Original Article

Phenotypic Detection of β-Lactamase-Producing Bacterial Pathogens and Antimicrobial Resistance Patterns in Bloodstream Infections at a Tertiary Care Hospital: A Cross-Sectional Study

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Abstract

Background: Bloodstream infections (BSIs) are considered great morbid and mortal events worldwide, especially in critically ill and immunocompromised patients in the hospital setting and are among the most serious infectious conditions encountered in clinical practice. If they are not diagnosed quickly and treated appropriately, these infections can rapidly develop into sepsis, septic shock and multiorgan dysfunction. The challenge of treating bloodstream infections is growing worldwide as more bacteria have become multidrug-resistant, especially those that are β-lactamase resistant, making the majority of standard antimicrobial therapies ineffective. Clinically important resistance mechanisms developed as an extension of β-lactamases are extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, metallo-β-lactamases (MBLs), and carbapenemases, and they have restricted therapeutic choices and negatively affected patient outcomes. Bacteriological profile of BSI, antimicrobial susceptibility pattern and phenotypically characterisation of the mechanism of resistance to β-lactam antibiotics in bacterial pathogens recovered from BSI. **Material and Methods:** This is a cross-sectional observational study performed in the Department of Microbiology of a Tertiary care hospital. Samples of blood were obtained from patients who had clinical suspicions of bloodstream infection and processed for isolation and identification of bacterial pathogens following standard microbiological procedures. Antimicrobial susceptibility testing was done by the Kirby–Bauer disc diffusion method according to the guideline set by the Clinical and Laboratory Standards Institute (CLSI). The phenotypic detection of ESBL production, production of AmpC β-lactamase and metallo-β-lactamase and carbapenemase activity were performed with the Double Disc Synergy Test and E-test. **Results:** A gram-negative bacillary bloodstream infection was found to be the most common pathogens, and Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter species were isolated as the major pathogens. Staphylococcus aureus and enterococci were also important pathogens, as they are gram positive. Significant levels of multidrug resistance were observed in gram-negative isolates, which were highly resistant to the cephalosporins, fluoroquinolones, and carbapenems. The presence of ESBL, AmpC β-lactamase, metallo-β-lactamase and carbapenemase-producing organisms was confirmed by phenotypic screening. Comparative evaluation showed perfect agreement between double disc synergy test and E-test for the detection of metallo-β-lactamase. **Conclusion:** The study underscores the significant burden of bloodstream pathogens with multiple resistance mechanisms that are specific to the β-lactam group, specifically Gram-negative bacilli with β-lactamase-mediated resistance mechanisms. Screening for the presence of resistance determinants and active surveillance of antimicrobials is crucial in optimizing antimicrobial therapy and preventing spread of resistance pathogens.

Keywords: The bloodstream infections, antimicrobial resistance, β-lactamase, ESBL, AmpC, metallo-β-lactamase, carbapenemase, and multidrug resistance.

Received: 01 June 2026 **Revised: 20 June 2026** **Accepted: 12 July 2026** **Published: 18 July 2026**

Introduction

Bloodstream infections (BSIs) are still a major public health problem in many parts of the world, and cause high morbidity and mortality, long hospital stays and high health care costs. Due to their very rapid onset to sepsis and septic shock, if not diagnosed and managed promptly enough, these infections are one of the most common reasons for severe systemic illnesses, especially in hospitalized, critically ill, and immunocompromised patients. Despite the advances of diagnostic and therapeutic strategies, bloodstream infections still have a significant clinical impact, as described by Costa and Carvalho.[1]

There are significant changes in the epidemiology of bloodstream infections in recent decades, and growing reports of predominance of Gram-negative bacteria in tertiary health care settings, especially in developing countries. Sonawane et al,[2]

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DOI:
10.21276/amit.2026.v13.i2.836

How to cite this article: Surje R, Jatav K, Jain AK, Gupta K. Phenotypic Detection of β-Lactamase-Producing Bacterial Pathogens and Antimicrobial Resistance Patterns in Bloodstream Infections at a Tertiary Care Hospital: A Cross-Sectional Study. Acta Med Int. 2026;13(2):1126-1131.

reported preponderance of Gram-negative bloodstream isolates from tertiary care hospital and another study conducted by Oyekale et al,[3] showed preponderance of Gram-negative organisms in bloodstream infections. Longer hospital stay, intensive care unit (ICU) unit admission, invasive medical procedures, indwelling catheterization and increased exposure to broad spectrum antimicrobial drugs have been cited as causes of the increased incidence of Gram-negative bacteremia.

Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter species are common causes of bloodstream infections, and are especially of concern because they can develop multidrug resistance. Kern and Rieg,[4] listed these organisms as among the best clinically relevant bloodstream pathogens in the entire world. Likewise, Costa and Carvalho,[1] highlight their relevance in the context of serious HABSI.

Gram-positive bacteria also continue to play an important role in causing bloodstream infections, especially those caused by Staphylococcus aureus and Enterococcus species. While Staphylococcus aureus is a highly virulent pathogen that is linked to invasive infections that result in significant mortality, enterococci are an opportunistic nosocomial pathogen with a high prevalence of acquired and intrinsic antimicrobial resistance. Girija and Mohammed Ali,[5] emphasised the still clinically important role of Gram-positive bloodstream pathogens in tertiary health care.

A key challenge is the rise of antimicrobial resistance, especially β-lactamase mediated resistance, in the treatment of bloodstream infection. β-lactam antibiotics being one of the most widely used in antimicrobial stewardship, are associated with their broad-spectrum activity and proven clinical efficacy. But the production of β-lactamase enzymes by bacteria has lately seriously compromised their effectiveness.

If you have extended-spectrum β-lactamases (ESBLs), they will cause failure of the commonly used cephalosporin drugs and monobactams. Hooja et al. [6] found a high proportion of Gram-negative ESBL-producers in clinical infections. AmpC β-lactamases make treatment more difficult as they can deactivate cephalosporins and cephamycins and are not very susceptible to conventional β-lactamase inhibitors.

More alarmingly, resistance to the carbapenems has become a phenomenon for Gram-negative organisms. In recent years, carbapenems have been touted as the „last resort“ drugs for multidrug-resistant infections, but carbapenem resistance has curtailed carbapenems' clinical utility. Along with the production of carbapenemases, reduction of membrane permeability and active efflux mechanisms, the resistance mechanism involves also metallo-β-lactamase activity. Chand et al,[7] highlighted the rising problem of CR Gram-negative pathogens and their treatment difficulties.

The presence of metallo-β-lactamases and carbapenemases is of special concern as they may be associated with MDR phenotypes and provide resistance to a number of β-lactam antibiotics including carbapenems. These resistance determinants are disseminated rapidly by plasmid-mediated horizontal gene transfer, making these a formidable infection control problem. Early detection of antimicrobial resistance mechanisms is essential for guiding appropriate antimicrobial therapy, preventing treatment failure, and limiting nosocomial transmission. In resource-limited microbiology laboratories, phenotypic detection methods remain practical, cost-effective, and accessible diagnostic tools. Conventional screening methods such as the Double Disc Synergy Test, E-test, and CARBA NP testing continue to play an important role in resistance detection. Given the increasing burden of antimicrobial resistance and the need for region-specific surveillance data, the present study was undertaken to evaluate the bacteriological spectrum of bloodstream infections, determine antimicrobial susceptibility patterns, and phenotypically detect important β-lactamase-mediated resistance mechanisms among bloodstream bacterial pathogens.

Materials and Methods

This hospital-based cross-sectional observational study was conducted in the Department of Microbiology, Index Medical College, Hospital & Research Centre, Indore, Madhya Pradesh, India, from January 2024 to January 2026. The study aimed to evaluate the bacteriological profile, antimicrobial susceptibility patterns, and resistance mechanisms of bloodstream pathogens among patients with suspected bloodstream infections.

Patients admitted to General Medicine, Paediatrics, Obstetrics and Gynaecology, Orthopaedics, General Surgery, and Intensive Care Units (ICUs) with clinical features suggestive of septicemia, bacteremia, pyrexia of unknown origin (PUO), or systemic infection were included. Inadequately collected, contaminated, or duplicate blood culture samples from the same infectious episode were excluded.

A total of 850 blood samples were collected under strict aseptic precautions and processed according to standard microbiological procedures. Positive cultures were sub-cultured on Selective media -blood Agar and indicator media- MacConkey Agar plates [Figure 1], and bacterial isolates were identified using Gram staining, colony morphology, and conventional biochemical methods.

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [Figure 2]. Gram-positive isolates were tested against penicillin, erythromycin, clindamycin, tetracycline, doxycycline, minocycline, ciprofloxacin, gentamicin, cefoxitin, vancomycin, and linezolid. Gram-negative isolates were tested against amikacin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, piperacillin/tazobactam, imipenem, meropenem, colistin, and polymyxin B.

Phenotypic detection of resistance mechanisms included extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, metallo-β-lactamase (MBL), and carbapenemase production. ESBL and AmpC detection were performed using standard phenotypic methods. MBL production was assessed by the Double Disc Synergy Test and confirmed by the E-test, while carbapenemase production was detected using the CARBA NP test [Figure 3]

Data were analyzed using appropriate statistical software. Categorical variables were expressed as frequencies and percentages, and associations were assessed using the Chi-square

test. A p-value <0.05 was considered statistically significant.

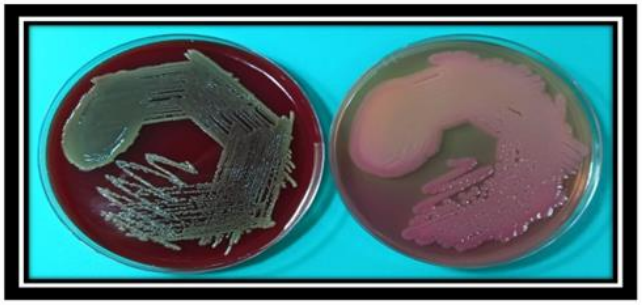


Figure 1: Isolation of organisms by the conventional culture method using the streak culture technique on Blood Agar and MacConkey agar.

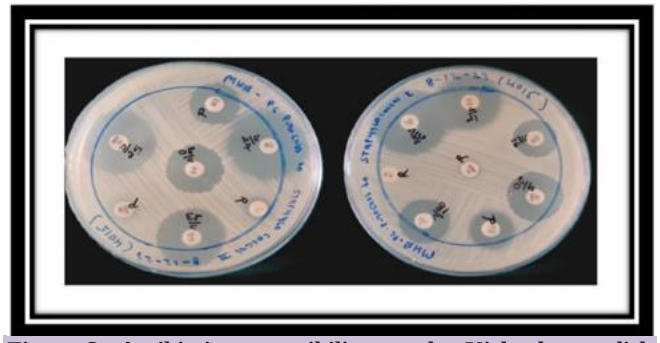


Figure 2: Antibiotic susceptibility test by Kirby bauer disk diffusion method.

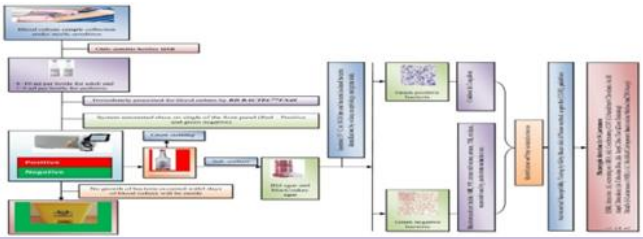


Figure 3: Current workflow of Microbiological Diagnosis in bloodstream infection

Results

Blood Culture Positivity and Distribution of Isolates

During the study period, a total of 850 blood culture samples were collected. Of these 655 (77.1%) samples were obtained from patients admitted to the Inpatient Department (IPD), whereas 195 (22.9%) were collected from patients attending the Outpatient Department (OPD) (Graph 4).

Out of the 850-blood culture samples,186 (21.9%) yielded significant bacterial growth, confirming microbiologically established bloodstream infections. The detection of culture-confirmed bacteremia highlights the ongoing clinical and epidemiological significance of bloodstream infections in hospitalized patients, particularly given their association with increased morbidity, prolonged hospital stays, and the need for prompt institution of targeted antimicrobial therapy.

Analysis of the culture-positive isolates revealed a predominance of Gram-negative bacilli, whereas Gram-positive cocci constituted a comparatively smaller proportion of the recovered pathogens. This distribution suggests a greater burden of Gram-negative bloodstream infections within the study population and is reflective of the evolving microbiological epidemiology observed in tertiary care healthcare settings, where Gram-negative organisms increasingly contribute to severe invasive infections.

The bacteriological profile demonstrated a heterogeneous distribution of etiological agents. Among Gram-negative pathogens, Escherichia coli was identified as the most frequently isolated organism, followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter species. Among Gram-positive isolates, Staphylococcus aureus emerged as the predominant pathogen, followed by Enterococcus species. The predominance of E. coli and K. pneumoniae is of clinical relevance, as these organisms are well-established causes of healthcare-associated bloodstream infections and are recognized for their substantial role in the dissemination of multidrug resistance, particularly through β-lactamase-mediated resistance mechanisms.

Antibiotic Susceptibility Pattern of Staphylococcus aureus

The antimicrobial susceptibility profile of Staphylococcus aureus (n=30) isolates demonstrated substantial resistance to several commonly used antibiotics (Table 1). Complete resistance was observed against penicillin, with all isolates (100.0%) demonstrating resistance, suggesting widespread β-lactamase-mediated resistance. High resistance was also observed against ciprofloxacin (70.0%), erythromycin (60.0%), and gentamicin (56.7%), indicating reduced effectiveness of these agents.

Moderate susceptibility was observed for clindamycin (56.7%), tetracycline (60.0%), doxycycline (63.3%), minocycline (63.3%), and cefoxitin (63.3%). The cefoxitin resistance pattern suggests the likely presence of methicillin-resistant Staphylococcus aureus among a proportion of isolates.

Importantly, all Staphylococcus aureus isolates were completely susceptible to vancomycin and linezolid, indicating preserved activity of these reserve therapeutic agents. Statistical analysis demonstrated a highly significant difference in susceptibility distribution among the tested antibiotics (Chi-square = 74.82; p < 0.0001).

Table 1: Antibiotic Susceptibility Pattern of Staphylococcus aureus Isolates.

Antibiotic	Resistant n (%)	Sensitive n (%)
Penicillin	30 (100.0)	0 (0.0)
Erythromycin	18 (60.0)	12 (40.0)
Clindamycin	13 (43.3)	17 (56.7)
Tetracycline	12 (40.0)	18 (60.0)
Doxycycline	11 (36.7)	19 (63.3)
Minocycline	11 (36.7)	19 (63.3)
Ciprofloxacin	21 (70.0)	9 (30.0)
Gentamicin	17 (56.7)	13 (43.3)

Vancomycin	0 (0.0)	30 (100.0)
Linezolid	0 (0.0)	30 (100.0)
Cefoxitin	11 (36.7)	19 (63.3)

Antibiotic Susceptibility Pattern of Enterococcus Species
The antimicrobial susceptibility profile of Enterococcus species (n=10) demonstrated variable susceptibility patterns [Table 2]. Complete susceptibility was observed to vancomycin and linezolid (100.0%), indicating preserved effectiveness of these agents.
Moderate susceptibility was observed for ampicillin (70.0%), penicillin (60.0%), and high-level gentamicin (60.0%). Equal proportions of susceptibility and resistance were observed for tetracycline, doxycycline, and minocycline, with each demonstrating 50.0% sensitivity and 50.0% resistance.
Statistical analysis showed that the observed susceptibility distribution was not statistically significant (Chi-square = 10.83; p = 0.146).

Table 2: Antibiotic Susceptibility Pattern of Enterococcus Species

Antibiotic	Sensitive n (%)	Resistant n (%)
Ampicillin	7 (70.0)	3 (30.0)
Penicillin	6 (60.0)	4 (40.0)
Tetracycline	5 (50.0)	5 (50.0)
Doxycycline	5 (50.0)	5 (50.0)
Minocycline	5 (50.0)	5 (50.0)
Vancomycin	10 (100.0)	0 (0.0)
Linezolid	10 (100.0)	0 (0.0)
Gentamicin (HLG)	6 (60.0)	4 (40.0)

Antibiotic Susceptibility Pattern of Gram-Negative Bacilli: The antimicrobial susceptibility pattern of Gram-negative bacilli (n=146) revealed an alarming degree of resistance [Table 3].
Gram-negative bacilli exhibited the highest resistance to ciprofloxacin (67.8%), followed by ceftazidime and ceftriaxone (65.1% each), and cefepime (58.9%). Moderate resistance was observed to piperacillin/tazobactam (43.8%), amikacin (41.1%), and both meropenem and imipenem (39% each).
Notably, all isolates were 100% susceptible to colistin and polymyxin B. The overall antimicrobial susceptibility pattern was statistically highly significant (Chi-square = 325.49; p < 0.0001).

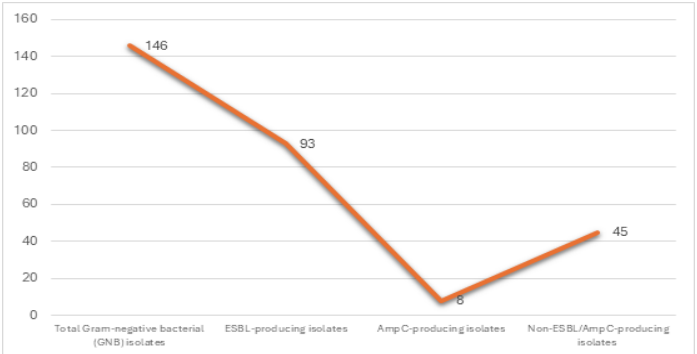
Table 3: Antibiotic Susceptibility Pattern of Gram-Negative Bacilli

Antibiotics	Resistant	Sensitive	Statistical analysis
Amikacin	60 (41.1%)	86 (58.9%)	Chi-square = 325.49
Ceftazidime	95 (65.1%)	51 (34.9%)	p-value <0.0001
Ceftriaxone	95 (65.1%)	51 (34.9%)	
Cefepime	86 (58.9%)	60 (41.1%)	
Ciprofloxacin	99 (67.8%)	47 (32.2%)	
Meropenem	57 (39%)	89 (61%)	
Imipenem	57 (39.7%)	89 (61%)	
Piperacillin/Tazobactam	64 (43.8%)	82 (56.2%)	
Colistin	0 (0.0%)	146 (100.0%)	
Polymyxin B	0 (0.0%)	146 (100.0%)	

Table 4: MBL detection among Carbapenem-Resistant Isolates (n=57)

Methods	Positive	Negative	p-value
DDST	19 (33.3%)	38 (67.7%)	
E-test	19 (33.3%)	38 (67.7%)	1.000

Phenotypic Detection of ESBL and AmpC β-Lactamase Production: Phenotypic screening identified a substantial proportion of Gram-negative isolates producing extended-spectrum β-lactamases (ESBLs), reflecting widespread resistance to third-generation cephalosporins. A significant number of isolates also demonstrated AmpC β-lactamase production, further highlighting the complexity of β-lactam resistance mechanisms in the study population.
These findings indicate a considerable burden of β-lactamase-mediated antimicrobial resistance among bloodstream pathogens. [Graph 1]



Graph 1: Distribution of Extended-Spectrum β-Lactamase (ESBL), AmpC Producers Among Isolated GNB

Metallo-β-Lactamase Detection: Comparative analysis of metallo-β-lactamase detection methods demonstrated identical detection rates between the Double Disc Synergy Test and E-test, indicating complete concordance between the two phenotypic methods (p = 1.000). This suggests that conventional phenotypic screening remains reliable for routine MBL detection. [Table 4]

Discussion

Bloodstream infections (BSIs) continue to be a significant clinical challenge globally and are linked with significant morbidity, mortality and extended hospital stays and health care resource utilisation, especially in the critically ill and immunocompromised. The emergence and spread of multidrug-resistant bacteria have made the management of bloodstream infections significantly more challenging that has restricted treatment options and made treatment failures more likely. The current study aimed to present an extensive bacteriological profile, antimicrobial susceptibility patterns and phenotypic β-lactamase mediated resistance pattern of bloodstream bacterial pathogens from a tertiary care hospital environment.

The predominant bloodstream pathogens in the present study were the Gram-negative bacilli. In Tertiary care centre, Sonawane et al,[2] have reported dominance of Gram-negative organisms as the blood stream pathogens. Similar reports were also made by Oyekale et al,[3] that Gram-negative bacteremia predominated and Khanal et al,[8] reported similar epidemiological trends. The high prevalence of Gram-negative microorganisms could be blamed on the rise in healthcare-associated infections, lengthier hospital stays, large use of invasive devices, intensive care unit admissions and the indiscriminate use of antibiotics.

Escherichia coli and Klebsiella pneumoniae were the most abundant Gram-negative isolates, which is in line with the results of Costa and Carvalho,[1] who found these two species as the more predominant ones responsible for BSI worldwide. Similar to prior reports, the authors of Kern and Rieg,[4] highlighted Enterobacterales as the primary group of bloodstream pathogens linked with serious systemic infections. These organisms have high clinical relevance, since they are a prevalent reservoir of genes that confer resistance to antimicrobial agents, including genes for the production of β-lactamases.

In present study the Gram-negative bacilli showed alarming high resistance to cephalosporins with ceftazidime, ceftriaxone and cefepime being the most resistant antianaerobic agents. The results are similar to those reported by Hooja et al,[6] who reported high numbers of cephalosporin resistance among ESBL-positive Gram-negative clinical bacterial isolates. In similar studies, Seni et al,[9] also reported a high level of resistance to cephalosporin in Enterobacterales from a bloodstream infection.

The high percentage of resistance to the third generation of cephalosporins points to high prevalence of extended-spectrum β-lactamase (ESBL) producing organisms in the study population. The ESBL-producing pathogens are an important therapeutic problem due to their ability to hinder the effectiveness of clinical use of the β-lactam antibiotic, and they frequently present with co-resistance to aminoglycosides and fluoroquinolones. Mohamed Abby et al,[10] have also reported significant resistance to ESBLs in clinical Klebsiella pneumoniae isolates.

The production of AmpC β-lactamase was also detected in Gram-negative isolates, provided another form of resistance to β-lactams. In addition, a higher proportion of A AmpC-producing organisms are resistant to any of the β-lactamase inhibitors, and are also poorly inhibited by traditional β-lactamase inhibitors, a feature that makes them especially problematic since these widely used antibiotics also include the cephalosporins and cephamycins. Similar results were reported by Chand et al,[7] highlighting the clinical relevance of AmpC-mediated resistance of Gram-negative organisms.

One of the most alarming results from the present work was the high rates of carbapenem resistance of Gram-negative isolates. Resistance to carbapenems is a substantial treatment disadvantage as these are regarded as last line of treatment in multidrug resistant Gram-negative infections. The global emergence of bloodstream pathogens resistant to carbapenems, as reported by Kern and Rieg,[4] and the relation of carbapenem resistance to poor clinical outcomes, as reported by Costa and Carvalho,[1] have been highlighted as concerns.

In the present study, all the gram-negative isolates were susceptible to colistin and polymyxin B while this was reported in multidrug resistant gram-negative isolates by Asati et al,[11] also. This is good news, but it must be noted that the use of polymyxins, which have known nephrotoxicity, neurotoxicity and the potential for resistance, is clinically problematic.

Staphylococcus aureus proved to be an important bloodstream isolate among Gram-positive pathogens. Both Kern and Rieg,[4] and Girija and Mohammed Ali,[12] reported the same observation that S. aureus was found to be a clinically significant pathogen associated with blood stream infections. No resistance to penicillin was found in the present study, which suggests that β-lactamase production is widespread. Similar results were reported by Oyekale et al,[3] who reported high levels of penicillin resistance in isolates of Staphylococcus aureus.

Multidrug resistance (to ciprofloxacin, erythromycin and gentamicin) was also seen in high numbers in S. aureus isolates implying decreased effectiveness of what are the more commonly prescribed. The cefoxitin resistance observed presents a likely case for the presence of methicillin-resistant Staphylococcus aureus (MRSA), which is a common therapeutic problem in hospital environments. The same was noted by Sharma et al.[13]

Importantly, all of the S. aureus isolates were sensitive to vancomycin and linezolid. These ~100% effective reserve antibiotics have been demonstrated against Gram-positive bloodstream pathogens by Girija and Mohammed Ali.[12]

The enterococci were also significant bloodstream pathogens in the present study. Moderate resistance to conventional antimicrobial agents was noted, with no resistance observed by vancomycin and linezolid. Kern and Rieg,[4] and Girija and Mohammed Ali,[12] found similar results. The lack of VRE in the present study was a positive development in view of the few options available for treatment of VRE infections.

ESBL production, AmpC β-lactamase production, metallo-β-

lactamase (MBL) production and carbapenemase production were highly prevalent among bloodstream isolates of Gram-negative bacteria, as detected by phenotypic methods. Multiple resistance mechanisms are a significant factor in complexity to treat antimicrobial agents and are also associated with poor clinical outcomes.

Excellent results from the concordance findings for the detection of MBL using the Double Disc Synergy Test and E-test confirm the reliability and usefulness of the traditional phenotypic screening tests in the diagnosis of MBL, especially in resource limited laboratories. Chand et al,[7] reported phenotyping to be similarly useful.

Overall, the results of the present study underscore the significant problem and high incidence of multidrug-resistant bloodstream pathogens, especially of Gram-negative bacilli with multiple β-lactamase-mediated resistance mechanisms. Such observations highlight the importance of ongoing microbiological monitoring, early identification of mechanisms of antimicrobial resistance, antibiotic susceptibility test (antibiogram) preparation at a particular healthcare setting, principled use of antibiotics, and effective infection prevention and control strategies.

Conclusion

The present study shows that MDR-BSI is a large problem in tertiary care hospitals and there is also a shift in favour of Gram-negative bacilli as compared to Gram-positive organisms. Across Gram negative isolates, cephalosporins, fluoro-quinolones and carbapenems showed resistance at a high rate, which is evidence of the increasing problem of antimicrobial resistance in bloodstream infections.

The presence of extended-spectrum β-lactamase, AmpC β-lactamase and metallo-β-lactamase and carbapenemase-producing organisms highlights the complexity of the resistance mechanisms involved in bloodstream pathogens. The dominance of NDM-mediated production of carbapenemase is an alarming epidemiological and therapeutic issue.

Vancomycin and linezolid are still effective against Gram-positive isolates and colistin and polymyxin B are still effective against Gram-negative isolates, which means there is still a role for the "last resort" antimicrobial agents; however, using these drugs does result in stewardship concerns.

The present results highlight the need for ongoing antimicrobial surveillance, prompt detection of antimicrobial resistance mechanisms by phenotypic testing, proper use of antimicrobial agents and rigorous infection control measures to reduce the prevalence of bloodstream pathogens with multiple antimicrobial resistance mechanisms and improve patient outcomes.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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