68.43%) was noted, likely attributable to borderline inoculum effects [15]. Baba et al. (2023), in their DST evaluation, reported 99.2% essential agreement for Gram-positive isolates with minor errors of 4.04% and major errors of 1.18% which is better than our Staphylococcal concordance of 88.67%, which may reflect differences in local resistance patterns and the proportion of multi-drug-resistant strains [19]. Ghosh et al. (2019) noted that Gram-positive organisms displayed resistance to Erythromycin, orally active Penicillins, Vancomycin, and Teicoplanin in their hospital antimicrobial sensitivity study, further emphasizing the importance of ongoing susceptibility surveillance for Staphylococci [27].

Concordance for *Enterococcus* Species

Enterococcus species (n=16) showed the highest overall categorical agreement in our study at 91.23%. Perfect 100% concordance was achieved for Linezolid, Ampicillin, Penicillin, Vancomycin, Ciprofloxacin, and Levofloxacin. Minor discordances were noted for Teicoplanin (68.75% concordance) and Gentamicin (50% concordance). Notably, both Penicillin and Vancomycin showed 0% sensitivity in both DST and conventional AST, indicating the presence of fully resistant Enterococcal strains which may represent Vancomycin-Resistant Enterococci (VRE). The 100% concordance for Linezolid with 100% sensitivity in both methods suggests that Linezolid remains a reliable therapeutic option for Enterococcal infections in our setting.

Enterococcal infections, particularly those caused by VRE, represent a significant challenge in hospital settings worldwide. Roy et al. (2020) noted that the resistance rate for VRE was only 2% in their study from North-East India, which is lower than what we appear to be observing in our setting [28]. Divyashanthi et al. (2015) found Linezolid to be 92.13% effective for Gram-positive organisms in their institution, supporting its use as a reliable drug against Enterococci [29]. Gupta et al. (2021) noted high sensitivity of Gram-positive organisms to Linezolid and Vancomycin in most isolates, though patterns varied across age groups [15]. Li et al. (2023) highlighted that Enterococcus faecalis exhibited greater antibiotic susceptibility than Enterococcus faecium excluding tetracycline, a nuance that warrants species-level susceptibility profiling in clinical practice [12]. Lokeshwari et al. (2021) observed that among Gram-negative Enterobacteriaceae the DST was highly reliable, and their study overall supports the utility of DST for Gram-positive cocci including Enterococci [24]. Quirino et al. (2021) developed a Microscan WA-based approach for direct AST from positive blood cultures and found it reduced turnaround time compared to traditional results, supporting the clinical application of direct susceptibility testing for Enterococcal infections [30]. Banerjee et al. (2021) further emphasized that rapid AST including for Gram-positive pathogens that can meaningfully improve sepsis care outcomes and antibiotic stewardship when adopted systematically [31].

Major and Minor Error Rates

In our study, the major error rates, defined as isolates reported as susceptible by DST but resistant by conventional AST which were low across all organism groups: Acinetobacter species 2.30%, Enterobacterales 4.86%, Staphylococcus species 3.80%, and Enterococcus species 1.26%. The minor error rates, defined as discrepancies between DST and AST in categorizing intermediate versus susceptible or resistant, were as follows: Acinetobacter species exhibited a rate of 10.32%, Enterobacterales 6.00%, Staphylococcus species 5.76%, and Enterococcus species 7.00%. No very major errors (where a resistant isolate is incorrectly called susceptible, potentially leading to treatment failure) were reported. These error rates fall within generally accepted thresholds as per Clinical and Laboratory Standards Institute (CLSI) guidelines, which recommend major error rates below 3% and very major error rates below 1.5% for acceptable performance.

A comparison with the prior literature revealed that our error rates were broadly consistent with those reported globally. Goel et al. (2015) reported minor errors at a rate of 12.5%, major errors at 3.33%, and very major errors at 0.47%, all of which fall within acceptable limits when employing disk diffusion DST [18]. Yakoob et al. (2018) reported an overall minor error rate of 55.17%, a major error rate of 15.5%, and a very major error rate of 0.8%, which were notably higher, reflecting the critical issue of inoculum standardization in direct disk diffusion testing [32]. Pan et al. (2018) documented a minor error rate of 2.63%, a major error rate of 0.24%, and a very major error rate of 0.24% for Gram-negative bacteria

when employing VITEK-2 direct testing. These rates are significantly lower than those observed with disk diffusion-based methods [25]. Baba et al. (2023) reported minor errors of 4.04% and major errors of 1.18% for Gram-positive isolates, and minor errors of 4% and major errors of 2% for Gram-negative isolates, with no very major errors [19]. Rishu et al. (2024) reported major errors at 1.54%, minor errors at 1.14%, and very major errors at 0.53%, which are among the lowest values documented [33]. The absence of very major errors in our study is a particularly encouraging finding, as these errors carry the greatest clinical risk. The relatively higher minor error rate for Acinetobacter (10.32%) in our study likely reflects the inherent challenges in direct disk diffusion testing for non-fermenters, which show variable growth rates in blood culture broth.

Clinical Significance: Time Advantage of DST

Beyond accuracy, one of the most important advantages of DST documented in our study, and consistently across the literature, is the time saving it offers. Traditional antimicrobial susceptibility testing (AST) necessitates an overnight incubation of isolated colonies prior to initiating susceptibility testing, resulting in a typical turnaround time of 48–72 hours from blood collection. In contrast, direct susceptibility testing (DST) conducted directly from positive blood culture broth can yield preliminary susceptibility results within 18-24 hours of blood culture positivity, which is a crucial timeframe for managing sepsis. Our study design involved processing positive blood cultures for DST immediately upon flagging, allowing early result generation that could be communicated to treating clinicians for timely de-escalation or escalation of antibiotic therapy.

Singh et al. (2024) specifically quantified this time advantage and noted a significant difference of 18-24 hours in time to report release between DST (Direct AST) and conventional AST for both Gram-negative and Gram-positive bacteria[13]. Rishu et al. (2024) similarly concluded that direct AST reduces turnaround time (TAT) by 24 hours, which they described as crucial for early initiation of appropriate antimicrobial therapy in patients with bloodstream infections[33]. Banerjee et al. (2021) conducted a comprehensive review of rapid AST trials and noted that interventions performed 1-2 days earlier in sepsis do not harm patients and in fact provide a valuable foundation for improved antibiotic stewardship [31]. Kumaresan et al. (2024) confirmed that VITEK-2 and MALDI-TOF MS together allow identification and full panel AST from positive blood cultures on the same day, cutting turnaround time by a day compared to traditional methods [26]. Titare et al. (2022) demonstrated that the TTC dye method significantly reduces the time required for early detection of blood culture positivity. Specifically, this unconventional approach achieved detection in 30 hours, compared to 51 hours with traditional methods [34]. Mazhar et al. (2025) in their Bangladesh study emphasized that DST from positive blood culture broth significantly shortens turnaround times and facilitates earlier antibiotic therapy initiation for sepsis patients [21]. Marino Miguélez et al. (2025) advanced this field by introducing a culture-

independent technique that employs microfluidic trapping and deep learning to identify E. coli and K. pneumoniae in spiked blood samples within a mere 2 hours [35]. This represents a significant step towards the future of ultrarapid diagnostics for bloodstream infections.

Broader Antibiotic Resistance Landscape

Our study also reflects the broader antibiotic resistance patterns observed in our region. The finding of high resistance to fluoroquinolones (zero sensitivity for Ciprofloxacin and Levofloxacin in Acinetobacter), significant carbapenem non-susceptibility in Enterobacterales (70% sensitivity suggesting 30% carbapenem resistance), and the presence of likely VRE in Enterococcal isolates collectively paints a picture of a high-resistance environment. These findings highlight the need for immediate and sustained antibiotic stewardship interventions, rational prescribing practices, and regular surveillance of local resistance patterns.

Gandra et al. (2016), analyzing a seven-year dataset (2008-2014) from India, highlighted alarming resistance trends including nalidixic acid resistance in Salmonella Typhi at 98%, carbapenem resistance in E. coli rising from 7.8% to 11.5%, and Klebsiella pneumoniae from 41.5% to 56.6% [17]. Datta et al. (2012) previously reported an increase in ESBL-producing E. coli from 40% to 61%, alongside a rise in carbapenem resistance in Klebsiella pneumoniae from 2.4% to 52% during the decade from 2000 to 2009. These findings, initially considered warning signs, have now become a clinical reality [16]. Divyashanthi et al. (2015) noted widespread resistance to Nalidixic acid (99%), Ampicillin (85.90%), and Cefuroxime (75%) in their study population. Roy et al. (2020) found that P. aeruginosa in their study was categorized as extensively drug-resistant (XDR), and 30% of S. aureus isolates were MRSA [29]. These studies collectively emphasize the critical need for early and precise susceptibility testing, which is directly addressed by drug susceptibility testing (DST) from positive blood cultures, as an integral component of a comprehensive strategy to combat antimicrobial resistance[29]. Our data, situated within this landscape, adds further evidence that DST is not merely a microbiological convenience but a clinical necessity in high-resistance settings.

CONCLUSION

This study evaluated the utility of Direct Susceptibility Testing (DST) from positive blood culture broths as an early method for antibiotic susceptibility reporting. A total of 256 positive blood cultures were processed, with Gram-negative bacilli predominating (78.90%), and Enterobacterales forming the largest isolate group (57.81%).

The overall categorical agreement between DST and conventional AST was high across all organisms, Enterococcus species 91.23%, Staphylococcus species 88.67%, Acinetobacter species 87.26%, and Enterobacterales 82.37%. Major error rates remained low across all groups, and notably, no very major errors were recorded, which is of significant clinical importance as such errors can directly lead to treatment failure.

DST offers a practical time advantage of 18-24 hours over conventional AST, which is critical in the early management of sepsis and bloodstream infections. In an environment characterized by the rising prevalence of multidrug resistance, as evidenced by our findings of significant carbapenem non-susceptibility in Enterobacterales and complete fluoroquinolone resistance in Acinetobacter, the implementation of early targeted therapy based on drug susceptibility testing (DST) results can significantly enhance patient outcomes and contribute to antibiotic stewardship initiatives.

Our study findings suggest that the routine integration of direct susceptibility testing (DST) from flagged positive blood culture bottles into standard microbiological practices at tertiary care hospitals should be implemented alongside conventional antimicrobial susceptibility testing (AST) for confirmatory reporting.

ABBREVIATIONS:

Bloodstream infections - (BSIs)

Antimicrobial susceptibility testing - (AST)

Direct susceptibility testing - (DST)

Antimicrobial resistance - (AMR)

Turnaround time - (TAT)

Gram-negative bacilli - (GNB)

Gram-positive cocci - (GPC)

In-patient department - (IPD)

Out-patient department - (OPD)

Coagulase-negative Staphylococci - (CoNS)

Escherichia coli - (*E. coli)*

Methicillin-resistant *Staphylococcus aureus* - (MRSA)

Methicillin-resistant coagulase-negative staphylococci - (MRCNS)

Vancomycin-Resistant Enterococci - (VRE)

Extensively drug-resistant - (XDR)

STATEMENTS AND DECLARATIONS:

Ethics Approval and Consent to Participate:

This study was approved by the School of Medical Sciences & Research Institutional Ethics Committee of Sharda University, Greater Noida, India (Ref. No. SU/SMS&R/76-A/2026/18). All procedures were performed in accordance with institutional ethical standards and the Declaration of Helsinki.

The requirement for informed consent to participate was not applicable, as the research utilized fully anonymized, retrospective clinical samples.

Consent for Publication:

Not applicable.

Availability of data and materials:

The datasets generated and analysed during the current study consist of patient clinical data stored on secure hospital servers and are not publicly available due to institutional policies, patient confidentiality, and ethics committee restrictions. In accordance with the decision of the institutional ethics board, these data cannot be shared.

Competing Interest:

The authors declare that there is no competing interest.

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Author Contributions:

KK- Conceived the study, managed the project, analysed the data, and drafted the manuscript.

SR- Contributed to study design, performed the laboratory investigations, provided clinical and diagnostic input on bloodstream infections and antimicrobial susceptibility

MPS- Supervised the project, contributed to data interpretation, reviewed and edited the manuscript for critical conceptual contribution

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