ORIGINAL ARTICLE

Antibiotic Resistance Pattern of Pathogens, Among Intensive Care Unit Patients: A Cross-Sectional Study at a Tertiary Care Hospital in Karachi, Pakistan

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ABSTRACT

Objective: To evaluate antibiotic resistance patterns of pathogens in patients admitted to the Intensive Care Unit.

Study Design: Retrospective cross-sectional study.

Place and Duration of Study: The study was conducted at Department of Microbiology, Combined Military Hospital (CMH), Karachi, Pakistan, from January 2021 to December 2021.

Methods: Patients with positive microbiological cultures from various specimens, collected during their stay in the Intensive Care Unit from January 2021 to December 2021, were included in the study. A non-probability consecutive sampling technique was used to enroll all eligible patients. Patients under 13 years, admitted for less than 48 hours, and referred from elsewhere were excluded.

At the patient's bedside, specimens were collected with aseptic measures and sent straightaway to the lab. Standard microbiological procedures with protocol were followed for culture, identification, and susceptibility testing. Statistical analysis was done by using SPSS version 25.

Results: The antimicrobial profile of Gram-positive cocci showed that all isolates of Staphylococcus aureus were Methicillin-resistant and sensitive to Linezolid and Vancomycin. Streptococcus pyogenes showed resistance (100%) to Ciprofloxacin, Doxycycline, Tigecycline, Clindamycin, Erythromycin, and Cotrimoxazole. 80% of Enterococcus species were resistant to Vancomycin; however, all isolates (100%) were susceptible to linezolid, Doxycycline, and Tigecycline.

All Gram-Negative Rod isolates were susceptible to polymyxin (100%). All Proteus mirabilis isolates were resistant to Ceftazidime, Co-trimoxazole, Doxycycline, and Tigecycline. Salmonella typhi isolates showed Extensive drug resistance. Acinetobacter baumannii isolates (100%) were resistant to Ceftriaxone, Meropenem, Piperacillin-Tazobactam, Amikacin, Gentamicin, Levofloxacin, and Co-trimoxazole. Among Klebsiella pneumoniae isolates, the resistance rates were 100% for Ceftriaxone, Meropenem, Piperacillin-Tazobactam, Amikacin, Gentamicin, Levofloxacin, and Doxycycline. 100% of E. coli isolates were resistant to Ceftriaxone, Piperacillin-Tazobactam, Doxycycline, Gentamicin, Levofloxacin, and Co-trimoxazole. Pseudomonas aeruginosa (60%) showed resistance to Meropenem and Piperacillin-Tazobactam; however, all isolates were resistant to Amikacin and Gentamicin.

Conclusion: Significant resistance patterns were observed in Gram-negative rods and Gram-positive cocci.

Keywords: *Antimicrobial Resistance, Drug Resistance, Intensive Care Units*

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Introduction

Antimicrobial resistance (AMR) has emerged as an alarming global health challenge, with low- and middle-income countries like Pakistan bearing a disproportionate burden due to factors such as unregulated antibiotic use, inadequate exacerbate control practices, and limited diagnostic facilities.[1,2]

In the intensive care units of tertiary care hospitals, where critically ill patients are most vulnerable, the prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens exacerbates morbidity, mortality, and healthcare costs.[3,4]

Recent studies from Karachi have highlighted alarming resistance patterns among ICU isolates. For instance, a cross-sectional study at the Indus Hospital & Health Network reported that among 1,785 carbapenem-resistant Gram-negative bacteria (CR-GNB) isolates, 1.7% were colistin-resistant, with *Klebsiella* species accounting for 77% of these cases.[5] Similarly, research from other tertiary care facilities in Karachi identified *Acinetobacter baumannii* as a predominant ICU pathogen, demonstrating high resistance to carbapenems and third-generation cephalosporins while maintaining susceptibility to tigecycline and polymyxin.[6]

These findings underscore the critical need for robust antimicrobial stewardship programs, routine surveillance, and adherence to infection control protocols to mitigate the spread of resistant pathogens in ICU settings.[7]

This article delves into current trends in antibiotic resistance among ICU patients at a tertiary care hospital in Karachi, analyzing the prevalence of MDR organisms and resistance patterns to inform future clinical management and public health policy in Pakistan.

Methods

This retrospective cross-sectional study was conducted over a period of one year, from January to December 2021 at Department of Microbiology, Combined Military Hospital (CMH), Karachi, Pakistan, from January 2021 to December 2021. using a non-probability consecutive sampling technique. The objective was to evaluate the antibiotic resistance patterns in ICU patients admitted to the Medical Intensive Care Unit (MICU) and Surgical Intensive Care Unit (SICU) of the hospital. Details of patients were collected from hospital records, including patient ID, age, gender, diagnosis, sample source, isolated pathogen, and the antibiotic susceptibility patterns. The study was approved by the Institutional Ethical Review Board of the hospital, vide certificate no: 132/2024/Trg/ERC, dated: 11th November 2021 to ensure compliance with ethical guidelines.

The inclusion criteria for the study were patients with positive microbiological cultures from clinical specimens (blood, urine, sputum, tracheal aspirates, pus, and wound swabs) collected during their ICU stay. The study excluded those patients who spent less than 48 hours in the ICU. Specimens were collected under aseptic conditions at the patient's bedside and immediately sent to the microbiology laboratory. Standard microbiological procedures were followed for culture, identification, and susceptibility testing. Cultures were inoculated onto MacConkey agar (MCA), chocolate agar (CA), or blood agar (BA) plates and incubated at 37°C for 18 to 24 hours. Blood cultures with positive growth were processed in an automated blood culture system (BactAlert 3D, bioMérieux, France). The identification of pathogens was based on colony morphology, Gram staining, and biochemical testing, in accordance with standard microbiological practices.

The Antimicrobial Susceptibility Test (AST) was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) plates. In the case of vancomycin, the E-strip was used to determine the minimum inhibitory concentration (MIC). Antimicrobial susceptibility testing (AST) results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[8,9] Salmonella isolates were considered XDR if resistant to ampicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, and ceftriaxone

Data from the patient records were entered into a relational database system designed for the study and exported into a spreadsheet for further analysis. The prevalence of antibiotic resistance was calculated by determining the percentage of positive bacterial cultures resistant to specific antibiotics. Statistical analysis was performed using SPSS version 25 for Windows.

Result

A total of 110 isolates were cultured from different clinical samples, including urine (27%), pus exudates (23%), respiratory (16%), blood (14%), tissue (11%), body fluids (5%), and cerebrospinal fluid (5%) (Table 1).

Table 1: Distribution of clinical samples for bacterial isolates (N = 110)

Types of samples	Frequency (%)
Urine	30 (27)
Exudates (e.g., pus, wound swabs, stitch line swabs)	25 (23)
Respiratory samples (e.g., BAL, tracheal aspirate, sputum, ET tube, nasal swabs)	18 (16)
Blood/CVP tip	15 (14)
Tissue	12 (11)
Body fluids (e.g., peritoneal, pleural)	5 (5)
CSF (Cerebrospinal fluid/ VP shunt)	5 (5)

Table 2: General characteristics of patients included in study (N = 110)

Factors	Frequency (%)
Gender	
Male	60 (54.5)
Female	50 (45.5)
Age range	
13–30 years	25 (22.7)
31– 45 years	40 (36.4)
46-65 years	45 (40.9)
Department	
MICU	55 (50)
SICU	55 (50)

Table 3: Shows the percentage of isolated bacteria from clinical samples (N = 110)

Isolates	Frequency (%)
Escherichia coli	20 (18)
Staphylococcus aureus, all MRSA	15 (14)
Acinetobacter baumannii	14 (13)
Klebsiella pneumoniae	13 (12)
Burkholderia cepacia	10 (9)
Enterococcus species	10 (9)
Pseudomonas aeruginosa	10 (9)
Enterobacter species	05 (5)
Salmonella typhi	05 (5)
Streptococcus pyogenes	04 (4)
Proteus mirabilis	02 (2)
Serratia species	02 (2)

Samples were received from ICUs of surgical (50%) and medical (50%) wards, including 25% of patients aged 13 to 30 years, whereas 85% were aged 31 to 65 years (Table 2).

Among GNR bacterial isolates, the most common species identified was E. coli (18%), followed by Acinetobacter baumannii (13%) and Klebsiella pneumoniae (12%), whereas among gram-positive cocci (GPC), the most common was Staphylococcus aureus (14%), followed by Enterococcus species (9%) and Streptococcus pyogenes (4%) (Table 3).

The antimicrobial profile of GPC showed that all

Staphylococcus aureus isolates were methicillin-resistant (MRSA) and sensitive to LNZ and VAN. Streptococcus pyogenes showed resistance (100%) to CIP, DOX, and CLI, whereas 50% of isolates were resistant to LEV, E, and COT. 80% of Enterococcus species were vancomycin-resistant (VRE); however, all isolates (100%) were susceptible to LNZ and TGC (Table 4).

Table 4: Shows antimicrobial susceptibility profile of gram-positive cocci (GPC) (N= 29)

Antibiotics tested	Susceptibility profile	*Staphylococcus aureus* All MRSA (N = 15)	*Streptococcus pyogenes* (N=4)	*Enterococcus* spp (N=10)
AK (N%)	Susceptible	0 (00)	NT	NT
	Resistant	15 (100)	-	-
P(N%)	Susceptible	0 (00)	4 (100)	NT
	Resistant	100	0 (00)	-
CRO (N%)	Susceptible	0 (00)	4 (100)	IR
	Resistant	15 (100)	0 (00)	IR
AMP (N%)	Susceptible	0 (00)	4 (100)	2 (20)
	Resistant	15 (100)	0 (00)	8 (80)
AUG (N%)	Susceptible	0 (00)	4 (100)	2 (20)
	Resistant	15 (100)	0 (00)	8 (80)
CLO (N%)	Susceptible	0 (00)	NT	NT
	Resistant	15 (100)		
CAP (N%)	Susceptible	0 (00)	NT	NT
	Resistant	15 (100)	-	-
CIP (N%)	Susceptible	0 (00)	0 (00)	0 (00)
	Resistant	15 (100)	4 (100)	10 (100)
CLI (N%)	Susceptible	0 (00)	0 (00)	IR
	Resistant	15 (100)	4 (100)	IR
COT (N%)	Susceptible	0 (00)	2 (50)	IR
	Resistant	15 (100)	2 (50)	IR
DOX (N%)	Susceptible	0 (00)	0 (00)	0 (00)
	Resistant	15 (100)	4 (100)	10 (100)
E (N%)	Susceptible	0 (00)	2 (50)	0 (00)
	Resistant	15 (100)	2 (50)	10 (100)
GEN (N%)	Susceptible	0 (00)	NT	NT
	Resistant	15 (100)	-	-
LNZ (N%)	Susceptible	15 (100)	4 (100)	10 (100)
	Resistant	0 (00)	0 (00)	0 (00)
VAN (N%)	Susceptible	15 (100)	4 (100)	2 (20)
	Resistant	0 (00)	0 (00)	8 (80)
TGC (N%)	Susceptible	0 (00)	NT	10 (100)
	Resistant	15 (100)	-	0 (00)
LEV (N%)	Susceptible	0 (00)	02 (50)	0 (00)
	Resistant	15 (100)	02 (50)	10 (100)

N = Number of isolates, NT = Not tested, AK = Amikacin, AMP = Ampicillin, AUG = Augmentin CAP=Chloramphenicol, CIP=Ciprofloxacin, CLO=Cloxacillin, CLI=Clindamycin, CRO=Ceftriaxone, DOX=Doxycycline, E=Erythromycin, GEN=Gentamicin, LEV=Levofloxacin, LNZ=Linezolid, P=Penicillin VAN=Vancomycin, COT=Co-trimoxazole, TGC=Tigecycline, spp=species.IR. Intrinsic Resistance

All GNR isolates were susceptible to PB (100%), except for Proteus species, Serratia species, and Burkholderia cepacia, which are intrinsically resistant to PB. All Proteus mirabilis isolates were resistant to CAZ, COT, DOX, and TGC; however, no isolate was resistant to CRO, MEM, TZP, AK, or GEN. Isolates of Burkholderia, Serratia, and Enterobacter species showed no resistance to MEM (0%). All Salmonella Typhi isolates were XDR (resistant to AMP, COT, CAP, CIP, and CRO) and sensitive only to MEM and AZM. Acinetobacter baumannii isolates (100%) were resistant to CRO, MEM, TZP, AK, GEN, LEV, and COT, whereas 43% of isolates were resistant to DOX and TGC. Among Klebsiella pneumoniae isolates, the resistance rates were 100% to CRO, MEM, TZP, AK, GEN, LEV, and DOX, whereas they were 39% to TGC and 77% to COT. 100% of E. coli isolates were resistant to CRO, TZP, DOX, LEV, GEN, and COT, whereas resistance against AK and MEM was 20% and 40%, respectively. Resistance of Pseudomonas aeruginosa to both MEM and TZP was 60%; however, all isolates were resistant to AK and GEN (Table 5).

Discussion

Antimicrobial resistance (AMR) remains a critical challenge in healthcare systems worldwide, particularly in intensive care units (ICUs), where patients are at increased risk of hospital-acquired infections and frequently require broad-spectrum antimicrobial therapy.[10] The current study from a tertiary care hospital in Karachi highlights concerning resistance profiles among both Gram-negative rods (GNR) and Gram-positive cocci (GPC), emphasizing the need for targeted stewardship and stringent infection control strategies.[11]

Among the 110 specimens collected from surgical and medical ICUs, the highest yield was from urine and pus samples, sites commonly associated with nosocomial infections. Predominant GNR included *Escherichia coli, Acinetobacter baumannii,* and *Klebsiella pneumoniae*, while *Staphylococcus aureus* and *Enterococcus* spp. dominated the GPC isolates. Similar pathogen distributions have been reported in South Asian ICU settings, reaffirming the regional burden of these organisms.[12,13]

High resistance rates were observed among GNR against β-lactams, particularly cephalosporins and piperacillin-tazobactam. This trend is consistent with , the increasing production of extended-spectrum β-lactamases (ESBLs), and the global spread of carbapenem-resistant Enterobacteriaceae (CRE).[14,15] While carbapenems, especially meropenem, retained partial efficacy, resistance among *K. pneumoniae* and *A. baumannii* was alarmingly high, thereby narrowing treatment options.[16]

Polymyxin B remained effective against most GNR, except for intrinsically resistant species such as *Proteus, Serratia,* and *Burkholderia*. Despite this, its nephrotoxicity and the risk of resistance emergence limit its role in empirical therapy.[17,18] *E. coli* isolates showed universal resistance to commonly prescribed antibiotics, including cephalosporins, doxycycline, levofloxacin, gentamicin, and cotrimoxazole, whereas resistance to meropenem and amikacin was lower (40% and 20%, respectively). Comparable resistance trends have been documented in Pakistan and India, largely linked to antibiotic misuse and weak infection control practices.[19,20]

Pseudomonas aeruginosa presented a particularly worrisome profile, with 60% resistance to meropenem and piperacillin-tazobactam, and 100% resistance to aminoglycosides, including amikacin and gentamicin. These findings mirror global reports highlighting *P. aeruginosa* intrinsic resistance mechanisms and genetic adaptability.[11]

The detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) further complicates ICU management. MRSA necessitates rigorous decolonization and hand hygiene practices, while VRE, identified in 80% of *Enterococcus* isolates in this study, remains associated with poor outcomes. Encouragingly, all VRE isolates were susceptible to linezolid and tigecycline, offering potential salvage options.[21]

Overall, the observed resistance patterns underscore the urgency of tailoring empirical therapy to local antibiograms rather than to standardized regimens. International frameworks strongly advocate antimicrobial stewardship, active surveillance, and adherence to infection prevention and control protocols to mitigate the threat of multidrug-resistant organisms. [22]

This was a single-center, retrospective, descriptive, cross-sectional study, which may limit the

Table 5: Shows antimicrobial susceptibility profile of GNR (N=81)

Antibiotics tested	Susceptibility profile	*S. typhi* All XDR N=5 (5%)	*A. baumannii* N=14 (13%)	*K. pneumoniae* N=13 (12%)	*E. coli* N=20 (18%)	*Enterobacter.* spp N=5 (5%)	*P. aeruginosa* N=10 (9%)	*P. mirabilis* N=2 (2%)	*Serratia* spp N=2 (2%)	*B. cepacia* N=10 (9%)
CRO (N%)	Susceptible	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	IR	2 (100)	0 (00)	0 (00)
	Resistant	5 (100)	14 (100)	13 (100)	20 (100)	5 (100)		0 (00)	2 (100)	10 (100)
MEM (N%)	Susceptible	5 (100)	0 (00)	0 (00)	12 (60)	5 (100)	4 (40)	2 (100)	2 (100)	10 (100)
	Resistant	0 (00)	14 (100)	13 (100)	8 (40)	0 (00)	6 (60)	0 (00)	0 (00)	0 (00)
AK (N%)	Susceptible	NT	0 (00)	0 (00)	16 (80)	5 (100)	0 (00)	2 (100)	0 (00)	0 (00)
	Resistant	-	14 (100)	13 (100)	4 (20)	0 (00)	10 (100)	0 (00)	2 (100)	10 (100)
GEN (N%)	Susceptible	NT	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	2 (100)	0 (00)	0 (00)
	Resistant	-	14 (100)	13 (100)	20 (100)	5 (100)	10 (100)	0 (00)	2 (100)	NT
LEV (N%)	Susceptible	NT	0 (00)	0 (00)	0 (00)	3 (60)	NT	2 (100)	2 (100)	6 (60)
	Resistant	-	14 (100)	13 (100)	20 (100)	2 (40)	-	0 (00)	0 (00)	4 (40)
TZP (N%)	Susceptible	NT	0 (00)	0 (00)	0 (00)	3 (60)	4 (40)	2 (100)	0 (00)	0 (00)
	Resistant	-	14 (100)	13 (100)	20 (100)	2 (40)	6 (60)	0 (00)	2 (100)	10 (100)
DOX (N%)	Susceptible	NT	8 (57)	0 (00)	0 (00)	0 (00)	IR	0 (00)	2 (100)	0 (00)
	Resistant	-	6 (43)	13 (100)	20 (100)	5 (100)	-	2 (100)	0 (00)	10 (100)
TGC (N%)	Susceptible	NT	8 (57)	8 (62)	20 (100)	0 (00)	IR	0 (00)	0 (00)	0 (00)
	Resistant	-	6 (43)	5 (38)	0 (00)	5 (100)	-	2 (100)	2 (100)	10 (100)
CT(N%)	Susceptible	NT	14 (100)	13 (100)	20 (100)	5 (100)	10 (100)	IR	IR	IR
	Resistant	-	0 (00)	0 (00)	0 (00)	0 (00)	0 (00)	-	-	-
COT (N%)	Susceptible	0 (00)	0 (00)	3 (23)	0 (00)	3 (60)	IR	0 (00)	2 (100)	7 (70)
	Resistant	5 (100)	14 (100)	10 (77)	20 (100)	2 (40)	-	2 (100)	0 (00)	3 (30)
CAZ (N%)	Susceptible	NT	NT	NT	NT	0 (00)	NT	0 (00)	0 (00)	5 (50)
	Resistant	-	-	-	-	5 (100)	-	2 (100)	2 (100)	5 (50)
AZM (N%)	Susceptible	5 (100)	NT	NT	NT	NT	NT	NT	NT	NT
	Resistant	0 (00)	-	-	-	-	-	-	-	-
AMP	Susceptible	0 (00)	NT	NT	0 (00)	NT	NT	NT	NT	NT
	Resistant	5 (100)	-	-	20 (100)	-	-	-	-	-
CIP	Susceptible	0 (00)	NT	NT	NT	NT	NT	NT	NT	NT
	Resistant	5 (100)	-	-	-	-	-	-	-	-
CAP	Susceptible	0 (00)	NT	NT	NT	NT	NT	NT	NT	NT
	Resistant	5 (100)	-	-	-	-	-	-	-	-

N = Number of isolates, IR = Intrinsic Resistance, NT=Not tested, AK=Amikacin, AMP= Ampicillin CAP=Chloramphenicol, CIP=Ciprofloxacin, CLO=Cloxacillin, CLI=Clindamycin, CRO=Ceftriaxone, DOX=Doxycycline, E=Erythromycin, GEN=Gentamicin, IMP=Imipenem, LEV=Levofloxacin, LNZ=Linezolid, MEM=Meropenem, CT =colistin, COT=Co-trimoxazole, TGC=Tigecycline, TZP=Piperacillin-Tazobactam, CAZ=Ceftazidime, AZM=Azithromycin, spp = Species, S. typhi = Salmonella typhi, A. baumannii = Acinetobacter baumannii, K. pneumoniae = Klebsiella pneumoniae, E. coli = Escherichia coli, Entero = Enterobacter, P. aeruginosa = Pseudomonas aeruginosa, B. cepacia = Burkholderia cepacia, P. mirabilis= Proteus mirabilis

generalizability of the findings. Only culture-positive isolates were included, which may introduce selection bias. Some organisms had small isolate numbers, limiting statistical robustness. Routine clinical data were utilized, and although standard protocols were followed, inherent variability in clinical practice may exist. Finally, molecular resistance mechanisms and correlations with patient outcomes were not assessed, limiting deeper insights into the drivers and clinical impact of antimicrobial resistance in this population.

Conclusion

This study identifies a high burden of antimicrobial resistance among ICU pathogens, particularly Gram-negative bacteria. The findings highlight the need for local antibiogram-guided therapy, continuous surveillance, and strengthened antimicrobial stewardship programs.

However, GPC, especially Staphylococcus and Enterococcus species, showed resistance to penicillin and macrolides. The identification of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) highlights the significance of treatment challenges faced by clinicians due to the development of antibiotic resistance. The increasing trends in antimicrobial resistance underscore the importance of antibiotic stewardship and continuous surveillance.

Findings of this study highlight the importance of surveillance reporting of routine antimicrobial susceptibility testing to stay updated on emerging antimicrobial resistance and to guide empirical treatment. Measures such as stringent infection control practices, regular surveillance, and judicious use of antimicrobials are therefore required to reduce the emergence of antimicrobial resistance and improve patients' clinical outcomes.

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Author Contributions

ST: Conception, design of the work, and approval for final submission
TK: Writing the original draft, proofreading, and approval for final submission
SHA: Manuscript writing for methodology design, investigation, and approval for final submission
SM: Data acquisition, curation, statistical analysis, and approval for final submission
FS: Revising, editing, supervising for intellectual content, and approving for final submission
SA: Validation of data, interpretation, write-up of results, and approval for final submission
ST is the nominated guarantor and takes full responsibility for the overall content and integrity of the work