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CAN RAPID DIRECT DISK DIFFUSION ANTIMICROBIAL SUSCEPTIBILITY TESTING FROM POSITIVE BLOOD CULTURES IN SEPSIS HELP IN EARLY THERAPEUTIC DECISION-MAKING

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ABSTRACT

Background: Sepsis is a life-threatening condition that requires prompt initiation of effective antimicrobial therapy to improve patient outcomes. Delayed antimicrobial susceptibility results often hinder timely treatment decisions, increasing the risk of morbidity and mortality. Conventional antimicrobial susceptibility testing (AST) methods typically require 24 to 48 hours following blood culture positivity, limiting rapid therapeutic intervention. Direct disk diffusion (dDD) performed directly from positively flagged blood culture broths offers a potential solution by providing susceptibility results 24 hours earlier. This study aims to evaluate the performance of direct disk diffusion testing for rapid antimicrobial susceptibility results in sepsis patients, comparing its accuracy to the reference disk diffusion while assessing error rates such as very major errors (VME), major errors (ME), and minor errors (mE).

Aim: To evaluate the performance of direct disk diffusion (dDD) for rapid antimicrobial susceptibility testing from positively flagged blood culture broths in sepsis patients and compare its accuracy with the reference disk diffusion method by assessing error rates (VME, ME, and mE).

Materials and Methods- A cross-sectional study was conducted in the Department of Microbiology, MMIMSR, Mullana, to assess the performance of direct disk diffusion testing directly from positively flagged blood culture broths. A total of 100 positively flagged blood culture bottles identified through an automated blood culture system were included. All positively flagged blood culture bottles were included in the study, while those containing multiple bacterial species or budding yeast cells, as confirmed by Gram staining, along with all negative blood culture bottles, were excluded from the analysis.

Results- Of 1167 blood cultures processed, 30.67% (358/1167) were positive, with 100 included in the study. Among these, 53% were Gram-positive cocci, predominantly Staphylococcus aureus (38/53; 71.69%), and 47% were Gram-negative bacilli, primarily Escherichia coli (23/47; 48.94%)

and Klebsiella spp. (12/47; 25.53%). The overall categorical agreement between direct disk diffusion and the reference method was 91.07%. Minor errors (2.94%), major errors (2.72%), and very major errors (2.13%) were observed. The highest minor error rate was in Acinetobacter spp. (2.5%), while major errors were most frequent in Acinetobacter spp. (5.83%) and Enterobacteriaceae (4%). Staphylococcus spp. showed the highest very major error rate (2.93%). The average reporting time for direct disk diffusion was 24 hours, with 60% of reports available 24 hours earlier than the reference method.

Conclusion- Prompt placement of blood culture bottles in instruments, timely removal, and rapid communication of Gram stain and antimicrobial susceptibility results to clinicians can significantly improve targeted therapy initiation. Direct testing of blood culture samples with Vitek 2 demonstrated favorable performance for microbial identification and antimicrobial susceptibility testing (AST) for both aerobic/anaerobic facultative Gram-negative bacteria and Gram-positive Staphylococcus and Enterococcus strains. AST results were available 18–24 hours sooner, enabling timely antibiotic therapy and enhancing antimicrobial stewardship efforts.

Introduction

Sepsis is a life-threatening condition characterized by the invasion of microorganisms or their toxins into the bloodstream, accompanied by a systemic inflammatory response that leads to significant morbidity and mortality in hospitalized patients (1). Individuals at higher risk include immunocompromised patients, those on mechanical ventilation, receiving renal replacement therapy, or undergoing recent surgery (2). The predominant causative agents include Gram-positive bacteria such as Staphylococcus aureus, coagulase-negative staphylococci, and Enterococcus faecalis, while Gram-negative pathogens are predominantly Escherichia coli, Klebsiella pneumoniae, Pseudomonas spp., and Acinetobacter baumannii (3). Additionally, non-albicans Candida species followed by Candida albicans are the most frequently isolated fungal pathogens. Bloodstream infections account for approximately 22% of hospitalized patients, with ICU cases comprising nearly 70% of this population, resulting in a mortality rate between 14% and 57% (4). Delayed initiation of appropriate antimicrobial therapy significantly worsens outcomes, underscoring the urgent need for timely diagnosis and treatment. Although blood culture remains the gold standard for diagnosing septicemia (5), about 40% of patients with bloodstream infections initially receive inappropriate antimicrobial therapy until positive culture results are obtained (6). Standard microbiological methods involve Gram staining, overnight sub-culturing on solid media, biochemical identification, and antimicrobial susceptibility testing (AST), requiring an additional 48 hours after a positive signal from automated systems such as BACTEC (7). To address this delay, direct susceptibility testing (DST) from positively flagged blood culture broths has gained recognition as a reliable method, endorsed by the British Society for Antimicrobial Chemotherapy (BSAC) but not yet standardized by the Clinical and Laboratory Standards Institute (CLSI) (8). DST reduces turnaround time (TAT) by approximately 24 hours, enabling earlier targeted therapy, improved clinical outcomes, reduced healthcare costs, and enhanced infection control. While previous studies have demonstrated good categorical agreement (CA) between DST and conventional AST, variability in CA and the potential for errors linked to antibiotic-pathogen combinations warrant further investigation. In this context, our study aims to assess the CA of direct disk diffusion testing with the standard reference method, emphasizing error analysis to provide practical guidance for accurate and prompt DST reporting in clinical microbiology laboratories (9).

MATERIALS AND METHODS

Study Design and Setting This cross-sectional study was conducted in the Department of Microbiology, MMIMSR, Mullana, between June 2020 and June 2021. The study population comprised 100 positively flagged blood culture bottles obtained using an automated blood culture system.

Inclusion Criteria All positively flagged blood culture bottles were included in the study.

Exclusion Criteria Blood culture bottles were excluded if they: Exhibited more than one type of bacteria or budding yeast cells on Gram staining, or Returned negative results on culture.

Sample Collection and Processing Blood samples (5 ml from adults and 2–3 ml from pediatric patients clinically suspected of bloodstream infections) were collected aseptically in BACTEC bottles and immediately incubated in the BACTEC system. Once flagged as positive, bottles were removed promptly and subjected to Gram staining.

Direct Disk Diffusion Testing (dDD) For direct antimicrobial susceptibility testing, four drops of blood culture broth were inoculated onto Mueller-Hinton agar plates and evenly streaked using a sterile swab. After an incubation period of 15–20 minutes to allow for pre-diffusion, antibiotic discs were applied based on the preliminary Gram stain results. Plates were then incubated at 37°C for 18–24 hours and the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

For Gram-negative bacilli, the following antibiotics were tested: ceftriaxone (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), imipenem (10 µg), ceftazidime (30 µg), cefoperazone-sulbactam (75/30 µg), aztreonam (30 µg), amoxicillin/clavulanate (20/10 µg), meropenem (10 µg), tigecycline (15 µg), and piperacillin-tazobactam (100/10 µg).

For Gram-positive cocci, testing was stratified by morphology:

- Isolates in clusters (Staphylococcus spp.) were tested with cotrimoxazole (1.25/23.75 µg), cefoxitin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), penicillin (10 units), and linezolid (30 µg).
- Isolates arranged in pairs (Enterococcus spp.) were tested with high-level gentamicin (120 µg), ampicillin (10 µg), tetracycline (30 µg), and vancomycin (30 µg).

The choice of antibiotics was based on local antibiograms and clinical relevance in the management of bloodstream infections.

Comparison with Routine Antimicrobial Susceptibility Testing (AST) To validate the performance of the direct disk diffusion test (dDD), a drop of the positive blood culture broth was also plated onto MacConkey Agar and Blood Agar. After incubation at 37°C for 24 hours, isolated colonies were processed following standard microbiological procedures. Routine AST was performed using the VITEK-2 system (bioMérieux, France) in accordance with the manufacturer's instructions.

Data Analysis and Error Categorization Results obtained by dDD (test method) were compared with those from routine AST (reference method) for categorical agreement. Categorical agreement was defined as identical susceptibility categorizations between the two methods. Discrepancies were classified as follows:

- **Minor Error (mE):** The reference method indicated sensitivity or resistance while the dDD yielded an intermediate result, or vice versa.
- **Major Error (ME):** The reference method indicated sensitivity, but dDD indicated resistance.
- **Very Major Error (VME):** The reference method indicated resistance, while dDD indicated sensitivity.

Statistical analysis was performed using [Statistical Software, e.g., SPSS version] to calculate the percentage agreement and error rates.

Result

A total of 1167 blood samples were collected during the study period, of which 358 (30.7%) tested positive for bloodstream infections. From these positive samples, 100 were selected for further

analysis using both the direct disc diffusion test (DDT) and conventional antimicrobial susceptibility testing (AST). Direct Gram staining was performed on all 100 samples.

Table I. Summary of Sample Data

Parameter	Number
Total samples received	1167
Total positive samples	358
DDT and Reference Method Performed	100
Direct Gram stain match with culture	100

*DDT: Direct Disc Diffusion Test

Table II Gender-wise distribution of patients

Gender	Number of Patients (n)	Percentage (%)
Male	53	53.0
Female	47	47.0
Total	**100**	**100.0**

A total of 100 patients were included in the study, comprising 53 (53.0%) males and 47 (47.0%) females, indicating a nearly equal gender distribution among the participants.

The age-wise distribution of the 100 patients revealed the highest proportion in the 61–70 years group (29.0%), followed by those aged >70 years (19.0%) and 51–60 years (15.0%). The neonatal group comprised 7.0% of the total, while only 1.0% of patients were in the 1–20 years age group. Chi-square goodness-of-fit testing against a uniform distribution (12.5 expected per group) showed statistically significant deviations in the 1–20 years ($p = 0.0011$) and 61–70 years ($p = 3.06 \times 10^{-6}$) groups. Other age groups did not show statistically significant variation from the expected distribution ($p > 0.05$).

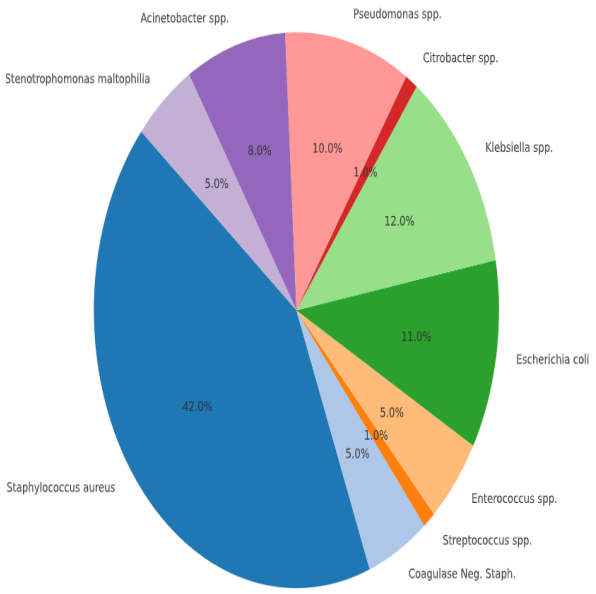
Table III. Age-wise distribution of patients with p-values

Age Group (years)	Number of Patients (n)	Percentage (%)	p-value
Neonates	7	7.0%	0.120
1–20	1	1.0%	0.0011 ★
21–30	6	6.0%	0.066
31–40	10	10.0%	0.480
41–50	13	13.0%	0.888
51–60	15	15.0%	0.480
61–70	29	29.0%	3.06×10^{-6} ★
>70	19	19.0%	0.066
Total	**100**	**100.0%**	

The time-to-positivity of blood culture bottles was assessed over a 96-hour period. The majority of bottles flagged positive between **6–12 hours (36%)** and **12–24 hours (32%)**, indicating early growth detection in most cases. A smaller proportion showed positivity within the first 0–6 hours (18%) and between 24–48 hours (12%). Only 1% of cultures flagged positive at 72 and 96 hours, respectively, suggesting that delayed positivity beyond 48 hours was uncommon.

Table IV. Time-to-positivity of blood culture bottles

Time Period (hours)	0–6	6–12	12–24	24–48	48–72	72–96
No. of Positivety Flagged Broth Bottles	18	36	32	12	1	1
Percentage (%)	18%	36%	32%	12%	1%	1%



A total of 100 organisms were isolated from positive blood culture bottles. The most frequently isolated pathogen was Staphylococcus aureus (42%), followed by Klebsiella spp. (12%) and Escherichia coli (11%). Other Gram-negative organisms included Pseudomonas spp. (10%), Acinetobacter spp. (8%), and Stenotrophomonas maltophilia (5%). Among Gram-positive organisms, Coagulase-negative Staphylococcus and Enterococcus species were each isolated in 5% of cases. Streptococcus species and Citrobacter spp. accounted for 1% each. The overall distribution is detailed in Table V.

Table V. Organism-wise distribution from positive blood culture bottles

Organism	Number of Isolates (n)	Percentage (%)
Staphylococcus aureus	42	42.0
Coagulase-negative Staphylococcus	5	5.0
Streptococcus species	1	1.0
Enterococcus species	5	5.0
Escherichia coli	11	11.0
Klebsiella spp.	12	12.0
Citrobacter spp.	1	1.0
Pseudomonas spp.	10	10.0
Acinetobacter spp.	8	8.0
Stenotrophomonas maltophilia	5	5.0
Total	**100**	**100.0**

The performance of direct disk diffusion testing was evaluated in comparison to the reference method across a total of 1,356 organism–antibiotic combinations. The overall **categorical agreement (CA)** was **91.07%**. Among Gram-positive organisms, Streptococcus species and Coagulase-negative

Staphylococcus demonstrated the highest agreement (100% and 95.38%, respectively). Enterococcus species also showed a high CA of 98.46%. For Gram-negative organisms, Stenotrophomonas maltophilia exhibited 96% agreement, followed by Citrobacter spp. (100%). Enterobacteriaceae, Pseudomonas spp., and Acinetobacter spp. showed CA values of 91.88%, 90%, and 90%, respectively. These findings suggest that the direct disk diffusion method provides reliable susceptibility results for a majority of the organism groups tested.

Table VI. Categorical agreement (CA) of direct disk diffusion test vs. reference method

Organism	**Combinations Tested (n × Ab)**	**Total (N)**	**Categorical Agreement (n, %)**
Staphylococcus spp.	42 × 13	546	506 (92.67%)
Coagulase-negative Staphylococcus	5 × 13	65	62 (95.38%)
Streptococcus species	1 × 5	5	5 (100%)
Enterococcus species	5 × 13	65	64 (98.46%)
Citrobacter spp.	1 × 10	10	10 (100%)
Enterobacteriaceae	23 × 15	345	317 (91.88%)
Pseudomonas spp.	10 × 15	150	135 (90%)
Acinetobacter spp.	8 × 15	120	108 (90%)
Stenotrophomonas maltophilia	5 × 10	50	48 (96%)
Total		**1356**	**1255 (91.07%)**

Minor categorical errors between the direct and reference disk diffusion methods were observed in **2.65% (n = 36)** of the total 1,356 organism–antibiotic combinations tested. Among Gram-positive organisms, Staphylococcus aureus showed the highest number of minor errors (2.3%, n = 13), followed by Enterococcus species (0.07%) and Coagulase-negative Staphylococcus (0.15%). No minor errors were detected for Streptococcus species or Citrobacter spp. Among Gram-negative organisms, minor errors were observed in Acinetobacter spp. (2.5%), Pseudomonas spp. (0.54%), and Enterobacteriaceae (0.69%). Stenotrophomonas maltophilia showed a minor error rate of 2.0%. The distribution of minor errors is summarized in Table VII.

Table VII. Minor error rates in categorical agreement between direct and reference disk diffusion testing

Organism	**Combinations Tested (n × Ab)**	**Total (N)**	**Minor Errors (n, %)**
Staphylococcus spp.	42 × 13	546	13 (2.3%)
Coagulase-negative Staphylococcus	5 × 13	65	2 (0.15%)
Streptococcus spp.	1 × 5	5	0 (0%)
Enterococcus spp.	5 × 13	65	1 (0.07%)
Citrobacter spp.	1 × 10	10	0 (0%)
Enterobacteriaceae	23 × 15	345	9 (0.69%)
Pseudomonas spp.	10 × 15	150	7 (0.54%)
Acinetobacter spp.	8 × 15	120	3 (2.5%)
Stenotrophomonas maltophilia	5 × 10	50	1 (2.0%)
Total		**1356**	**36 (2.65%)**

Major errors (ME), defined as the misclassification of susceptible isolates as resistant, were observed in **2.72% (n = 37)** of the total 1,356 organism–antibiotic combinations tested. Among Gram-positive organisms, Staphylococcus aureus exhibited the highest number of major errors (2.01%, n = 11),

followed by Coagulase-negative Staphylococcus (0.07%). No major errors were reported for Streptococcus species, Enterococcus species, or Citrobacter spp.

Among Gram-negative organisms, Acinetobacter spp. demonstrated the highest rate of major error (5.83%, n = 7), followed by Enterobacteriaceae (4.0%, n = 14), Stenotrophomonas maltophilia (2.0%), and Pseudomonas spp. (0.23%). The distribution of major errors across organisms is summarized in Table VIII.

Table VIII. Major error rates in categorical agreement between direct and reference disk diffusion testing

Organism	Combinations Tested (n × Ab)	Total (N)	Major Errors (n, %)
Staphylococcus spp.	42 × 13	546	11 (2.01%)
Coagulase-negative Staphylococcus	5 × 13	65	1 (0.07%)
Streptococcus species	1 × 5	5	0 (0%)
Enterococcus species	5 × 13	65	0 (0%)
Citrobacter spp.	1 × 10	10	0 (0%)
Enterobacteriaceae	23 × 15	345	14 (4.0%)
Pseudomonas spp.	10 × 15	150	3 (0.23%)
Acinetobacter spp.	8 × 15	120	7 (5.83%)
Stenotrophomonas maltophilia	5 × 10	50	1 (2.0%)
Total		**1356**	**37 (2.72%)**

Very major errors (VMEs), defined as the false classification of resistant isolates as susceptible, were detected in **2.06% (n = 28)** of the 1,356 total organism–antibiotic combinations tested. Among Gram-positive organisms, Staphylococcus aureus showed the highest VME rate of **2.93%** (n = 16). No very major errors were identified for Coagulase-negative Staphylococcus, Streptococcus species, Enterococcus species, or Citrobacter spp.

In Gram-negative organisms, VMEs were observed in Enterobacteriaceae and Pseudomonas spp. (both **0.38%**, n = 5 each), while Acinetobacter spp. showed a VME rate of **1.66%** (n = 2). No VMEs were recorded in Stenotrophomonas maltophilia. The organism-wise distribution of very major errors is provided in Table IX.

Table IX. Very major error (VME) rates in categorical agreement between direct and reference disk diffusion testing

Organism	Combinations Tested (n × Ab)	Total (N)	Very Major Errors (n, %)
Staphylococcus spp.	42 × 13	546	16 (2.93%)
Coagulase-negative Staphylococcus	5 × 13	65	0 (0%)
Streptococcus species	1 × 5	5	0 (0%)
Enterococcus species	5 × 13	65	0 (0%)
Citrobacter spp.	1 × 10	10	0 (0%)
Enterobacteriaceae	23 × 15	345	5 (0.38%)
Pseudomonas spp.	10 × 15	150	5 (0.38%)
Acinetobacter spp.	8 × 15	120	2 (1.66%)
Stenotrophomonas maltophilia	5 × 10	50	0 (0%)
Total		**1356**	**28 (2.06%)**

Very major errors (VMEs), defined as the false classification of resistant isolates as susceptible, were detected in **2.06% (n = 28)** of the 1,356 total organism–antibiotic combinations tested. Among Gram-

positive organisms, Staphylococcus aureus showed the highest VME rate of **2.93%** (n = 16). No very major errors were identified for Coagulase-negative Staphylococcus, Streptococcus species, Enterococcus species, or Citrobacter spp.

In Gram-negative organisms, VMEs were observed in Enterobacteriaceae and Pseudomonas spp. (both **0.38%**, n = 5 each), while Acinetobacter spp. showed a VME rate of **1.66%** (n = 2). No VMEs were recorded in Stenotrophomonas maltophilia. The organism-wise distribution of very major errors is provided in Table IX.

Table IX. Very major error (VME) rates in categorical agreement between direct and reference disk diffusion testing

Organism	**Combinations Tested (n × Ab)**	**Total (N)**	**Very Major Errors (n, %)**
Staphylococcus spp.	42 × 13	546	16 (2.93%)
Coagulase-negative Staphylococcus	5 × 13	65	0 (0%)
Streptococcus species	1 × 5	5	0 (0%)
Enterococcus species	5 × 13	65	0 (0%)
Citrobacter spp.	1 × 10	10	0 (0%)
Enterobacteriaceae	23 × 15	345	5 (0.38%)
Pseudomonas spp.	10 × 15	150	5 (0.38%)
Acinetobacter spp.	8 × 15	120	2 (1.66%)
Stenotrophomonas maltophilia	5 × 10	50	0 (0%)
Total		**1356**	**28 (2.06%)**

The performance of the direct disk diffusion test was evaluated against the reference disk diffusion method across 23 Enterobacteriaceae isolates for various antibiotics. Categorical agreement (CA) was highest for **Meropenem**, achieving **100% agreement**. High CA rates (≥95%) were also observed for **Ciprofloxacin**, **Ampicillin**, **Piperacillin-Tazobactam**, **Ceftriaxone**, **Amikacin**, and **Tigecycline**.

Notably, **Cefepime** exhibited the lowest CA at **78.26%**, accompanied by a **21.73% minor error rate**. **Cefotaxime** and **Ertapenem** both demonstrated a CA of **86.95%**, each with a **13.04% major error rate**. **Amoxyclavulanic Acid** showed a CA of **86.95%**, with **8.69% major errors** and **4.34% minor errors**.

The detailed performance metrics, including categorical disagreements (minor, major, and very major errors), are presented in Table X.

Table X. Performance of direct disk diffusion test compared to reference disk diffusion test for Enterobacteriaceae

Antibiotic	**CA, n (%)**	**Minor Error, n (%)**	**Major Error, n (%)**	**Very Major Error, n (%)**
Ciprofloxacin	22 (95.65%)	0	1 (4.34%)	0
Ampicillin	22 (95.65%)	1 (4.34%)	0	0
Piperacillin-Tazobactam	22 (95.65%)	0	0	1 (4.34%)
Amoxyclavulanic Acid	20 (86.95%)	1 (4.34%)	2 (8.69%)	0
Cefuroxime	21 (91.30%)	0	1 (4.34%)	1 (4.34%)
Cefepime	18	5 (21.73%)	0	0

	(78.26%)			
Ceftriaxone	22 (95.65%)	0	0	1 (4.34%)
Cefotaxime	20 (86.95%)	0	3 (13.04%)	0
Ceftazidime	21 (91.30%)	0	1 (4.34%)	1 (4.34%)
Imipenem	21 (91.30%)	1 (4.34%)	1 (4.34%)	0
Meropenem	23 (100%)	0	0	0
Gentamycin	21 (91.30%)	1 (4.34%)	1 (4.34%)	0
Amikacin	22 (95.65%)	0	0	1 (4.34%)
Ertapenem	20 (86.95%)	0	3 (13.04%)	0
Tigecycline	22 (95.65%)	0	1 (4.34%)	0

The performance of the direct disk diffusion test was evaluated against the reference disk diffusion method across 10 Pseudomonas species isolates for various antibiotics. Categorical agreement (CA) was **100%** for **Ciprofloxacin**, **Cefepime**, **Cefotaxime**, and **Tigecycline**. High CA rates (≥90%) were observed for **Ampicillin**, **Piperacillin-Tazobactam**, **Cefuroxime**, **Ceftriaxone**, **Ceftazidime**, **Imipenem**, **Meropenem**, and **Ertapenem**.

Notably, **Amikacin** exhibited the lowest CA at **70%**, accompanied by **10% minor errors**, **10% major errors**, and **10% very major errors**. **Amoxyclavulanic Acid** and **Gentamycin** both demonstrated a CA of **80%**, each with a **20% minor error rate**.

The detailed performance metrics, including categorical disagreements (minor, major, and very major errors), are presented in Table XI.

Table XI. Performance of direct disk diffusion test compared to reference disk diffusion test for Pseudomonas species

Antibiotic	**CA, n (%)**	**Minor Error, n (%)**	**Major Error, n (%)**	**Very Major Error, n (%)**
Ciprofloxacin	10 (100%)	0	0	0
Ampicillin	9 (90%)	0	0	1 (10%)
Piperacillin-Tazobactam	9 (90%)	1 (10%)	0	0
Amoxyclavulanic Acid	8 (80%)	2 (20%)	0	0
Cefuroxime	9 (90%)	0	1 (10%)	0
Cefepime	10 (100%)	0	0	0
Ceftriaxone	9 (90%)	0	1 (10%)	0
Cefotaxime	10 (100%)	0	0	0
Ceftazidime	9 (90%)	1 (10%)	0	0
Imipenem	9 (90%)	0	0	1 (10%)
Meropenem	9 (90%)	0	0	1 (10%)
Gentamycin	8 (80%)	2 (20%)	0	0
Amikacin	7 (70%)	1 (10%)	1 (10%)	1 (10%)
Ertapenem	9 (90%)	0	0	1 (10%)
Tigecycline	10 (100%)	0	0	0

This table provides a comprehensive overview of the agreement and discrepancies between the direct and reference disk diffusion tests for Pseudomonas species across various antibiotics.

The performance of the direct disk diffusion test was evaluated against the reference disk diffusion method across 8 Acinetobacter species isolates for various antibiotics. Categorical agreement (CA) was **100%** for **Cefuroxime**, **Cefotaxime**, **Imipenem**, **Gentamycin**, and **Tigecycline**. High CA rates (≥87.5%) were observed for **Ampicillin**, **Piperacillin-Tazobactam**, **Amoxyclavulanic Acid**, **Cefepime**, **Ceftriaxone**, **Ceftazidime**, **Meropenem**, and **Ertapenem**.
Notably, **Ciprofloxacin** and **Amikacin** exhibited the lowest CA at **75%**. **Ciprofloxacin** had **12.5% minor errors** and **12.5% major errors**, while **Amikacin** had **25% major errors**.
The detailed performance metrics, including categorical disagreements (minor, major, and very major errors), are presented in Table XII.

Table XII. Performance of direct disk diffusion test compared to reference disk diffusion test for Acinetobacter species

Antibiotic	CA, n (%)	Minor Error, n (%)	Major Error, n (%)	Very Major Error, n (%)
Ciprofloxacin	6 (75%)	1 (12.5%)	1 (12.5%)	0
Ampicillin	7 (87.5%)	1 (12.5%)	0	0
Piperacillin-Tazobactam	7 (87.5%)	0	1 (12.5%)	0
Amoxyclavulanic Acid	7 (87.5%)	0	1 (12.5%)	0
Cefuroxime	8 (100%)	0	0	0
Cefepime	7 (87.5%)	0	0	1 (12.5%)
Ceftriaxone	7 (87.5%)	0	1 (12.5%)	0
Cefotaxime	8 (100%)	0	0	0
Ceftazidime	7 (87.5%)	0	0	1 (12.5%)
Imipenem	8 (100%)	0	0	0
Meropenem	7 (87.5%)	0	1 (12.5%)	0
Gentamycin	8 (100%)	0	0	0
Amikacin	6 (75%)	0	2 (25%)	0
Ertapenem	7 (87.5%)	1 (12.5%)	0	0
Tigecycline	8 (100%)	0	0	0

The performance of the direct disk diffusion test was evaluated against the reference disk diffusion method across 42 Staphylococcus species isolates for various antibiotics. Categorical agreement (CA) was **100%** for **Ciprofloxacin** and **Tetracycline**. High CA rates (≥90.47%) were observed for **Penicillin**, **Oxacillin**, **Gentamicin**, **Clindamycin**, **Linezolid**, **Daptomycin**, **Teicoplanin**, **Vancomycin**, and **Cotrimoxazole**. Notably, **Levofloxacin** and **Erythromycin** exhibited the lowest CA at **85.71%** and **88.09%**, respectively.□

Table XIII. Performance of direct disk diffusion test compared to reference disk diffusion test for Staphylococcus species

Antibiotic	CA, n (%)	Minor Error, n (%)	Major Error, n (%)	Very Major Error, n (%)
Penicillin	39 (92.85%)	0	1 (2.38%)	2 (4.76%)
Oxacillin	39 (92.85%)	0	1 (2.38%)	2 (4.76%)

Gentamicin	39 (92.85%)	2 (4.76%)	0	1 (2.38%)
Ciprofloxacin	42 (100%)	0	0	0
Levofloxacin	36 (85.71%)	2 (4.76%)	1 (2.38%)	3 (7.14%)
Erythromycin	37 (88.09%)	1 (2.38%)	0	4 (9.51%)
Clindamycin	38 (90.47%)	0	1 (2.38%)	3 (7.14%)
Linezolid	39 (92.85%)	1 (2.38%)	2 (4.76%)	0
Daptomycin	38 (90.47%)	0	4 (9.52%)	0
Teicoplanin	41 (97.61%)	1 (2.38%)	0	0
Vancomycin	40 (95.2%)	2 (4.76%)	0	0
Tetracycline	42 (100%)	0	0	0
Cotrimoxazole	40 (95.2%)	0	2 (4.76%)	0

This table provides a comprehensive overview of the agreement and discrepancies between the direct and reference disk diffusion tests for Staphylococcus species across various antibiotics.

The performance of the direct disk diffusion test was evaluated against the reference disk diffusion method across 13 antibiotic tests involving 5 Enterococcus species isolates. Categorical agreement (CA) was **100%** for all antibiotics except **Erythromycin**, which exhibited a CA of **92.30%** with a minor error rate of **7.70%**.

Table XIV. Performance of direct disk diffusion test compared to reference disk diffusion test for Enterococcus species

Antibiotic	**CA, n (%)**	**Minor Error, n (%)**	**Major Error, n (%)**	**Very Major Error, n (%)**
Penicillin	13 (100%)	0	0	0
Oxacillin	13 (100%)	0	0	0
Gentamicin	13 (100%)	0	0	0
Ciprofloxacin	13 (100%)	0	0	0
Levofloxacin	13 (100%)	0	0	0
Erythromycin	12 (92.30%)	1 (7.70%)	0	0
Clindamycin	13 (100%)	0	0	0
Linezolid	13 (100%)	0	0	0
Daptomycin	13 (100%)	0	0	0
Teicoplanin	13 (100%)	0	0	0
Vancomycin	13 (100%)	0	0	0
Tetracycline	13 (100%)	0	0	0
Cotrimoxazole	13 (100%)	0	0	0

This table provides a comprehensive overview of the agreement and discrepancies between the direct and reference disk diffusion tests for Enterococcus species across various antibiotics.

The study assessed the time required for final report dispatch using the direct disk diffusion (DDD) test versus the VITEK method across various samples. The DDD test consistently demonstrated a reduction in reporting time compared to the VITEK method, with time differences ranging from 24

to 96 hours. Notably, for 19 samples, the DDD test reported results within 24 hours, whereas the VITEK method required up to 48 hours, indicating a 24-hour time difference. Similarly, for 6 samples, the DDD test took less than 24 hours, while the VITEK method took up to 72 hours, resulting in a 48-hour difference. The detailed distribution of time differences across all samples is presented in Table XV.

Table XV. Time taken by direct disk diffusion test and VITEK method for final report dispatch

Time taken by DDD (hours)	Time taken by VITEK (hours)	Difference in time (hours)	Total Samples (n)
<24	<48	24	19
<24	<72	48	6
<24	<120	96	1
<48	<72	24	33
<48	<96	48	22
<48	<120	72	5
<72	<96	24	6
<72	<120	48	5
<96	<120	24	1
<96	<144	48	1
<144	<168	24	1

This table illustrates the comparative efficiency of the DDD test over the VITEK method in terms of time to final report dispatch across various sample groups.

Discussion

The present study was conducted in the Department of Microbiology at MMIMSR, Mullana, Ambala, to compare the performance of the direct disk diffusion test (DDDT) with the reference VITEK-2 method using 100 positively flagged blood culture bottles from both IPD and OPD patients. Out of 1167 blood cultures received during the study period, 69.32% (809) were sterile, and 30.67% (358) showed growth. Ultimately, 100 samples (8.56%) were included after excluding five cultures due to discordance between the direct Gram stain and culture smear, with five additional cultures incorporated subsequently.

Our findings are in concordance with those reported by Rajshekar et al. [4], who received 17,215 blood cultures with 71.9% sterile cultures, and by Daley et al. [20], who reported an 83% sterility rate among 41,096 blood cultures. These studies underscore the inherent challenges in isolating pathogens from blood cultures, particularly in settings with high contamination or sterility rates.

The demographic profile of the patients revealed a nearly equal gender distribution (53% male and 47% female) as shown in Table II. The age distribution (Table III) indicated a broad range, with neonates comprising 7%, young adults (21–30 years) 6%, and the highest number of patients in the 61–70 years age group (29%). These findings reflect the diverse patient population attending our institution.

Regarding the time-to-positivity, Table IV demonstrates that a significant proportion of blood culture bottles flagged positive within the first 24 hours (18 bottles within 6 hours, 36 within 12 hours, and 32 between 12 and 24 hours). In contrast, very few bottles flagged positive beyond 48 hours. This rapid detection by the BACTEC system is critical for timely initiation of targeted therapy.

Table V details the organism distribution, where 53% of isolates were Gram-positive cocci and 47% were Gram-negative bacilli. Among Gram-positive isolates, Staphylococcus aureus was predominant (71.69%), with other organisms including Enterococcus spp. and coagulase-negative staphylococci (CONS) each accounting for 9.4%, and a small percentage of methicillin-resistant staphylococci (7.5%) and streptococci (1.88%). For Gram-negative bacilli, Klebsiella species (25.53%) and

Escherichia coli (23.40%) were the most common, followed by Pseudomonas spp. (21.27%), Acinetobacter spp. (17.07%), Stenotrophomonas maltophilia (10.63%), and Citrobacter spp. (2.12%). These results are comparable to those reported by Rajshekar et al. [4], who observed a similar distribution among Enterobacteriaceae, non-fermenters, and Gram-positive cocci.

In terms of antimicrobial susceptibility testing (AST), a total of 1356 organism–antibiotic combinations were evaluated (Table VI). The overall categorical agreement (CA) between DDDT and VITEK-2 was 91.07%. CA for individual groups ranged from 100% for Streptococcus spp. to 92.67% for Staphylococcus spp. and 95.38% for CONS, while Enterococcus spp. showed a CA of 98.46%. Among Gram-negative organisms, CA was 91.88% for Escherichia coli and Klebsiella spp., 90% for both Pseudomonas spp. and Acinetobacter spp., and 96% for Stenotrophomonas maltophilia. These findings are consistent with earlier studies by Coyle et al. [12] and L. Coorevit et al. [19], who reported high CA values for Gram-positive cocci, and with Rajshekar et al. [4], who observed CA values exceeding 95% for key organism groups.

Categorical discrepancies were further analyzed. As shown in Table VII, the overall minor error (mE) rate was 2.94%, with the highest mE observed in Acinetobacter spp. (2.50%) followed by Staphylococcus spp. (2.3%) and Stenotrophomonas maltophilia (2%). In contrast, the minor error rates for Enterobacteriaceae and Pseudomonas spp. were 0.69% and 0.54%, respectively, while CONS and Enterococcus spp. showed minimal errors (0.15% and 0.07%, respectively). These findings are within the acceptable range reported by Edelmann et al. [14] and others.

Table VIII summarizes the major error (ME) rates, with an overall ME of 2.72%. The highest ME rates were noted in Acinetobacter spp. (5.83%), followed by Enterobacteriaceae (4.0%) and Staphylococcus spp. (2.01%), whereas Pseudomonas spp. and CONS exhibited minimal errors. Similarly, Table IX reports the very major error (VME) rate as 2.06%, with Staphylococcus spp. showing the highest VME (2.93%) and lower rates in Gram-negative groups (0.38–1.66%). These error rates compare favorably with the literature, where ME and VME values have been reported in the range of 0.1% to 3.33% [22, 37]. Detailed evaluations for specific organism groups are provided in Tables X to XIV. For instance, in the Enterobacteriaceae group (Table X), CA ranged from 78.26% for Cefepime to 100% for Meropenem, with acceptable levels of minor, major, and very major errors. For Pseudomonas spp. (Table XI) and Acinetobacter spp. (Table XII), CA values were 90% and 96%, respectively, although Amikacin in Pseudomonas showed lower agreement (70%) with corresponding error rates. Similarly, Table XIII shows that Staphylococcus spp. had a total CA of 92.67%, with perfect agreement for Ciprofloxacin and Tetracycline, while Table XIV demonstrates that Enterococcus spp. achieved 100% CA for most antibiotics except for a slight deviation with Erythromycin.

Furthermore, Table XV highlights the practical advantage of DDDT in reducing the turnaround time for AST reports. On average, DDDT provided final reports within 24 hours, with 60% of samples dispatched 24 hours earlier than the VITEK-2 method, 34% dispatched 48 hours sooner, and a few reports even 72 to 96 hours faster. These findings reinforce those of Bazzi Ali et al. [6], who reported a similar time advantage with the direct disk diffusion method.

In summary, the DDDT not only offers a high degree of categorical agreement comparable to that of the automated VITEK-2 system but also provides a significant reduction in reporting time. This rapid turnaround is critical for initiating timely and appropriate antimicrobial therapy, thereby potentially improving patient outcomes. The error rates observed in this study are within acceptable limits and corroborate findings from several previous studies. However, further multicentric evaluations and standardization of direct testing methods are warranted to enhance its clinical utility.

Summary

The study was performed in the Department of Microbiology at MMIMSR, Mullana, Ambala, to compare the performance of the direct disk diffusion test (DDDT) with the reference VITEK-2 system using 100 positively flagged blood culture bottles from IPD and OPD patients. Out of 1167 blood

cultures received between September 2020 and March 2021, 30.67% (358) were positive, of which 100 samples were ultimately included after excluding discrepant Gram stain results.
Key findings include:

Time to Positivity: BACTEC flagged 86 bottles within 24 hours, 12 within 48 hours, and only one each at 72 and 96 hours.

- **Demographics:** Among the 100 patients, 53 were male and 47 female. The age distribution ranged from 7 neonates to 19 patients over 70 years, with the highest representation in the 61–70 years group (29%).
- **Organism Distribution:** Gram staining revealed 53% Gram-positive cocci and 47% Gram-negative bacilli. Among Gram-positive isolates, Staphylococcus aureus (42%) was predominant, followed by coagulase-negative staphylococci and Enterococcus spp. (each 5%), while among Gram-negative bacilli, Klebsiella spp. (12%) and Escherichia coli (11%) were common. Non-fermenters included Pseudomonas spp. (10%), Acinetobacter spp. (8%), and Stenotrophomonas maltophilia (5%).
- **Antimicrobial Susceptibility Testing:** A total of 1356 organism–antibiotic combinations were tested. Overall categorical agreement (CA) between the DDDT and VITEK-2 methods was 91.07%. CA was 92.67% for Staphylococcus spp., 95.38% for coagulase-negative staphylococci, 100% for Streptococcus spp., and 98.46% for Enterococcus spp. Among Gram-negative organisms, CA was 91.88% for Enterobacteriaceae (with 100% agreement for Citrobacter spp.), 90% for both Pseudomonas spp. and Acinetobacter spp., and 96% for Stenotrophomonas maltophilia.
- **Categorical Discrepancies:** Minor errors were observed in 2.65% of tests, with the highest rates in Acinetobacter spp. (2.50%) and Staphylococcus spp. (2.30%). Major errors accounted for 2.72% of tests, with the greatest discrepancies noted in Acinetobacter spp. (5.83%) and Enterobacteriaceae (4.0%). Very major errors were 2.06% overall, predominantly seen in Staphylococcus spp. (2.93%).
- **Antibiotic-Specific Performance:** Within the Enterobacteriaceae group, CA ranged from 78.26% for Cefepime to 100% for Meropenem, while for non-fermenters and Gram-positive cocci, CA values varied between 70% and 100% depending on the antibiotic. Notably, DDDT provided AST reports 24–96 hours sooner than VITEK-2, with the majority (60%) dispatched 24 hours earlier.

Overall, the direct disk diffusion method demonstrated high categorical agreement with the automated VITEK-2 system while offering significantly faster turnaround times, supporting its utility as an effective and rapid alternative for antimicrobial susceptibility testing in clinical microbiology.

Conclusion

Routine blood culture practice is optimized when bottles are promptly placed on the instrument (within 2 hours of collection), removed immediately after flagging positive, and when direct Gram stain and antimicrobial susceptibility results are rapidly communicated to clinicians. This approach enables early initiation of targeted therapy, which is a crucial antimicrobial stewardship intervention. In our study, direct testing of blood culture samples with VITEK-2 demonstrated favorable performance for both microbial identification and AST for aerobic/anaerobic facultative Gram-negative bacteria as well as for Gram-positive Staphylococcus and Enterococcus strains. Notably, AST results were available on average 18–24 hours sooner than with conventional methods, facilitating timely optimization of antimicrobial therapy and potentially improving patient outcomes.

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