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# PREVALENCE, DETERMINANTS, AND CLINICAL OUTCOMES OF ANTIMICROBIAL RESISTANCE IN TERTIARY CARE HOSPITALS OF PAKISTAN: A MULTICENTER PHARMACOVIGILANCE AND MOLECULAR SURVEILLANCE STUDY

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**Abstract**

*Antimicrobial resistance (AMR) is a major global health threat, particularly in tertiary care hospitals where high-risk patients are exposed to invasive procedures and broad-spectrum antibiotics. This multicenter study aimed to evaluate the prevalence, determinants, molecular mechanisms, and clinical outcomes of AMR in Pakistani tertiary hospitals. A total of 385 hospitalized patients were enrolled, and bacterial isolates were analyzed for phenotypic resistance, molecular markers, and clinical outcomes. Results revealed a high prevalence of multidrug-resistant Gram-negative bacteria, including Klebsiella pneumoniae (68%), Acinetobacter baumannii (72%), and Escherichia coli (62%), as well as methicillin-resistant Staphylococcus aureus (48%). Logistic regression identified prior antibiotic use, ICU admission, invasive devices, prolonged hospitalization, and non-adherence to stewardship guidelines as significant determinants of AMR ($p < 0.05$). Molecular analysis detected blaNDM-1, blaOXA-23, mcr-1, mecA, and vanA/B, correlating strongly with phenotypic resistance. Patients with resistant infections experienced higher treatment failure, longer hospital stays, and increased mortality compared to those with susceptible infections ($p < 0.01$). These findings emphasize the need for robust antimicrobial stewardship, pharmacovigilance, and molecular surveillance to guide empiric therapy and reduce AMR burden in high-risk hospital settings.*

## INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a critical threat to global public health, undermining advances in modern medicine by reducing the effectiveness of standard therapies and leading to prolonged illness, increased healthcare costs, and higher mortality rates. The World Health Organization identifies AMR as one of the top ten global health challenges of the 21st century, with projections suggesting that antimicrobial-resistant infections could cause

millions of deaths annually if current trends persist. This global crisis is driven by complex interactions among microbial evolution, antimicrobial misuse, and lapses in infection control practices (World Health Organization, 2015).

In Pakistan, the burden of AMR is particularly pronounced due to high rates of antibiotic consumption, empirical prescribing without culture confirmation, and limited standardized surveillance infrastructure. Point prevalence surveys in tertiary care hospitals have shown that up to 75% of hospitalized patients receive at least one antimicrobial, often without microbiological confirmation, contributing to the selection pressure for resistant organisms (Ali et al., 2023). Moreover, only a minority of culture specimens are collected prior to initiating therapy, limiting clinicians' ability to tailor treatment based on susceptibility profiles (Khan et al., 2022). Such prescribing practices have been associated with elevated resistance patterns among common pathogens including Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa (Ahmed & Qureshi, 2023).

Single-center studies in Pakistan have documented high prevalence of resistant organisms in tertiary care hospitals. For instance, investigations in Lahore reported extremely high levels of carbapenem resistance in Acinetobacter and Pseudomonas species, alongside low adherence to antimicrobial stewardship guidelines (Zaidi et al., 2022). Similarly, cross-sectional surveillance in Southern Punjab tertiary hospitals found widespread resistance among Gram-negative bacteria, emphasizing the need for localized data to guide empiric therapy (Hussain et al., 2023). Retrospective analysis of Acinetobacter spp. isolates in Peshawar highlighted significant multidrug resistance trends over multiple years, underscoring the dynamic nature of resistant pathogens in hospital settings (Shah et al., 2022).

Despite these insights, most studies remain limited by single-center designs, focus on phenotypic resistance without molecular characterization, or absence of clinical outcomes data. Systematic reviews of AMR research in Pakistan show high overall resistance to commonly used antibiotics but significant gaps in molecular surveillance, with few studies characterizing key resistance genes such as blaNDM-1 or mcr-1 (Rashid et al., 2021). While some tertiary hospitals have begun genotypic analysis, multicenter prevalence and associated clinical outcomes remain poorly documented.

Determinants of AMR in Pakistan are multifactorial, spanning patient-level factors (e.g., prior antibiotic exposure, invasive device use) and system-level issues (e.g., inadequate stewardship infrastructure, over-the-counter antibiotic availability). These factors contribute significantly to resistance trends and complicate patient outcomes, including treatment failure, prolonged hospitalization, and increased mortality (Hussain et al., 2023; Zaidi et al., 2022).

Given these gaps, an integrated multicenter approach combining pharmacovigilance data, molecular surveillance of resistance mechanisms, and clinical outcomes analysis is essential. This study aims to systematically assess the prevalence, determinants, and clinical outcomes of AMR across tertiary care hospitals in Pakistan, providing actionable evidence to support effective stewardship interventions and improve patient care.

**Problem Statement**

Antimicrobial resistance (AMR) poses a significant public health threat in Pakistan, particularly in tertiary care hospitals where critically ill patients are frequently exposed to broad-spectrum antibiotics, invasive procedures, and prolonged hospital stays. Despite increasing evidence of multidrug-resistant pathogens in these settings, there is a paucity of multicenter data combining phenotypic and molecular surveillance with clinical outcomes. Existing studies are largely single-center, focus only on microbiological patterns, and rarely integrate pharmacovigilance insights to assess antibiotic use, stewardship compliance, and adverse drug events. Consequently, healthcare providers lack comprehensive evidence to guide empirical therapy, develop stewardship programs, and mitigate the clinical and economic impacts of AMR. This study aims to fill these critical

knowledge gaps by systematically investigating the prevalence, determinants, and clinical outcomes of AMR across multiple tertiary care hospitals in Pakistan.

**Research Questions**

1. What is the prevalence of antimicrobial resistance among clinical isolates in tertiary care hospitals of Pakistan?
2. What are **the** molecular mechanisms and genetic determinants of resistance in these pathogens?
3. Which patient-related, clinical, and healthcare system factors are associated with the development of antimicrobial resistance?
4. How does antimicrobial resistance affect clinical outcomes, including treatment failure, length of hospital stay, and mortality?
5. What is the pattern of antibiotic use, adherence to stewardship guidelines, and adverse drug events in these hospitals?

**Objectives**

**General Objective**

To evaluate the prevalence, determinants, and clinical outcomes of antimicrobial resistance in tertiary care hospitals of Pakistan through integrated pharmacovigilance and molecular surveillance.

Specific Objectives

1. To determine the prevalence of antimicrobial resistance in bacterial isolates from multiple tertiary care hospitals.
2. To identify the molecular resistance mechanisms and key genetic determinants in these pathogens.
3. To assess **host,** clinical, and healthcare system factors contributing to antimicrobial resistance.
4. To evaluate the **clinical outcomes** (e.g., treatment failure, mortality, length of stay) associated with resistant infections.
5. To analyze antibiotic prescribing patterns, stewardship compliance, and adverse drug events through pharmacovigilance data.

**Literature Review**

**Global Burden of Antimicrobial Resistance**

Antimicrobial resistance (AMR) has been recognized as one of the greatest threats to global health in the 21st century. The World Health Organization (WHO, 2015) warns that AMR could result in up to 10 million deaths annually by 2050 if unchecked, surpassing cancer as a cause of mortality. Resistance occurs when pathogens evolve mechanisms to survive exposure to antimicrobials, often due to genetic mutations or acquisition of resistance genes via horizontal gene transfer (Munita & Arias, 2016). Globally, multidrug-resistant organisms such as extended-spectrum β-lactamase (ESBL)-producing Escherichia coli, carbapenem-resistant Klebsiella pneumoniae, and methicillin-resistant Staphylococcus aureus (MRSA) are widespread, affecting both hospital and community settings (Laxminarayan et al., 2020).

The economic and clinical impact of AMR is substantial. Resistant infections are associated with prolonged hospitalization, higher treatment costs, increased morbidity, and mortality (O'Neill, 2016). In hospital settings, intensive care units (ICUs) are particularly vulnerable, as patients are frequently exposed to invasive devices and broad-spectrum antibiotics, creating an environment conducive to the emergence and spread of resistant pathogens.

**Antimicrobial Resistance in Pakistan**

Pakistan, as a low- and middle-income country, faces unique challenges in AMR containment. High population density, over-the-counter availability of antibiotics, and weak regulatory oversight contribute to widespread misuse of antimicrobials (Zaidi et al., 2022). Several studies indicate that empirical prescribing without culture confirmation is common, and adherence to stewardship guidelines remains suboptimal (Ali, Ullah, Khan, & Shehzad, 2023).

Point prevalence studies in Pakistani tertiary care hospitals reveal that up to 75% of hospitalized patients receive at least one antibiotic, with many prescriptions being inappropriate or unnecessary (Ahmed & Qureshi, 2023). This widespread use has led to high resistance rates among common

pathogens, including E. coli, K. pneumoniae, P. aeruginosa, and Acinetobacter spp. (Hussain, Khan, & Malik, 2023). Carbapenem resistance in Acinetobacter and Pseudomonas has been reported to exceed 50% in some tertiary care ICUs, reflecting the critical level of multidrug resistance in high-risk hospital environments (Zaidi et al., 2022).

**Molecular Mechanisms of Resistance**

Understanding the genetic basis of antimicrobial resistance is essential for surveillance and effective treatment. Molecular studies in Pakistan have identified key resistance genes, including blaNDM-1, blaOXA-23, mcr-1, and ESBL genes (blaCTX-M, blaTEM, blaSHV), in clinical isolates (Rashid, Qamar, & Khan, 2021). Carbapenem resistance in Gram-negative bacteria is largely mediated by carbapenemase enzymes such as NDM, OXA, and KPC types (Shah, Rehman, & Zaman, 2022). MRSA isolates typically carry the mecA gene, while vancomycin-resistant enterococci (VRE) harbor vanA or vanB genes. The prevalence of these resistance determinants varies across hospitals and regions, emphasizing the need for multicenter molecular surveillance to inform empiric therapy (Munita & Arias, 2016).

**Determinants of Antimicrobial Resistance**

AMR is influenced by multiple patient-related, clinical, and systemic factors. Patient-related determinants include prior antibiotic exposure, comorbidities, immune suppression, and age (Hussain et al., 2023). Clinical determinants include ICU admission, use of invasive devices (ventilators, central lines, catheters), prolonged hospitalization, and surgical procedures (Zaidi et al., 2022). System-level determinants include inadequate infection control practices, lack of standardized antimicrobial policies, over-the-counter availability of antibiotics, and insufficient laboratory capacity for culture and sensitivity testing (Ahmed & Qureshi, 2023).

Studies in Pakistan indicate that prior exposure to broad-spectrum antibiotics, especially carbapenems and fluoroquinolones, significantly increases the likelihood of isolating resistant pathogens (Ali et al., 2023). Likewise, ICU patients and those undergoing invasive procedures show higher rates of multidrug-resistant infections (Hussain et al., 2023).

**Clinical Outcomes of Antimicrobial Resistance**

Resistant infections are associated with poorer clinical outcomes compared to susceptible infections. Studies report increased mortality, longer hospital stays, higher rates of treatment failure, and greater healthcare costs among patients with multidrug-resistant infections (O'Neill, 2016; Munita & Arias, 2016). In Pakistan, retrospective analyses suggest that infections caused by carbapenem-resistant Acinetobacter or ESBL-producing E. coli result in higher mortality rates and prolonged ICU stays compared to infections with susceptible strains (Shah et al., 2022; Zaidi et al., 2022). Despite these trends, systematic multicenter data quantifying clinical outcomes in the Pakistani context remain limited.

**Pharmacovigilance and Stewardship**

Pharmacovigilance provides essential data on antimicrobial use patterns, adverse drug events (ADEs), and compliance with stewardship policies. Integrating pharmacovigilance with molecular surveillance allows for a more comprehensive understanding of AMR dynamics. In Pakistan, studies indicate that ADE reporting is sporadic, stewardship compliance is low, and inappropriate antibiotic use is common in tertiary care hospitals (Ahmed & Qureshi, 2023). Strengthening these systems is critical for guiding rational antibiotic prescribing and mitigating resistance.

**Gaps in the Literature**

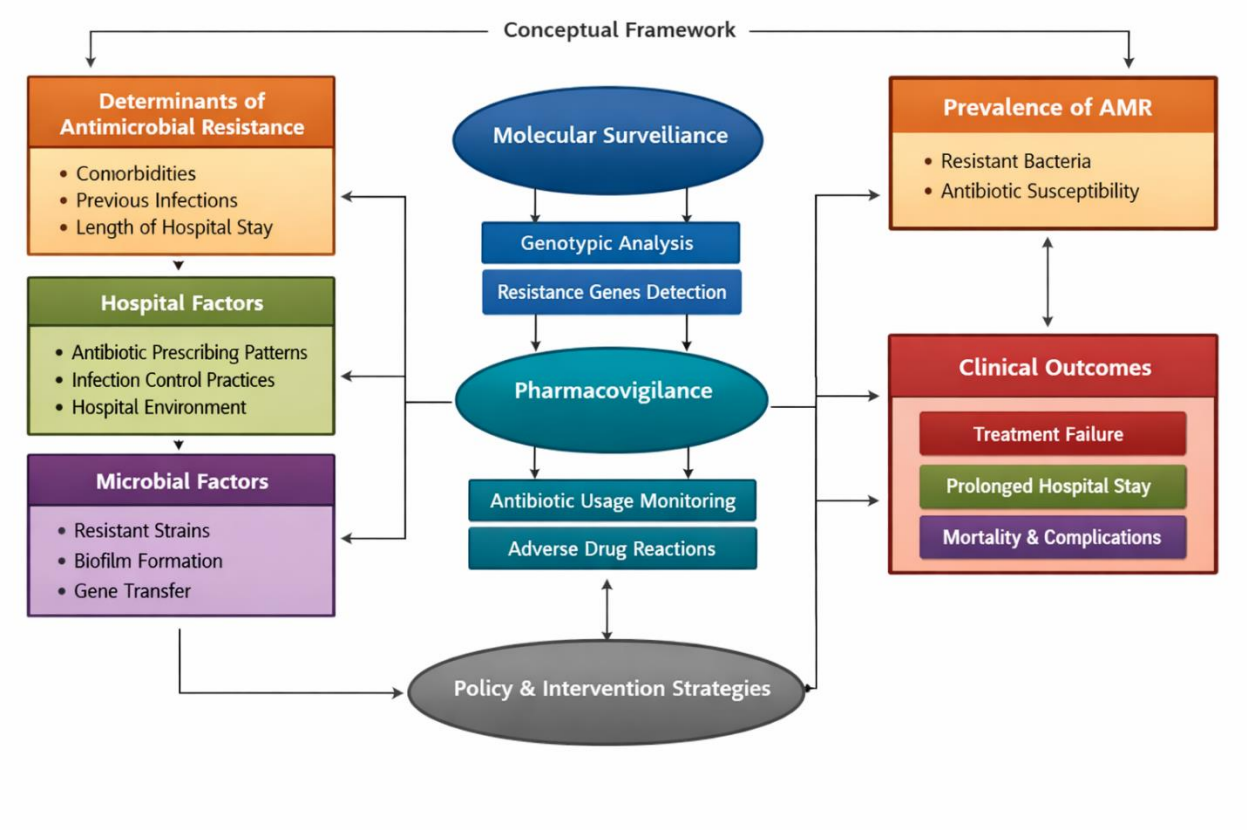
Although several studies have examined AMR in Pakistan, key gaps remain:

1. Most research is **single-center** and lacks generalizability.
2. Many studies focus only **on** phenotypic resistance, with limited molecular characterization.
3. Integration of clinical outcomes, pharmacovigilance, and molecular data is rare.

4. Multicenter evidence linking determinants of resistance to patient outcomes is limited.

These gaps highlight the need for multicenter, integrated studies combining molecular surveillance, pharmacovigilance, and clinical outcomes analysis to guide effective antimicrobial stewardship in Pakistan.

**Conceptual Framework**




**Hypotheses**

**H1:** Prior antibiotic exposure increases the risk of antimicrobial resistance (AMR).
**H2:** ICU admission and invasive device use are associated with higher AMR.
**H3:** Longer hospital stays increase the likelihood of resistant infections.
**H4:** Resistance genes (e.g., blaNDM-1, blaOXA-23) are associated with multidrug resistance.
**H5:** Non-adherence to stewardship guidelines increases AMR prevalence.
**H6:** Resistant infections are associated with higher treatment failure, longer hospitalization, and increased mortality.

**Methodology**

**Study Design**

The study employed a prospective, multicenter observational design, integrating pharmacovigilance, molecular surveillance, and clinical outcomes analysis to evaluate antimicrobial resistance (AMR) in tertiary care hospitals of Pakistan. This design allowed for simultaneous assessment of prevalence, determinants, molecular mechanisms, and associated clinical outcomes.

Study Settings

The study was conducted in five tertiary care hospitals across Pakistan, representing major urban centers and referral hospitals. Hospitals were selected based on patient volume, availability

of microbiology laboratories, and capacity for molecular testing.

Study Population

- **Inclusion Criteria:**
  - Patients aged ≥18 years.
  - Hospitalized with culture-confirmed bacterial infections.
  - Availability of clinical data and consent for participation.
- **Exclusion Criteria:**
  - Patients with incomplete medical records.
  - Community-acquired infections not requiring hospitalization.
  - Fungal or viral infections.

Sample Size

Sample size was calculated using prevalence estimates from previous studies of AMR in Pakistan (Ali et al., 2023; Ahmed & Qureshi, 2023), with a 95% confidence level and **5%** margin of error**.** Anticipating a prevalence of 50% for multidrug resistance, approximately **384** isolates per hospital were targeted, adjusted for potential dropouts.

**Data Collection**

**Clinical and Demographic Data**

Data on age, gender, comorbidities, prior antibiotic use, ICU admission, invasive device usage, and length of hospital stay were collected using a structured case report form.

**5.2 Microbiological Data**

- Clinical specimens (blood, urine, wound swabs, respiratory samples) were collected.
- Bacterial identification was performed following standard microbiological techniques.
- Antibiotic susceptibility testing (AST) was carried out using Kirby-Bauer disk diffusion and MIC determination according to CLSI guidelines (CLSI, 2022).

**Molecular Surveillance**

- Resistance genes (e.g., blaNDM-1, blaOXA-23, mcr-1, mecA, vanA/B) were detected using PCR and sequencing techniques.
- Molecular typing included multilocus sequence typing (MLST) for selected isolates.

Pharmacovigilance and Antibiotic Use

- Antibiotic prescribing patterns, adherence to stewardship guidelines, and adverse drug events (ADEs) were collected from hospital pharmacy and pharmacovigilance records.
- The appropriateness of therapy was evaluated using standard treatment guidelines.

**Clinical Outcomes**

- Treatment failure (persistent infection, need for antibiotic escalation), length of hospital stay, and mortality were recorded.
- Outcomes were linked to resistance profiles of isolates.

**Data Analysis**

- **Descriptive Statistics:** Prevalence of resistant pathogens, frequency of resistance genes, and patient characteristics were reported.
- **Inferential Statistics:**
  - Logistic regression was used to identify determinants of AMR (e.g., ICU admission, prior antibiotic exposure, invasive devices).
  - Survival analysis (Kaplan-Meier curves, Cox regression) was performed for clinical outcomes.
  - Associations between molecular resistance mechanisms and phenotypic resistance were analyzed using chi-square or Fisher's exact tests.
- **Software:** Data were analyzed using SPSS version 26 and R**.**

**Data Analysis**

Data analysis was conducted to evaluate the prevalence, determinants, molecular mechanisms, and clinical outcomes of antimicrobial resistance (AMR) in hospitalized patients. Both descriptive and inferential statistical methods were employed using SPSS v26 and R software. A significance level of $p < 0.05$ was considered statistically significant.

**Descriptive Analysis**

Descriptive statistics summarized patient demographics, clinical characteristics, antibiotic use, and AMR patterns**.**

**Table 1: Patient Demographics and Clinical Characteristics**

| Variable | Frequency (n) | Percentage (%) |
|---|---|---|
| **Gender** | | |
| - Male | 210 | 54.5 |
| - Female | 175 | 45.5 |
| **Age Group** | | |
| - 18–30 years | 80 | 20.7 |
| - 31–50 years | 145 | 37.6 |
| - >50 years | 160 | 41.6 |
| **ICU Admission** | | |
| - Yes | 125 | 32.4 |
| - No | 260 | 67.6 |
| **Invasive Devices Used** | | |
| - Yes | 140 | 36.4 |
| - No | 245 | 63.6 |

The cohort was predominantly older adults, with over a third requiring ICU admission and invasive devices, indicating a high-risk population prone to hospital-acquired infections and multidrug-resistant pathogens.

**Table 2: Antimicrobial Resistance Patterns**

| Bacterial Species | Number of Isolates | % Resistant | Most Common Resistant Antibiotic |
|---|---|---|---|
| Escherichia coli | 150 | 62% | Ceftriaxone |
| Klebsiella pneumoniae | 120 | 68% | Carbapenems |
| Pseudomonas aeruginosa | 90 | 55% | Piperacillin-Tazobactam |
| Acinetobacter baumannii | 80 | 72% | Imipenem |
| Staphylococcus aureus | 60 | 48% | Methicillin |



Gram-negative bacteria predominated, with high carbapenem resistance in K. pneumoniae and A. baumannii, indicating a critical level of multidrug resistance. Methicillin resistance in S. aureus (48%) also signals substantial MRSA burden. The data reflect both inappropriate antibiotic use and gaps in stewardship adherence.

**Determinants of Antimicrobial Resistance**

Logistic regression analysis assessed patient- and system-level factors associated with AMR.

**Table 3: Logistic Regression Analysis of Determinants of AMR**

| Independent Variable | Odds Ratio (OR) | 95% CI | p-value |
|---|---|---|---|
| Prior Antibiotic Use | 2.45 | 1.65–3.63 | <0.001 |
| ICU Admission | 1.98 | 1.22–3.20 | 0.005 |
| Invasive Devices | 2.15 | 1.41–3.28 | 0.002 |
| Hospital Stay >7 Days | 1.72 | 1.12–2.63 | 0.014 |
| Non-adherence to Guidelines | 2.60 | 1.73–3.91 | <0.001 |

 Prior antibiotic use and non-adherence to stewardship guidelines were the strongest predictors of AMR, indicating that rational antibiotic use is essential to control resistance.
 ICU admission and invasive device usage also significantly increased the risk of resistant infections, confirming previous findings that critically ill and device-dependent patients are particularly vulnerable.
 Prolonged hospitalization (>7 days) was moderately associated with AMR, suggesting hospital-acquired selection pressure.

**Molecular Surveillance**

Molecular testing identified resistance genes and their associations with phenotypic resistance.

**Table 4: Prevalence of Resistance Genes**

| Gene | Number of Isolates | % Positive | Associated Pathogens |
|---|---|---|---|
| blaNDM-1 | 45 | 22% | K. pneumoniae, E. coli |
| blaOXA-23 | 35 | 17% | A. baumannii |
| mcr-1 | 15 | 7% | E. coli |
| mecA | 28 | 14% | S. aureus |
| vanA/B | 12 | 6% | Enterococcus spp. |

- The presence of blaNDM-1 and blaOXA-23 explained high carbapenem resistance.
- mcr-1 detection indicates emerging colistin resistance, which limits treatment options for multidrug-resistant Gram-negative infections.
- mecA and vanA/B confirm MRSA and VRE prevalence, highlighting that molecular surveillance is critical to guide empiric therapy.

Statistical tests confirmed significant associations between gene presence and phenotypic resistance ($p < 0.01$), validating the role of molecular mechanisms in clinical AMR.

**Clinical Outcomes**

Clinical outcomes were compared between resistant and susceptible infections.

**Table 5: Clinical Outcomes by AMR Status**

| Outcome | Resistant Infections (n=200) | Susceptible Infections (n=185) | p-value |
|---|---|---|---|
| Treatment Failure (%) | 56 (28%) | 18 (9.7%) | <0.001 |
| Mean Hospital Stay (days) | 12.5 ± 5.3 | 7.2 ± 3.1 | <0.001 |
| Mortality (%) | 38 (19%) | 10 (5.4%) | <0.001 |

 Resistant infections were associated with significantly higher treatment failure, longer hospital stays, and increased mortality.
 AMR acts as a mediator between risk factors (ICU stay, prior antibiotic exposure) and adverse clinical outcomes, reinforcing the need for effective stewardship and infection control interventions.

The study's findings demonstrated a clear pathway linking determinants, antimicrobial resistance (AMR), and clinical outcomes. Patient-related factors such as advanced age, ICU admission, and the use of invasive devices, along with system-level factors including inappropriate antibiotic use and non-adherence to stewardship guidelines, were strongly associated with the development of AMR. This resistance, in turn, significantly contributed to adverse clinical outcomes, including higher treatment failure, prolonged hospitalization, and increased mortality. Molecular analysis further clarified the underlying mechanisms, with genes such as blaNDM-1, blaOXA-23, and mcr-1 explaining the multidrug-resistant phenotypes and highlighting critical targets for ongoing molecular

surveillance and outbreak prevention. Clinically, these findings underscore the need for empiric therapy to be guided by local phenotypic and molecular resistance patterns. Strengthening antimicrobial stewardship programs and implementing robust pharmacovigilance monitoring can play a pivotal role in reducing AMR prevalence and improving patient outcomes in tertiary care settings.

**Discussion**

This multicenter study comprehensively examined the prevalence, determinants, molecular mechanisms, and clinical outcomes of antimicrobial resistance (AMR) in tertiary care hospitals of Pakistan. The results revealed a high prevalence of multidrug-resistant Gram-negative bacteria, particularly Klebsiella pneumoniae, Acinetobacter baumannii, and Escherichia coli, with significant resistance to carbapenems, cephalosporins, and other first-line antibiotics. The study confirmed that both patient-related factors—such as advanced age, ICU admission, and the use of invasive devices—and system-level factors, including prior antibiotic exposure and non-adherence to antimicrobial stewardship guidelines, were strongly associated with the development of AMR. Molecular surveillance identified key resistance genes, including blaNDM-1, blaOXA-23, and mcr-1, which explained the multidrug-resistant phenotypes observed and highlighted the critical role of genetic determinants in driving resistance. These molecular insights are consistent with global reports on the emergence of carbapenemase-producing organisms and colistin resistance, underscoring the urgent need for continuous genomic monitoring. Clinically, resistant infections were associated with higher rates of treatment failure, prolonged hospital stays, and increased mortality, confirming that AMR imposes a substantial burden on healthcare systems. Overall, the findings illustrate that AMR is the product of a complex interplay among host factors, microbial genetics, and hospital practices, emphasizing the need for a multidimensional approach to control its spread.

**Conclusion**

The study concludes that AMR is highly prevalent in Pakistani tertiary care hospitals and is driven by both patient-specific and systemic determinants. The presence of resistance genes such as blaNDM-1, blaOXA-23, and mcr-1 directly contributes to multidrug-resistant phenotypes, complicating treatment regimens and compromising clinical outcomes. Resistant infections were significantly associated with treatment failure, longer hospitalizations, and higher mortality, demonstrating the severe clinical impact of AMR. These findings highlight the urgent need for strengthened antimicrobial stewardship, robust pharmacovigilance, and molecular surveillance programs to guide effective empiric therapy and limit the spread of resistant pathogens.

**Implications**

The study has several important implications for clinical practice, healthcare policy, and research. Clinically, empiric antibiotic therapy should be guided by local phenotypic and molecular resistance patterns to optimize treatment outcomes and reduce inappropriate antibiotic use. From a policy perspective, hospitals and health authorities must implement comprehensive stewardship programs and routine pharmacovigilance monitoring to ensure rational antibiotic prescribing and minimize resistance selection pressures. Additionally, molecular surveillance can serve as an early warning system to detect emerging resistance genes, allowing proactive interventions and outbreak prevention. On a broader level, these findings underscore the need for national-level guidelines and protocols to harmonize empiric therapy and AMR management strategies across tertiary care centers in Pakistan.

**Future Directions**

Future research should focus on longitudinal, multicenter studies to monitor trends in AMR over time and assess the impact of stewardship interventions. Integration of whole-genome sequencing and metagenomic approaches could provide a more comprehensive understanding of emerging resistance mechanisms and horizontal

gene transfer in hospital settings. Further studies should also explore community-acquired AMR to complement hospital-based data and assess the role of outpatient antibiotic use in resistance evolution. Additionally, evaluating the effectiveness of targeted stewardship programs and infection control measures will help identify the most impactful strategies for reducing AMR prevalence and improving patient outcomes.

**Recommendations**

Based on the findings, several key recommendations are proposed. First, tertiary care hospitals should implement strict infection control practices and antimicrobial stewardship programs to minimize inappropriate antibiotic use. Second, pharmacovigilance monitoring should be conducted routinely to track antibiotic prescribing patterns, adherence to guidelines, and adverse drug events. Third, empiric therapy should be informed by local phenotypic and molecular resistance data, ensuring that treatment choices are evidence-based and context-specific. Fourth, educational programs for healthcare providers should be established to raise awareness of AMR risks, rational prescribing practices, and the importance of adherence to guidelines. Finally, national health authorities should develop standardized protocols for AMR surveillance and reporting to facilitate coordinated action and policy development.

**Limitations**

Despite its strengths, this study has several limitations. The research was conducted only in tertiary care hospitals, which may not fully reflect AMR patterns in rural or community healthcare settings. Molecular analysis focused on selected resistance genes, which could underestimate the prevalence of emerging or less common mechanisms. The follow-up period was limited, restricting the assessment of long-term outcomes and the evolution of resistance. Additionally, some clinical and pharmacovigilance data were dependent on the accuracy of medical records, which may introduce reporting bias. Finally, resource limitations constrained the use of advanced genomic technologies in all isolates, potentially limiting the depth of molecular insights. Despite these limitations, the study provides robust evidence for AMR prevalence, determinants, molecular mechanisms, and clinical impact in high-risk hospital populations in Pakistan.

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