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Prevalence of nosocomial infections caused by multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae* encoded by different genes

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ABSTRACT

Background: Nosocomial infections caused by multi-drug resistant *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae (K. pneumoniae)* are a growing public health concern. These bacteria, commonly found in healthcare settings, possess various resistance genes that enable them to evade multiple antibiotics, leading to increased morbidity and mortality. Understanding the prevalence and genetic mechanisms of these pathogens is essential for improving infection control and treatment strategies. **Methods:** This study collected 150 isolates of *E. coli* and *K. pneumoniae* from various clinical samples of hospitalized patients. The samples were cultured and incubated, and the isolates were identified and tested for antimicrobial sensitivity using both conventional and automated methods via the BD Phoenix M50 system. Resistant isolates were further analyzed for specific resistance genes through PCR to detect the presence of KPC, IMP, VIM, NDM, and OXA-48 genes, using the GeneXpert System. All procedures were conducted at the Armed Forces Labs for Medical Research and Blood Bank. **Results:** Out of 203 samples, 8 samples showed no growth, 150 isolates showed *E. coli* and *K. pneumoniae*, and 45 isolates showed other species. 23.3% of the 150 isolates were sensitive, while 76.7% contained MDR organisms. Among MDR, 65 out of 115 (56.5%) were ESBLs and 50 out of 115 (43.5%) were carbapenem-resistant. The majority of carbapenem-resistant isolates contained one antimicrobial resistance gene, 24% had two antimicrobial resistance genes, and only 6% had all three. The most common genes were NDM (66%), OXA-48 (58%), and KPC (12%). **Conclusion:** This study demonstrated that antimicrobial susceptibility testing revealed increased resistance to most antibiotics in *K. pneumoniae* isolates, particularly carbapenem resistance. In contrast, ESBL resistance was much greater in *E. coli* isolates. In addition, *K. pneumoniae* and *E. coli*-associated nosocomial infections contained an increased number of resistance genes.

Introduction

The *Enterobacteriaceae* family, a large group of Gram-negative rods primarily found in the colons of humans and animals, includes many organisms that are part of the normal flora. While facultative anaerobes from this family are common in the large intestine, they are outnumbered by

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anaerobes like Bacteroides. Despite sharing a taxonomic classification, members of *Enterobacteriaceae* cause a wide range of diseases through different pathogenetic mechanisms**[1]**.

The most significant members of this family, *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), account for about 80% of isolates and are responsible for numerous human diseases, including pneumonia, urinary tract infections, surgical site infections, meningitis, sepsis, endotoxic shock, and diarrhea**[2]**. These bacteria are major contributors to healthcare-associated infections (HAIs), with the excessive and irresponsible use of antibiotics leading to the rise of multidrug-resistant (MDR) strains. Nearly 20% of all reported bacterial infections are caused by MDR bacteria**[3]**.

The development of antibiotic resistance in *E. coli* and *K. pneumoniae* involves multiple mechanisms, including the production of enzymes like extended-spectrum β-lactamases (ESBLs) and carbapenemases. These enzymes degrade a broad range of β-lactam antibiotics, rendering them ineffective. Additionally, resistance can arise through mutations in target sites, reduced permeability of bacterial cell walls, and active efflux pumps that expel antibiotics from the cell. The genetic plasticity of these organisms, facilitated by plasmids and transposons, allows them to rapidly acquire and disseminate resistance genes, posing a significant challenge to infection control**[4]**.

The increasing prevalence of extended-spectrum β-lactamase (ESBL) and carbapenem resistance in *E. coli* and *K. pneumoniae* represents a global health threat. Monitoring these resistant bacteria is crucial for understanding their prevalence among hospitalized patients**[5]**. Rapid methods for determining antimicrobial susceptibility are vital for public health, ensuring the appropriate use of antimicrobial agents and limiting the spread of resistant bacteria**[6]**.

Antibiotic stewardship programs play a critical role in combating the rise of MDR organisms such as *E. coli* and *K. pneumoniae*. These programs aim to optimize the use of antibiotics to treat infections effectively while minimizing the development of resistance. By promoting the appropriate selection, dosage, and duration of antimicrobial therapy, these initiatives help preserve the efficacy of existing antibiotics**[7]**.

The global rise in carbapenem-*resistant E. coli* and *K. pneumoniae* is particularly concerning. These bacteria produce carbapenemases, which contribute to resistance, with the most common carbapenemase genes being KPC, IMP, VIM, NDM, and OXA-48. These genes, often located on plasmids, can be horizontally transferred between *E. coli* and *K. pneumoniae* during conjugation. Monitoring these carbapenemase-producing genes is crucial in both nosocomial and community-acquired infections, given their significant role in antimicrobial resistance**[8]**.

Rapid diagnostic testing for antimicrobial resistance is essential in managing infections caused by *E. coli* and *K. pneumoniae*. Traditional culture-based methods can take several days to yield results, during which time patients may receive inappropriate or ineffective treatments. Rapid diagnostic tools, such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), allow for the timely identification of resistance genes, enabling clinicians to tailor antibiotic therapy more precisely**[9]**.

Materials and Methods

This cross-sectional study was conducted between October 2022 and July 2023 at various Armed Forces hospitals and included patients who developed infections after admission, regardless of their gender, age, or comorbidities. The research focused on 150 non-duplicate *Escherichia coli* and *K. pneumoniae* isolates collected from different samples, including urine, wound swabs, sputum, and peripheral blood.

Inclusion and exclusion criteria:

Patients diagnosed with infections that were neither acquired nor incubated at the time of admission were included in the study, while those who had infections that were acquired or incubated upon admission were excluded.

Conventional identification and antibiotic susceptibility testing of bacterial pathogens:

Direct films were prepared from the samples, stained with Gram stain, and examined under a light microscope using an oil immersion lens. The samples were then cultured on different media, including blood agar (prepared by sterilizing nutrient agar and adding blood), MacConkey's agar, and CLED agar for urine samples. These cultures were incubated aerobically at 37°C for 18-24 hours.

Bacteriological identification of the colonies was performed as follows:

• The colonies were first examined with the naked eye, assessing their morphology on different culture media and the characteristic pigment on MacConkey's agar.

• Films were prepared from various colonies, stained with Gram stains, and examined to observe the morphology of the organisms and their Gram reaction.

• Pure cultures were obtained from these colonies on nutrient agar plates, and the organisms were systematically identified through microscopic examination of Gram-stained films.

• For Gram-negative bacilli, identification was further confirmed using conventional biochemical reactions, including sugar fermentation and the oxidase test.

Antimicrobial susceptibility testing was conducted on Mueller-Hinton agar plates using a 0.5 McFarland standard suspension of the pathogen, with results interpreted according to CLSI guidelines.

Automated identification and sensitivity procedures:

The method for automated identification and sensitivity testing of *E. coli* and *K. pneumoniae* involved using The BD Phoenix™ Automated Microbiology System. This system enables rapid identification and antimicrobial susceptibility testing by utilizing BD Phoenix combination panels, which can handle up to 50 tests simultaneously. The testing process included a sealed tray with micro-wells containing dried reagents, where one side of the panel was dedicated to bacterial or yeast identification, and the other to antibiotic sensitivity with varying antimicrobial concentrations and controls. The procedure required specific components such as BD Phoenix panels, broths, indicator solutions, and the BD Phoenix Inoculation Station, among other lab supplies.

After inoculation, the system automatically processes the samples, providing rapid identification and sensitivity results.

Detection of resistance gene:

The detection of KPC, IMP, VIM, NDM, and OXA-48 genes responsible for antimicrobial resistance in *E. coli* and *K. pneumoniae* was performed using the GeneXpert System device. This system automates the process, which includes sample preparation, DNA extraction, amplification, and real-time PCR detection. The device utilizes specific cartridges containing the necessary reagents to identify the presence of these resistance genes.

The system automatically detects the presence of these resistance genes by amplifying specific gene sequences and analyzing the results. This method provided rapid and accurate identification of antimicrobial resistance, aiding in appropriate treatment decisions.

Statistical analysis

Data analysis was performed using the IBM SPSS software package version 26.0. (Armonk, New York: IBM Corporation). Quantitative data were described as range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The significance of the difference between groups was assessed with a two-tailed Student's t test. Qualitative factors were examined using the chi squared $\chi 2$ test. *p*-values ≤ 0.05 were considered statistically significant.

Results

This study involved 203 patient samples from nosocomial infections. After conventional culture, 8 samples showed no growth, while 195 isolates were obtained and subjected to automated culture and sensitivity testing. Of these, 150 isolates were identified as *E. coli* and *K. pneumoniae*, and the remaining 45 were other species as shown in **figure (1)**.

Among the various types of specimens collected, urine samples were the most frequent, accounting for 36.7%, followed by sputum samples at 31.3%. Pus samples were the least common, comprising only 6.7% of the total. *K. pneumoniae* was most commonly isolated from urine (56%) while *E. coli* from sputum (42%) as illustrated in **table (1)**.

Table 2 shows the antimicrobial susceptibility testing of 150 isolates. These isolates showed high resistance to many antibiotics. Cephalosporins, penicillins, and fluoroquinolones had the highest resistance rates, with 88% resistance to cefazolin and cefuroxime, 98% to ampicillin, and 96.67% to ciprofloxacin and levofloxacin. Carbapenems showed moderate resistance, with 36% resistant to ertapenem. Aminoglycosides like gentamicin had 40% resistance, while nitrofurantoin had lower resistance at 27.33%. These findings indicate significant antibiotic resistance, posing challenges for effective treatment.

The automated analysis of the 150 isolates, illustrated in **table (3)**, revealed a similar identification results that *E. coli* was the more prevalent organism, comprising 59.3% of the samples, while *K. pneumoniae* accounted for 40.7%. A significant majority of the isolates, 76.7%, were classified as MDR, with only 23.3% being non-MDR. Among the MDR isolates, 56.5% were found to produce extended-spectrum beta-lactamases (ESBL), and 43.5% were resistant to carbapenems. These findings highlight the high prevalence of antimicrobial resistance in the studied bacterial populations.

The antibiotic sensitivity testing and MDR comparison between *E. coli* and *K. pneumoniae* isolates revealed significant differences as shown in **table (4)**. *E. coli* showed a lower proportion of MDR cases (71.9%) compared to *K. pneumoniae* (83.6%). However, within the 115 MDR isolates, *E. coli* had a significantly higher prevalence of ESBL production (79.7%) compared to *K. pneumoniae* (27.5%). Conversely, *K. pneumoniae* exhibited a much higher rate of carbapenem resistance (72.5%) compared to *E. coli* (20.3%) highlighting the varying resistance patterns between the two bacterial species.

The comparison of antimicrobial susceptibility between *E. coli* and *K. pneumoniae* isolates presented in **figure (2)** showed that *K. pneumoniae* generally had higher resistance rates across most antibiotics. Specifically, *K. pneumoniae* was more resistant to aminoglycosides, carbapenems, cephalosporins, and Nitrofurantoin compared to *E. coli*. In contrast, *E. coli* isolates demonstrated relatively lower resistance rates, particularly to amikacin and carbapenems. This indicates a significant disparity in resistance profiles between the two bacteria.

Table 5 summarizes the genetic study of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates. Most isolates (70%) had one resistance gene, with *E. coli* showing a slightly higher rate. Two resistance genes were found in 24% of isolates, all in *K. pneumoniae*, and three genes in 6%, exclusively in *E. coli*. OXA-48 was present in 58% of isolates, NDM in 66%, and KPC in 12%. There were no significant differences between the two bacteria for OXA-48 and NDM, but KPC was more common in *E. coli* without statistical significance.

Table 1. Type of specimen among the whole study group.

(N= 150)		N	*E. coli*	*K. pneumoniae*	%
specimen	Urine	55	21(24%)	34(56%)	36.7%
	Sputum	47	38(42%)	9 (15%)	31.3%
	Wound swab	20	9(10%)	11(18%)	13.3%
	Blood	18	14(16%)	4 (6%)	12.0%
	Pus	10	7 (8%)	3 (5%)	6.7%
	Total	150	89	61	

Table 2. Antimicrobial susceptibility testing for isolates.

(N= 150)		Resistant	Intermediate	Sensitive
		N (%)	N (%)	N (%)
Aminoglycosides	Amikacin	36 (24%)	0 (0%)	114 (76%)
	Gentamicin	60 (40%)	0 (0%)	90 (60%)
Carbapenems	Ertapenem	54 (36%)	0 (0%)	96 (64%)
	Imipenem	50 (33.33%)	0 (0%)	100 (66.67%)
	Meropenem	53 (35.33%)	0 (0%)	97 (64.67%)
Cephalosporins	Cefazolin	132 (88%)	4 (2.67%)	14 (9.33%)
	Cefuroxime	132 (88%)	0 (0%)	18 (12%)
	Ceftazidime	112 (74.67%)	13 (8.67%)	25 (16.67%)
	Ceftriaxone	129 (86%)	3 (2%)	18 (12%)
	Cefepime	119 (79.33%)	11 (7.33%)	20 (13.33%)
	Ceftolozane-tazobactam	60 (40%)	3 (2%)	87 (58%)
Penicillins	Ampicillin	147 (98%)	0 (0%)	3 (2%)
	Amoxicillin-clavulanate	77 (51.33%)	27 (18%)	46 (30.67%)
	Piperacillin-tazobactam	52 (34.67%)	12 (8%)	86 (57.33%)
Nitrofurantoin		41 (27.33%)	13 (8.67%)	96 (64.0%)
Fluoroquinolones	Ciprofloxacin	145 (96.67%)	0 (0%)	5 (3.33%)
	Levofloxacin	145 (96.67%)	0 (0%)	5 (3.33%)

Table 3. Automated method findings for the whole isolates.

(N= 150)			N	%
Automated method	Organism	*E-coli*	89	59.3%
		Klebsiella pneumoniae	61	40.7%
	Interpretation	Non MDR	35	23.3%
		MDR	115	76.7%
	MDR	ESBL	65	56.5%
		Carbapenem resistant	50	43.5%

Table 4. Interpretation of antibiotic sensitivity testing and MDR between *E. coli* and *K. pneumoniae* isolates.

		E.coli	*Klebsiella pneumoniae*	Chi-Square test	
		N (%)	N (%)	*p*-value	Sig.
Interpretation (N= 150)	Non-MDR	25 (28.1%)	10 (16.4%)	0.096	NS
	MDR	64 (71.9%)	51 (83.6%)		
MDR (N= 115)	ESBL	51 (79.7%)	14 (27.5%)	<0.001	S
	Carbapenem resistant	13 (20.3%)	37 (72.5%)		

Table 5. Genetic study among *E. coli* and *K. pneumoniae* carbapenem resistant isolates.

		Total (N= 50)	*E. coli* (N= 13)	*Klebsiella pneumoniae* (N= 37)	Test of significance	
		N (%)	N (%)	N (%)	*p*-value	Sig.
Resistance's Genes	One gene	35 (70.0%)	10 (76.9%) [a]	25 (67.6%) [a]	0.002[(F)]	S
	Two genes	12 (24.0%)	0 (0%) [a]	12 (32.4%) [b]		
	Three genes	3 (6.0%)	3 (23.1%) [a]	0 (0%) [b]		
OXA-48		29 (58.0%)	6 (46.2%)	23 (62.2%)	0.314[(C)]	NS
NDM		33 (66.0%)	10 (76.9%)	23 (62.2%)	0.499[(F)]	NS
KPC		6 (12.0%)	3 (23.1%)	3 (8.1%)	0.173[(F)]	NS

[(F)] Fisher's Exact test of significance.
[(C)] Chi-Square test of significance.
* Each subscript letter denotes a subset of Group categories whose column proportions do not differ significantly from each other at the 0.05 level.

Figure 1. Distribution of bacterial isolates from nosocomial infections.

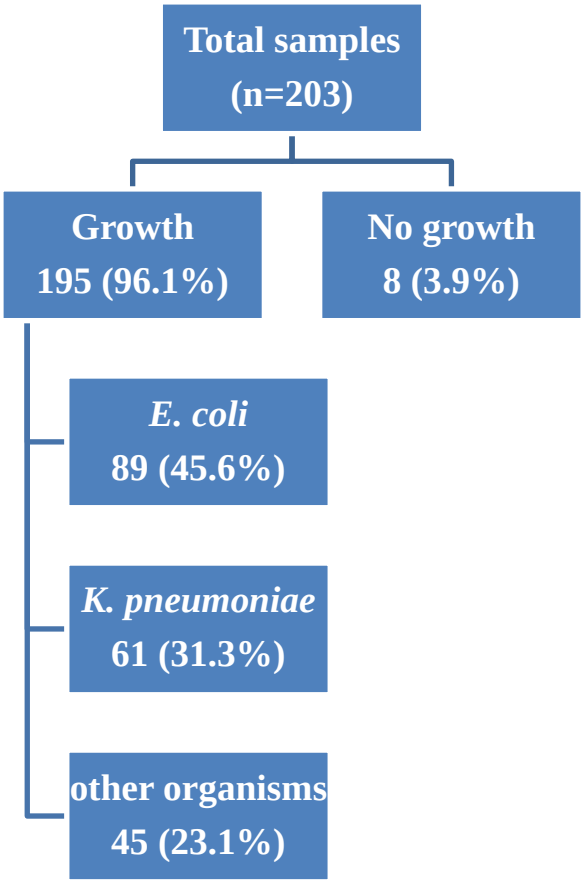
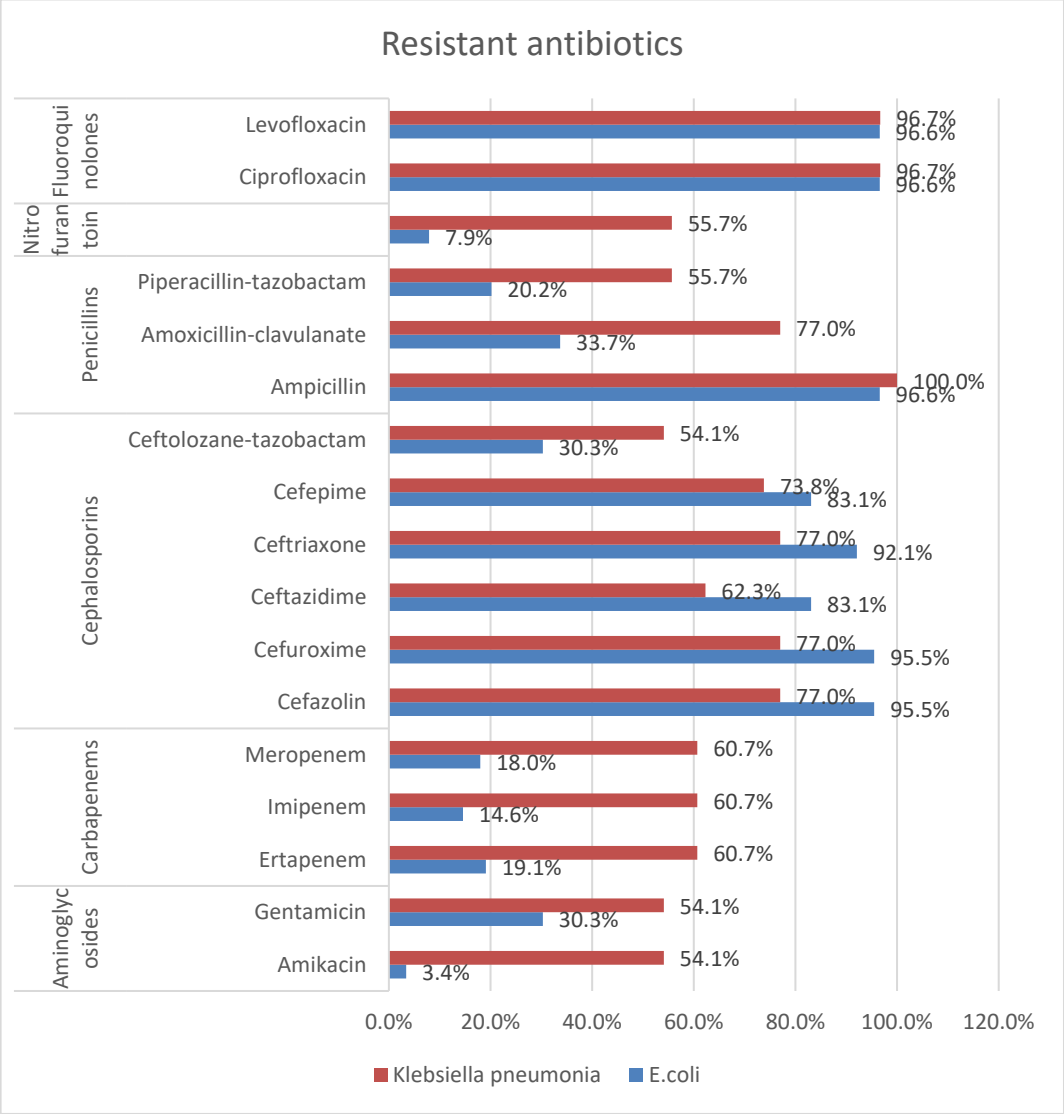


Figure 2. Comparison of antimicrobial susceptibility testing between *E. coli* and *K. pneumoniae* isolates



Discussion

The incidence of MDR *E. coli* and *K. pneumoniae* has significantly risen among hospital-acquired infections, with most displaying ESBL characteristics. This trend poses an added challenge for the treatment and management of these critical infections [10].

This study analyzed 150 *E. coli* and *K. pneumoniae* isolates, with urine being the most common sample type (36.7%), consistent with [11], who reported urinary tract infections as the most frequent hospital-acquired infections.

Klebsiella pneumonia was most isolated from urine samples (56%), followed by wound swab samples (18%) which come in agreement with [12] who reported that, among 468 bacterial isolates obtained from various clinical specimens, 50.8% of *K. pneumonia* was isolated from urine.

[13] also reported the isolation of 88 *K. pneumoniae* samples, with the majority (94.3%) from urine, four (4.5%) from sputum, and one (1.1%) from blood.

In this study, antimicrobial susceptibility testing of the isolates showed varying levels of sensitivity: Amikacin 76%, gentamicin 60%, ertapenem 64%, imipenem 66.7%, and meropenem 64.7%, with lower sensitivity to other antibiotics such as cefazolin (9.33%) and ampicillin (2%). These findings align with [14], who found that *E. coli* isolates were mostly resistant to ampicillin (84.8%) but highly susceptible to imipenem (97.8%). Similarly, [13] reported that *K. pneumoniae* isolates were most effectively treated with neomycin and other antibiotics like imipenem and amikacin, showing over 70% effectiveness.

After antimicrobial susceptibility testing, 76.7% of the samples were identified as MDR, with 56.5% being ESBL producers and 43.5% resistant to carbapenems, while 23.3% were non-MDR. These findings align with previous studies. For instance, [12] found *K. pneumoniae* was a significant cause of nosocomial infections in ICU patients, particularly in burn units. [14] reported high antibiotic resistance in ESBL-producing *E. coli* and *K. pneumoniae*. [15] noted that 19% of *E. coli* and 18% of *K. pneumoniae* isolates were MDR, showing 55%-85% resistance to multiple antibiotics. [16] observed high resistance to ceftriaxone, ceftazidime, and other antibiotics in 39 isolates. Similarly, [17] reported that 57.3% of *K. pneumoniae* isolates were MDR, with high resistance to aminoglycosides like amikacin and gentamicin.

Our results showed that carbapenem resistance was significantly higher in *K. pneumoniae* isolates, while ESBL resistance was more prevalent in *E. coli* isolates ($p < 0.001$). These findings align with those of [12] and [14]. [12] reported that 59% of *K. pneumoniae* isolates were ESBL producers and 77% were MDR, with a significant portion linked to hospital-acquired infections. [14] found MDR prevalence to be 82.5% in *E. coli* and 60.3% in *K. pneumoniae*, with MDR rates higher in ESBL-producing isolates.

Susceptibility testing of resistant *E. coli* isolates showed varying resistance levels, with the highest resistance observed for cefazolin and cefuroxime (95.5% each), while the lowest was for Amikacin (3.4%). In comparison, *K. pneumoniae* isolates exhibited higher resistance across most antibiotics, with complete resistance to ampicillin and significant resistance to carbapenems (60.7%) and cephalosporins. These findings align with previous studies, such as those by [18], [19], [12], [20], and [21] which reported high resistance rates for various antibiotics, particularly in multidrug-resistant and ESBL-producing strains.

Regarding genetic studying of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates, 70% of samples had one resistance gene, 24% had two, and 6% had three. The most common genes identified were NDM (66%), OXA-48 (58%), and KPC (12%). These findings align with several studies, such as those by [14], [22], and [20], which also reported the prevalence of similar resistance genes in *E. coli* and *K. pneumoniae* isolates. For instance, [14] noted the co-occurrence of blaSHV-1, blaNDM-1, and blaOXA-1 in both bacteria, while [22] found high rates of blaCTX-M, blaTEM, blaOXA, and blaSHV genes in their isolates. These studies emphasize the widespread presence of resistance genes, particularly in strains associated with MDR, posing significant challenges to treatment.

Conclusion

This research confirmed that antimicrobial susceptibility testing identified a significant level of resistance to many antibiotics in *K. pneumoniae* isolates, with carbapenem resistance being especially pronounced. Furthermore, *E. coli* isolates exhibited a notably higher level of ESBL resistance. The study also detected a greater presence of resistance genes in *K. pneumoniae*, and *E. coli* strains linked to HAIs, underscoring the challenge these pathogens pose in clinical settings.

Recommendation

It is recommended that antibiotics be used carefully and appropriately in the treatment of nosocomial infections to prevent further resistance. Prior to administering antibiotics, thorough antibiotic susceptibility testing should be conducted to ensure the most effective treatment is selected. Additionally, it is important to closely examine the clinical characteristics of the patient populations, including their treatment protocols and outcomes, to inform best practices. Conducting studies with larger sample sizes is also advised to enhance the reliability of the findings.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest. All authors of this study have participated in the article preparation. All authors have approved the final article.

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