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Colistin and Carbapenem Resistance in Gram-Negative Bacteria: An Emergent Threat

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ABSTRACT:

The rise of Gram-negative bacteria that resist both colistin and carbapenem antibiotics creates serious problems for clinical laboratories and patient treatment. This research examined how effectively automated laboratory systems can identify these developing resistance patterns in medically important Gram-negative bacteria. **Methods:** Researchers conducted a forward-looking observational study in hospitals throughout Erbil, Iraq from November 2024 to March 2025, studying 350 clinical samples taken from patients between 1 and 90 years old. Specimens included urine (295), swabs (17), blood (16), stool (14), and sputum (8) samples. Bacterial identification and antimicrobial susceptibility testing were performed using automated systems.

Seven Gram-negative bacterial species were identified, with *Escherichia coli* predominating (183 isolates, 52.3%). Alarming carbapenem resistance emergence was detected, particularly in *Klebsiella pneumoniae* (48.10% imipenem, 37.97% meropenem resistance). Most critically, emerging colistin resistance was documented in typically susceptible organisms: *Escherichia coli* (1.64%), *Klebsiella pneumoniae* (3.80%), and *Pseudomonas aeruginosa* (6.67%), representing a dangerous evolutionary step toward pan-drug resistance. Female patients showed significantly higher infection rates (65.43% vs 34.57%, p=0.0222).

The emergence of colistin resistance in previously susceptible Gram-negative bacteria, combined with extremely high carbapenem resistance rates, signals a critical evolution toward untreatable infections. This emerging resistance pattern demands immediate intervention through enhanced surveillance, antimicrobial stewardship, and infection control measures to prevent widespread dissemination of pan-drug resistant organisms.

Keywords: Colistin resistance, Emergence, Carbapenem resistance, AST, pan-drug resistance.

1 INTRODUCTION

The emergence and rapid dissemination of carbapenem and colistin-resistant Gram-negative bacteria represent one of the most pressing challenges in contemporary clinical microbiology and infectious disease management [1]. These bacteria that resist multiple drugs have completely changed how we approach antibiotic treatment, making it necessary to develop advanced detection techniques and thorough monitoring programs [2].

Carbapenemantibiotics, such as imipenem, meropenem, and ertapenem, have traditionally been the foundation for treating serious infections caused by Gram-negative bacteria. Their ability to work against a wide range of bacteria and resist breakdown by many β-lactamase enzymes made them the preferred treatment for critically ill patients with complicated infections [3]. However, the widespread appearance of bacteria that produce carbapenemase enzymes, especially among Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, has seriously reduced the effectiveness of these essential antibiotics [4]. The World Health Organization still classifies carbapenem-resistant Enterobacteriaceae as a high-priority threat, emphasizing the critical need for better diagnostic methods [5].

Recent monitoring data shows concerning resistance levels, with carbapenemase production making up more than 50% of carbapenem-resistant Enterobacteriaceae cases, especially among Klebsiella pneumoniae strains. The worldwide spread demonstrates complicated patterns of how resistance mechanisms distribute globally [6].

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At the same time, the development of colistin resistance has created an even more troubling situation. Colistin, a polymyxin antibiotic previously limited to topical applications because of its kidney and nerve toxicity, made a comeback as a "final option" treatment for carbapenem-resistant infections [7]. The appearance of colistin (polymyxin E) resistance mechanisms has raised the frightening possibility of bacteria resistant to all drugs that are essentially impossible to treat with currently available antibiotics [8].

What makes today's situation especially worrying is when carbapenem and colistin resistance combine within the same bacterial strain. This combination creates medical situations where infections involve bacteria resistant to nearly every available antibiotic, presenting extraordinary treatment challenges [9]. Research consistently shows that patients with these resistant bacterial infections face longer hospital stays, higher medical costs, and significantly increased death rates [10].

The medical importance of quick and accurate detection cannot be emphasized enough. Delayed identification of carbapenem or colistin resistance can result in treatment failures, increased deaths, extended hospital stays, and greater spread within healthcare facilities [11]. Every hour of delay in getting the correct diagnosis and proper antibiotic treatment raises death rates by roughly 8%, making understanding of resistance mechanisms essential for patient care [12].

The biological processes behind carbapenem resistance include several routes such as carbapenemase enzyme production, changes in outer membrane channels, increased efflux pump activity, and modifications in penicillin-binding proteins [13]. These processes can happen alone or together, creating complicated resistance patterns that need careful analysis [14].

Colistin resistance involves changes to bacterial cell wall components, especially lipopolysaccharide structures, which modify where colistin antibiotics can attach [15]. These changes can develop through different cellular control systems and can spread between bacterial populations, creating difficulties for detection and treatment [16].

The distribution patterns of resistant Gram-negative bacteria vary considerably across different geographic areas, healthcare environments, and patient groups. Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumoniae, are the most common causes of hospital-acquired infections and show growing rates of carbapenem and colistin resistance worldwide [17]. Pseudomonas aeruginosa naturally resists many antibiotics and easily develops additional resistance mechanisms, making it an especially difficult pathogen in intensive care units [18]. *Acinetobacter baumannii* has emerged as a critical nosocomial pathogen with remarkable ability to survive in hospital environments and acquire multiple resistance mechanisms simultaneously [19].

This comprehensive evaluation addresses the critical need for understanding resistance mechanisms in the era of antimicrobial resistance. The emergence of carbapenem and colistin resistance represents a critical evolutionary step toward untreatable bacterial infections that threatens the foundation of antimicrobial therapy [20].The detection of these resistance patterns represents a sentinel event requiring immediate intervention to prevent the spread of untreatable bacterial infections.

2 MATERIALS AND METHODS

SPECIMEN COLLECTION

Between November 2024 and March 2025, we collected 350 clinical specimens from patients at Erbil hospitals. Our comprehensive approach included multiple specimen types to examine bacterial infections across various body sites. All specimens were processed within two hours of collection to maintain bacterial quality. When immediate processing was not possible, samples were refrigerated at 4°C to maintain bacterial viability while preventing contamination or overgrowth of non-target organisms.

URINE SAMPLES

Urine specimens were obtained from patients with suspected urinary tract infections using the clean-catch midstream method. Patients cleansed their urethral area before collecting the middle portion of their urine stream to minimize contamination from normal skin bacteria.

BLOOD SAMPLES

Blood was drawn using strict sterile procedures and placed into BacT/Alert bottles designed for automated blood culture systems. These specialized bottles were inserted into automated monitoring systems that continuously detect bacterial growth through metabolic byproducts.

WOUND SWABS

Burn wound specimens were collected using sterile cotton swabs and proper aseptic techniques. We systematically swabbed the wound surface using sterile collection devices while avoiding healthy tissue to ensure adequate sampling of potential pathogens.

STOOL SPECIMENS

Stool samples were obtained from patients with gastrointestinal infections presenting with diarrhea, abdominal pain, or other enteric symptoms. Samples were collected in sterile containers with transport media to preserve bacterial viability during transport.

SPUTUM SAMPLES

Respiratory specimens were collected from patients with suspected lower respiratory tract infections, preferably as early morning samples. Patients were instructed on proper collection techniques to ensure specimens representative of lung infections rather than upper respiratory secretions.

SAMPLE PRESERVATION

For bacterial storage, bacterial isolates were grown on MacConkey agar plates at 37°C overnight, then several colonies were mixed with Brain heart infusion (BHI) broth containing 20% glycerol in small tubes. These glycerol stock samples were frozen at -20°C for short-term storage up to 6 months or at -80°C for long-term preservation lasting several years while keeping the bacteria viable.

SPECIMENS PROCESSING

All clinical specimens subjected to primary isolation by carefully streaking them onto blood agar plates containing 5% blood and MacConkey agar plates using standard bacteriological streaking methods (continuous streaking for urine samples and quadrant streaking for the rest of the samples). This approach ensured proper colony separation and allowed this study to assess morphological characteristics effectively. The inoculated plates were then incubated aerobically at 35±2°C for 18-24 hours under optimal atmospheric conditions. After incubation, the test was performed preliminary differentiation between Gram-positive and Gram-negative bacteria by observing their characteristic growth patterns. This study revealed identified Gram-negative organisms by their ability to grow well on MacConkey agar, while most Gram-positive bacteria showed inhibited or absent growth on this selective medium. And further differentiated organisms through their lactose fermentation patterns on MacConkey agar.

BACTERIAL IDENTIFICATION

From the primary culture plates, we carefully selected well-isolated, morphologically distinct colonies that we suspected to be Gram-negative bacteria based on their growth characteristics. We prepared standardized bacterial suspensions by emulsifying pure colonies in 0.45% sterile saline solution and adjusted the turbidity to match 0.5-0.63 McFarland standards using visual comparison. This step was crucial to ensure the consistent inoculum density required for accurate performance of the Vitek2 system. We then processed the confirmed Gram-negative isolates using the Vitek2 automated identification and antimicrobial susceptibility testing system (bioMérieux, Durham, NC, USA), following the manufacturer's specifications and our established laboratory protocols.

We inoculated Vitek2 identification cards, specifically designed for Gram-negative bacteria, with our standardized bacterial suspensions and loaded them into the automated system for biochemical identification. This process typically required 4-8 hours for complete species identification and confidence level determination.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Concurrently, we performed antimicrobial susceptibility testing using Vitek2 AST-GN cards that contained a comprehensive panel of clinically relevant antibiotics. Our testing panel included carbapenem agents such as meropenem, imipenem, and colistin, allowing us to detect resistance patterns to these critical last-resort antimicrobial agents.

We interpreted all antimicrobial susceptibility results according to current Clinical and Laboratory Standards Institute (CLSI, 2024) breakpoints to determine carbapenem and colistin resistance patterns accurately. Our data analysis involved systematic recording of resistance profiles for each bacterial isolate, calculation of prevalence rates of carbapenem and colistin resistance across the different clinical specimen types we tested, and comprehensive analysis of co-resistance patterns. This approach allowed us to identify potentially dangerous multidrug-resistant organisms that could pose significant clinical and epidemiological threats to patient care and public health.

STUDY CRITERIA

The study only included Gram-negative bacteria that were collected from patients in Erbil, Kurdistan, Iraq. And excluded any Gram-positive bacteria, samples from other cities or regions, and bacteria that had already been tested for antibiotic resistance in previous studies.

ETHICAL APPROVAL

A scientific ethics committee reviewed and approved the study proposal at the Medical Microbiology department, College of Health and Science, Koya University, with reference number DMMB-8-24 dated November ,1, 2024.

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism version 10.0.0 (GraphPad Software, San Diego, CA). Categorical variables were expressed as frequencies and percentages, while continuous variables were presented as means with standard deviations and standard error of the mean. The chi-square test of independence was utilized to assess associations between categorical variables, with Fisher's exact test applied when expected cell counts were less than five. Statistical significance was defined as $p<0.05$.

3 RESULTS

STUDY POPULATION AND BACTERIAL ISOLATES

During the study period from November 2024 to March 2025, 350 clinical specimens yielded Gram-negative bacteria from patients aged 1-90 years (mean age 39.18 ± 19.94 years). The specimen distribution included urine samples (295, 84.3%), swabs (17, 4.9%), blood (16, 4.6%), stool (14, 4.0%), and sputum (8, 2.3%) samples, reflecting the predominance of urinary tract infections in the study population.

BACTERIAL SPECIES DISTRIBUTION AND DEMOGRAPHICS

Seven Gram-negative bacterial species were identified, with *Escherichia coli* predominating (183 isolates, 52.3%), followed by *Klebsiella pneumoniae* (79 isolates, 22.6%) and *Pseudomonas aeruginosa* (45 isolates, 12.9%). The remaining species included *Proteus mirabilis* (16, 4.6%), *Burkholderia cepacia* (12, 3.4%), *Acinetobacter baumannii* (9, 2.6%), and *Enterobacter cloacae* (6, 1.7%) (**Table 1A**).

Significant demographic patterns emerged across the bacterial isolates. Female patients showed significantly higher overall infection rates (229/350, 65.4%) compared to males (121/350, 34.6%, p=0.0222). This female predominance was particularly pronounced for *Escherichia coli* infections, affecting 134 females versus 49 males (73.2% vs 26.8%, p=0.0005), consistent with established urinary tract infection epidemiology. *Klebsiella pneumoniae* also showed female predominance (54.4% vs 45.6%, p=0.0444), while *Pseudomonas aeruginosa* demonstrated no significant gender preference (p=0.5074). *Acinetobacter baumannii*, *Proteus mirabilis*, *Burkholderia cepacia*, and *Enterobacter cloacae* showed no significant sex-based differences. To provide clearer visualization and better interpretation of the data, (**Figure 1**) were generated following the (**Table 1A**), highlighting the main trends and comparisons observed in the study.

Age distribution analysis revealed distinct epidemiological patterns (**Table 1B**). *Escherichia coli* infections peaked in young adults aged 19-35 years (79 cases, 43.2%) and middle-aged adults 36-64 years (65 cases, 35.5%), with significantly fewer cases in adolescents 13-18 years (4 cases, 2.2%) (p=0.0168). *Klebsiella pneumoniae* showed predominant clustering in the 36-64 age group (39 cases, 49.4%) followed by young adults (27 cases, 32.9%), though this pattern did not reach statistical significance (p=0.1111). *Pseudomonas aeruginosa* was fairly evenly distributed across age groups (p=0.1883). *Burkholderia cepacia* demonstrated significant age-related clustering (p=0.0010), appearing primarily in middle-aged (4 cases) and elderly patients (4 cases), with minimal representation in younger age groups. *Acinetobacter baumannii*, *Proteus mirabilis*, and *Enterobacter cloacae* showed no significant age-related patterns. To provide clearer visualization and better interpretation of the data, (**Figure 2**) were generated following the (**Table 1B**), highlighting the main trends and comparisons observed in the study.

Table 1A. Bacterial species distribution by sex

Bacterial type	Total	Female	Male	P-value
Escherichia coli	183	134	49	0.0005***
Klebsiella pneumoniae	**79**	**43**	**36**	**0.0444***
Pseudomonas aeruginosa	**45**	**27**	**18**	**0.5074**
Acinetobacter baumannii	**9**	**6**	**3**	**>0.9999**
Proteus mirabilis	**16**	**8**	**8**	**0.2839**
Burkholderia cepacia	**12**	**5**	**7**	**0.1235**
Enterobacter cloacae	**6**	**3**	**3**	**0.6700**
Total	**350 (100%)**	**229 (65.4%)**	**121 (34.6%)**	**0.0222***

"A p-value of less than 0.05 was considered statistically significant."

Table 1B. Age distribution of bacterial species

Bacterial type	0-12	13-18	19-35	36-64	65-90	P-value
Escherichia coli	15	4	79	65	20	0.0168*
Klebsiella pneumoniae	**2**	**5**	**27**	**39**	**6**	0.1111
Pseudomonas aeruginosa	**5**	**3**	**14**	**14**	**9**	0.1883
Acinetobacter baumannii	**0**	**0**	**4**	**5**	**0**	0.5737
Proteus mirabilis	**2**	**2**	**5**	**6**	**1**	0.4940
Burkholderia cepacia	**0**	**3**	**1**	**4**	**4**	0.0010**
Enterobacter cloacae	**0**	**0**	**0**	**5**	**1**	0.1922
Total	**24**	**17**	**130**	**138**	**41**	0.0030**

"A p-value of less than 0.05 was considered statistically significant."

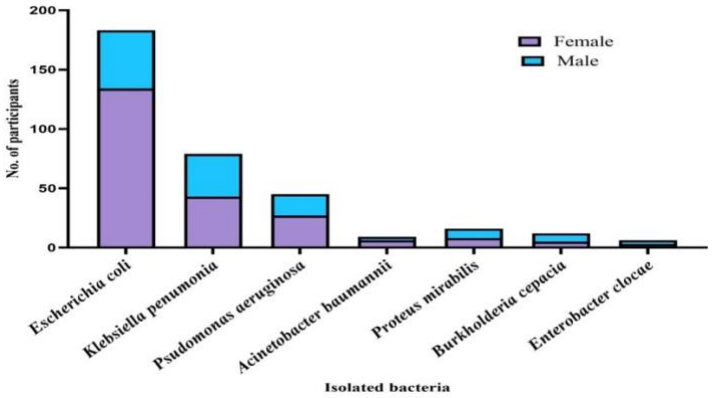

FIGURE 1. Sex distribution of bacterial pathogens

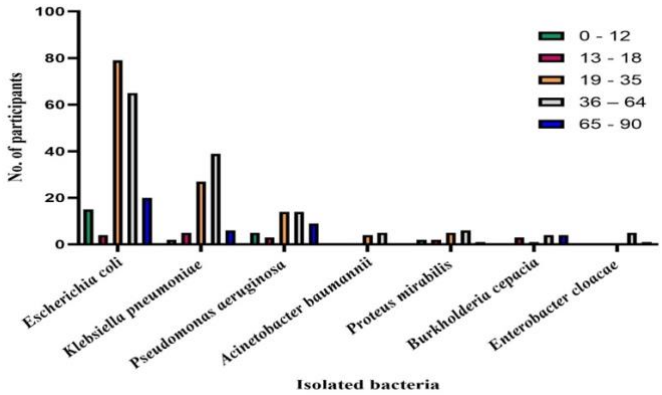

FIGURE 2. Age-related epidemiological distribution of Gram-negative bacterial infections

ANTIMICROBIAL RESISTANCE PATTERNS: CRITICAL EMERGENCE IN LAST-RESORT ANTIBIOTICS

Antimicrobial susceptibility testing revealed alarming resistance patterns, particularly affecting last-resort antibiotics essential for treating multidrug-resistant infections (**Table 2**). The emergence of resistance to both carbapenem and colistin antibiotics represents a critical threat to clinical management of serious Gram-negative infections.

CARBAPENEM RESISTANCE: COMPROMISING FIRST-LINE DEFENSE

Carbapenem resistance varied dramatically between bacterial species, with *Klebsiella pneumoniae* demonstrating the highest resistance rates. Imipenem resistance reached 48.10% (38/79 isolates) in *Klebsiella pneumoniae* compared to 16. 94% (31/183) in *Escherichia coli* ($p<0.0001$). Meropenem resistance followed similar patterns, affecting 37.97% (30/79) of *Klebsiella pneumoniae* isolates versus 12.57% (23/183) of *Escherichia coli* isolates ($p=0.0007$). *Pseudomonas aeruginosa* showed intermediate carbapenem resistance rates of 28.89% for both imipenem and meropenem (13/45 isolates each).

Burkholderia cepacia exhibited extensive imipenem resistance (91.67%, 11/12 isolates), reflecting the intrinsic carbapenem resistance characteristic of this species. *Acinetobacter baumannii* and *Proteus mirabilis* demonstrated moderate carbapenem resistance rates ranging from 25.00% to 43.75%.

COLISTIN RESISTANCE: THE FINAL THERAPEUTIC BARRIER BREACHED

Most critically, emerging colistin resistance was documented in typically susceptible organisms, representing a dangerous evolutionary step toward pan-drug resistance. Colistin resistance emerged in 1.64% (3/183) of *Escherichia coli* isolates, 3.80% (3/79) of *Klebsiella pneumoniae* isolates, and 6.67% (3/45) of *Pseudomonas aeruginosa* isolates ($p<0.0001$ across species). While these percentages appear low, any emergence of colistin resistance in these historically susceptible species signals a critical shift in resistance evolution.

Acinetobacter baumannii showed notably higher colistin resistance at 22.22% (2/9 isolates), while *Proteus mirabilis* (12.50%), *Burkholderia cepacia* (41.67%), and *Enterobacter cloacae* (16.67%) demonstrated intrinsic or acquired colistin resistance consistent with their natural resistance profiles.

STANDARD THERAPEUTIC CLASSES: WIDESPREAD RESISTANCE COMPROMISING EMPIRICAL THERAPY

The extensive resistance patterns observed across standard antibiotic classes fundamentally compromise empirical therapy options, forcing clinicians toward increasingly narrow therapeutic choices and last-resort agents.

Beta-lactam Antibiotics: This foundational antibiotic class showed concerning resistance patterns across all agents tested. Ampicillin demonstrated the highest resistance rates, particularly in *Escherichia coli* (69.95%) and *Klebsiella pneumoniae* (40.51%), effectively eliminating it as empirical therapy for these common pathogens. The addition of beta-lactamase inhibitors provided variable improvement: ampicillin/sulbactam showed dramatically reduced resistance in most species except *Proteus mirabilis* (50.00%), while amoxicillin/clavulanic acid maintained moderate effectiveness against *Escherichia coli* and *Klebsiella pneumoniae* (45.36% and 44.30% resistance respectively). Piperacillin/tazobactam, often considered a broad-spectrum option, showed concerning resistance rates across all major pathogens, particularly *Klebsiella pneumoniae* (58.23%) and *Acinetobacter baumannii* (55.56%).

Cephalosporin Antibiotics: Generation-specific resistance patterns revealed evolving bacterial adaptation strategies. First-generation cefazolin showed moderate resistance in *Escherichia coli* (39.34%) and *Klebsiella pneumoniae* (45.57%), while maintaining excellent activity against *Proteus mirabilis* (0% resistance). Third-generation agents demonstrated variable effectiveness: ceftriaxone resistance was particularly high in *Escherichia coli* (56.28%), while ceftazidime showed even higher resistance in *Klebsiella pneumoniae* (64.56%). Fourth-generation cefepime provided improved activity against most organisms but showed extensive resistance in *Burkholderia cepacia* (83.33%). The newest fifth-generation agent, ceftolozane/tazobactam, maintained excellent activity against Enterobacteriaceae but showed concerning resistance in *Pseudomonas aeruginosa* (26.67%) and *Proteus mirabilis* (50.00%).

Fluoroquinolone Antibiotics: Once considered highly effective broad-spectrum agents, fluoroquinolones now show extensive resistance that severely limits their clinical utility. Ciprofloxacin resistance exceeded 50% in most major pathogens: *Escherichia coli* (54.10%), *Klebsiella pneumoniae* (68.35%), and *Pseudomonas aeruginosa* (62.22%), with *Burkholderia cepacia* showing near-universal resistance (91.67%). Levofloxacin demonstrated slightly better activity but still showed significant resistance in *Escherichia coli* (38.25%) and *Pseudomonas aeruginosa* (28.89%).

Aminoglycoside Antibiotics: These agents showed variable but concerning resistance patterns. Gentamicin resistance ranged from moderate in *Escherichia coli* (35.52%) too high in *Pseudomonas aeruginosa* (48.89%) and *Burkholderia cepacia* (83.33%). Amikacin, often reserved for resistant infections, showed lower overall resistance rates but still

demonstrated significant resistance in *Pseudomonas aeruginosa* (48.89%) and *Burkholderia cepacia* (75.00%), limiting its utility for these problematic pathogens.

Alternative Therapeutic Classes: Sulfonamide combinations and nitrofurans showed mixed effectiveness. Trimethoprim/sulfamethoxazole demonstrated high resistance rates in *Proteus mirabilis* (62.50%) and *Klebsiella pneumoniae* (55.70%), while maintaining better activity against *Pseudomonas aeruginosa* (15.56%). Nitrofurantoin, primarily used for urinary tract infections, showed excellent activity against *Escherichia coli* (16.94% resistance) but high resistance in *Klebsiella pneumoniae* (55.70%), reflecting its limited systemic activity. And for more visualization. The heat map visualization (**Figure 3**) provides a comprehensive visual representation of antimicrobial resistance patterns across all seven Gram-negative bacterial isolates, effectively demonstrating the critical narrowing of effective antimicrobial options. This visualization reveals several key clinical insights that highlight the urgency of the resistance crisis.

Table 2. resistant-pattern of isolated gram -negative bacteria from clinical samples

Antibiotic groups	Antibiotics	Isolated Gram-negative bacteria from clinical samples							P-value
		Escherichia coli (n=183)	*Klebsiella pneumonia* (n=79)	*Pseudomonas aeruginosa* (n=45)	*Acinetobacter baumannii* (n=9)	*Proteus mirabilis* (n=16)	*Burkholderia cepacia* (n=12)	*Enterobacter cloacae* (n=6)	
β-lactam Antibiotics	Ampicillin/Sulbactam	20 (10.93%)	9 (11.39%)	1 (2.22%)	1 (11.11%)	8 (50.00%)	0 (0.00%)	1 (16.67%)	<0.0001****
	Piperacillin/Tazobactam	79 (43.17%)	46 (58.23%)	20 (44.44%)	5 (55.56%)	5 (31.25%)	5 (41.67%)	3 (50.00%)	0.3034
	Amoxicillin/ Clavulanic Acid	83 (45.36%)	35 (44.30%)	6 (13.33%)	1 (11.11%)	5 (31.25%)	0 (0.00%)	2 (33.33%)	0.0001***
	Ampicillin	128 (69.95%)	32 (40.51%)	5 (11.11%)	1 (11.11%)	4 (25.00%)	0 (0.00%)	1 (16.67%)	<0.0001****
1st Generation	Cefazolin	72 (39.34%)	36 (45.57%)	6 (13.33%)	1 (11.11%)	0 (0.00%)	5 (41.67%)	1 (16.67%)	0.0002***
3rd Generation	Ceftazidime	77(42.08%)	51 (64.56%)	14 (31.11%)	4 (44.44%)	8 (50.00%)	0 (0.00%)	2 (33.33%)	0.0002***
4th Generation	Ceftriaxone	103 (56.28%)	34 (43.04%)	4 (8.89%)	4 (44.44%)	2 (12.50%)	5 (41.67%)	2 (33.33%)	<0.0001****
5th Generation	Cefepime	48 (26.23%)	40 (50.63%)	17 (37.78%)	4 (44.44%)	4 (25.00%)	10 (83.33%)	2 (33.33%)	<0.0001****
	Ceftolozane/Tazobactam	8 (4.37%)	6 (7.59%)	12 (26.67%)	0 (0.00%)	8 (50.00%)	0 (0.00%)	1 (16.67%)	<0.0001****
Carbapenems	Imepenem	31 (16.94%)	38 (48.10%)	13 (28.89%)	3 (33.33%)	7 (43.75%)	11 (91.67%)	3 (50.00%)	<0.0001****
	Meropenem	23 (12.57%)	30 (37.97%)	13 (28.89%)	3 (33.33%)	4 (25.00%)	3 (25.00%)	1 (16.67%)	0.0007***
Aminoglycosides	Amikacin	43 (23.50%)	24 (30.38%)	22 (48.89%)	3 (33.33%)	3 (18.75%)	9 (75.00%)	2 (33.33%)	0.0097**
	Gentamicin	65 (35.52%)	33 (41.77%)	22 (48.89%)	4 (44.44%)	6 (37.50%)	10 (83.33%)	2 (33.33%)	0.0609
Fluoroquinolones	Ciprofloxacin	99 (54.10%)	54 (68.35%)	28 (62.22%)	5 (55.56%)	8 (50.00%)	11 (91.67%)	4 (66.67%)	0.0923
	Levofloxacin	70 (38.25%)	19 (24.05%)	13 (28.89%)	1 (11.11%)	3 (18.75%)	6 (50.00%)	1 (16.67%)	0.0438*
Polymyxin	Colistin	3 (1.64%)	3 (3.80%)	3 (6.67%)	2 (22.22%)	2 (12.50%)	5 (41.67%)	1 (16.67%)	<0.0001****
Sulfonamides	Trimethoprim/Sulfamethoxazole	84 (45.90%)	44 (55.70%)	7 (15.56%)	5 (55.56%)	10 (62.50%)	4 (33.33%)	3 (50.00%)	0.0004***
Nitrofurans	Nitrofurantoin	31 (16.94%)	32 (55.70%)	7 (15.56%)	0 (0.00%)	2 (12.50%)	1 (8.33%)	2 (33.33%)	0.0003***

*Statistical significance: *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001

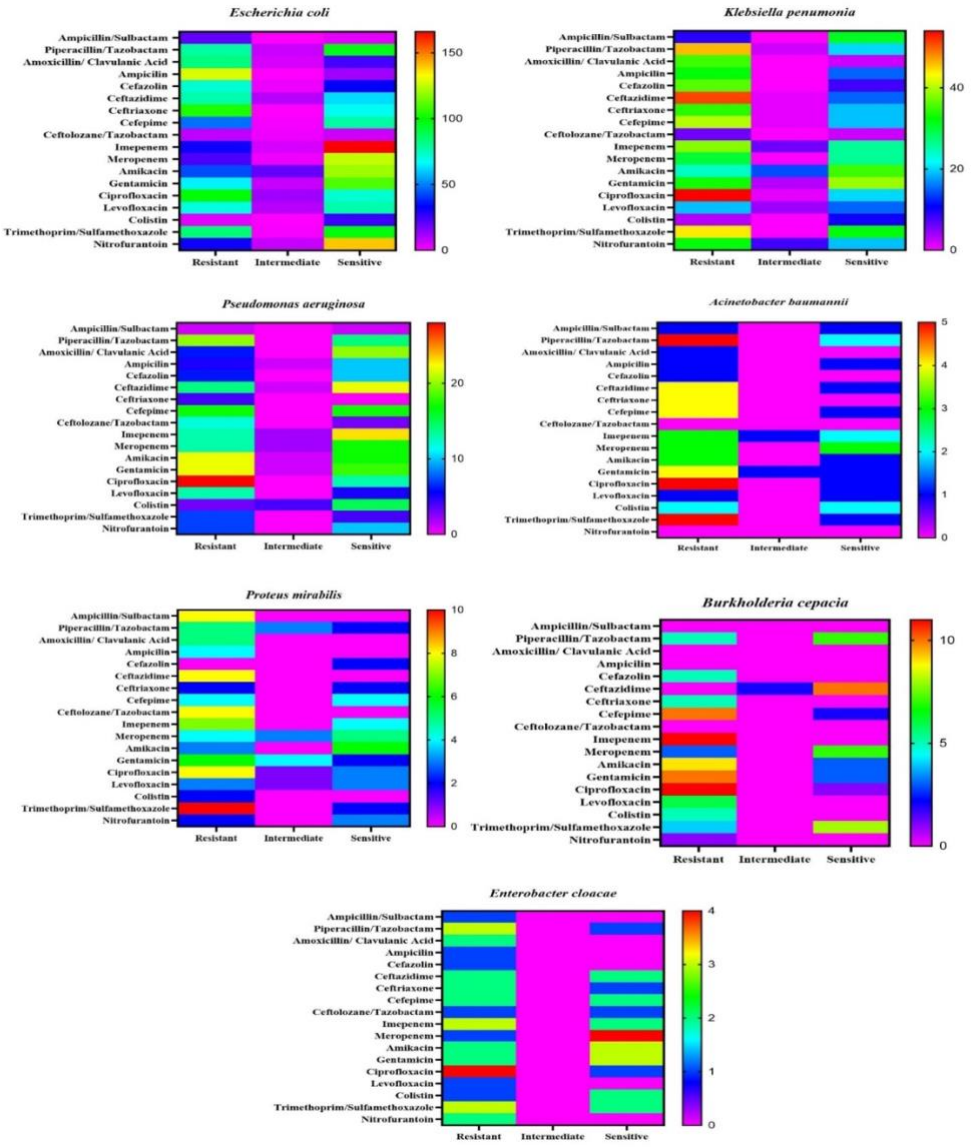

FIGURE 3. Heat map visualization of antibiotic resistance patterns in clinical Gram-negative isolates

CLINICAL SAMPLE DISTRIBUTION

Clinical sample analysis revealed significant gender-based distribution patterns ($p<0.0001$) that inform infection control strategies (**Table 3**). Urine samples comprised the vast majority of specimens (295/350, 84.3%), with females providing significantly more urine samples (204) compared to males (91), consistent with the higher prevalence of urinary tract infections in women. Blood samples, though less frequent (16 total), showed female predominance (12 vs 4 males), potentially indicating more severe infections or different healthcare-seeking behaviors. Conversely, stool samples (14 total) and sputum samples (8 total) showed male predominance, reflecting different infection patterns and occupational exposures. Swab samples (17 total) were fairly evenly distributed between genders. These findings reflect typical clinical patterns where women predominantly present with urinary tract infections while men more commonly have gastrointestinal and respiratory infections requiring bacterial testing. To provide clearer visualization and better interpretation of the data, (**Figure 4**) were generated following the (**Table 3**), highlighting the main trends and comparisons observed in the study.

Table 3. Clinical sample distribution by sex

Sample source	Total	Female	Male	Overall P-value
Urine	295	204	91	<0.0001****
Swab	17	6	11	
Blood	16	12	4	
Stool	14	2	12	
Sputum	8	2	6	

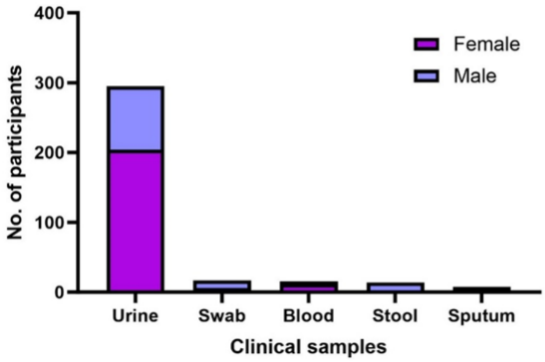

FIGURE 4. Gender distribution across clinical sample types

CRITICAL CONVERGENCE OF RESISTANCE PATTERNS

The convergence of carbapenem and colistin resistance within bacterial populations creates clinical scenarios approaching pan-drug resistance. K. pneumoniae demonstrated the most concerning pattern, with 48.10% showing carbapenem resistance while simultaneously developing colistin resistance in 3.80% of isolates. This convergence eliminates the sequential therapeutic approach traditionally used in clinical practice. *Burkholderia cepacia* showed the most extensive resistance profile, with high resistance rates across virtually all antibiotic classes including 91.67% imipenem resistance and 41.67% colistin resistance, confirming its notorious multidrug-resistant nature and limited therapeutic options.

CLINICAL IMPLICATIONS

The resistance patterns documented represent a critical evolution toward untreatable infections across all seven bacterial species studied. The emergence of colistin resistance in previously susceptible species (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) indicates active circulation of resistance mechanisms. Combined with extremely high carbapenem resistance rates, particularly in *Klebsiella pneumoniae*, these findings suggest empirical therapy failure rates approaching 50% for serious infections. The extensive resistance across multiple antibiotic classes forces clinicians to rely increasingly on last-resort antibiotics, accelerating the development of pan-drug resistance.

4 DISSCUSSION

Colistin represents our final therapeutic refuge against carbapenem-resistant gram-negative infections, making resistance to this agent particularly devastating. The bacterial pathogens identified in our research present varying colistin susceptibility profiles with profound clinical consequences. Colistin resistance develops through multiple pathways, including chromosomal mutations that alter lipopolysaccharide structure and horizontally transferable resistance genes, notably the mcr family [21].

Our study documented the emergence of colistin resistance in traditionally susceptible Gram-negative bacteria, with rates of 1.64% in *Escherichia coli*, 3.80% in *Klebsiella pneumoniae*, and 6.67% in *Pseudomonas aeruginosa*. These findings are consistent with recent global surveillance data showing increasing colistin resistance prevalence. A research reported similar colistin resistance emergence in clinical isolates, with rates ranging from 1.8% to 15.2% across different geographic regions [22]. However, our rates are lower than those reported by Another research, who documented 16.1% colistin resistance in *Klebsiella pneumoniae* compared to 6% in 2013, suggesting our study may be capturing earlier stages of resistance emergence [23].

The intrinsic colistin resistance observed in *Proteus mirabilis* (12.50%) and *Burkholderia cepacia* (41.67%) aligns with expected patterns. it is important to note that Proteus species, Burkholderia species, and Enterobacter species exhibit intrinsic resistance to colistin due to their naturally occurring lipopolysaccharide modifications and membrane characteristics that prevent colistin binding [24]. This intrinsic resistance explains the higher baseline resistance rates observed in these organisms compared to typically susceptible species like *Escherichia coli* and *Klebsiella pneumonia*. The emergence of colistin resistance has attracted global concern and led to enhanced surveillance programs for monitoring resistance trends in clinical settings [9], while international health organizations have implemented new guidelines for colistin use to preserve its effectiveness as a last-resort antibiotic [25].

The carbapenem resistance rates observed in our study were alarming, particularly for *Klebsiella pneumoniae* showing 48.10% imipenem resistance and 37.97% meropenem resistance. These findings exceed global averages reported in recent systematic reviews. A meta-analysis [26] reported pooled carbapenem resistance rates of approximately 49% for imipenem and 53.2% for meropenem in *Klebsiella pneumoniae* globally [26]. Conversely, data from the WHO's GLASS program show lower global resistance rates of 10.63% for imipenem and 12.34% for meropenem in carbapenem-resistant *Klebsiella pneumoniae* [4]. The higher rates in our study likely reflect regional factors and healthcare-associated transmission patterns specific to our setting.

Our findings of 16.94% imipenem resistance and 12.57% meropenem resistance in *Escherichia coli* show notable regional variation compared to other studies from Iraq and neighboring regions. As in other report a significantly higher overall prevalence of carbapenemase-producing Escherichia coli of 47.3% in Erbil City, Kurdistan Region, with 16.2% being Metallo beta-lactamase producers, substantially exceeding your observed resistance rates [27]. In contrast, a study from Baghdad found lower carbapenem resistance rates of 19.6% among *Escherichia coli* isolates with both metallo-β-lactamase and carbapenemase activity [28], which more closely aligns with our findings. These geographic variations suggest significant heterogeneity in carbapenem resistance patterns across Iraq.

The gender distribution observed in our study showed significant female predominance overall (65.43% vs 34.57%, p=0.0222), particularly for *Escherichia coli* (73.2% vs 26.8%, p=0.0005). This observation reinforces well-documented epidemiological trends regarding urinary tract infections in women. The anatomical vulnerability of females to ascending urinary infections stems from their shorter urethra and the proximity of urethral opening to potential bacterial reservoirs [29]. However, recent epidemiological studies suggest this gender gap may be narrowing in certain populations, with reporting that gender accounts for only 0.28% of variation in antimicrobial resistance patterns globally [30].

Focusing on clinical samples significant gender differences in bacterial infections in Kurdistan, Iraq, are revealed by the distribution of clinical samples (overall P<0.0001). Urine samples (204/295) were dominated by females, which is in line with global UTI trends caused by anatomical factors and made worse by local sanitation issues [31]. The regional prevalence of anemia and obstacles to accessing healthcare are likely the causes of the especially high blood infection disparity (12/16 females) [32, 33]. In contrast, males predominated in the sputum (6/8), swab (11/17), and stool (12/14) samples, indicating different hygiene practices and occupational exposures (e.g., farming, construction) [34, 35]. The overall gender distribution is statistically significant (P<0.0001), highlighting the fact that these patterns are not the result of chance variation but rather systemic differences. These results demonstrate the pressing need for gender-specific public health initiatives in Kurdistan, including programs for women to prevent UTIs and men to be safe at work, as well as improved access to healthcare that tackles the root causes of these disparities. On the other hand, our findings show female predominance in urine samples, recent epidemiological data reveals important exceptions to this pattern. The incidence of UTI in men approaches that of women only in men older than 60 years, suggesting that age significantly modifies gender-based infection patterns. In elderly populations, healthcare-associated factors and prostatic conditions create different risk profiles that may not reflect your younger cohort findings [36]. While our finding of female predominance in blood infections (12/16 females) contrasts sharply with established epidemiological patterns. Large-scale population studies consistently demonstrate male predominance in bloodstream infections, with men showing 41% higher risk of first-time BSI (Bloodstream Infections) compared to women [37]. This male predominance is particularly pronounced in healthcare-associated bloodstream infections and among patients with MRSA bacteremia [38]. Our small sample size (n=16) may represent a statistical anomaly rather than a true regional pattern.

The age-stratified analysis revealed *Escherichia coli* peak prevalence in the 19-35 age group (43.17% of isolates), followed by the 36-64 group (35.5%). This finding contrasts with established epidemiological patterns showing higher Escherichia coli infection risk in vulnerable populations. Clinical evidence demonstrates that people at greatest risk include newborns, young children, and adults over 65 years, with invasive *Escherichia coli* disease incidence steadily increasing with age [39]. Although *Escherichia coli* historically exhibits lower carbapenem resistance compared to other Enterobacteriaceae, the emergence of carbapenemase-producing strains has created new clinical challenges. The age-related patterns we observed align with healthcare exposure risks, where older populations typically harbor higher rates of resistant organisms due to cumulative hospital stays and invasive medical procedures [40]. Our younger demographic concentration may reflect healthcare-seeking behaviors or local epidemiological factors.

Klebsiella pneumoniae showed predominant concentration in the 36-64 age group (49.37% of isolates), which aligns with recent studies confirming median patient ages of 68.9 years for *Klebsiella pneumoniae* bacteremia [41]. This age distribution supports established patterns showing elderly populations have increased susceptibility to carbapenem-resistant strains [42].

Burkholderia cepacia demonstrated significant age-related clustering (p=0.0010), concentrating in older age groups. likely because they are more frequently admitted to ICUs and exposed to strong antibiotics. A study linked its rise to the heavy use of colistin—an antibiotic *Burkholderia cepacia* naturally resists. This makes treatment difficult and highlights the need to protect high-risk patients with better antibiotic practices [43]. *Burkholderia cepacia*: Pediatric and Young Adult Focus Contrary to your finding of *Burkholderia cepacia* clustering in older age groups, extensive clinical research demonstrates this organism's particular significance in pediatric and young adult populations. In people with cystic fibrosis, *Burkholderia cepacia* can cause severe lung infections that lead to accelerated lung damage, with most cases occurring in children and adolescents [44].

However, *Acinetobacter baumannii* showed concentration in middle-aged adults (36-64 years) rather than expected elderly predominance. This contrasts with recent research demonstrating that *Acinetobacter baumannii* pneumonia poses a serious threat specifically to elderly populations, with patients aged 60 or older at higher risk for ventilator-associated infections [45]. The median age in *Acinetobacter baumannii* studies typically ranges 61-62 years, suggesting our sample may underrepresent elderly patients who are most vulnerable to this opportunistic pathogen.

Our demographic patterns showing highest infection burden in young adults (19-35 years) contrast with some studies showing elderly predominance in resistant infections. However, this may reflect local healthcare utilization patterns and exposure risks specific to our population [46].

Our colistin resistance findings in *Acinetobacter baumannii* (22.22%) are consistent with global trends. Islam et al. (2024) reported that colistin-resistant *Acinetobacter baumannii* strains represent a significant public health threat, particularly in healthcare settings with high selective pressure [47]. However, some studies report higher resistance rates, with certain regions showing up to 55% colistin resistance in *Acinetobacter baumannii* [48].

The convergence of high carbapenem resistance with emerging colistin resistance in our study creates concerning implications for clinical management. Recent studies have documented the emergence of strains resistant to both antibiotic classes, effectively creating pan-drug resistant organisms [49]. This convergence is particularly evident in our *Klebsiella pneumoniae* isolates, where high carbapenem resistance (48.10%) for imepenem and (37.97%) for meropenem coincides with emerging colistin resistance (3.80%).

The WHO's 2024 surveillance report documented global emergence of hypervirulent *Klebsiella pneumoniae* carrying carbapenemase genes in all WHO regions, supporting our findings of widespread resistance [50]. Regional studies from Iraq have documented mcr-mediated colistin resistance emergence, indicating that our findings reflect broader Middle Eastern resistance trends [51].

The clinical implications of our findings are significant, as patients infected with carbapenem-resistant organisms experience longer hospital stays, higher healthcare costs, and increased mortality rates compared to those with susceptible infections [52]. The emergence of colistin resistance in our setting indicates active circulation of resistance mechanisms, creating potential for rapid horizontal dissemination through healthcare facilities.

Our findings underscore the urgent need for enhanced surveillance, antimicrobial stewardship, and infection control measures. The convergence of carbapenem and colistin resistance represents a critical evolutionary step toward pan-drug resistant organisms that threatens the foundation of antimicrobial therapy [53].

CONCLUSION

The emergence of colistin resistance among traditionally susceptible Gram-negative bacteria represents a critical evolutionary milestone demanding immediate intervention. This study documented the concerning emergence of colistin resistance in *Escherichia coli* (1.64%), *Klebsiella pneumonia* (3.80%), and *Pseudomonas aeruginosa* (6.67%), Organisms historically considered reliably susceptible to this last-resort antibiotic. Any detection of colistin resistance in these species signals a fundamental shift in antimicrobial resistance evolution, as colistin serves as the final therapeutic option for carbapenem-resistant infections.

The convergence of emerging colistin resistance with extremely high carbapenem resistance rates, particularly in *Klebsiella pneumonia* (48.10% imipenem, 37.97% meropenem), creates the potential for truly pan-drug resistant organisms essentially untreatable with current antimicrobial agents. This convergence represents the emergence of a perfect storm in antimicrobial resistance evolution, where loss of both carbapenem and colistin activity eliminates viable treatment options for serious Gram-negative infections.

The mere presence of colistin resistance in routine patient samples indicates these "superbugs" are already circulating in our healthcare facilities, suggesting widespread transmission between patients.

Time is running out. We urgently need better antibiotic stewardship, enhanced surveillance systems, and stronger infection control measures. Without immediate action, we risk entering an era where common infections become deadly once again.

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CONFLICTS OF INTEREST

No conflict of interest.

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