

# Trends in Antibiotic Resistance among Gram-Negative Rods in a Tertiary Care Hospital, Rawalpindi

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## Abstract

**Background:** A global health concern, antimicrobial resistance (AMR) results from the overuse and misuse of antibiotics over the past 60 years, leading to more resistant microorganisms and limited treatment options, imposing significant societal costs.

**Objective:** To evaluate the patterns of antibiotic resistance in common Gram-negative rods isolated from the clinical samples during the study period from January 2021 to December 2023, according to CLSI guidelines 2023.

**Materials and Methods:** The data was collected from retrospective record review and prospective data from October 2023 to December 2023 of commonly isolated Gram-negative rods with confirmed 4486 cases from various clinical samples.

**Results:** According to descriptive statistics in 2021, 2022, and 2023, the percentage of *E. coli* is 35.01% to 30.03% to 34.96%. *Klebsiella spp.* was 16.54%, 40.96%, 42.51% respectively. *Pseudomonas spp.* dropped from 29.13% to 21.47% and rose to 49.40% in 2023. *Proteus spp.* 4.10%, 41.9%, and 53.0%. Resistance to *E. coli* and *Klebsiella spp.* decreased in response to frontline antibiotics 60%, 57%, 28% for *E. coli* and 30.60%, 27.70%, 17% for *Klebsiella spp.*, respectively, suggesting potential efficacy. The Penicillin like Amoxicillin demonstrated a marked rise in resistance levels, 87.20% in 2023. The resistance to aminoglycosides in *Pseudomonas spp.* decreased significantly in 2023, 37.50% and 14.50%, respectively; yet resistance to Ciprofloxacin increased to 50.60%.

**Conclusion:** The dynamic evolution of resistance among bacterial species to various drugs advocates for selective antibiotic use and continuous surveillance to control the spread of resistant diseases.

**Keywords:** Gram-Negative Bacteria; Drug Resistance; Microbial Sensitivity Tests; Escherichia coli; Klebsiella; Proteus

## Introduction

Enterobacteriaceae, a gram-negative bacilli family, is increasingly resistant to antibiotics, with definitions in both clinical and microbiological contexts.<sup>1</sup> Microbiologically, resistance involves a genetically specified mechanism that can be acquired or modified. In clinical settings, the organism is classified as susceptible or resistant based on laboratory cut-off values, with resistance indicating a high likelihood of therapeutic failure and the bacteria's ability to replicate against the drug. The most common measure of the resistance of pathogen is minimum inhibitory concentration (MIC), the lowest concentration required to prevent the replication of bacteria.<sup>2</sup> Selman Waksman, a prominent researcher in the field of actinomyces in the early 12<sup>th</sup> century described the term antibiotic, a chemical compound produced from certain microorganism to kill the other organism.<sup>3</sup>

The global increase in resistant Gram-negative infections over the past decade has led to a global priority list for new antibiotics, focusing on *Pseudomonas aeruginosa* and Carbapenem-resistant *Enterobacteriaceae*.<sup>4</sup> *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are significant drug-resistant pathogens, with *A. Baumannii* strains showing high resistance to Imipenem. *P. aeruginosa* frequently causes nosocomial infections due to its high virulence and treatment resistance. Among bloodstream isolates, *E. Coli* ranks third, followed by *Proteus mirabilis*, *Enterobacter spp.*, and *Klebsiella pneumoniae*. For nosocomial pneumonia, *E. Coli*

is third as well, with *Serratia spp.*, *Enterobacter spp.*, and *K. pneumoniae* following. In urinary tract infections, *E. Coli* is the prevalent pathogen, with *K. pneumoniae* in fourth place, *Enterobacter spp.* in sixth place, and *P. mirabilis* in seventh place.<sup>5</sup>

The rising issue of AMR threatens modern medical progress, with approximately 40% of the AMR health burden stemming from infections resistant to critical antibiotics such as Carbapenems and Colistin. Addressing this challenge, the Global Action Plan on Antimicrobial Resistance (GAP), endorsed by the World Health Assembly in 2015, emphasizes increased investment in new antibiotics, vaccines, and therapies for AMR as a key strategy.<sup>6</sup> *Pseudomonas* and *Enterobacteriaceae* are among Gram-negative bacteria with multidrug-resistant properties, contributing to innate and acquired multidrug resistance (MDR), as highlighted by the U.S. Centers for Disease Control and Prevention.<sup>7</sup>

The WHO has classified antibiotic-resistant diseases into critical, high, and medium priorities, with a significant prevalence of Gram-negative bacteria.<sup>8</sup> It is projected that antibiotic resistance may lead to 10 million deaths annually by 2050. Japan reports a 10-15% medication resistance rate, while China and South Korea have rates around 30%.<sup>9</sup> *E. coli's* resistance to Cotrimoxazole and Fluoroquinolones varies across Europe and the Mediterranean, with higher rates observed in Japan and Australia (90%).<sup>10</sup> Research indicates reduced exposure to specific antibiotics raises

susceptibility to UTIs. Increasing anti-microbial resistance, particularly in *E. coli* and Enterobacteriaceae, necessitates robust surveillance to address the threat posed by *K. pneumoniae*, especially in Asia.

The objective of the study was to evaluate the trends in antibiotics resistance patterns of the most common Gram-negative rods according to the CLSI guidelines 2023 from January 2021 to December 2023 in a major tertiary care hospital in Rawalpindi.

## Materials and Methods

This research implemented an Observational study design, using data collected from Antimicrobial Susceptibility Testing (AST) reports of commonly isolated Gram-negative rods isolates from various clinical samples. The study included 4486 confirmed cases. The retrospective data from January 2021 to September 2023 was gathered from the Hospital's Information and Management System (HIMS) and prospective data from October 2023 to December 2023 was also collected from the Pathology Lab of Benazir Bhutto Hospital. This study will adhere to the ethical guidelines of IRB (Institutional Review Board) of Rawalpindi Medical University (Reference# RMU/IAHS/14/2025 Dated: 21/05/2025), maintaining patient confidentiality and obtaining necessary permissions from the hospital's ethics committee. Inclusion criteria includes non-duplicate isolates collected from various clinical samples, including wound swabs, pus swabs, blood cultures, and respiratory specimens. Only the most common Gram-

negative bacteria were included in my research studies. Sample reports with incomplete or missing AST data, improper storage and transport, contaminated and repeated samples are excluded from the study.

The study uses various culture media, consumables, and biochemical to analyze *E. coli* colonies. For the prospective part, samples were processed in biosafety cabinets, and pathogen growth is inoculated on culture media plates. The plates are incubated at 37° C 24 hours, with Gram-negative rods growing best at 35-37° C. Slides are labeled, de-waxed, emulsified, heat fixed, and stained with crystal violet stain, iodine, ethanol, and safranin.

Tests are conducted on isolated Gram-negative rods using the first-line antibiotics Augmentin (AUG 30), Imipenem (IPM 10 µg), Ciprofloxacin (CIP 5 µg), Gentamycin (CN 10 µg), Tazobactam (TZP TZP 110 µg), Ceftazidime (CAZ 30 µg), and Co-trimoxazole (SXT 25 µg). Cefoperazone (CFP 75 µg) and Fosfomycin (FOS 200 µg), second line antibiotics, are tested against resistant Gram-negative rods if first line drugs are shown to be resistant.

By measuring the widths of the zones of inhibition, one can ascertain the sensitivity, resistance, or intermediate resistance of the commonly isolated Gram-negative rods to these antibiotics, in accordance with the Latest Clinical and Laboratory Standards Institute (CLSI) 2023 interpretative criteria.<sup>11</sup>

Data analysis is done using SPSS software version 25, and descriptive statistics are used to compile antibiotic resistance information for Gram-negative rods collected during a three-years period.

## Results

The study comprised verified cases of frequently recovered Gram-negative rod isolates from the pathology lab of the Hospital in Rawalpindi of the Benazir Bhutto Hospital in Rawalpindi, from January 1, 2021, to December 30, 2023. The study included 4486 positive examples of these rods that were Gram negative.

According to descriptive statistics, Comparison of resistance pattern of *Escherichia coli* over three years' period, the percentage of *E. Coli* dropped from 35.01% in 2021 to 30.03% in 2022 and again rose to 34.96% in 2023. In Comparison of resistance pattern of *Klebsiella Spp.* over three years' period, *Klebsiella Spp* percentage was 16.54% in 2021, rose to 40.96% in 2022, and then increased to 42.51% in 2023. *Pseudomonas spp's* percentage also first dropped, going from 29.13% in 2021 to 21.47% in 2022 and again rose to 49.40% in 2023. *Proteus Spp.* increased from 4.10%, 41.9%, and 53.0% of the total in 2021, 2022, and 2023. Table 1 shows frequency distribution of Gram-negative over 3 years' period.

**Table 1** Frequency Distribution of Commonly Isolated Gram-Negative Rods Over 3 Years' Period

Organism	2021(n)	2022 (n)	2023 (n)
<b>E. coli</b>	710 (35.01%)	609 (30.03%)	709 (34.96%)
<b>Klebsiella spp</b>	128 (16.54%)	317 (40.96%)	329 (42.51%)
<b>Psuedomonas spp</b>	407 (29.13%)	300 (21.47%)	690 (49.40%)
<b>Proteus spp</b>	4 (4.10%)	41 (41.90%)	53 (54%)

**Table 2** Table of Gram-negative Rods Markedly isolated from different specimen are shown below:

Organisms	Specimen	2021 (n)	2022 (n)	2023 (n)
<b>E. Coli</b>	<b>Pus</b>	274	225	325
	<b>Urine</b>	427	439	376
	<b>Blood</b>	9	6	8
<b>Klebsiella Spp</b>	<b>Pus</b>	58	201	191
	<b>Urine</b>	58	111	121
	<b>Blood</b>	12	5	17
<b>Pseudomonas Spp</b>	<b>Pus</b>	324	224	376
	<b>Urine</b>	71	71	314
	<b>Blood</b>	19	18	0
<b>Proteus Spp</b>	<b>Pus</b>	2	39	53
	<b>Urine</b>	2	2	0

Crucially, resistance to *E. coli* and *Klebsiella* spp. decreased to frontline antibiotics including Ciprofloxacin, Meropenem, and Ceftriaxone, with rates of 60%, 57%, and 28% for *E. coli* and 30.60%, 27.70%, and 17% for *Klebsiella* spp., respectively, suggesting potential efficacy against these pathogens. In contrast, Penicillins like Amoxicillin demonstrated a marked rise in resistance levels, 87.20% in 2023. Moreover, resistance to aminoglycosides like fosfomycin and amikacin in *Pseudomonas* species decreased significantly in 2023, with percentages of

37.50% and 14.50%, respectively; yet resistance to ciprofloxacin dramatically increased to 50.60%. The given table below shows the yearly percentage statistics for different line antibiotics according to CLSI guidelines 2023. Amikacin in *Pseudomonas* species decreased significantly in 2023, with percentages of 37.50% and 14.50%, respectively; yet resistance to ciprofloxacin dramatically increased to 50.60%. The given table 3 below shows the yearly percentage statistics for different line antibiotics according to CLSI guidelines 2023.

**Table 3** Yearly Percentage Stats of Different Line Antibiotics According to CLSI Guidelines 2023.

ANTIBIOTICS (CLSI guidelines 2023)	ANNUAL PERCENTAGE DISTRIBUTION		
	2021	2022	2023
<i>E. Coli</i>			
1 <sup>st</sup> line Flouroquinolones (Ciprofloxacin)	60%	57%	57%
Carbopenem, Meropenem (MEM)	16.60%	10.30%	16.10%
2 <sup>nd</sup> line Cephalosporins, Ceftriaxone (CRO)	80%	76%	28%
3 <sup>rd</sup> line Aminoglycosides Amikacin (AK)	16.60%	16.60%	16.10%
Mono-bactams Azotreaonam (ATM)	83.30%	65.90%	63.20%
<i>Klebsiella Spp</i>			
1 <sup>st</sup> line Meropenem (MEM)	30.60%	27.70%	17%
Ceftriaxone (CRO)	82%	80.70%	62.10%
2 <sup>nd</sup> line antibiotics Amoxicillin (AMC)	80%	82.30%	87.20%
3 <sup>rd</sup> line Aminoglycosides, Amikacin (AK)	20.10%	35.20%	17.50%
<i>Pseudomonas Spp</i>			

<b>1<sup>st</sup> line, Amikacin (AK)</b>	42.80%	38.50%	37.50%
<b>Fosfomycin (FOS)</b>	41.10%	31.70%	14.50%
<b>Ciprofloxacin (CIP)</b>	12.50%	21.25%	50.60%
<b>2<sup>nd</sup> line Mono-bactams, Aztreonam (ATM)</b>	47%	42%	64%
<b><i>Proteus Spp</i></b>			
<b>1<sup>st</sup> line Cefipime (FEP)</b>	33%	35.70%	36.50%
<b>Penicillins, Amoxicillin (AMC)</b>	62.50%	65.90%	66.50%

## Discussion

*Escherichia coli*, the most prevalent Gram-negative bacterial pathogen, presents a clinical and epidemiological challenge. In the last ten years, a large number of high-risk, multidrug-resistant bacteria have surfaced, primarily due to the growing selective pressure imposed on by the use of antibiotics.<sup>12</sup> Antimicrobial resistance (AMR) in *E. Coli* has noticeably increased during the last 20 years. Similarly, Fluoroquinolone (FQ) resistance was detected in over 20% of urine isolates from 18 European nations in a 2018 investigation. TMP-SMX resistance was detected in 32.7% of the *E. Coli* isolates from outpatient urine samples. The CLSI guidelines for 2023 show a decline in antibiotic resistance against *Escherichia coli*, with Ciprofloxacin and Ceftriaxone showing potential improvements. The *E. coli* in urine has become resistant to many antibiotics. However, globally there is no consistent decline in *E. coli* resistance. According to a retrospective study by Rama Alkhalwaldeh 27% have resistance to at least one antibiotic for

UTI.<sup>13</sup> Thus, ongoing challenges require integrated strategies and stewardship.

Over the past six years, resistance patterns against *Klebsiella spp.* have significantly increased, with Imipenem resistance reaching 19% by 2015, indicating a significant rise in broad-spectrum antibiotic resistance.<sup>14</sup> The *Klebsiella Spp.* findings of this investigation aligned with those of a study carried out from September 2018 to March 2019 at two significant hospitals in Hamadan, west of Iran. *Klebsiella Spp.* isolates were sensitive to Colistin, with Cefotaxime exhibiting the highest level of antibiotic resistance (92%). In accordance with our current investigation, this data also demonstrates that clinical isolates from inpatient departments are more common than those from outpatient departments.<sup>15</sup>

In Quetta, Baluchistan, at the Bolan Medical Complex, another study was carried out. Urine and sputum samples from clinical patients (n = 107) were gathered and treated with selected culture media to isolate *Klebsiella Spp.* Amoxicillin, Cefixime, Amoxicillin-Clavulanic

Acid, Cefotaxime, and Ceftriaxone were all resistant to the isolates. A 32-year-old female patient's tracheal secretion and endotracheal tube were found to contain a carbapenem resistant strain of *Klebsiella* Spp in June 2015 when the patient was in the intensive care unit of the tertiary hospital at the Pakistan Institute of Medical Sciences, Islamabad. It was discovered that isolates were only sensitive to Tigecycline and Colistin and were multidrug resistant.<sup>16</sup> *Pseudomonas* species infections in hospitals have exacerbated antibiotic resistance globally. Between 2022 and 2017, resistance to all tested antibiotics decreased, with a significant drop in ICU isolates. P is not greater than 0.001. However, carbapenems production increased in isolates resistant to Carbapenem.<sup>17</sup>

Antimicrobial resistance against *Proteus* species is increasing to Cephalosporins, penicillin, and Amoxicillin (AMC) between 2021 and 2023, highlighting the need for vigilant antibiotic stewardship and continuous surveillance. AMR causes 9% of global fatalities, with 12.7 million direct and 495 million indirect deaths in 2019, accounting for 13.7 million deaths.<sup>18</sup> Current guidelines for treating antimicrobial-resistant (AMR) disease are a priority for the Infectious Diseases Society of America (IDSA). Gram-negative bacteria (GNBs) that exhibit antimicrobial resistance (AMR) represent a serious risk to public health.<sup>19</sup>

The World Health Organization (WHO) has designated ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

*Pseudomonas aeruginosa*, and *Enterobacter* spp.—as the highest priority pathogens.<sup>20</sup> Antimicrobial resistance, a growing concern, is causing 10 million deaths annually by 2050. It's categorized into intrinsic, acquired, genetic, and DNA transfer mechanisms. In 2019, it caused 5 million casualties. By 2050, antibiotic inefficiency is predicted to be the cause of 90% of mortality in Asia and Africa.<sup>21</sup>

The recent study reveals that antibiotic susceptibility patterns in gram-negative bacteria from China's emergency departments. TZP resistance rates decreased from 2016 to 2019, while *E. coli* showed less than 10% resistance to various antibiotics. *K. pneumoniae* showed 6.4% resistance to COL and less than 20% resistance to AMK.<sup>22</sup> The WHO reports that only 18 new antibiotics have been approved since 2014, highlighting the urgent need for new antibiotics. Artificial intelligence (AI) has significantly improved the effectiveness of finding new antibiotics. Between 2014 and 2021, 18 medications, including one for highly drug-resistant TB, were licensed and made accessible. These antibiotics were approved by various agencies, including the FDA, EU, India, and Japan.<sup>23</sup>

The use of antibiotic substitutes as antimicrobials is positively impacted by the seven medications' clinical trial success, as there is currently no medication available on the market. As an innovative strategy to counteract antimicrobial resistance, some researchers are now looking at biopharmaceuticals as an alternative to antibiotics<sup>24</sup>. Antibiotic-resistant bacterial

infections could cause 10 million global deaths by 2050, potentially leading to a pandemic.<sup>25</sup> Big pharmaceutical firms' shift from antibiotic development exacerbates the urgency. Scientific literature shows obstacles and other methods for finding new antibiotic alternatives, highlighting the need for urgent action.

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### Conclusion

The primary findings of the study show that resistance rates for common Gram-negative rods increased significantly from January 2021 to December 2023, interpreted according to CLSI guidelines 2023. This highlights the necessity of selective antibiotic use and ongoing surveillance. These Gram-negative rods show declining antibiotic resistance, sensitivity to Aminoglycosides, and Carbapenem. In *Klebsiella* spp., amoxicillin resistance rose to 87.20%. While *Pseudomonas* spp. are more abundant in pus and blood samples, *E. coli* is highly prevalent in urine samples. Ciprofloxacin resistance in *Pseudomonas* spp. surged to 50.60% showing reduced sensitivity by 2023. Hence rising prevalence of pathogens with increased resistances is a challenge. It is

recommended that new study should be according to CLSI guidelines 2024. The study also advocates selective antibiotic use like local antibiotic guidelines and continuous surveillance to control the spread of resistant diseases.

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