MAK HILL Publications Research Journal of Medical Sciences

Research Article
doi: 10.36478/makrjms.2025.2.168.173

Comparative Patterns of Antimicrobial Susceptibility Testing by Direct Susceptibility and Conventional Suscepbility Testing of Blood Culture Isolates from a Tertiary Care Centre from Dibrugarh, Assam

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Key Words
Direct antimicrobial susceptibility testing, blood culture, disk diffusion septicemia

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Received: 05 November 2024
Accepted: 07 January 2025
Published: 21 January 2025

Citation: Chimanjita Phukan, Prapti Bora, Sumita Boro, Arunjyoti Sarmah, Rupsikha Gogoi and Reema Nath, 2025. Comparative Patterns of Antimicrobial Susceptibility Testing by Direct Susceptibility and Conventional Suscepbility Testing of Blood Culture Isolates from a Tertiary Care Centre from Dibrugarh, Assam. Res. J. Med. Sci., 19: 168-173, doi: 10.36478/ makrjms. 2025.2.168.173

ABSTRACT

Early initiation of appropriate antimicrobial agents is essential for adequate management of septicemia. Antimicrobial susceptibility reporting by conventional methods require a minimum of 24-48 hours after flagging positive. Direct antimicrobial susceptibility testing can reduce the turn around time by 24 hours. To compare the difference in susceptibility patterns of blood culture isolates between conventional antimicrobial susceptibility testing and primary drug sensitivity method in sepsis cases and to find the very major, major and minor errors in the antibiotics tested for Gram negative and Gram positive isolates, amongst the two methods. Blood culture bottles flagged positive by BacT/ALERT systems were analyzed by Grams stain. Samples showing monomicrobial growth were further processed for primary drug susceptibility testing by disk diffusion method, following the CLSI 2022 guidelines. Conventional culture and sensitivity testing were also carried out simultaneously. The number of very major errors, major errors and minor errors were calculated for each antimicrobial agent, along with the concordance rate between conventional and direct susceptibility testing. Out of 120 positive cultures, 68 (56.7%) were Gram negative isolates and 44 (36.7%) were Gram positive isolates. Most commonly isolated Gram negative and Gram positive organisms were Escherichia coli and Staphylococcus aureus, respectively. Slightly high discrepencies were seen for Enterobacterales testing against Ciprofloxacin, Meropenem and Beta lactam/Beta lactamase inhibitors, non enterobacterales against Ampicillin/sulbactam, Meropenem and Amikacin, Staphylococci species against Cefoxitin and Enterococci species against Ampicillin and Tetracycline. Primary drug susceptibility testing enables physicians to initiate appropriate antimicrobial therapy at least 24 hours earlier compared to conventional methods. This can significantly reduce mortality in patients with septicemia.

INTRODUCTION

Sepsis remains one of the major causes of mortality and morbidity in hospitals, affecting approximately 2% of all hospitalized patients and up to 70% of patients admitted in the Intensive Care Unit[1]. Mortality increases in patients in septic shock with every hour of delay in antibiotic administration following onset of shock. Culture based methods are considered as gold standard for identification of pathogens causing blood stream infections[2]. Once the culture flags positive, conventional antimicrobial susceptiblity testing by disk diffusion method requires a minimum of 24-48 hours for reporting final susceptibility patterns. Moreover, initial empirical antibiotics can be inadequate or unnecessarily broad spectrum, leading to poor outcomes. Early administration of appropriate antibiotic increases the survival chances in patients with sepsis[2,3]. Timely and early identification of pathogen and their antibiotic susceptibility pattern helps clinicians in early diagnosis and also reduces hospital care related costs[4]. Also it aids in optimizing the timing of initiation of the appropriate antibiotics thereby reducing time to antibiotic de-escalation and facilitating timely switch over to oral antibiotics[2]. Primary drug sensitivity testing is a phenotypic approach which uses a non standardized inoculum directly from the positive blood culture. This method can provide antibiotic susceptibility reports approximately 24 hours earlier compared to those performed from pure subcultures by disk diffusion method[5,6].

Aims and Objectives:

- To compare the difference in susceptibility patterns of blood culture isolates between conventional antimicrobial susceptibility testing and primary drug sensitivity methods.
- To find the very major, major and minor errors in the antibiotics tested for Gram negative and Gram positive isolates, amongst the two methods.

MATERIALS AND METHODS

The study was a prospective analytical study conducted at Department of Microbiology, Assam Medical College and Hospital, Dibrugarh from November 2022-May 2023. A total of 120 positive blood culture samples were included in the study. Blood cultures with polymicrobial growth or showing growth of fungi were excluded from the study. Blood culture bottles which were received were incubated in BacT/ALERT automated system. When the system flagged positive indicating growth in blood culture bottles, bottle was removed and an aliquot of sample was used in preparation of smear. Gram staining was performed for all samples which flagged positive and smears showing single morphological forms were included in the study.

For Gram negative bacterial isolates, one drop (20µl) of blood from the broth was added to 5 ml of sterile water to prepare the inoculum. A sterile cotton swab was dipped into it and rotated against the walls of the tube to remove any extra inoculum. The inoculum was then spread uniformly over the surface of the Mueller Hinton agar plate with the help of the swab[4]. The following antimicrobial disks, procured from Hi Media Laboratories, Mumbai, India, were tested for *Enterobacterales*. Ampicillin (10 µg), Cefazolin (30µg), Amoxicillin-clavulanate (20/10µg), Ceftriaxone(30µg), Ciprofloxacin (5µg), Ampicillin/sulbactum (5µg), Trimethoprim/sulfamethoxazole (1.25/23.75µg), Gentamicin (10µg), Meropenem (10µg), Imipenem (10µg, Piperacillin/tazobactum (10µg), Cefepime (30µg), Amikacin (30µg), Tetracycline (30µg), Ccefuroxime (30µg), Tobramycin (10µg). For *Pseudomonas aeruginosa*, the following antimicrobials were tested: Amikacin(30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ampicillin/ sulbactam (5µg), Ceftazidime (30µg), Cefepime (30µg), Piperacillin/ tazobactam(10µg), Tobramycin (10µg), Meropenem (10µg), Imipenem (10µg). For Acinetobacter species, the following antimicrobials were tested. Cefepime(30µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Ampicillin/sulbactam (5µg), Gentamicin (120 µg), Amikacin (30µg), Piperacillin/ tazobactam (10µg), Meropenem(10µg), Imipenem (10µg), Tobramycin (10µg), Ttrimethoprim/ sulfamethaxazole (1.25/23.75µg), Minocycline (30µg). For Gram positive isolates, three drops (60 µl) of blood was added to 5ml of sterile water. A sterile cotton swab was dipped into it and any excess inoculum was removed by rotating the swab against the walls of the container. The inoculum was then spread evenly over the surface of the Mueller Hinton agar plate with the help of the swab[4]. The following panels of antimicrobial disks were used., For Staphylococcus species. Cefoxitin (30µg), Clindamycin (2µg), Tetracycline (30µg), Erythromycin (5µg), Linezolid (30µg), Penicillin (10units), Trimethoprim/ sulfamethaxazole (1.25/23.75µg), Ciprofloxacin (5µg).

For Enterococcus Species: Ampicillin (10µg), Penicillin (10µg), high level Gentamicin (120µg), Tetracycline (30µg), Vancomycin (30µg) and Linezolid (30µg) were used. The zone inhibition were interpreted as Susceptibile (S), Intermediate (I), Resistant (R) as per CLSI guidelines 2022, for both Gram negative and Gram positive isolates[7]. For each bottle positive flagged by the BacT/ALERT system, a subculture was done on Mac Conkey agar, blood agar and chocolate agar, procured from Hi Media Laboratories Limited and incubated at 35°C overnight to obtain pure isolated colonies. These colonies were inoculated in Muller-Hinton broth to make a suspension equivalent to a 0.5 McFarland standard and used for conventional antimicrobial susceptibility testing by disk diffusion method.

The quality control strains used were Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* 29212 and Pseudomonas aeruginosa ATCC 27853. Susceptibility results obtained from both direct antibiotic susceptibility testing was compared with conventional susceptibility test. Following definitions were used to classify the errors between antibiotics tested by the two methods[4]:

- **Essential Agreement or Minor Errors:** Standard method is susceptible (S) or.
- resistant (R) and DAST is intermediate (I)., alternatively, standard method is.
- intermediate (I) and DAST is susceptible (S) or resistant (R).
- Major errors., standard method yields susceptible (S) result whereas DAST yields resistance (R).
- **Very Major Errors:** Standard method is resistance (R) and DAST yields susceptible.

RESULTS AND DISCUSSIONS

Out of 1100 blood samples received during the study time period, positive culture was obtained in 120 cases (10.9%). Out of the positive cultures, 68 (56.7%) were Gram negative isolates, 44 (36.7%) were Gram positive isolates while 8 (6.6%) isolates were found to be contaminants. The most frequent Gram negative isolate and Gram positive isolate amongst positive cultures were Escherichia coli and Staphylococcus aureus respectively. The Gram negative organisms were broadly divided into *Enterobacterales* and *Non-enterobacterales*. Among the *Enterobacterales*, the organisms isolated were *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* species. The number of errors observed when susceptibility results of primary drug sensitivity was compared with conventional drug sensitivity for Enterobacterales were-2 very major errors, 2 major errors and 3 minor errors. Very major discrepancy was observed in antibiotics Meropenem and Ciprofloxacin. Major errors were observed in case of Ciprofloxacin and Amikacin. Minor errors were seen in case of Piperacillin-tazobactam, Meropenem and Amikacin. The non-enterobacterales category included the organisms Pseudomonas aeruginosa and Acinetobacter species, which showed a total of 3 major errors and 4 minor errors. Major error was seen in case of amikacin and ampicillin/sulbactam and minor errors were seen in meropenem, ampicillin/sulbactam and amikacin. Among the Gram positive organisms isolated, Staphylococcus aureus and Coagulase negative Staphylococci together showed a total of 4 major errors (13.3%). Antibiotics showing major errors were penicillin, cefoxitin and ciprofloxacin. *Enterococcus* group showed a total of 1 major error (7.1%) and 1 minor error (7.1%). Major error was seen in case of ampicillin and minor error was seen in case of tetracycline. No very major errors were observed in the Gram positive isolates.

Blood stream infections are associated with high mortality rate if appropriate antibiotic therapy is not initiated on time[7]. Administration of inappropriate or inadequate antibiotics is associated with increased overall mortality in patients with septicemia[8]. In this study, we compared the differences in susceptibility patterns of blood culture isolates by conventional antimicrobial susceptibility testing and primary drug susceptibility testing. Percentage of minor errors, major errors and very major errors were calculated for different antimicrobials for both the methods. In our study, blood culture positivity rate was 10.9%, which was a similar finding with study done by Lamy[9] (5%-13%) and Sarode[10] (10.6%). Majority of the isolates were Gram negative organisms (56.7%) which is a comparable finding in study done by Kumar[11], where 65.2% of the isolates were Gram negative organisms. For *Enterobacterales,* very major errors were observed in case of Ciprofloxacin (2.17%) and meropenem (2.17%). Comparable findings were seen in studies by Menon[12] (Ciprofloxacin 2.7%, Meropenem 1.4%) and Roshni[13] (Ciprofloxacin 6.6%). Major errors were seen in case of piperacillin/ tazobactam and ampicillin/sulbactam. Major error by piperacillin/tazobactam was also a consistent finding in studies by Menon[13] (2.8%) and Rajshekar[14] (5.5%). Minor errors were seen in piperacillin/tazobactam, amikacin and meropenem. Comparable findings were seen in studies conducted by Roshni[13], Rajshekar[14], Noman[15], Lokeshwari[16], Chandrasekaran[17]. Gentamicin and Ceftriaxone showed no errors in our study, which is a discordant finding in studies by

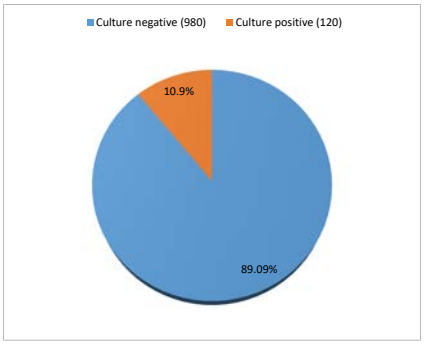

Fig.1 Percentage of Culture Positive Isolates

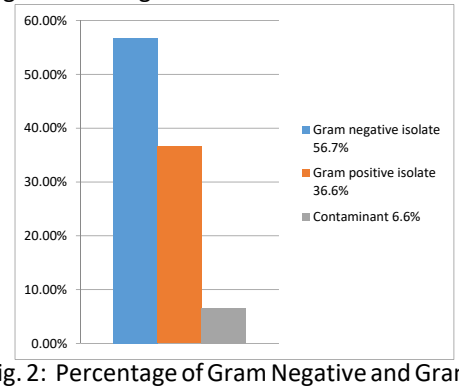

Fig. 2: Percentage of Gram Negative and Gram Positive Isolates

Table 1: Comparison of Susceptibility Results by Direct Susceptibility Testing and Conventional Susceptibility Testing of Enterobacterales (n=46)

Antimicrobials tested	Minor error	Major error	Very major error	Concordance rate
Amikacin	1 (2.17%)	0	0	97.83%
Amoxycillin-clavulanate	0	0	0	100%
Ampicillin	0	0	0	100%
Cefepime	0	0	0	100%
Ceftriaxone	0	0	0	100%
Ciprofloxacin	0	1 (2.17%)	1(2.17%)	95.66%
Ampicillin/ Sulbactum	0	1 (2.17%)	0	97.83%
Trimethoprim/ Sulfamethoxazole	0	0	0	100%
Meropenem	1(2.17%)	0	1(2.17%)	95.66%
Imipenem	0	0	0	100%
Gentamicin	0	0	0	100%
Piperacillin/ Tazobactam	1(2.17%)	0	0	97.83%
Cefuroxime	0	0	0	100%
Tobramycin	0	0	0	100%
Cefoxitin	0	0	0	100%
Cefazolin	0	0	0	100%
Tetracycline	0	0	0	100%

Table2: (A) Comparison of Susceptibility Results by Direct Susceptibility Testing and Conventional Susceptibility Testing of Non-Enterobacterales (n=14) Pseudomonas aeruginosa

Antimicrobials tested	Minor error	Major error	Very major error	Concordance rate (%)
Amikacin	1 (7.14%)	1 (7.14%)	0	85.72%
Gentamicin	0	0	0	100%
Ciprofloxacin	0	0	0	100%
Ampicillin/sulbactam	1 (7.14%)	0	0	92.86%
Ceftazidime	0	0	0	100%
Cefepime	0	0	0	100%
Piperacillin/ tazobactam	0	0	0	100%
Tobramycin	0	0	0	100%
Meropenem	1 (7.14%)	0	0	92.86%
Imipenem	0	0	0	100%
Aztreonam	0	0	0	100%

Table 2: (B) Comparison of Susceptibility Results by Direct Susceptibility Testing and Conventional Susceptibility Testing of Non-Enterobacterales (n=08) Acinetobacter Species

Antimicrobials tested	Minor error	Major error	Very major error	Concordance rate (%)
Cefepime	0	0	0	100%
Ceftazidime	0	0	0	100%
Ciprofloxacin	0	0	0	100%
Ampicillin/sulbactam		1(12.5%)	0	87.5%
Gentamicin	0	0	0	100%
Amikacin	0	1 (12.5%)	0	87.5%
Piperacillin/ tazobactam	0	0	0	100%
Meropenem	1 (12.5%)	0	0	87.5%
Imipenem	0	0	0	100%
Tobramycin	0	0	0	100%
Trimethoprim-sulfamethoxazole	0	0	0	100%
Minocycline	0	0	0	100%

Table 3: Comparison of Susceptibility Results by Direct Susceptibility Testing and Conventional Susceptibility Testing of Staphylococcus Aureus and Coagulase Negative Staphylococci (n=30)

Antimicrobials tested	Minor error	Major error	Very major error	Concordance rate (%)
Penicillin	0	1 (3.3%)	0	96.7%
Cefoxitin	0	2 (6.6%)	0	93.4%
Ciprofloxacin	0	1 (3.3%)	0	96.7%
Trimethoprim/ Sulfamethoxazole	0	0	0	100%
Erythromycin	0	0	0	100%
Linezolid	0	0	0	100%
Clindamycin	0	0	0	100%
Tetracycline	0	0	0	100%

Table 4: Comparison of Susceptibility Results by Direct Susceptibility Testing and Conventional Susceptibility Testing of Enterococci Species (n=14)

Antimicrobials tested	Minor error	Major error	Very major error	Concordance rate (%)
Ampicillin	0	1 (7.1%)	0	92.9%
High level Gentamicin	0	0	0	100%
Vancomycin	0	0	0	100%
Penicillin	0	0	0	100%
Tetracycline	1 (7.1%)	0	0	92.9%
Linezolid	0	0	0	100%

Rajshekar *et al.* (Gentamicin 4.1% and Ceftriaxone 2.5%) and Menon *et al.* (Gentamicin 2.8% and Ceftriaxone 4.3%). For the non-enterobacterales, our study showed the highest rate of discrepancy in Amikacin, followed by ampicillin/sulbactam and meropenem. In the above mentioned study by Rajshekar[14], amikacin showed high discrepencies for Pseudomonas aeruginosa and Acinetobacter species,

which is comparable to our study. In the study conducted by Rajshekar *et al,* Gentamicin showed a total of 15 (7.8%) errors and 7 (4.8%) errors for Pseudomonas species and Acinetobacter baumannii respectively. Ceftazidime showed a total of 8 (4.1%) and 3 (2%) errors for *Pseudomonas aeruginosa* and Acinetobacter species respectively. Gentamicin and Ceftazidime showed no errors for Pseudomonas aeruginosa and Acinetobacter species in our study. In our study, high discrepancy was observed in case of Cefoxitin followed by Ciprofloxacin and Penicillin for Staphylococcus species. Comparable findings were observed in studies by Rajshekar[14] (Cefoxitin 4.9% major errors, Ciprofloxacin 0.7% major errors, Penicillin 0.7% major errors and 0.7% very major errors) and Menon[12] (Cefoxitin 3% major errors) In case of Enteroccocus, Ampicillin (3.2%) showed major errors, which was comparable to our study. However, antimicrobials like Erythromycin (50% minor errors) showed discrepencies for Staphylococci species and high level Gentamicin (4.4% major errors), Vancomycin (2.9% major errors) showed discrepencies for Enterococci species respectively in the above studies, which is a dissimilar finding in our study.

CONCLUSION

Direct susceptibility testing had concordant susceptibility patterns when compared to conventional susceptibility testing. The overall concordance rate was >85% in all the categories of organisms. Standardization of direct susceptibility testing can further reduce the discrepencies in susceptibility patterns. This can significantly reduce the reporting time and also have a positive impact on antibiotic stewardship along with better clinical outcomes.

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