

Beyond treatment: Escalating crisis of multidrug-resistant gram-negative pathogens, transmission dynamics and potential risk factors in a tertiary care hospital

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ABSTRACT

Objective: To assess the frequency, antimicrobial susceptibility and risk factors (prior hospitalization, antibiotic use, age ≥ 60 years) for emergence of multidrug-resistance Gram negative rods (MDR-GNRs) in a tertiary care hospital in Bahawalpur.

Material and Methods: This analytical cross-sectional study was conducted in the Department of Pathology at a tertiary-care hospital in Bahawalpur, Pakistan, from January 2023 to February 2025. Risk factors including age, prior hospitalization, and antibiotic use were assessed. Blood, urine, tissue, respiratory, pus, and body fluid specimens were processed, and organisms were identified by colony morphology, differential media, and standard biochemical tests. Antimicrobial resistance was interpreted according to CLSI criteria, with zone diameter cutoffs used to define resistance, including ≤ 19 mm for meropenem or imipenem to confirm carbapenem resistance.

Results: Data of 396 hospitalized patients yielding Gram negative pathogens were analyzed, of which 201(50.75%) isolates were MDR-GNRs. The most prevalent isolate was *E. coli* 196/396 (49.5%) followed by *Pseudomonas aeruginosa* 76/396 (19.2%), whereas the most prevalent MDR-GNRs were *Acinetobacter baumannii* 54.7% and *Klebsiella spp* 42.8%. Statistical analysis revealed a concerning prevalence of MDR-GNRs with statistically significant association to prior hospitalization (OR=14.87), prior antibiotic use (OR=7.34) and age ≥ 60 (OR=3.39).

Conclusion: High MDR-GNRs frequency linked to prior hospitalization, antibiotic exposure, and old age highlights urgent need for infection control measures and antimicrobial stewardship.

Keywords: Antibiotic use, Antimicrobial stewardship, Multi-drug resistant Gram-negative rods, Infection control, Tertiary care Hospital Bahawalpur.

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INTRODUCTION

Multi-drug-resistant Gram-negative rods (MDR-GNRs) are creating a crisis situation in healthcare set ups around the globe. The alarming ability of these organisms to resist multiple antibiotics, including carbapenems, which are considered the last line of defense [1] against severe bacterial infections has become an emergency. The World Health Organization (WHO), because of the growing threat, has designated MDR-GNRs as priority pathogens that demand immediate attention and action [2]. In recent years, MDR-GNRs have been on the rise in various regions, including Pakistan, where the burden of antibiotic resistance is already substantial. Studies have shown that MDR-GNRs can spread rapidly within healthcare settings, making it essential to

understand the risk factors, track their prevalence and understand their transmission dynamics [3][4].

This study focuses on evaluating the risk factors associated with the increasing prevalence of MDR-GNRs, in a tertiary care hospital in Bahawalpur, Pakistan. Bahawalpur, being a significant urban center in South Punjab, provides a unique context to investigate the risk factors of prior hospitalization, prior antibiotic use and the extent of MDR-GNRs in different clinical specimens from different age groups admitted in the hospital. By examining the patient population, this research aims to identify potential risk factors contributing to transmission of MDR-GNRs and informs evidence-based strategies to combat this growing public health concern. Understanding the prevalence and transmission dynamics of MDR-GNRs is crucial for developing effective infection control policies and antibiotic stewardship programs [5]. The findings of this study aim at contributing to the national and global efforts to combat antibiotic resistance to protect the vulnerable population.

MATERIAL AND METHODS

This analytical cross-sectional study was conducted at a tertiary care diagnostic facility of CMH

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Bahawalpur, Pakistan, over a period of 26 months from January 2023 to February 2025 after Ethical Review Committee approval certificate vide letter number 000232 dated 26th January 2023. The sample size was calculated using the Cochran formula for prevalence studies, with 34.98% expected prevalence (p) [6], 95% confidence level ($Z = 1.96$), and 5% margin of error (e). This yielded a minimum sample size of 349, which was adjusted to 396. Clinical specimens (n=396) were collected from patients admitted to the hospital during the study period. Key clinical and demographic risk factors, including age, gender, prior hospitalization, and recent antibiotic use, were investigated and analyzed for their association with multi-drug resistance in hospitalized patients. Patients were stratified in two age groups, ≤ 59 and ≥ 60 years. Specimen sources included blood, urine, tissue, respiratory, sputum, broncho-alveolar lavage, pus, and body fluid. Risk factors and their association with the isolation of specific Gram-negative bacteria were investigated. Specimens were included if they were collected from patients who had complete demographic and clinical information, were admitted to the hospital during the study period, and if they showed growth of Gram-negative rods on culture. Specimens were excluded from the study if they were duplicate isolates from the same patient, had no growth or contamination, or the patient had incomplete demographic or clinical information.

Specimens were processed using standard microbiological techniques. Briefly, blood and body fluids were inoculated into blood culture bottles (Becton Dickinson, USA) and incubated at 37°C for a maximum of 5 days. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar and incubated at 37°C for 24-48 hours. Other specimens were cultured on blood agar and MacConkey agar plates and incubated at 37°C for 24-48 hours. Isolates were identified using colony morphology, Gram staining, and biochemical tests. Oxidase test was used for Non-Lactose Fermenting (NLF) colonies to differentiate *Pseudomonas aeruginosa* from other GNRs. API 10 S strips (bioMérieux, France) was used for Lactose fermenting (LF) and Oxidase negative NLF colonies. Antibiotic sensitivity testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates (Oxoid, UK). Imipenem (10 µg) and meropenem (10 µg) discs were used for phenotypic confirmation of carbapenem resistance. Other antibiotics discs used in the study included gentamicin (10 µg), amikacin (30 µg), ceftazidime (30

µg), ceftriaxone (30 µg), piperacillin-tazobactam (110 µg) and ciprofloxacin (5 µg). CLSI guidelines were used to interpret the results. An isolate resistant to one antibiotic in at least three different classes of antibiotics was taken as MDR. Zone diameter of ≤ 14 mm for gentamicin (10 µg), ≤ 16 mm amikacin (30 µg), ≤ 17 mm ceftazidime (30 µg), ≤ 19 mm ceftriaxone (30 µg), ≤ 20 mm piperacillin-tazobactam (110 µg) and ≤ 21 mm for ciprofloxacin (5 µg) were taken as resistant [7]. Zone diameter ≤ 19 mm for imipenem or meropenem disk diffusion were taken as phenotypic confirmation of carbapenem resistance [8].

SPSS version 26 (IBM, USA) was used to analyze the data. Quantitative variables like age was presented with Mean $\pm$ Standard deviation (SD), while qualitative variables like type of organism and resistance patterns were estimated in frequencies and percentages. Chi-square test was used to compare categorical variables (e.g., presence or absence of prior hospitalization, antibiotic use, and old age ≥ 60 years) and to find association between MDR and Non-MDR groups. A p value < 0.05 was considered significant. The risks of MDR were calculated by Odds ratio for prior hospitalization, prior antibiotic use and old age (≥ 60 years).

RESULTS

In this study clinical and demographic data of 396 hospitalized patients yielding Gram negative pathogens were analyzed. The samples comprised of 206 (52%) males and 190 (48%) females. The mean age of subjects was 43.7 (26) years, ranging from 1 year to 90 years with median age of 45 years. Out of these 201(50.75%) isolates were MDR-GNRs and 195 (49.25%) were Non-MDRs. The mean and median ages of the MDR groups were 55.32 and 63 years respectively, while mean and median ages of the non-MDR group were 32.3 and 24 years respectively. The most prevalent isolate was *E. coli* 196/396 (49.5%) followed by *Pseudomonas aeruginosa* 76/396 (19.2%), *Acinetobacter baumannii* 53/396 (13.4%), *Klebsiella spp* 42/396 (10.6%), and *Proteus spp* 23/196 (5.8%). Descriptive statistics, such as percentages, were used to summarize prevalence and susceptibility patterns (e.g., carbapenem and other antibiotics resistance rates). Carbapenem resistance among the GNRs isolated was 25.5 % (101/396) for imipenem and 22.9% (91/396) for meropenem. Carbapenem resistance was most prevalent in *Acinetobacter baumannii* 29/53 (54.7%) followed by *Klebsiella spp* 18/42 (42.8%), *Pseudomonas aeruginosa* 18/76 (23.7%), and *E. coli*

28/196 (14.3%). Resistance to other antibiotics as per organism is given in Table-I.

Multi-drug Resistance (MDR) is defined as resistance to ≥ 3 antibiotic classes (carbapenems, aminoglycosides, cephalosporins, fluoroquinolones, beta-lactamase inhibitors). Overall MDR rate in our study was 201/396 (50.8%). MDR rate by organism is given in Figure-I. It was highest in *Acinetobacter baumannii* (83%), followed by *Klebsiella spp* (57.1%), *Pseudomonas aeruginosa* (47.3%), and *E.coli* (43.1%). A significant number of patients 49 (12.3%) had prior history of hospitalization. Out of 49 patients, specimen of 46 (93.8%) patients yielded growth GNRs resistant to carbapenems. Furthermore, 60 (15.2%) patients had used some sort of antibiotic in the previous 3 months. Out of 60 patients, specimen of 53 (88.3%) patients yielded growth of MDR-GNRs and 49 (81.6%) isolates were GNRs resistant to carbapenems. Old age (≥ 60 years) was also a significant predictor of emergence of MDR-GNRs.

When compared by Chi-square test prior hospitalization, antibiotic use and old age were significantly associated with MDR (p value <0.001). Prior hospitalization history within last 6 months was present in 49 (12.3%) patients. Of these specimens 46 (93.8%) yielded growth of GNRs resistant to carbapenems. Antibiotic use in the previous 3 months was noted in 60 (15.2%) patients, of these 53 (88.3%) were with MDR infections. 144 (36.36) patients were ≥ 60 years age, of these 112 (78.7%) had MDR infection. Risk of MDR estimated by odds ratios (ORs) with 95% confidence intervals (CIs) was higher in patients with prior hospitalization (14.87, 95% CI 4.70-47.03), antibiotic use (7.34, 95% CI 3.4-15.75), and old age ≥ 60 years (3.39, 95% CI 2.4-4.7) and were independent predictors for development of MDR. Results of association between risk factors of prior hospitalization, prior antibiotic use, old age and MDR by Chi-square Test and Odds ratio are given in Table-II.

Table-I: Resistance pattern of organisms to non-carbapenems antibiotics.

Antibiotic	<i>E. coli</i> (n=196)	<i>P. aeruginosa</i> (n=76)	<i>A.baumannii</i> (n=53)	<i>Klebsiella spp</i> (n=42)	<i>Proteus spp</i> (n= 23)
Gentamicin	36.2% (71/196)	46% (35/76)	71.6% (38/53)	45.2% (19/42)	34.7% (8/23)
Amikacin	25.5% (50/196)	39.4% (30/76)	58.4% (31/53)	35.7% (15/42)	30.4% (7/23)
Ceftazidime	47.9% (94/196)	40.7% (31/76)	69.8% (37/53)	66.6% (28/42)	17.3% (4/23)
Ceftriaxone	45.9% (90/196)	NA	67.9 (36/53)	57.1% (24/42)	39.1% (9/23)
Pipracillin-tazobactam	35.2% (69/196)	31.5% (24/76)	71.6% (38/53)	50% (21/42)	17.3% (4/23)
Ciprofloxacin	65.3% (128/196)	72.3% (55/76)	79.2% (42/53)	71.4% (30/42)	56.5% (13/23)

Table II: Association of Risk Factors with MDR Pathogens estimated by chi square test and Odds ratio.

Antibiotic	<i>E. coli</i> (n=196)	<i>P. aeruginosa</i> (n=76)	<i>A.baumannii</i> (n=53)	<i>Klebsiella spp</i> (n=42)	<i>Proteus spp</i> (n= 23)
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Pipracillin-tazobactam	35.2% (69/196)	31.5% (24/76)	71.6% (38/53)	50% (21/42)	17.3% (4/23)
Ciprofloxacin	65.3% (128/196)	72.3% (55/76)	79.2% (42/53)	71.4% (30/42)	56.5% (13/23)

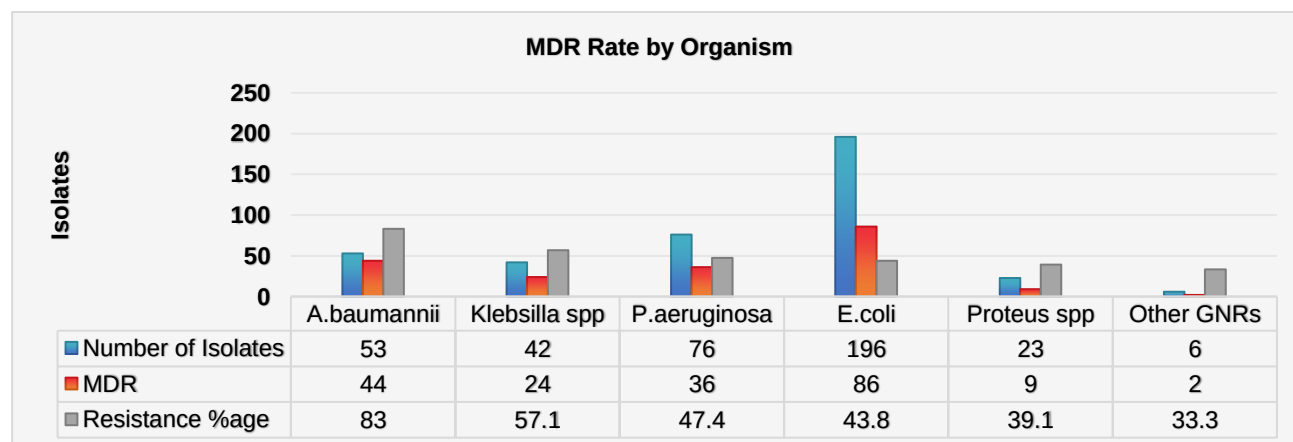


Figure-I: MDR rates among different isolates.

DISCUSSION

This study analyzed the burden of MDR-GNRs in a tertiary care hospital in Bahawalpur Pakistan. The findings exposed an alarming prevalence of MDR, especially among the elderly and those with previous use of antibiotics or prior hospitalization history. Our study results generally align with the global trends in low-and-middle-income-countries (LMICs). Globally, MDR-GNRs have been emerging as a critical public health challenge. The injudicious use of antibiotics is escalating the crisis. Multiple studies [9], [10] have shown that the overuse of antibiotics in healthcare including self-medication and the antibiotic usage in livestock is driving us to the precipice of pre-antibiotic era. The growing resistance to the last-resort antibiotics, such as carbapenems is especially a matter of huge concern [11]. The higher prevalence of MDR-GNRs in this study reveals an urgent need for requisite and robust infection control measures as well as antibiotic stewardship programs to curtail the alarming spread of the resistant pathogens in resource limited countries like Pakistan.

Globally, *E. coli*, *P. aeruginosa* and *A.baumannii* are usually the most frequently involved bacteria in infections due to their widespread prevalence as well as their ability to develop antimicrobial resistance mechanisms [12], [13]. The predominance of *E. coli* in our study is particularly concerning because it is one of the leading causes of both community-acquired as well as hospital-acquired infections. Moreover, their resistance to carbapenems particularly limits treatment options because of resistance to other antibiotics. Carbapenem resistance rates (meropenem: 22.9% and imipenem: 25.5%) in our study were highest for *Acinetobacter baumannii* (54.7%) and *Klebsiella spp* (42.8%). These findings are consistent with studies from Iran by Sholeh *et al* [14].

In our study prior hospitalization was strongly associated with MDR-GNRs (OR:14.87, 95% CI: 4.70-47.03), a finding consistent with the above-mentioned study from Iran in which the OR was found to be 4.2. A similar study from Bangladesh [15] had an OR of 3.3. The odds ratio in our study is comparatively higher and this discrepancy might be due to differences in local infection control practices prolonged hospitalizations, overcrowding, or inadequate sanitation.

Similarly, in our study prior antibiotic use increased MDR risk by 7.34-fold, mirroring trends in studies in Vietnam [16] with OR = 3.1–4.5. Iranian study [17] by Moghadam pour M *et al* showed similar results with

OR of 5.1. Our study revealed 81.6% of patients with recent antibiotic exposure in the last three months yielding growth of carbapenem-resistant isolates, suggesting empiric carbapenem overuse. Such over use practices have been documented in studies from Sri Lanka [18] and India [19]. Our findings also emphasize clinical misuse which are likely due to imprudence towards or lack of knowledge about the Antimicrobial Stewardship in our country.

Elderly patients (≥ 60 years) had 3.39-fold higher odds of MDR-GNRs infection (95% CI: 3.4-15.75), a risk factor less explored in other regional studies. It is possible that presence of multiple comorbidities like renal failure and diabetes etc along with frequent and prolonged hospitalizations, and aging immune system might be the main drivers rather than merely old age itself. Still our findings align with data from developed countries, where elderly patients are disproportionately affected by MDR-GNRs infections [20], [21].

Our study reinforces the global AMR crisis but underscores region-specific drivers. For example, our data emphasize clinical misuse and hospital-based transmission rather than community acquired resistance for which agricultural misuse is the main factor as reported from China [22] and India [23]. The inclination to use carbapenem empirically in re-hospitalized patients due to high resistance to other antibiotics may be a prominent factor in our set ups.

Our study is limited by its lack of molecular characterization of resistance genes and single-center design but it will definitely help in focusing on targeted interventions, enhanced surveillance and age-stratified antibiotic stewardship. Our hospitals should prioritize hospital infection control measures, tailored antimicrobial strategies and monitor the use of precious antibiotics to de-escalate the crisis of antimicrobial resistance in MDR pathogens.

CONCLUSION

Escherichia coli, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella spp* are predominant clinical isolates emerging as MDR-GNRs. The ever-growing AMR necessitates urgent actions and a multifaceted approach to tackle this menace. Elderly population, those with prior use of antibiotics and hospitalization history require closer monitoring for antimicrobial resistance. Stringent implementation of these strategies will not only improve antibiotic utilization and patient outcomes but will also mitigate the frightful threat of antimicrobial resistance.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Shamshad Ali: Project conception and development, study design, drafting the manuscript, final approval, accountable for all aspects of publication

Inam Ullah Khan: Substantial contribution to the project development, interpretation of the result and critical review of important intellectual content, final approval, accountable for all aspects of publication

Muhammad Anwar: Statistical analysis, critical reviews and result interpretation, final approval, accountable for all aspects of publication

Mohsin Hussain Nizamani: Data Collection, literature review, results interpretation, final approval, accountable for all aspects of publication

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