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Research Article

Bacteriological profile and antimicrobial resistance of blood culture isolates

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Abstract

Bloodstream infections are common, often life-threatening infections associated with substantial morbidity and mortality, particularly when caused by multidrug-resistant organisms. They are particularly more prevalent in vulnerable populations, such as elderly patients, children and immunocompromised individuals. The increasing antimicrobial resistance among these pathogens have added difficulty in the process of infection control and patient management. The main objective of this study was to investigate the prevalent microbial pathogens isolated from blood cultures and determine their antimicrobial susceptibility pattern. 400 blood culture samples were obtained from various departments of hospital and processed in bioMérieux BACT/ALERT® automated microbial detection system. Sub-culturing of positive cultures, and further identification of bacterial species was done using standard microbiological as well as biochemical tests. Antibiotic susceptibility testing was conducted in line with Clinical and Laboratory Standards Institute directions. Data analysis was performed using Statistical Package of Social Sciences (SPSS) and Microsoft Excel. Among 400 samples, there were 27 culture positive (6.75%). Out of these, 20 (74.1%) were females and 7 (25.9%) were males. Gram-negative bacteria were predominant compared to Gram-positive bacteria. Commonly isolated organisms included *Burkholderia cepacia*, *Salmonella typhi*, methicillin-resistant coagulase-negative *staphylococci*, and extensively drug-resistant *Salmonella typhi*. High resistance rates were observed against common antibiotic agents including ceftriaxone, ampicillin, co-trimoxazole and augmentin. Conversely, most of the isolates were sensitive to Carbapenems, linezolid, vancomycin and azithromycin, making them reliable treatment options. Although the culture positivity rate was low, there was a very high prevalence of multi drug-resistant organisms. Therefore, rational use of antibiotics, effective infection control policies and updated local antibiograms are essential to enhance treatment effects and overcome the increasing resistance.

Keywords: Bacteriological Profile, Antimicrobial Resistance, Blood Culture, Multidrug Resistance, Antibiogram.

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Introduction

Bloodstream infections (BSIs) are caused by entry of viable microbial pathogens into

the bloodstream. Such infections trigger the host immune response and result in life-threatening complications such as sepsis and septic shock or even multi-organ failure. They are linked with consequences such as high morbidity and mortality especially when caused by multidrug-resistant and extensively drug-resistant organisms. Such infections most commonly affect vulnerable populations, such as elderly patients, children and immunocompromised individuals [1].

Although significant progress has been achieved in the domains of therapeutics and supportive clinical care, the increasing antimicrobial resistance of these microbial pathogens to the widely used antibiotics has complicated the infection control and patient management. Antimicrobial resistance (AMR) is now considered to be one of the most serious global public health concerns. According to recent studies in Pakistan, resistant infections increase the financial and morbidity burden associated with health care due to prolong hospitalization and poor patient outcome [2].

Over the past two decades, multidrug resistance (MDR) and extensively drug-resistance (XDR) have developed in a multitude of pathogens posing a serious threat to health care. This growing resistance against widely used antibiotics such as beta-lactams, fluoroquinolones and carbapenems has diminished the effectiveness of traditional treatment options. The increasingly common rise of resistance in bacterial infections in the neonatal population makes the problem even more complex, requiring specific antibiotics management approaches [3].

Previous studies in Pakistan indicated the high resistance in methicillin-resistant *Staphylococcus aureus* to beta-lactams, but vancomycin and linezolid often remain effective treatment options, this showed the limited availability and application of effective antimicrobials against resistant infections[4]. Similar results have also been reported for Gram-positive and Gram-negative organisms being resistant to commonly used antibiotics such as ampicillin, cephalosporins and co-trimoxazole, while maintaining sensitivity to carbapenems and aminoglycosides [5 - 6].

The inadequacy of infection control practices, as well as limited antimicrobial stewardship is in many cases among low- and middle-income countries, notably Asian and South American countries aggravating the burden of resistant bloodstream infections [7]. Furthermore, due to lack of resources and finances, access to advanced diagnostic technologies and effective treatment options is restricted, which makes infection management difficult [8].

There are several studies which have investigated bloodstream infections, but limited up-to-date regional data available on bacteriological profiles and patterns of antimicrobial resistance. Therefore, regular review of blood culture isolates, infection control and development of local antibiograms with scientific support would be significant to ensure proper treatment regimens.

The aim of the present study was to identify the bacteriological profile of the microorganisms isolated from blood culture samples and access their antimicrobial susceptibility patterns in blood stream infection suspected patients. The results will help to inform decision-making for empirical treatment, improving antimicrobial stewardship and effective infection control strategies, and will serve as valuable local information on the prevalence and resistance trends of pathogens.

Methods

Study design and setting

This cross-sectional study was conducted in a tertiary care hospital equipped with standard diagnostic and microbiological facilities, for a duration of one year. The study aimed to evaluate the trends of bacteriological profile and antimicrobial resistance of blood culture isolates obtained from patients with suspected bloodstream infections.

Sample collection and microbial cultivation

A total of 400 blood samples were collected from patients admitted to various wards in the hospital. Sampling was done under aseptic conditions. To reduce the contamination of venipuncture site, alcohol and tincture of iodine were applied at the site. Blood samples were collected using sterile syringes, and approximately 3–5 mL of blood was collected per patient depending on the age of patient.

Blood samples were then inoculated into bioMérieux BACT/ALERT® aerobic and anaerobic culture bottles. These bottles were then loaded into the BACT/ALERT® 3D automated microbial detection system (bioMérieux, Marcy l'Etoile, France). The automated system detects microbial growth through colorimetric sensors that identify the carbon dioxide (CO_2) production as a metabolic byproduct of microbial activity. After incubation at 37°C for up to 7 days, bottles showing positive signal were sub-cultured immediately, otherwise they were considered negative.

Culture media preparation and quality assurance

Different types of growth media such as Blood agar, MacConkey agar, and Mueller-Hinton agar were prepared under strict aseptic laboratory conditions. For maintaining sterility, media plates were incubated at 37°C for 24 hours and then used for diagnostic applications. Culture media preparation and quality control procedures were conducted in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations [9].

Sub-culturing and microbial identification

Positive culture bottles were sub-cultured onto blood agar and MacConkey agar plates and incubated at 37°C for 24 hours. Following incubation, colony morphologies were examined and Gram staining technique was used for preliminary classification of the isolates into Gram-positive or Gram-negative to enable differentiation in identification.

Bacterial isolates were further identified into species using standard biochemical tests and commercial identification kits. Gram-positive isolates were differentiated on the basis of catalase, coagulase and DNase tests. Gram-negative isolates were differentiated using oxidase test and API 10S identification system (bioMérieux).

Antibiotic susceptibility evaluation

Antibiotic susceptibility testing (AST) was performed using Kirby-Bauer disc diffusion method on Mueller-Hinton Agar according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

The discs inoculated with antibiotics were aseptically placed on inoculated media plates and incubated at 37°C for 24 hours. The measurement of zones of inhibition was done in millimeter with a standardized measuring scale. Results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) ratings.

Ethical approval

The study was approved by the Institutional

Review Board (IRB) of the hospital. Patient confidentiality was maintained and no extra procedures were done other than standard laboratory investigations.

Data analysis

Patient demographics, type of microorganism and antimicrobial susceptibility results were recorded into Microsoft Excel and statistically analyzed using Statistical Package for the Social Sciences (SPSS).

Frequencies, percentages, and distribution patterns of the isolated microorganisms and resistance pattern were calculated using descriptive statistics.

Results

Culture positivity rate

A total of 400 blood samples were obtained, and cultured. Among them, 27 samples showed microbial growth, and the rate of culture positivity was 6.75%. The remaining 373 samples (93.25%) had no microbial growth. Figure 1 shows the distribution in form of pie chart.

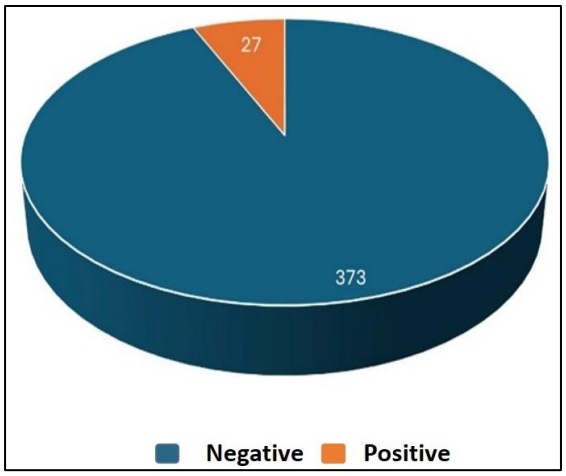


Figure 1: Blood culture positivity rate.

Demographic characteristics

Out of 400 patients, the study population included 241 (60.25%) females and 159 (39.75%) males. Out of the 27 culture-positive cases, 20 (74.1%) were females and 7 (25.9%) were males. Patients were divided into three age units: days, months, and years. Figure 2 shows the above given demographic features.

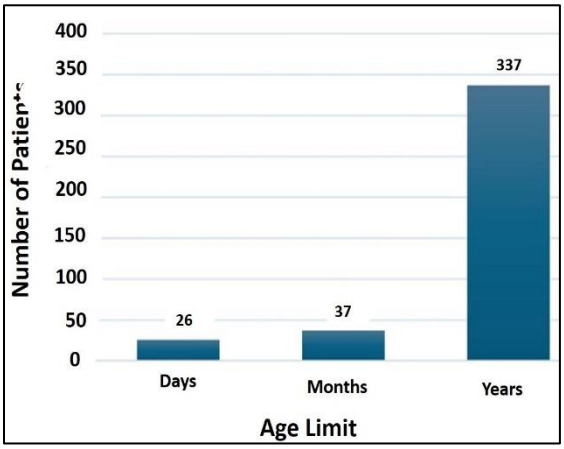


Figure 2: Distribution of patients by age unit.

The majority of patients (337; 84.25%) were categorized in years, followed by 37 (9.25%) in months and 26 (6.5%) in days. This categorization allowed the better interpretation of trends in the infection through various life stages.

Blood culture positivity by gender

These results in Table 1 indicated that female patients had a greater prevalence of bloodstream infections.

Table 1: Gender-wise blood culture results

Gender	Negative [n]	Positive [n]	Total [n]	Positivity [%]
Female	221	20	241	8.3
Male	152	7	159	4.4
Total	373	27	400	—

Distribution of isolated pathogens

A total of 27 microbial isolates belonging to ten different microbial species were identified. Gram-negative bacilli were predominant in the study population as shown in Table 2.

Table 2: Frequency and distribution of organisms isolated from positive blood cultures (n=27).

Organism	Frequency [n]	Percentage [%]
Burkholderia cepacia	5	18.5
Salmonella typhi	5	18.5
MR-CoNS	5	18.5
Salmonella typhi [XDR]	4	14.8
Escherichia coli	2	7.4
Candida spp.	2	7.4
Acinetobacter baumannii	1	3.7
Enterobacter cloacae	1	3.7
Klebsiella oxytoca	1	3.7
MRSA	1	3.7
Total	27	100

The most prevalent isolates were *Burkholderia cepacia, Methicillin-resistant Coagulase-negative Staphylococci* (MRCoNS), and *Salmonella typhi* and its XDR species. Less common isolated organisms included *Escherichia coli, Candida spp., Enterobacter cloacae, Acinetobacter baumannii, Methicillin-resistant Staphylococcus aureus* (MRSA) and *Klebsiella oxytoca.* Given in figure 3.

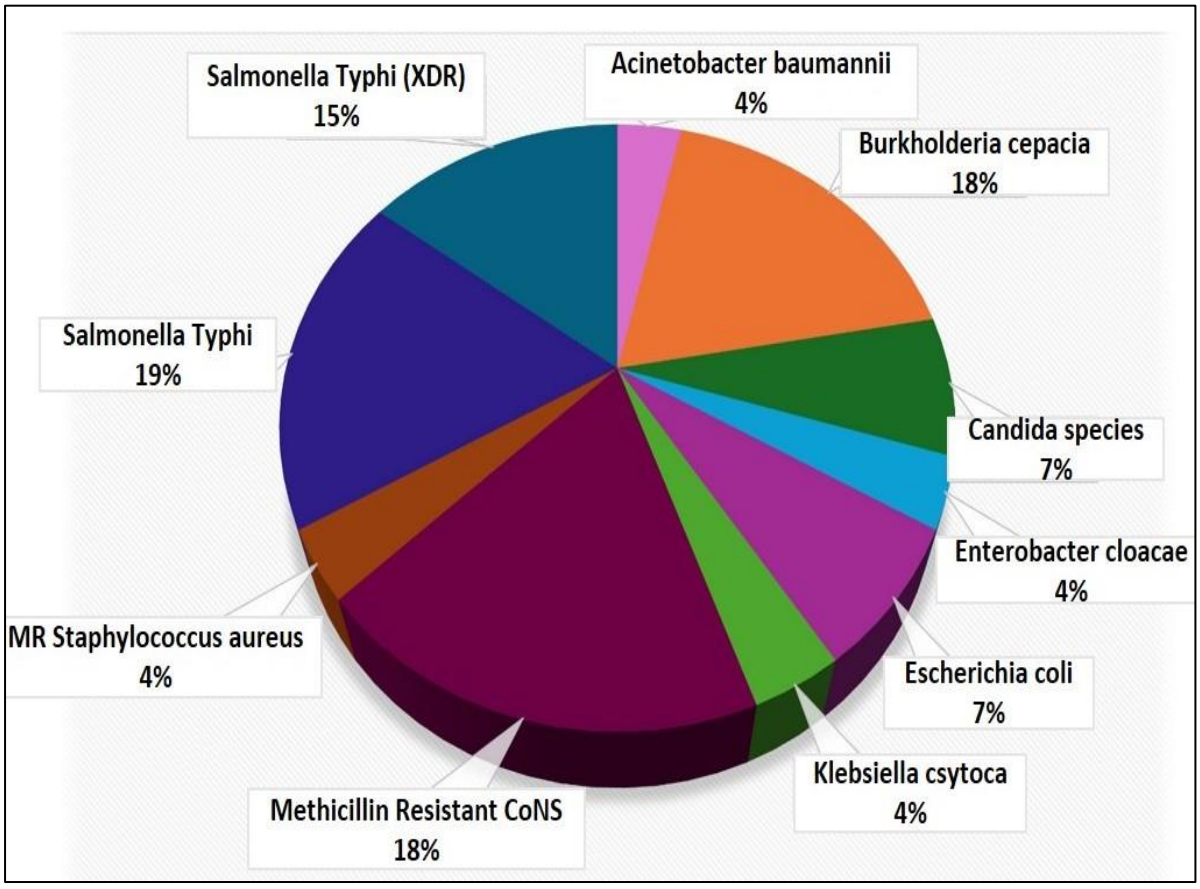


Figure 3: Proportional distribution of microbial isolates recovered from positive blood cultures (n=27).

Antibiotic susceptibility profile

High resistance rates were observed against commonly used antibiotics including: Ceftriaxone (≈90%), Ampicillin (88%), Co-trimoxazole (85%) and Augmentin (82%). Cephalosporins such as ceftazidime and cefepime had moderate resistance. Aminoglycosides (amikacin, gentamicin) and Fluoroquinolones (ciprofloxacin) showed variable sensitivity. Carbapenems like imipenem, meropenem were highly effective against the majority of the isolates. Newer therapeutic agents including azithromycin, linezolid, and vancomycin were highly sensitive (>85%), which made them valuable treatment options. Tetracyclines such as doxycycline, minocycline, and tigecycline had an intermediate sensitivity indicating their use with limited efficiency in bloodstream. In fungal isolates, fluconazole showed resistance in approximately 50% of cases. On the other hand, amphotericin B and voriconazole exhibited good sensitivity with less resistance, results shown Table 3 and Figure 4.

Table 3: Antibiotic susceptibility pattern and multi-drug resistance (MDR) pattern among isolates (n = 27).

Organism	Total Isolates (n)	MDR (Yes/No)	Highly Resistant to	Highly Sensitive to
Burkholderia cepacia	5	Yes	AMP, CRO, SXT, CIP, AMC	IPM, MEM, AK, AZM
Salmonella typhi (XDR)	4	Yes (XDR)	CRO, CIP, SXT, AMP, AMC	IPM, MEM, AK, AZM
Salmonella typhi	5	Yes	AMP, CRO, CIP	IPM, MEM, GEN
Acinetobacter baumannii	1	Yes	CRO, AMP, CIP, SXT, GEN	IPM, MEM, DOX
Enterobacter cloacae	1	Yes	AMP, CRO, AMC	IPM, AK
Escherichia coli	2	Yes	CRO, AMP, CIP, SXT	IPM, AK, TGC
Klebsiella oxytoca	1	Yes	CRO, CIP, AMP	AK, PB, TGC
Methicillin-Resistant CoNS	5	Yes	PEN, MET, AMP, CRO	VAN, LZD, AK
MR Staphylococcus aureus	1	Yes	PEN, AMP, CRO	VAN, LZD, GEN
Candida species	2	No	—	AMB, VOR, FLU

Abbreviations: AMP =Ampicillin; CRO = Ceftriaxone; SXT = Co-trimoxazole; CIP = Ciprofloxacin; AMC = Amoxicillin clavulanate; IPM = Imipenem; MEM = Meropenem; AK = Amikacin; AZM = Azithromycin; GEN = Gentamicin; DOX = Doxycycline; TGC = Tigecycline; PB = Polymyxin B; VAN = Vancomycin; LZD = Linezolid; PEN = Penicillin; MET = Methicillin; AMB = Amphotericin B; VOR = Voriconazole; FLU = Fluconazole.

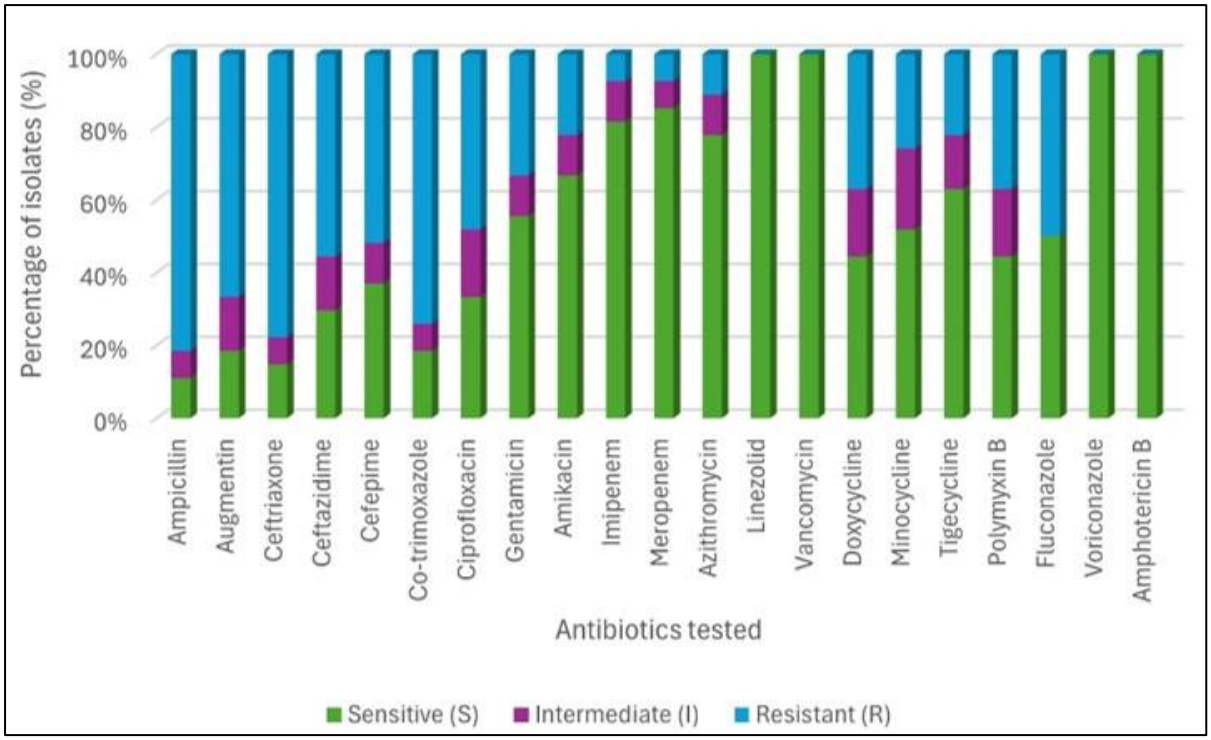


Figure 4: Pattern of antibiotic susceptibility among commonly administered antibiotic.

Overall, the results showed a significant decrease in the effectiveness of first-line antibiotics that are commonly used. This is a call to keep monitoring antimicrobials, regularly updating hospital antibiograms and implementing rational use of antibiotics to reduce the prevalence of spread of resistant organisms.

Discussion

The present study aimed to evaluate the bacteriological profile and antimicrobial susceptibility of blood culture specimens collected from patients with suspected blood stream infections. The culture positivity rate in this study was relatively low, which could be explained by previous antibiotic use, non-bacterial causes of fever or poor timing of specimen collection. However, the presence of multidrug-resistant (MDR) organisms is clinically relevant because these organisms have been found to be closely linked to poor treatment outcomes such as severe illness, hospitalization, and healthcare costs.

In the present study, Gram-negative organisms were found to be predominant, exceeding the frequency of Gram-positive isolates. The predominance of Gram-negative pathogens is similar to that found in other studies from Pakistan and other developing nations, where *Enterobacterales*, *Staphylococcus aureus*, and *Salmonella spp.* are common bloodstream pathogens. The observations highlight the need for up-to-date local surveillance data for timely, appropriate antimicrobial treatment and highlight the need for continuous local surveillance.

Additionally, the detection of methicillin-resistant organisms indicates a potential for hospital-acquired resistant infections, thus reinforcing the importance of improved infection prevention and antimicrobial stewardship. Previous investigations have similarly reported that isolates of MRSA were resistant to beta-lactam antibiotics while maintaining sensitivity to vancomycin and linezolid. This implies that few antibiotics are available to treat Gram-positive resistant infections and such effective therapeutic options should be used carefully [4].

The presence of XDR *Salmonella typhi* confirmed the previous reports on increasing resistance to commonly used drugs from Pakistan. Such resistance substantially limits effective therapeutic options for *enteric* fever and *Salmonella* infections [10]. The high level of first-line antibiotic resistance seen in this study aligns with the patterns of resistance reported in earlier studies, in which the use of first-line antibiotics was frequent and inappropriate, resulting in decreased first-line antibiotic efficacy [3, 11].

On the other hand, most of the multidrug-resistant isolates were sensitive to carbapenems such as imipenem and meropenem. These findings are consistent with previous reports that identified carbapenems as effective therapeutic options for severe multidrug resistant infections [12]. However, excessive use of these last-line drugs can promote further resistance development; therefore, administration of these drugs must be closely monitored.

Linezolid, azithromycin and vancomycin were highly active against resistant isolates particularly Gram-positive bacteria. Similar observations have been reported for resistant staphylococcal infections [4]. These antimicrobials should be preserved for confirmed resistant cases to maintain their activity.

Antimicrobial resistance also has an economic impact of increased costs in healthcare systems. Resistant infections generally require prolonged hospitalization, expensive treatment, medications and supportive therapies. A recent study from Pakistan also determined increased treatment cost among patients with resistant infections as compared to treatment in susceptible infections [2].

The present study has some limitations. The findings may not be generalizable to the broader population due to the relatively low number of culture-positive cases and a single-center study design. Moreover, the absence of access to better diagnostic tools like molecular characterization of resistance genes, which could have provided more details in the understanding of mechanisms of antimicrobial resistance.

Despite some of these drawbacks, this study offers local information on the bacteriological pro-file and antimicrobial susceptibility of bloodstream infection isolates. The results indicate that it is important to adopt evidence-based antibiotic prescription, ongoing surveillance and creation of localized antibiograms, as well as use of efficient infection control policies to counteract the alarming rise of multidrug-resistant blood infections.

Conclusion

Bloodstream infections continue to be a significant clinical challenge, especially with the development of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens such as *Burkholderia cepacia*, *Salmonella typhi*, *Escherichia coli* and *methicillin-resistant coagulase-negative staphylococci* (MR-CoNS).

Empirical treatment options are limited by high rates of resistance to most common first line antibiotics, such as ceftriaxone, ampicillin, augmentin and co-trimoxazole. In comparison, the carbapenems, linezolid, vancomycin and azithromycin, all showed strong antimicrobial activity against most resistant isolates. The results here highlight the necessity to implement effective antimicrobial stewardship programs, rational use of antibiotics, quick diagnostic tools, largescale infection control interventions and continuous local surveillance using hospital-based antibiograms. Future studies should be conducted using a large multicentered approach and molecular detection methods

to further understand emerging resistance mechanisms as well as infection control mechanisms. Specific clinical interventions and ongoing long-term monitoring are crucial to maintain the efficacy of existing therapeutic strategies and improve patient outcomes.

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