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ANTIBIOTIC RESISTANCE TRENDS IN BLOOD CULTURE ISOLATES FROM HOSPITALS IN FAISALABAD

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Abstract

Antimicrobial resistance (AMR) in bloodstream infections (BSIs) presents a critical threat to global health, complicating treatment and increasing mortality. This retrospective, cross-sectional study characterized AMR patterns in bacterial isolates from a tertiary care hospital in Pakistan. Standard microbiological methods were used to identify isolates from blood cultures, and antimicrobial susceptibility testing was performed via Kirby-Bauer disc diffusion against a comprehensive panel of antibiotics, following CLSI 2024 guidelines. The results revealed high rates of multidrug resistance among key pathogens. Gram-positive isolates, particularly methicillin-resistant Staphylococcus aureus (MRSA), showed significant resistance to beta-lactams and macrolides (>80%), though remained largely susceptible to vancomycin (85%), linezolid (88%), and chloramphenicol (82%). Gram-negative isolates exhibited even more alarming resistance, with high rates to carbapenems (e.g., 83% for meropenem), third-generation cephalosporins (98–100%), and fluoroquinolones (60%). Despite this, colistin (85% susceptible) and tigecycline (91% susceptible) retained efficacy against most Gram-negative isolates. These findings underscore a severe and evolving AMR landscape in BSIs within the studied region, emphasizing the urgent need for robust antimicrobial stewardship and evidence-based empirical therapy. Sustained surveillance and prudent antibiotic use are essential to mitigating further resistance spread and guiding clinical practice.

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most pressing global public health crises of the modern era, fundamentally undermining the efficacy of conventional therapeutic agents and transforming previously manageable bacterial infections into chronic, life-threatening conditions (do Vale, 2022). The proliferation of multidrug-resistant (MDR) pathogens escalates healthcare costs and substantially elevates the risks associated with routine medical interventions, including surgery, chemotherapy, and organ transplantation (Alara & Alara, 2024). Without effective antibiotics, the foundational advances of modern medicine are in jeopardy, potentially regressing to a pre-antibiotic era where common infections and minor injuries again become fatal (do Vale, 2022; Salam et al., 2023).

Nowhere are the consequences more acute than in the context of bloodstream infections (BSIs), where delays in appropriate therapy due to resistance directly correlate with increased mortality (Holmbom, 2021). Gram-negative pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* frequently exhibit extended-spectrum beta-lactamase (ESBL) production and carbapenem

resistance, while Gram-positive threats like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) continue to challenge therapeutic options (Manandhar, 2022).

The epidemiology of AMR is markedly heterogeneous, exhibiting significant regional variation influenced by local antibiotic prescribing practices, infection control standards, and surveillance capabilities (Coque et al., 2023). For instance, ESBL rates among Gram-negative isolates exceed 60% in some regions, such as India, while countries with stringent antibiotic stewardship programs, notably in Europe, report comparatively lower resistance rates (Kashef Hamadani, 2023). In Pakistan, recent surveillance indicates a troubling increase in resistance among BSIs, with rising resistance to cephalosporins, fluoroquinolones, and carbapenems, coupled with high MRSA prevalence (Riaz et al., 2024).

Confronting this crisis requires robust, continuous local surveillance to inform empirical treatment guidelines and stewardship policies. This study aims to characterize the current AMR patterns among bacterial isolates recovered from bloodstream infections in a major Pakistani metropolis. Through a comprehensive analysis of pathogen distribution and susceptibility profiles, this study seeks to generate critical evidence to guide empirical therapy, support the development of targeted interventions, and ultimately contribute to curbing the further spread of antimicrobial resistance.

MATERIALS AND METHODS

Study Design and Duration

This study employed a retrospective, cross-sectional design to analyze blood cultures. Data was collected from three major healthcare institutions in Faisalabad (Allied Hospital-II, National Hospital and Children Hospital) to ensure broad geographic coverage within the city. Data was collected from January-2025 to May-2025, and was analyzed to determine the prevalence and patterns of antimicrobial resistance among bacterial isolates from patients with bloodstream infections. The objective was to characterize local resistance trends to guide evidence-based therapeutic and stewardship interventions.

Sample Collection and Processing

Clinical blood samples were aseptically collected from patients presenting with sepsis symptoms. Following standard protocols, samples were inoculated into broth-based culture bottles and incubated at 37°C for 24–48 hours. Growth-positive cultures were subsequently sub-cultured onto blood agar and MacConkey agar to isolate and differentiate bacterial pathogens.

Identification of Isolates

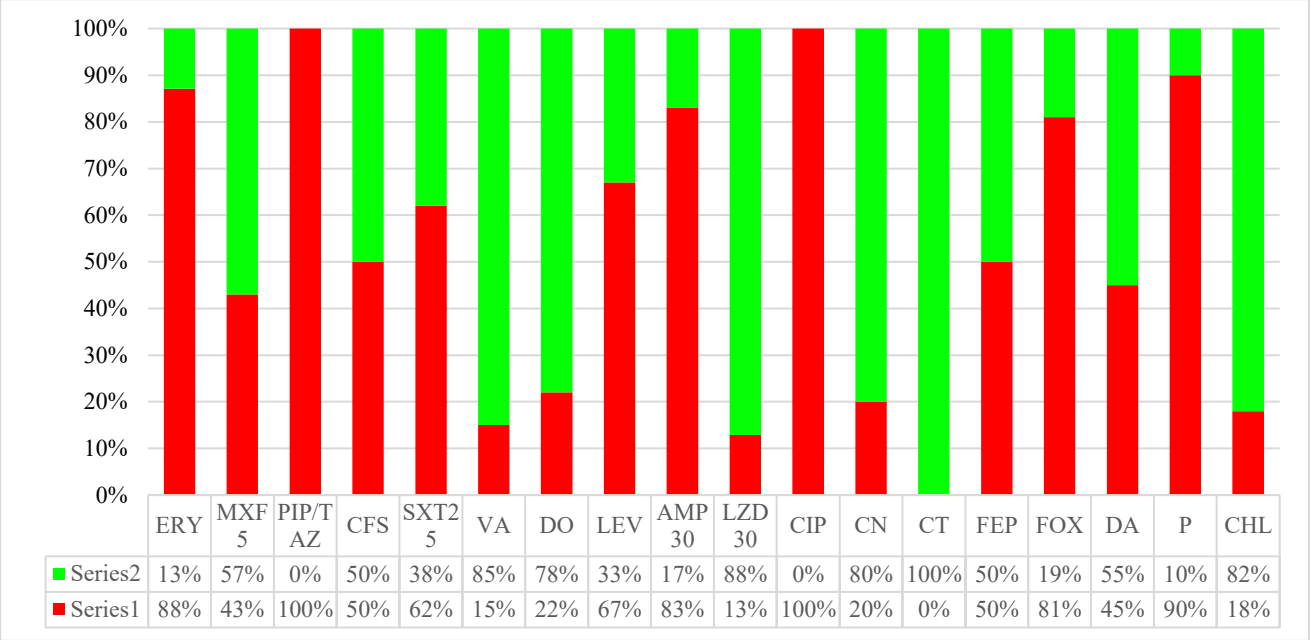
Bacterial isolates were differentiated using Gram staining, with Gram-positive organisms appearing purple and Gram-negative organisms as pink. Initial characterization was confirmed through standard biochemical testing. Coagulase-negative staphylococci without clinical significance were excluded from the analysis. Species identification was achieved using organism-specific protocols. Suspected *Staphylococcus* isolates underwent catalase, coagulase, and DNase testing to differentiate *S. aureus*. Members of the Enterobacteriaceae family were characterized using urease, indole, and Triple Sugar Iron (TSI) tests. For complex or ambiguous isolates, precise identification was obtained using the standardized API 20E (Enterobacteriaceae) and API Staph. (*Staphylococcus*) systems.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed via the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, as per Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. A panel of antibiotics was selected based on the isolated organism and included agents from the beta-lactam, aminoglycoside, fluoroquinolone, carbapenem, and other relevant classes: Antimicrobial susceptibility testing was performed using a comprehensive panel of antibiotic disks from major drug classes, including penicillins (Ampicillin, Penicillin), beta-lactam/beta-lactamase inhibitors (Amoxicillin-clavulanate, Piperacillin-tazobactam, Cefoperazone-sulbactam), cephalosporins (Cefuroxime, Cefoxitin, Ceftriaxone, Cefixime, Cefepime, Cefpodoxime), and carbapenems (Imipenem, Meropenem). The testing also incorporated aminoglycosides (Gentamicin,

Amikacin), fluoroquinolones (Ciprofloxacin, Norfloxacin, Levofloxacin, Moxifloxacin), macrolides (Erythromycin, Azithromycin, Clarithromycin), glycopeptides (Vancomycin), tetracyclines (Doxycycline, Tigecycline), sulfonamides (Trimethoprim-sulfamethoxazole), oxazolidinones (Linezolid), lincosamides (Clindamycin), amphenicols (Chloramphenicol), and polymyxins (Colistin).

Data Collection and Analysis



	ERY	MXF 5	PIP/TAZ	CFS	SXT25	VA	DO	LEV	AMP 30	LZD 30	CIP	CN	CT	FEP	FOX	DA	P	CHL
Series2	13%	57%	0%	50%	38%	85%	78%	33%	17%	88%	0%	80%	100%	50%	19%	55%	10%	82%
Series1	88%	43%	100%	50%	62%	15%	22%	67%	83%	13%	100%	20%	0%	50%	81%	45%	90%	18%

All microbiological laboratory results, including patient clinical information, isolated microorganisms, and their corresponding resistance profiles, were systematically documented. Data were compiled in Microsoft Excel and analyzed using descriptive statistical methods, including frequencies and percentages, to summarize resistance patterns. The analysis compared resistance profiles between Gram-positive and Gram-negative bacteria and computed multidrug resistance (MDR) rates.

RESULTS

For every organism-antibiotic combination, resistance patterns and percentages were calculated, and resistance trends were graphically represented to explain the clinical implications. Gram-positive and Gram-negative organisms are compared.
and doxycycline may serve as effective alternatives for MRSA infections.

Gram-Positive Organisms

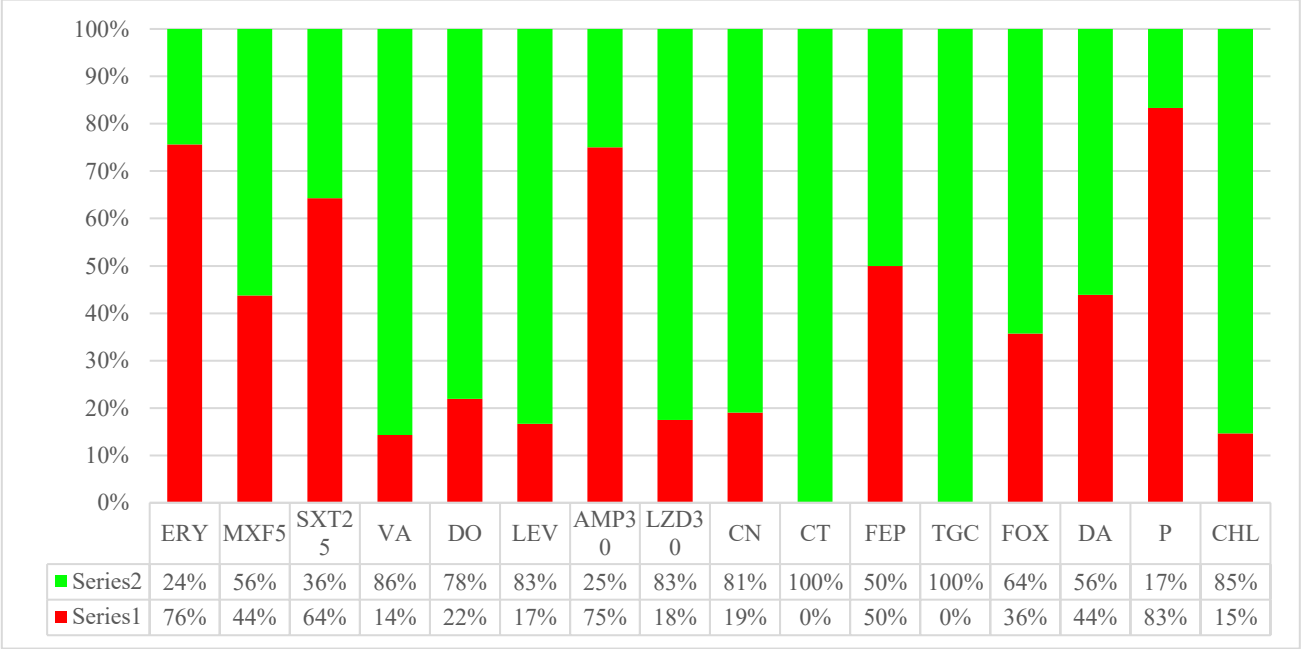
Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates exhibited a characteristic multidrug-resistant phenotype, demonstrating high resistance rates to beta-lactams and macrolides. Specifically, resistance was observed to penicillin (90%), erythromycin (87.5%), and cefoxitin (81.3%). Moderate resistance was noted for levofloxacin (67%) and clindamycin (45%).

In contrast, susceptibility remained high to linezolid (88%), vancomycin (85%), chloramphenicol (82%), gentamicin (82%), and doxycycline (78%). These results confirm that vancomycin and linezolid remain first-line therapeutic options, while chloramphenicol

Series 1: Resistance % **for** Methicillin-resistant *Staphylococcus aureus* (MRSA).
Series 2: Sensitivity % for Methicillin-resistant *Staphylococcus aureus* (MRSA).

Coagulase-negative *Staphylococcus* (CoNS) isolates demonstrated significant multidrug resistance. High resistance rates were observed against penicillin (83%), erythromycin (76%), and ampicillin (75%), indicating substantial ineffectiveness of beta-lactams and macrolides. Moderate resistance was recorded for trimethoprim-sulfamethoxazole (64%) and clindamycin (44%).

Susceptibility remained high for key therapeutic agents, including vancomycin (86% susceptible), linezolid (83%), chloramphenicol (85%), doxycycline (78%), and gentamicin (81%). Notably, susceptibility results for colistin and tigecycline were based on a single isolate and are not considered reliable.

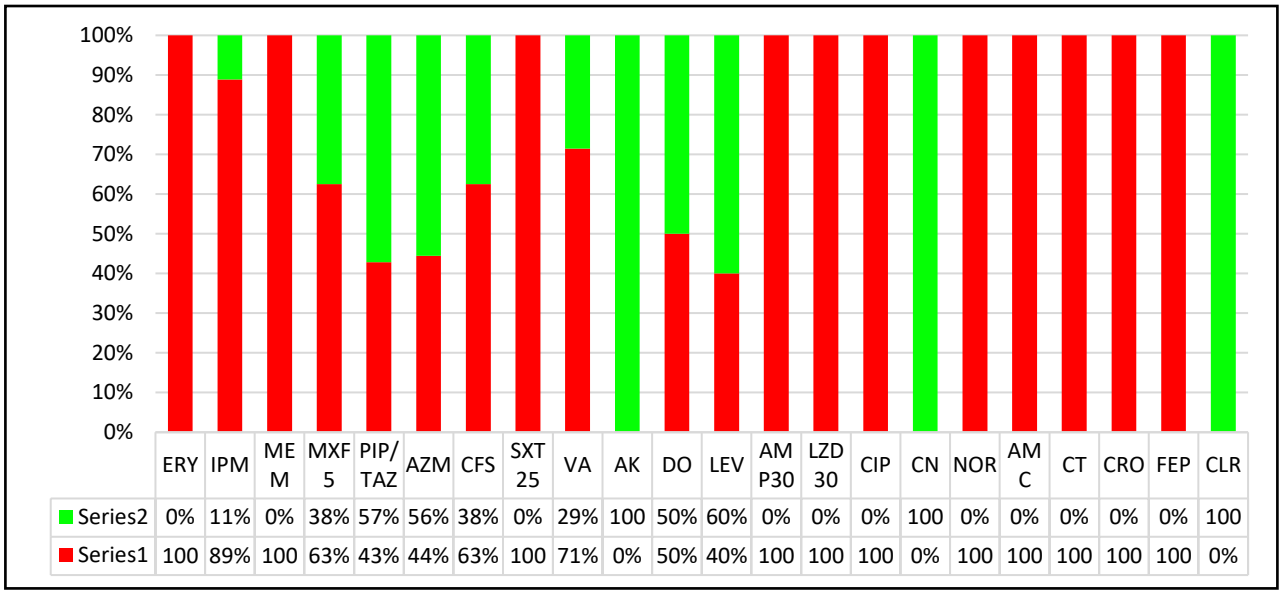


	ERY	MXF5	SXT25	VA	DO	LEV	AMP30	LZD30	CN	CT	FEP	TGC	FOX	DA	P	CHL
Series2	24%	56%	36%	86%	78%	83%	25%	83%	81%	100%	50%	100%	64%	56%	17%	85%
Series1	76%	44%	64%	14%	22%	17%	75%	18%	19%	0%	50%	0%	36%	44%	83%	15%

Series 1: Resistance % for *Staphylococcus* coagulase-negative (SCoN).
Series 2: Sensitivity % for *Staphylococcus* coagulase-negative (SCoN).

Gram-Negative Organisms

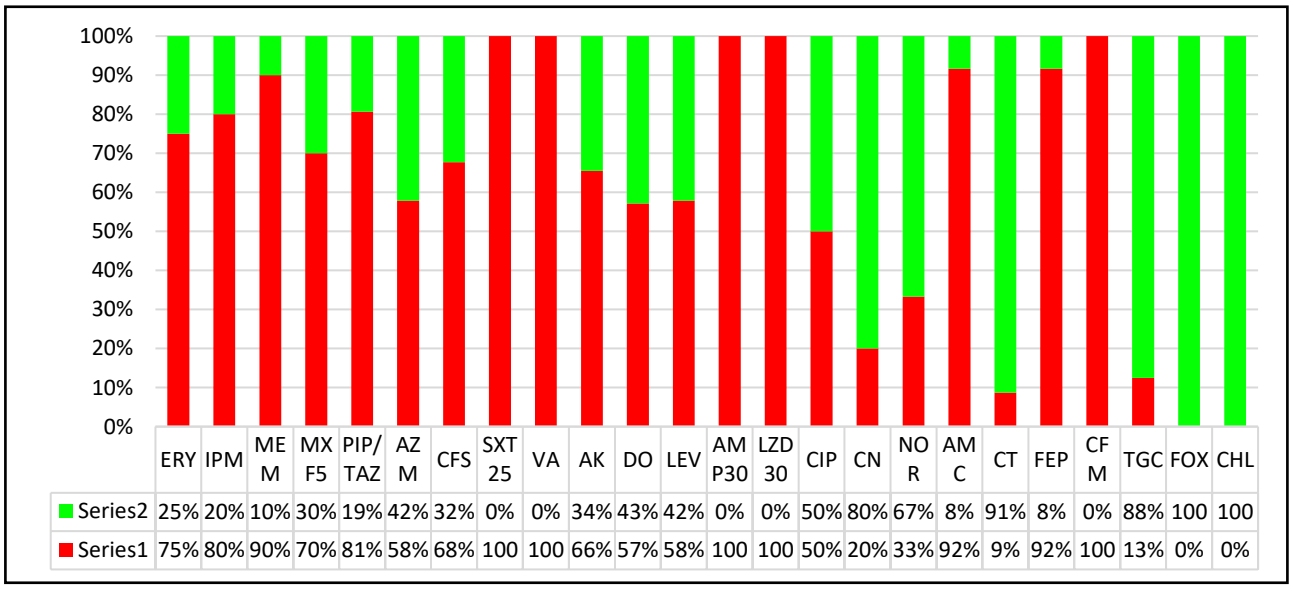
The analyzed *Escherichia coli* isolates demonstrated extensive multidrug resistance, though these findings are constrained by limited sample sizes for most antibiotics. Notably high resistance rates were observed to carbapenems (imipenem 89%, meropenem 100%), ampicillin (100%), and third-generation cephalosporins (ceftriaxone and cefepime, 100%). Fluoroquinolone resistance was also prevalent, with complete resistance to ciprofloxacin and norfloxacin. In contrast, amikacin exhibited 100% susceptibility (n=9).



Series 1: Resistance % **for** *Escherichia coli*.
Series 2: Sensitivity % for *Escherichia coli*.

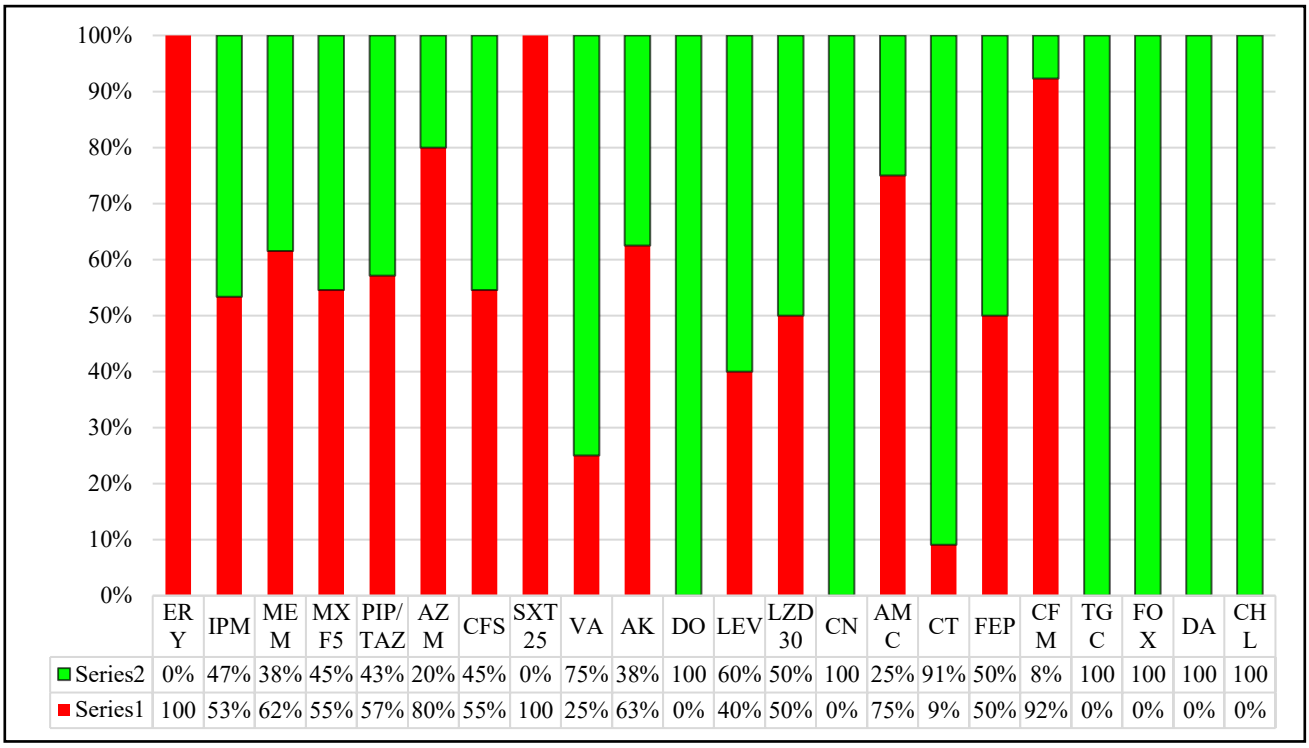
Klebsiella pneumoniae isolates exhibited alarming multidrug resistance, particularly to beta-lactams and carbapenems. Resistance to meropenem and imipenem was 90% and 80%, respectively. High resistance was also observed to cephalosporins, including cefepime (92%) and cefixime (100%), and to piperacillin-tazobactam (81%).

In contrast, colistin remained highly effective (91% susceptible), and tigecycline showed promising activity (88% susceptible), supporting their use as last-resort agents. Aminoglycoside activity was variable, with amikacin resistance at 66%.



Series 1: Resistance % **for** *Klebsiella pneumonia.*
Series 2: Sensitivity % for *Klebsiella pneumonia.*

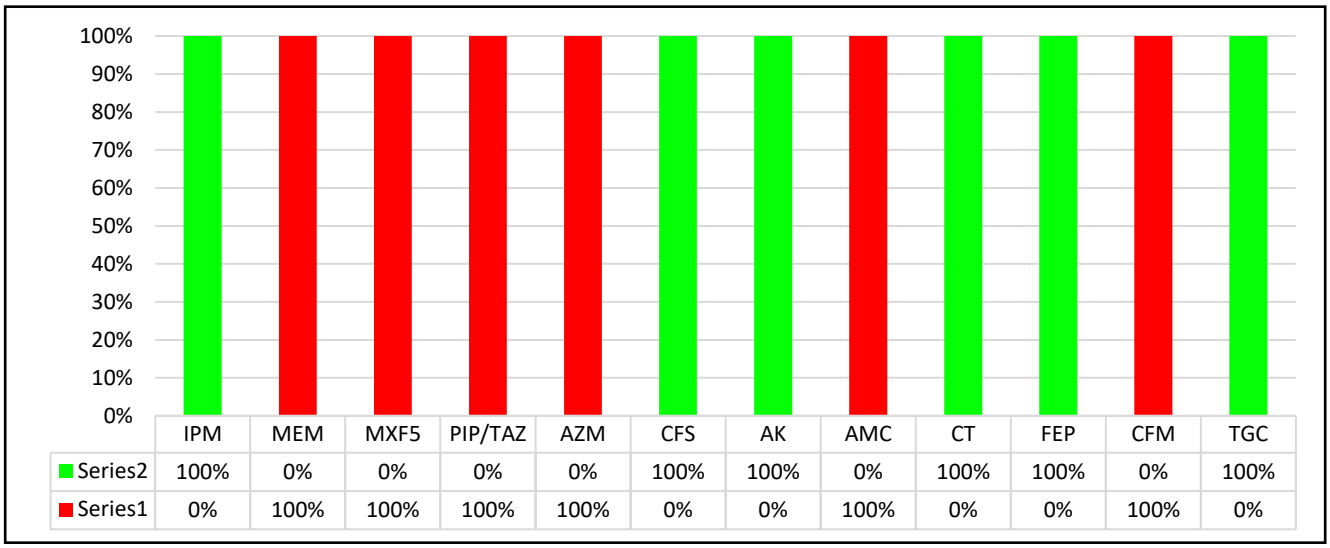
This investigation analyzed the susceptibility of 16 *Pseudomonas aeruginosa* blood culture isolates, revealing significant resistance to multiple antimicrobial classes. Carbapenem resistance was substantial, with 62% of isolates resistant to meropenem and 53% to imipenem. Resistance to broad-spectrum beta-lactams was also notable, including piperacillin-tazobactam (57%) and cefoperazone-sulbactam (55%). Among fluoroquinolones, moxifloxacin and levofloxacin resistance was 55% and 40%, respectively. Amikacin resistance was observed in 63% of isolates. In contrast, colistin remained highly effective, with a susceptibility rate of 91%.



	ERY	IPM	MEM	MXF5	PIP/TAZ	AZM	CFS	SXT25	VA	AK	DO	LEV	LZD30	CN	AMC	CT	FEP	CFM	TGC	FOX	DA	CHL
Series2	0%	47%	38%	45%	43%	20%	45%	0%	75%	38%	100	60%	50%	100	25%	91%	50%	8%	100	100	100	100
Series1	100	53%	62%	55%	57%	80%	55%	100	25%	63%	0%	40%	50%	0%	75%	9%	50%	92%	0%	0%	0%	0%

Series 1: Resistance % **for** *Pseudomonas aeruginosa.*
Series 2: Sensitivity % for *Pseudomonas aeruginosa.*

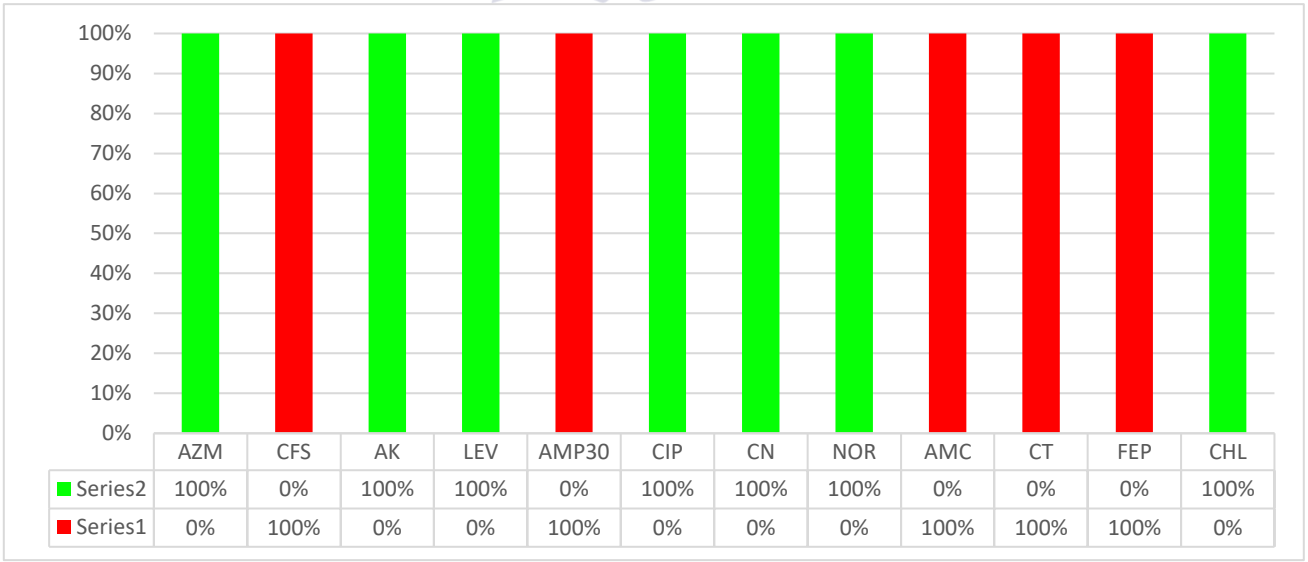
Both *Acinetobacter baumannii* isolates exhibited 100% resistance to meropenem, moxifloxacin, piperacillin-tazobactam, azithromycin, amoxicillin-clavulanate, and cefixime, suggesting a potential multidrug-resistant phenotype. Conversely, both isolates showed 100% susceptibility to imipenem, colistin, amikacin, cefoperazone-sulbactam, cefepime, and tigecycline.



Series 1: Resistance % **for** *Acinetobacter baumannii*.
Series 2: Sensitivity % for *Acinetobacter baumannii*.

Both *Salmonella Typhi* isolates exhibited complete resistance (100%) to ampicillin, cefoperazone/sulbactam, amoxicillin-clavulanate, colistin, and cefepime, suggesting significant ineffectiveness among beta-lactam agents.

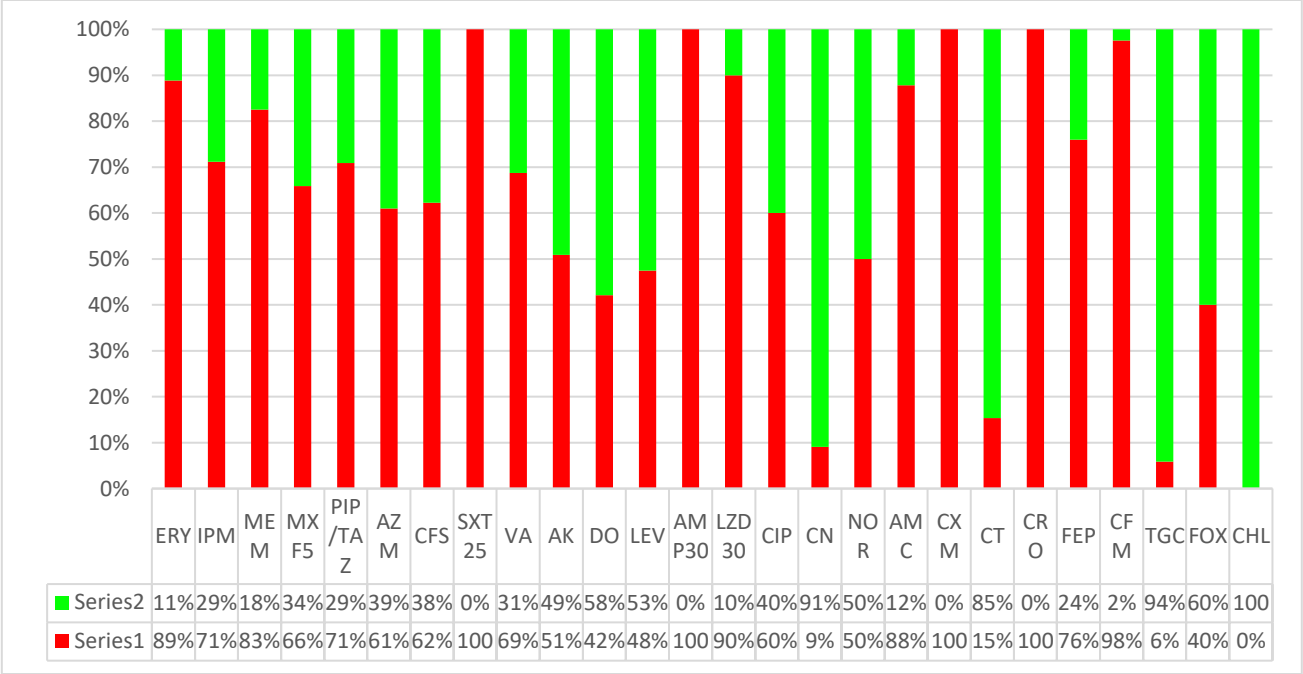
In contrast, susceptibility remained high (100%) to azithromycin, amikacin, levofloxacin, ciprofloxacin, gentamicin, norfloxacin, and chloramphenicol, indicating these may represent viable therapeutic alternatives.



Series 1: Resistance % **for** *Salmonella Typhi*.
Series 2: Sensitivity % for *Salmonella Typhi*.

The study revealed alarmingly high resistance rates among Gram-negative isolates. Carbapenem resistance was substantial, with 83% of isolates resistant to meropenem and 71% to imipenem. Resistance to third-generation cephalosporins was nearly universal, including cefixime (98%) and ceftriaxone (100%). High resistance was also observed to beta-lactam/beta-lactamase inhibitors such as amoxicillin-clavulanate (88%) and piperacillin-tazobactam (71%), as well as to fluoroquinolones like ciprofloxacin (60%).

Despite these trends, several antibiotics have retained efficacy. Colistin demonstrated high susceptibility (85%), affirming its role as a last-line agent. Aminoglycosides showed variable activity, with gentamicin maintaining 91% susceptibility, though amikacin was less effective (49% susceptible). Tigecycline also showed promising activity (91% susceptible).



	ERY	IPM	MEM	MXF5	PIP/TAZ	AZM	CFS	SXT25	VA	AK	DO	LEV	AMP30	LZD30	CIP	CN	NOR	AMC	CXM	CT	CRO	FEP	CFM	TGC	FOX	CHL
Series2	11%	29%	18%	34%	29%	39%	38%	0%	31%	49%	58%	53%	0%	10%	40%	91%	50%	12%	0%	85%	0%	24%	2%	94%	60%	100
Series1	89%	71%	83%	66%	71%	61%	62%	100	69%	51%	42%	48%	100	90%	60%	9%	50%	88%	100	15%	100	76%	98%	6%	40%	0%

Series 1: Resistance rates among Gram-negative isolates.
Series 2: Sensitivity rates among Gram-negative isolates.

DISCUSSION

The findings of this study paint a concerning picture of the high prevalence of antimicrobial resistance (AMR) among both Gram-positive and Gram-negative bacterial isolates from bloodstream infections, aligning with the global AMR crisis. The stark contrast in resistance patterns between these groups underscores the necessity for pathogen-specific therapeutic strategies and robust antimicrobial stewardship programs (Zjajo, 2024).

Among Gram-positive organisms, the high resistance rates in *Staphylococcus aureus* and coagulase-negative staphylococci to beta-lactams and macrolides are particularly alarming. The confirmation of methicillin resistance in over 80% of *S. aureus* isolates (via cefoxitin testing) signifies a challenging treatment landscape. However, the consistently high susceptibility to vancomycin and linezolid reinforces their status as cornerstone therapies for serious MRSA infections. The retained efficacy of older agents like chloramphenicol and doxycycline offers valuable alternative options for less severe cases or in resource-limited settings, potentially helping to preserve last-resort drugs.

The scenario is considerably more critical for Gram-negative bacteria. The high prevalence of carbapenem resistance in *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* is a major public health threat, as it effectively dismantles the primary defense against multidrug-resistant (MDR) Gram-negative infections. The near-universal resistance to third- and fourth-generation cephalosporins and fluoroquinolones further narrows the viable treatment options. This escalating resistance underscores the dire consequences of decades of antibiotic overuse and highlights the rapid horizontal transfer of resistance genes, such as those encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases.

In this grim context, colistin and tigecycline emerge as crucial last-line agents, demonstrating commendable in vitro efficacy against most Gram-negative isolates. However, their use is fraught with challenges, including toxicity (colistin) and uncertain pharmacokinetics (tigecycline). The variable susceptibility to aminoglycosides, such as the stark difference between amikacin and gentamicin, emphasizes the indispensable role of ongoing and precise local susceptibility testing to guide empiric therapy.

This study's limitations, including small sample sizes for some species-antibiotic combinations, preclude broad generalizations but serve as a critical snapshot of local resistance trends. The data unequivocally argue for the immediate implementation of enhanced infection control measures, strict antibiotic policies to curb the misuse of broad-spectrum drugs, and continued investment in surveillance to monitor the evolution of these dangerous resistance patterns. The development of novel antibacterial agents remains an urgent priority to avert a post-antibiotic era.

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