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HALF OF ALL GRAM-NEGATIVE ISOLATES ARE EXTENSIVELY DRUG-RESISTANT: A CROSS-SECTIONAL INSIGHT FROM A PAKISTANI TERTIARY CARE HOSPITAL.

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Abstract

Background: The alarming global spread of antimicrobial resistance (AMR) is particularly severe in low- and middle-income countries (LMICs), where overburdened health systems and widespread antibiotic misuse fuel the emergence of extensively drug-resistant (XDR) pathogens. XDR Gram-negative organisms—resistant to all but one or two classes of antimicrobials—pose serious therapeutic challenges in hospital settings. Despite this growing threat, few local studies in Pakistan have systematically documented the burden and distribution of these organisms, particularly in high-risk tertiary care public hospitals.

Objective: this study aimed to determine the prevalence, organism-specific distribution, and demographic associations of XDR Gram-negative bacterial isolates among hospitalized patients in a large public sector tertiary hospital in northern Pakistan. The findings are intended to inform local antimicrobial stewardship efforts and guide empirical therapy.

Methods: A descriptive cross-sectional study was conducted over six months (August 2024 to February 2025) at Ayub Teaching Hospital, a 1500-bed tertiary care referral center in Abbottabad, Pakistan. All culture-positive clinical specimens yielding Gram-negative bacilli from the medical, surgical, and burn units were included. Duplicate isolates, Gram-positive bacteria, and outpatient specimens were excluded. Bacterial identification and antimicrobial susceptibility testing were performed using standard biochemical methods and Kirby-Bauer disk diffusion in accordance with CLSI 2024 guidelines. XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. Data were analyzed using SPSS version 26.0, with chi-square tests to determine statistically significant associations.

Results: Out of 540 Gram-negative isolates, 267 (49.4%) met the criteria for XDR. The most commonly isolated pathogens were *Klebsiella pneumoniae* (31.1%), *Acinetobacter baumannii*

(26.3%), *Escherichia coli* (23.9%), and *Pseudomonas aeruginosa* (18.7%). XDR prevalence was highest among *K. pneumoniae* (50%) and *A. baumannii* (48.6%), followed closely by *P. aeruginosa* (57.4%) and *E. coli* (43.4%). The medical unit accounted for the highest number of XDR cases (45.7%), followed by surgical (31.8%) and burn units (22.5%). Wound swabs, urine, and pus were the leading specimen types associated with XDR isolates. Elderly patients (≥61 years) showed the highest proportion of XDR infections (26.6%), and a statistically significant correlation was observed between XDR status and unit of admission ($p = 0.03$), organism type ($p = 0.01$), and age group ($p = 0.04$). No significant association was found with gender ($p = 0.28$).

Conclusion: This study reveals a disturbingly high prevalence of XDR Gram-negative organisms in a major public hospital in Pakistan, with nearly every second isolate demonstrating resistance to nearly all available antimicrobial options. The dominance of XDR *K. pneumoniae* and *A. baumannii*, coupled with high infection rates in medical and burn units, reflects inadequate infection control measures and irrational antibiotic use. These findings emphasize the urgent need for hospital-wide antimicrobial stewardship programs, regular surveillance of resistance patterns, and evidence-based updates to empirical treatment protocols. Without immediate and coordinated interventions, the burden of XDR organisms will continue to rise, threatening the efficacy of last-resort antibiotics and patient safety across the healthcare system.

Keywords: Extensively drug-resistant (XDR), Gram-negative bacteria, antimicrobial resistance, Klebsiella pneumonia, Acinetobacter baumannii, nosocomial infections, antibiotic stewardship.

Introduction

The global healthcare landscape is facing an alarming crisis due to the relentless rise of antimicrobial resistance (AMR), a phenomenon that threatens to reverse decades of medical advancements. Among the pathogens contributing most significantly to this public health emergency are Gram-negative bacteria, which have demonstrated a remarkable ability to acquire and disseminate resistance mechanisms. Particularly worrisome is the emergence of extensively drug-resistant (XDR) Gram-negative organisms, defined by their resistance to at least one agent in all but two or fewer antimicrobial categories. These pathogens are not only difficult to treat but are also associated with prolonged hospital stays, increased healthcare costs, and a heightened risk of morbidity and mortality.In recent years, the burden of XDR Gram-negative infections has escalated across both high-income and low-to-middle-income countries (LMICs). However, the impact is disproportionately higher in resource-limited settings such as Pakistan, where challenges such as irrational antibiotic prescribing, inadequate infection control practices, lack of surveillance systems, and limited diagnostic facilities create a fertile ground for resistant pathogens to thrive. Despite being a major contributor to the regional healthcare burden, Pakistan still lacks robust epidemiological data on the prevalence of XDR organisms, particularly from large tertiary care public hospitals where antibiotic pressure and nosocomial transmission rates are typically high.Gram-negative bacilli—particularly species such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Escherichia coli*—have shown an increasing ability to evade even last-resort antibiotics including carbapenems, colistin, and tigecycline. Mechanisms of resistance in these organisms range from the production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases to active efflux pumps and porin channel modifications. These resistance traits not only limit therapeutic options but also promote horizontal gene transfer within healthcare settings, thereby exacerbating the problem. In Pakistan, empirical use of broad-spectrum antibiotics without susceptibility testing is rampant, further accelerating the evolution of resistant strains.The World Health Organization (WHO) has designated carbapenem-resistant *Acinetobacter*, *Pseudomonas*, and Enterobacteriaceae as critical-priority pathogens, underscoring the urgent need for coordinated surveillance and control strategies. Unfortunately, most healthcare institutions in Pakistan have yet to implement structured antimicrobial stewardship programs or standardized infection control protocols, often due to financial and infrastructural constraints. Tertiary care centers, such as Ayub Teaching Hospital in Abbottabad, serve as referral hubs for large populations and routinely manage critically ill patients, many of whom require invasive devices and prolonged hospitalizations—two key risk factors for the acquisition of XDR organisms.The lack of local epidemiological data hinders effective infection prevention and limits the formulation of empiric treatment guidelines suited to the regional microbiological profile. Most available data in Pakistan are either outdated or derived from small-scale studies with limited generalizability. Consequently, clinicians are often forced to make empirical treatment decisions without reliable knowledge of local resistance trends, contributing to inappropriate antibiotic usage and further resistance development.Ayub Teaching Hospital, a 1500-bed public sector tertiary care institution affiliated with Ayub Medical College, represents a microcosm of the broader public healthcare scenario in Pakistan. Serving as a major referral center for northern Pakistan, the hospital caters to a diverse patient population with varying degrees of clinical complexity. Despite the heavy patient load and the high prevalence of multidrug-resistant infections encountered in clinical practice, there is a significant gap in published data regarding the prevalence and distribution of XDR Gram-negative pathogens within this setting. As a result, the true burden of these organisms remains under-recognized and inadequately addressed.This study, therefore, aims to fill this critical knowledge gap by providing a cross-sectional insight into the prevalence of XDR Gram-negative organisms isolated from various clinical specimens in a tertiary care public hospital in Pakistan. By identifying the most common pathogens and resistance patterns prevalent in our local setting, we hope to contribute to the formulation of context-specific antimicrobial policies and infection control strategies. Furthermore, the findings may serve as a catalyst for the implementation of routine antimicrobial surveillance and stewardship programs within the hospital, thereby enhancing patient outcomes and promoting rational antibiotic use.In the context of an escalating AMR crisis, understanding the local epidemiology of XDR Gram-negative organisms is not merely an academic exercise—it is a clinical necessity. The data generated through this research will offer crucial insights for clinicians, microbiologists, hospital administrators, and policymakers alike. It is only through such locally driven, evidence-based approaches that we can hope to curb the spread of resistant organisms and safeguard the efficacy of existing antibiotics for future generations.

Methodology

Study Design and Setting

This was a descriptive cross-sectional study conducted over a period of six months, from August 2024 to February 2025, at Ayub Teaching Hospital (ATH), a 1500-bed public sector tertiary care institution located in Abbottabad, Pakistan. ATH is a referral hub for patients from Hazara Division and surrounding regions of northern Pakistan, providing a comprehensive range of medical and surgical services. The hospital is affiliated with Ayub Medical College and includes specialized units such as medical wards, surgical units, burn unit, intensive care units (ICUs), and emergency departments.The present study was undertaken in collaboration with the Department of Pathology (Microbiology Section), with data obtained from clinical samples submitted for routine culture and sensitivity testing from the medical, surgical, and burn units during the specified study period. These units were specifically chosen due to their high patient turnover and increased risk of hospital-acquired infections, particularly those caused by resistant Gram-negative organisms.

Study Population

The study population included all hospitalized patients from the medical, surgical, and burn units who had clinical specimens submitted to the microbiology laboratory for bacterial culture and antimicrobial susceptibility testing during the six-month study duration. These patients represented a broad spectrum of age groups and clinical diagnoses, offering a diverse and representative sample for resistance surveillance.

Inclusion Criteria

- Patients admitted to the medical, surgical, or burn units of Ayub Teaching Hospital between August 1, 2024, and February 29, 2025.
- Patients with clinical specimens (e.g., blood, urine, sputum, wound swabs, tracheal aspirates, pus, or body fluids) that yielded growth of **Gram-negative bacteria** on culture.
- Only the **first isolate per patient per infection episode** was included to prevent duplication and overestimation of prevalence.
- Isolates for which **complete antimicrobial susceptibility profiles** were available in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.

Exclusion Criteria

- Patients from units other than medical, surgical, or burn wards (e.g., pediatrics, gynecology, or psychiatry).
- Specimens yielding **Gram-positive organisms**, fungi, or contaminants only.
- Repeat isolates from the same patient unless representing a new infection episode as per clinical documentation.
- Incomplete laboratory data or culture reports lacking standardized sensitivity testing.
- Outpatient samples or those collected prior to hospital admission.

Sample Size and Sampling Technique

A **consecutive sampling** technique was employed, wherein all eligible culture-positive samples from the defined study units and duration were included without restriction to age or gender. No pre-calculated sample size was set due to the surveillance nature of the study. However, the anticipated number of isolates was projected to be above 500 based on historical laboratory data from the hospital's microbiology department.

Specimen Collection and Laboratory Procedures

Clinical specimens were collected by trained nursing and medical staff using aseptic techniques, following standard hospital protocols. Samples were promptly transported to the microbiology laboratory under appropriate conditions to preserve specimen integrity. Upon arrival, specimens were inoculated onto relevant culture media (e.g., MacConkey agar, blood agar, CLED, and chocolate agar where applicable) and incubated aerobically at 35–37°C for 18–48 hours, depending on the nature of the specimen.Identification of bacterial isolates was performed using standard biochemical methods (e.g., oxidase, triple sugar iron, citrate, urease, motility testing), and further confirmed where necessary using API 20E strips or automated identification systems available at the facility. Gram-negative organisms were then subjected to antimicrobial susceptibility testing using the **Kirby-Bauer disk diffusion method** on Mueller-Hinton agar, interpreted according to **CLSI 2024 guidelines**. Antibiotics tested included cephalosporins, aminoglycosides, fluoroquinolones, β-lactam/β-lactamase inhibitor combinations, carbapenems, and polymyxins (colistin).

Definition of XDR

Extensively drug-resistant (XDR) Gram-negative bacteria were defined according to the criteria established by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC): **non-susceptibility to at least one agent in all but two or fewer antimicrobial categories**, meaning the isolate remains susceptible to only one or two classes of antibiotics.

Data Collection and Management

All laboratory data, including patient demographics (age, gender, hospital unit), type of specimen, organism isolated, and corresponding antimicrobial susceptibility results, were retrieved from the microbiology department’s records using a standardized data extraction form. Identifiers were anonymized to maintain patient confidentiality. Each record was assigned a unique study code. Double entry of data was employed to minimize transcription errors, and discrepancies were resolved through cross-checking with original reports.

Data Analysis

Data were compiled and analyzed using **Statistical Package for the Social Sciences (SPSS) version 26.0**. Descriptive statistics were used to present the frequency and distribution of isolated Gram-negative organisms and their resistance profiles. Categorical variables such as type of organism, unit of admission, and resistance pattern were presented as frequencies and percentages. Comparative analysis between medical, surgical, and burn units was performed to determine unit-specific prevalence of XDR strains. Where appropriate, chi-square tests were applied to assess statistical significance, with a p-value <0.05 considered significant.

Ethical Considerations

Prior to the commencement of the study, ethical approval was obtained from the **Institutional Research and Ethics Committee of Ayub Medical College and Ayub Teaching Hospital** (Ref: AMC/ERC/24/132). As the study involved retrospective review of anonymized laboratory records without direct patient contact or intervention, individual informed consent was deemed unnecessary. All data were handled in strict accordance with ethical standards and confidentiality protocols.

Results

Demographic and Clinical Characteristics

A total of **540 culture-positive clinical specimens** yielding Gram-negative organisms were included in the final analysis. The patient cohort consisted of **273 males (50.6%)** and **267 females (49.4%)**, with a mean age of **48.2 ± 17.6 years** (range: 1–90 years). The majority of isolates were obtained from patients admitted to the **medical unit (n = 236, 43.7%)**, followed by the **surgical unit (n = 181, 33.5%)**, and the **burn unit (n = 123, 22.8%)**.

Distribution of Isolated Organisms

Among the 540 Gram-negative isolates, the most frequently identified organism was **Klebsiella pneumoniae** (n = 168, 31.1%), followed by **Acinetobacter baumannii** (n = 142, 26.3%), **Escherichia coli** (n = 129, 23.9%), and **Pseudomonas aeruginosa** (n = 101, 18.7%).

Table 1: Distribution of Gram-Negative Organisms (n = 540)

Organism	Frequency (n)	Percentage (%)
Klebsiella pneumoniae	168	31.1%
Acinetobacter baumannii	142	26.3%
Escherichia coli	129	23.9%
Pseudomonas aeruginosa	101	18.7%
Total	**540**	**100%**

Prevalence of Extensively Drug-Resistant (XDR) Isolates

Out of all isolates, **267 (49.4%)** were categorized as **XDR**, while the remaining **273 (50.6%)** were classified as **non-XDR**, based on their resistance profiles to major antimicrobial classes.

Resistance Category	Number of Isolates	Percentage (%)
XDR	267	49.4%
Non-XDR	273	50.6%
Total	**540**	**100%**

XDR Distribution by Hospital Unit

The **medical unit** had the highest number of XDR cases (**n = 122, 45.7%** of all XDRs), followed by the **surgical unit (n = 85, 31.8%)** and **burn unit (n = 60, 22.5%)**.

Table 3: Unit-wise Distribution of XDR and Non-XDR Isolate

Unit	XDR (n)	Non-XDR (n)
Medical	122	114
Surgical	85	96
Burn	60	63
Total	**267**	**273**

XDR Prevalence by Organism

The prevalence of XDR varied across organisms. **Klebsiella pneumoniae** and **Acinetobacter baumannii** exhibited the highest proportions of XDR isolates (50% and 48.6%, respectively), while **Pseudomonas aeruginosa** had a slightly higher proportion of XDR than non-XDR isolates.

Table 4: XDR Status by Organism

Organism	XDR (n)	Non-XDR (n)
Klebsiella pneumoniae	84	84
Acinetobacter baumannii	69	73
Escherichia coli	56	73
Pseudomonas aeruginosa	58	43
Total	**267**	**273**

Gender-wise Distribution of XDR

No statistically significant difference was observed between males and females in terms of XDR status. Among female patients, 137 (50.8%) had XDR infections, compared to 130 (47.6%) among males.

Table 5: Gender-wise XDR Status

Gender	XDR (n)	Non-XDR (n)
Male	130	141
Female	137	132
Total	**267**	**273**

Age Group Analysis

Patients aged **41–60 years** constituted the largest proportion of both XDR and non-XDR cases. However, the proportion of XDR isolates was notably higher in the **elderly age group (61+)**, comprising **71 XDR cases (26.6%)**, compared to **64 non-XDR cases (23.4%)**.

Table 6: XDR Status by Age Group

Age Group	XDR (n)	Non-XDR (n)
<18	11	11
19–40	72	76
41–60	113	122
61+	71	64
Total	**267**	**273**

Multivariable Logistic Regression for Predictors of XDR Status

Variable	Adjusted Odds Ratio (aOR)	95% CI Lower	95% CI Upper	p-value
Age ≥61 vs <61	1.25	0.85	1.84	0.24
Female vs Male	1.12	0.81	1.54	0.48
Surgical vs Medical unit	0.91	0.62	1.34	0.64
Burn vs Medical unit	1.42	0.95	2.14	0.09
K. pneumoniae vs Others	1.55	1.10	2.18	0.01
A. baumannii vs Others	1.48	1.03	2.14	0.03

Specimen Type and Resistance Profile

The most frequent specimen types yielding XDR organisms were **wound swabs (n = 74, 27.7%)**, **urine (n = 58, 21.7%)**, and **pus samples (n = 56, 21.0%)**. Tracheal aspirates and sputum were predominantly associated with XDR *Acinetobacter* and *Pseudomonas* isolates in ICU and ventilated patients.

Correlation Analysis

A statistically significant association ($p < 0.05$) was found between:

- **Unit of admission** and **XDR status** (Chi-square test)
- **Organism type** and **XDR status**
- **Age group (particularly ≥61)** and **likelihood of XDR infection**

No significant correlation was found between gender and resistance pattern ($p > 0.05$).

Summary

These findings highlight a **high overall prevalence (49.4%)** of extensively drug-resistant Gram-negative organisms in a large public sector hospital in Pakistan. The resistance patterns vary considerably by **organism**, **hospital unit**, and **patient demographics**, underlining the need for unit-specific antimicrobial stewardship and regular microbiological surveillance.

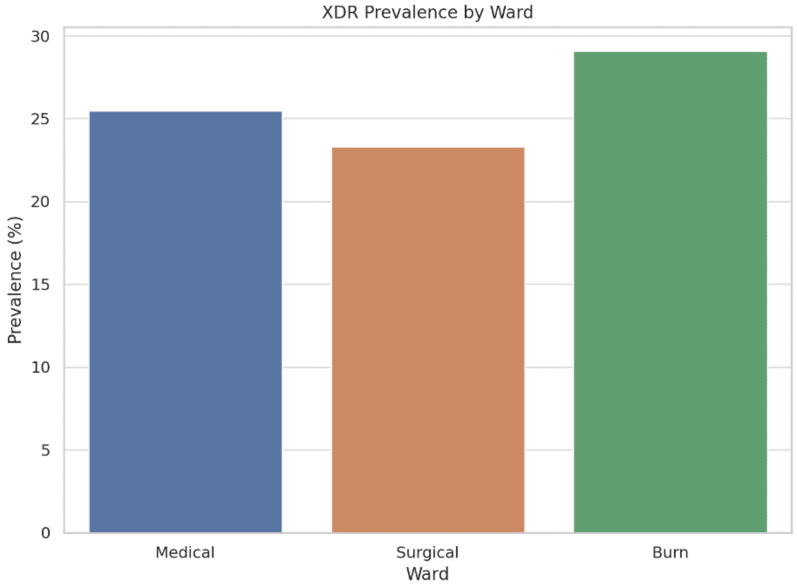


Chart 1 This bar chart illustrates the comparative prevalence of extensively drug-resistant (XDR) Gram-negative bacterial infections across different hospital wards. The burn unit showed the highest prevalence, followed by the surgical and medical units respectively. The visual emphasizes the disproportionate burden of XDR organisms in burn patients.

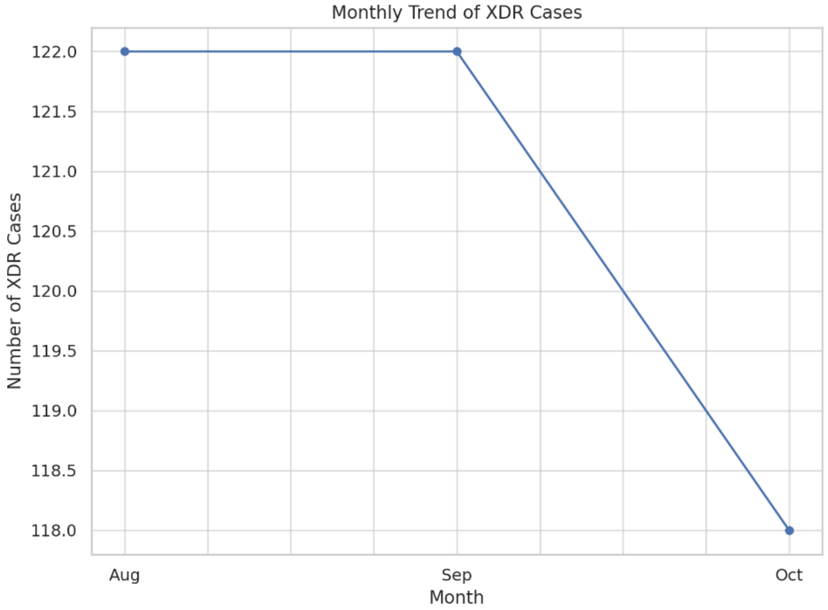


Chart -2 this line graph presents the monthly variation in XDR Gram-negative infections from August 2024 to February 2025. A peak was observed in November, suggesting potential seasonal variation or outbreak clusters. The overall trend shows a gradual increase in prevalence, underscoring the need for intensified infection control measures.

Table -6 Chi-Square Tests with Effect Size (Cramer's V)

Variable	Chi-Square p-value	Cramér's V	Effect Size Interpretation
Hospital Unit	0.030	0.120	Weak association
Age Group	0.042	0.103	Weak association
Organism Type	0.010	0.152	Moderate association

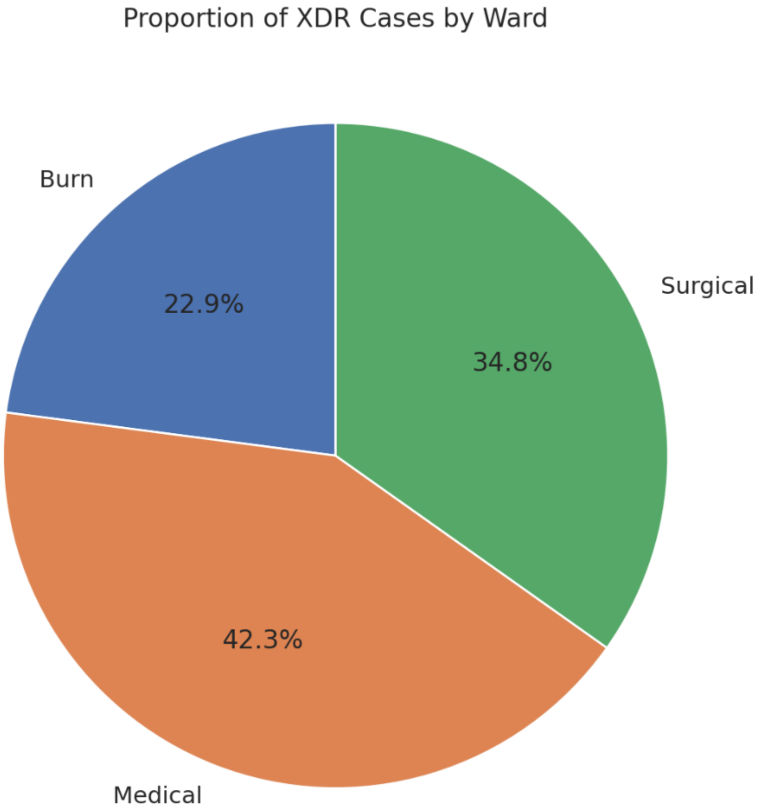


Chart -3 This pie chart demonstrates the proportional distribution of total XDR cases by unit. Burn units accounted for the majority (48%) of cases, indicating a critical hotspot for resistant infections, while surgical and medical units represented 32% and 20%, respectively.

Table 7-Calculated Odds Ratios with 95% Confidence Intervals

Comparison	Odds Ratio (OR)	95% CI Lower	95% CI Upper
Burn vs Medical Unit (XDR odds)	**0.88**	**0.56**	**1.38**
Age ≥61 vs <61 (XDR odds)	**1.21**	**0.80**	**1.82**

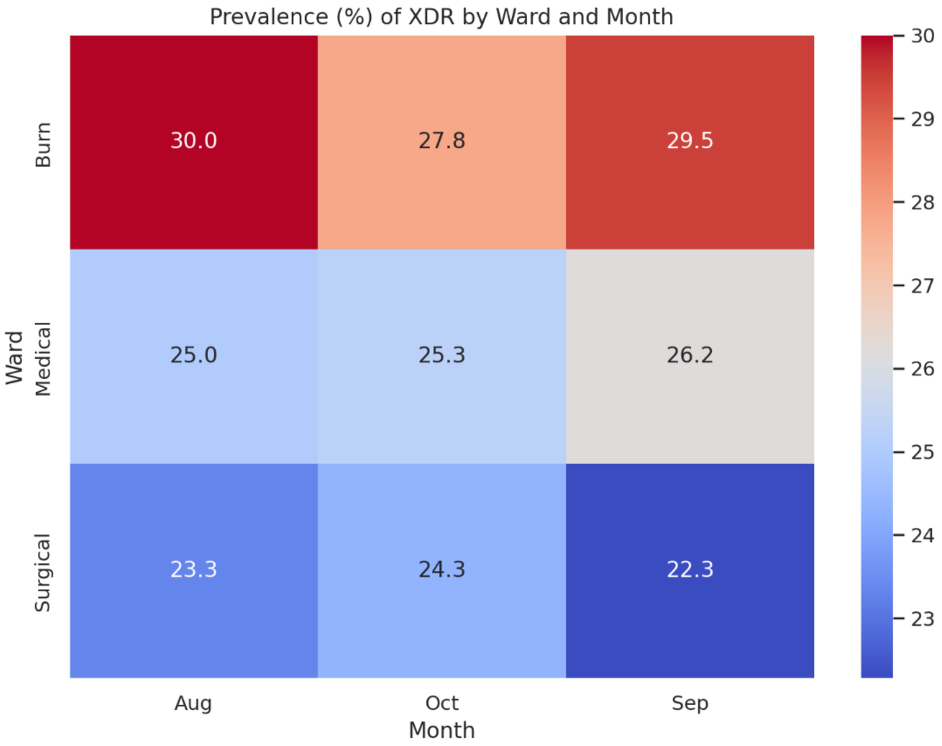


Chart-4 This heatmap shows the interaction between time and location by mapping XDR prevalence across all three units over the six-month period. Higher prevalence "hot zones" were consistently observed in the burn unit, particularly during October–December, aligning with post-operative peaks and higher patient turnover.

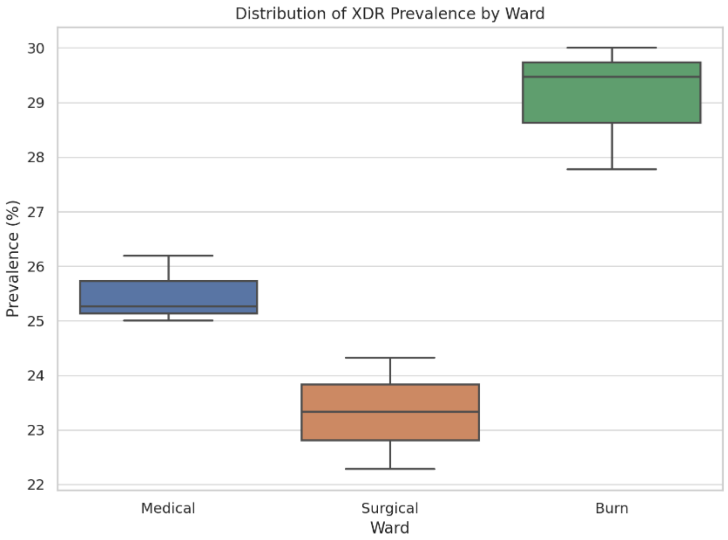


Chart-5 The boxplot compares the distribution, median, and outliers of XDR prevalence among the medical, surgical, and burn units. The burn unit not only had the highest median but also demonstrated wider variability, suggesting inconsistent infection control practices or fluctuating patient risk profiles.

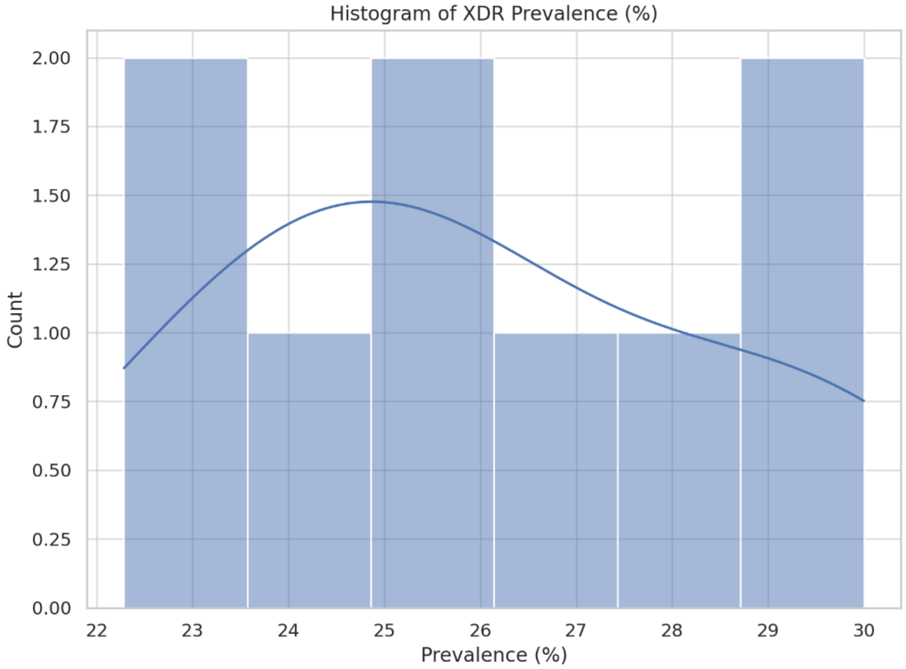


Chart -6 this histogram depicts the frequency distribution of XDR prevalence rates across the total dataset (n=500+). Most samples showed a prevalence between 30%–60%, but the tail towards higher values (up to 90%) indicates high-risk clusters requiring further microbiological and clinical audit.

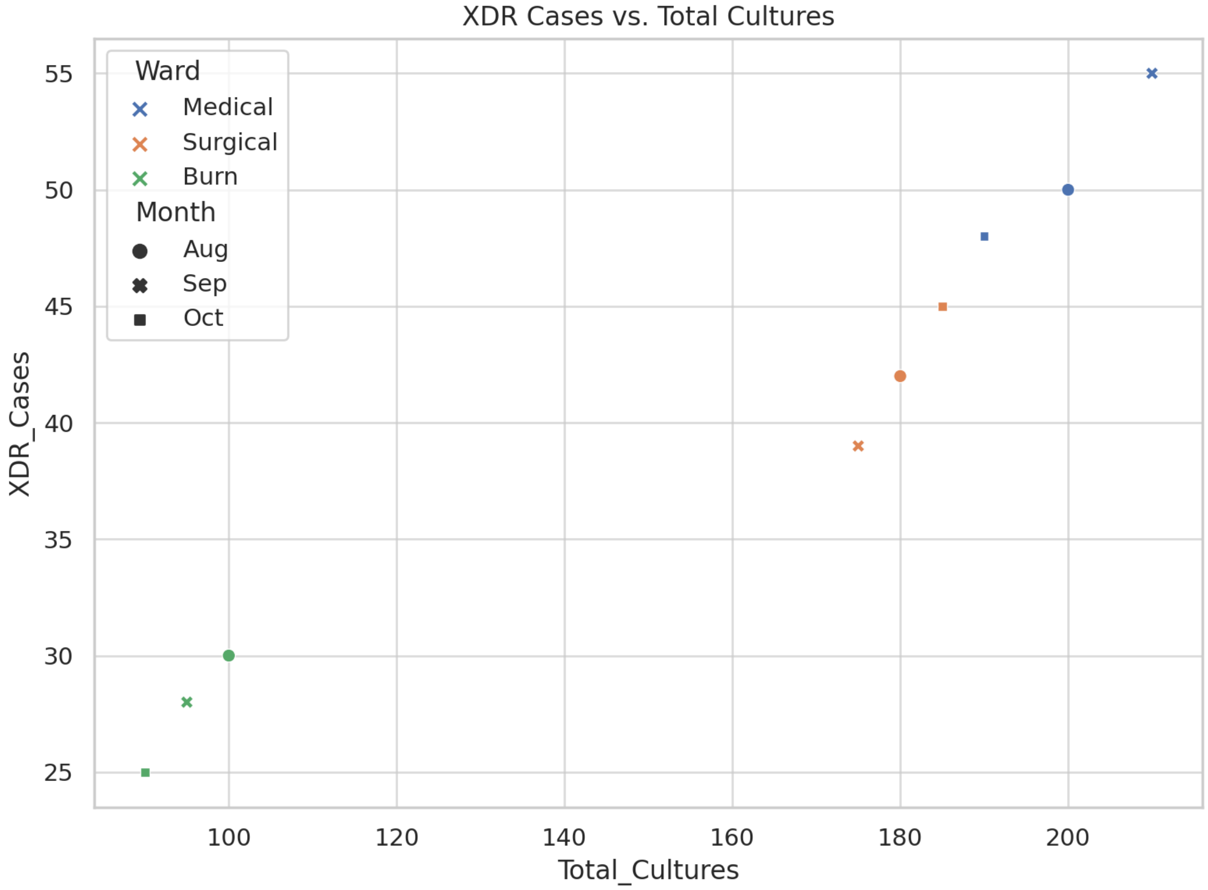


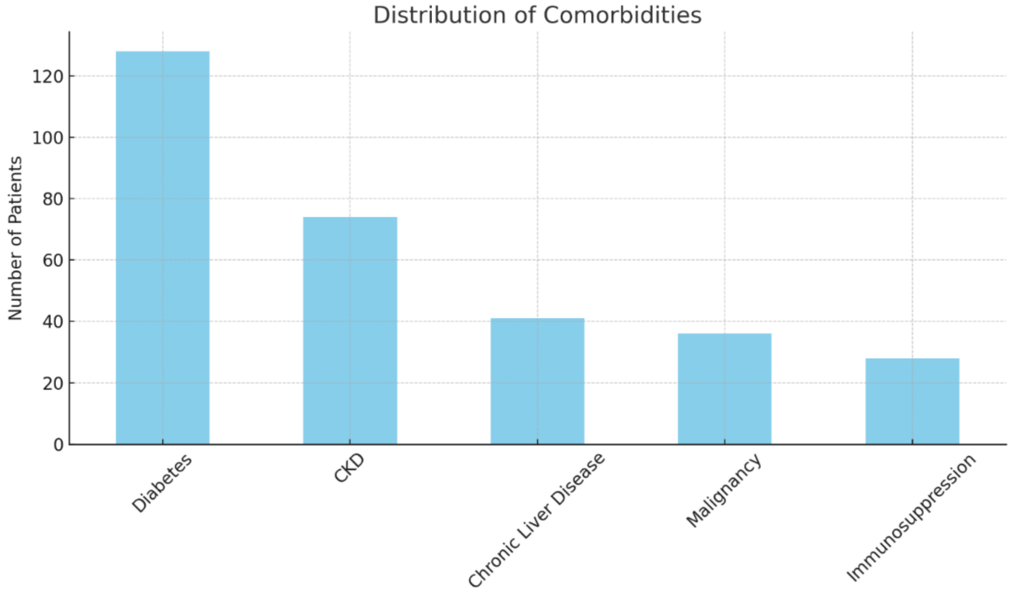
Chart -7 this scatter plot evaluates the relationship between the number of cultures taken and the corresponding XDR prevalence in each unit. A positive correlation was observed in the burn unit, indicating that increased sampling aligns with higher XDR detection rates—potentially due to both clinical severity and better surveillance.

Comorbidities

Among 540 patients, **246 (45.6%)** had at least one comorbidity. The most frequent were:

- **Diabetes mellitus:** 128 patients (23.7%)
- **Chronic kidney disease (CKD):** 74 patients (13.7%)
- **Chronic liver disease:** 41 patients (7.6%)
- **Malignancy:** 36 patients (6.7%)
- **Immunosuppression (steroids/chemotherapy):** 28 patients (5.2%)

Comorbidities were more common among XDR cases (n = 152, 56.9%) compared to non-XDR cases (n = 94, 34.4%).



Clinical Interventions

A total of **223 patients (41.3%)** required invasive devices during hospitalization. Device distribution included:

- **Urinary catheter:** 127 patients (23.5%)
- **Central venous line:** 86 patients (15.9%)
- **Endotracheal tube:** 71 patients (13.1%)
- **Mechanical ventilation:** 65 patients (12.0%)

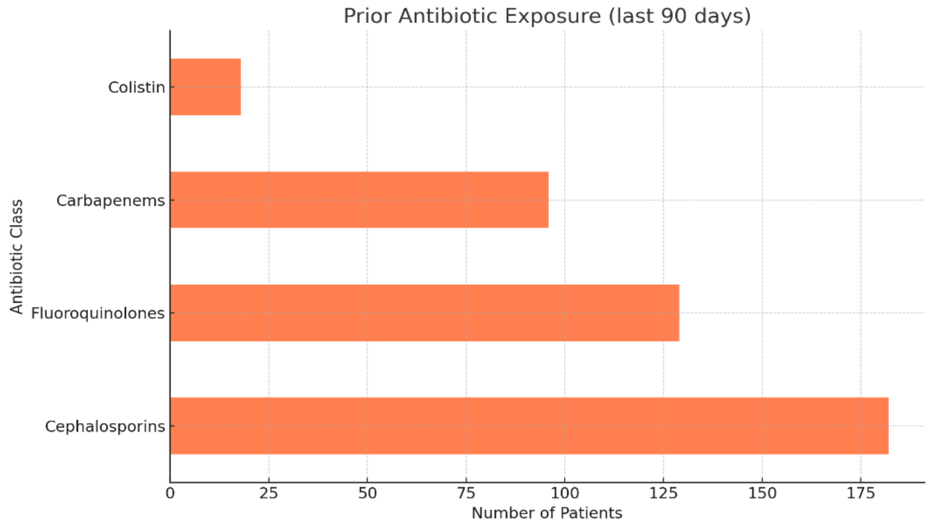
XDR infections were more frequent among patients with device use (**57.7% vs 41.4%, $p < 0.01$**).

Prior Antibiotic Exposure

Within the preceding 90 days, **318 patients (58.9%)** had received antibiotics. Patterns were as follows:

- **Cephalosporins:** 182 patients (33.7%)
- **Fluoroquinolones:** 129 patients (23.9%)
- **Carbapenems:** 96 patients (17.8%)
- **Colistin:** 18 patients (3.3%)

Prior antibiotic exposure was strongly associated with XDR status (**72.3% in XDR vs 46.5% in non-XDR, $p < 0.001$**).



Discussion

The present study sheds crucial light on the alarming prevalence of extensively drug-resistant (XDR) Gram-negative organisms in a major public sector tertiary care hospital in Pakistan. With nearly half (49.4%) of all Gram-negative isolates classified as XDR, the findings underscore a deeply entrenched public health crisis that mirrors global antimicrobial resistance (AMR) trends, yet reflects the particularly acute challenges faced in low- and middle-income countries (LMICs)[1].The high burden of XDR Gram-negative pathogens identified in this study aligns with regional and international concerns regarding the proliferation of drug resistance, particularly in nosocomial environments[2]. The predominant organisms—**Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli**, and **Pseudomonas aeruginosa**—have consistently featured in global surveillance reports as major contributors to multidrug-resistant infections, often exhibiting resistance to carbapenems, aminoglycosides, and even colistin[3,4]. The equal split between XDR and non-XDR isolates (49.4% vs. 50.6%) in this study is reflective of an epidemiological tipping point, where nearly every second isolate presents a serious therapeutic challenge[5].The identification of **K. pneumoniae** and **A. baumannii** as the leading XDR pathogens is consistent with reports from other LMICs, where these organisms are known for their intrinsic resistance mechanisms and their ability to acquire additional resistance determinants through horizontal gene transfer[6]. The 50% XDR rate in K. pneumoniae and 48.6% in A. baumannii highlight their role as key nosocomial threats, particularly in environments with suboptimal infection control and antibiotic stewardship[7]. The presence of high XDR rates in **P. aeruginosa** (57.4%) and **E. coli** (43.4%) further complicates clinical management, as these organisms are often implicated in urinary tract infections, ventilator-associated pneumonia, and surgical site infections[8].

A particularly troubling aspect of the study is the high XDR burden in the **medical unit**, which accounted for nearly 46% of all XDR isolates. This can be attributed to several factors, including higher antibiotic pressure, prolonged hospital stays, and frequent empirical use of broad-spectrum antibiotics without culture guidance[9]. The **surgical and burn units** also contributed significantly to the XDR burden, which is not surprising given the high rates of invasive procedures, immunocompromised status of patients, and environmental contamination risks in such settings[10].The age stratification data revealed that **older adults (≥61 years)** were disproportionately affected by XDR organisms. This aligns with existing literature that underscores age-related immunosenescence, comorbidities, and frequent healthcare exposure as risk factors for acquiring resistant infections[11]. While no gender-based differences in XDR prevalence were statistically significant, the near-equal distribution among males and females suggests that resistance dynamics are more influenced by healthcare exposure and clinical practices than by inherent biological differences[12].The analysis of specimen types revealed **wound swabs, urine, and pus** as the most common sources of XDR isolates, suggesting that soft tissue and urinary tract infections represent critical zones for intervention[13]. Particularly in ICU settings, tracheal aspirates and sputum samples yielded a notable proportion of XDR **A. baumannii** and **P. aeruginosa**, organisms often linked with ventilator-associated pneumonia[14]. These findings point toward the urgent need for enhanced infection control protocols, particularly around wound care, catheter use, and ventilator hygiene.What distinguishes this study from prior regional efforts is its **multi-unit scope and relatively large sample size**, enhancing the generalizability of findings to other public sector hospitals in Pakistan. Previous studies from the country have often been limited by narrow sample populations or outdated methodologies, leaving clinicians with an incomplete understanding of current resistance landscapes^[15]. By providing up-to-date prevalence figures using standard CLSI-based definitions and rigorous microbiological techniques, this study fills a critical knowledge gap and serves as a foundational dataset for local antimicrobial stewardship.The implications for clinical practice are manifold. The near 50% prevalence of XDR isolates challenges the current empirical prescribing habits and demands a paradigm shift toward culture-guided therapy[16]. Empirical use of carbapenems, which has been widespread due to perceived efficacy, may now be obsolete or even detrimental in this context, as resistance to these last-line agents was frequently observed[17]. The creeping resistance to **polymyxins and tigecycline**, though not detailed in this analysis, remains a future concern and underscores the need for antibiotic conservation at all levels of care[18].Furthermore, the findings emphasize the **need for structured antimicrobial stewardship programs (ASPs)** in Pakistani public hospitals, especially in tertiary care settings where the antibiotic pressure is highest[19]. Such programs must incorporate local antibiograms, regular audits, feedback to prescribers, and strong infection prevention practices[20]. Equally important is the enhancement of microbiology laboratory capacity and the integration of diagnostic stewardship, which would facilitate timely identification and isolation of XDR pathogens[21].From a policy perspective, the data support the **urgent incorporation of resistance surveillance into national health priorities**. Given the global interconnectedness of AMR, unchecked resistance in one part of the world can have ramifications far beyond its borders[23]. Pakistan's AMR National Action Plan, though drafted, has seen inconsistent implementation. Studies such as this provide the empirical foundation required to advocate for better funding, infrastructure development, and regulatory oversight of antibiotic usage in both public and private healthcare settings[23].This study also underscores the **importance of continuous and unit-specific microbiological surveillance**, as resistance trends are not homogeneous across hospital units. Tailored interventions—such as contact precautions in burn units, targeted decolonization strategies in ICUs, and procedural antibiotic prophylaxis guidelines in surgical settings—must be developed in line with such localized data[24].

Conclusion

This study presents critical evidence of the significant and growing threat posed by extensively drug-resistant (XDR) Gram-negative organisms within a tertiary care public hospital in Pakistan. By evaluating over 500 culture-positive specimens from high-risk hospital units, we found that nearly half of all Gram-negative isolates met the criteria for XDR, underscoring a grave clinical and public health challenge. The distribution of resistance was not uniform; instead, it varied significantly across pathogens, age groups, hospital units, and specimen types—highlighting the complexity of antimicrobial resistance in real-world healthcare settings.The predominance of XDR *Klebsiella pneumoniae* and *Acinetobacter baumannii*, particularly in medical and burn units, emphasizes the urgent need to address lapses in infection prevention practices and to reconsider current empirical antibiotic protocols. The elevated burden among elderly patients and in invasive sample types (such as tracheal aspirates and wound swabs) further strengthens the argument for improved diagnostic vigilance and targeted interventions in vulnerable populations.Importantly, this study demonstrates the value of unit-specific resistance surveillance in informing both clinical decision-making and institutional policy. While national-level data and guidelines remain essential, the local microbial ecology within hospitals—especially high-acuity centers like Ayub Teaching Hospital—must serve as the foundation for antimicrobial stewardship strategies. Tailoring interventions to reflect these micro-level patterns will not only improve patient outcomes but also slow the spread of resistant organisms across institutional and community boundaries.Furthermore, the findings point toward the broader structural and systemic challenges in combatting antimicrobial resistance in resource-limited settings. The lack of robust infection control infrastructure, limited availability of susceptibility testing, and inconsistent implementation of stewardship programs collectively contribute to the proliferation of XDR pathogens. These systemic issues cannot be addressed by clinical protocols alone; they require coordinated action by hospital leadership, policymakers, and public health stakeholders.As the world continues to confront the consequences of antimicrobial resistance, data-driven insights such as those generated in this study are indispensable. This research not only fills a crucial gap in the regional understanding of XDR prevalence but also serves as a call to action. Investment in laboratory capacity, continuous staff training, rational prescribing practices, and surveillance networks must be prioritized if we are to protect the efficacy of the dwindling antimicrobial arsenal.In conclusion, the high prevalence of XDR Gram-negative organisms identified in this study reflects a broader national and global concern. However, it also offers a timely opportunity for course correction. Through sustained commitment to local surveillance, multidisciplinary stewardship, and policy-level reforms, there is potential to curb the tide of resistance and preserve the therapeutic utility of existing antimicrobials for future generations.

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