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Molecular detection of some capsular Serotypes Genes in multidrug resistance Klebsiella pneumonia classical and hyper virulent in Iraqi patients

Hamza Jabbar Jabour [*1], Nada H. A. Al-Mudallal [1b]

[1a] Medical Microbiology Department, College of Medicine, Al-Iraqia University, Baghdad, Iraq
Email address: hamza.j.jebur@aliraqia.edu.iq,
[1b] Medical Microbiology Department, College of Medicine, Al-Iraqia University, Baghdad, Iraq
Email: Nada_Mudallal@aliraqia.edu.iq,
https://orcid.org/0000-0003-4972-3990

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* Corresponding Author: Hamza Jabbar Jabour
Email: hamza.j.jebur@aliraqia.edu.iq

REVIEWER
Professor Hana Kadum Shanan
hanakadum@mu.edu.iq

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Abstract

Klebsiella pneumoniae is an opportunistic pathogenic bacterium and a major cause of nosocomial infections. This study aimed to detect selected capsular serotype genes in multidrug-resistant hypervirulent (hvKp) and classical (cKp) *K. pneumoniae* isolates from urinary tract infection (UTI) cases using conventional polymerase chain reaction (PCR). This cross-sectional study included 250 urine samples collected from male and female patients aged 18–65 years presenting with UTI symptoms. The study was conducted at Medical City Hospital, Baghdad, Iraq, from October 2023 to February 2024. Samples were cultured on MacConkey agar, nutrient agar, blood agar, and UTI medium for bacterial isolation. Identification was performed using standard biochemical tests and confirmed by the VITEK 2 compact system for antibiotic susceptibility profiling. The string test was used to differentiate hvKp from cKp strains. Capsular serotype genes K1 and K2 were detected in selected multidrug-resistant *K. pneumoniae* isolates using conventional PCR. Of the 250 urine samples, 201 (84.6%) showed positive bacterial growth. The mean patient age was 54 ± 12 years, with a higher prevalence of UTIs in females. Escherichia coli and *K. pneumoniae* were the most frequently isolated pathogens. Among the 62 *K. pneumoniae* isolates, 21 (33.9%) were identified as hvKp and 41 (66.1%) as cKp. Antibiotic susceptibility testing showed that 34% of cKp isolates were multidrug-resistant (MDR), while 90.4% of hvKp isolates exhibited MDR profiles. Molecular analysis of 42 selected MDR *K. pneumoniae* isolates (21 cKp and 21 hvKp) revealed that 19 of the cKp isolates harbored capsular genes—K1 in 11 isolates (57.9%) and K2 in 8 (42.1%). Among hvKp isolates, 18 carried capsular genes, with K1 in 7 isolates (39%) and K2 in 9 (50%). Conclusion: Hypervirulent *K. pneumoniae* isolates showed a higher frequency of multidrug resistance and extended-spectrum β-lactamase (ESBL) production compared to classical strains. The K1 and K2 capsular serotypes were predominant among hvKp isolates, while both were also present in cKp strains but at varying proportions.

Keywords: Classical K. pneumoniae, MDR, HvKp, Serotyping.

Introduction

Klebsiella pneumoniae is an opportunistic pathogenic bacterium and is considered one of the main causes of nosocomial infection. Hypervirulent *K. pneumoniae* (hvKp) has emerged as a pathogen of global importance (1). Two pathotypes termed *classical K. pneumoniae* (cKp) and *hypervirulent K. pneumoniae* (hvKp) are presently circulating, each of which presents unique challenges for the clinician (2). Despite the reality that

hvKp infections are growing more widespread outside of Asia; however, cKp has long been considered as the most typical source of sickness in the Western countries (3). The considerable diversity and relatively low virulence of cKp isolates may explain why they are more common in hospitalized and immunocompromised patients (4). Multidrug-resistant cKp (MDR-cKp) strains, which are resistant to clinically relevant antibiotics in three or more classes, constitute a clinically significant fraction. Extended-spectrum beta-lactamases (ESBLs), such as CTX-M-15 and SHV-12, are commonly produced by MDR-cKp bacteria and provide resistance to third-generation cephalosporins (5). Despite being extremely pathogenic, hvKp isolates are typically sensitive to drugs. Through the inhibition of phagocytosis, opsonization, and complement-mediated death, capsules enable the bacterium to withstand the host's innate immune response (6). CKp have extensively acquired mobile genetic elements that encode antimicrobial resistance genes (7). Extended-spectrum beta-lactamase (ESBL) and carbapenemase-encoding K. pneumoniae (together referred to as MDR-Kp) are globally disseminated and cause infections that are often difficult to treat, placing MDR-Kp high on current lists of significant threats to public health by the CDC and the WHO (8). The multidrug resistance of MDR-Kp and corresponding antimicrobial susceptibility of hvKp is an important distinction between these two pathotypes, and a review of the literature revealed scarce publications worldwide, especially in Iraq. So, the aim of current study molecular detection of capsular serotype genes using PCR of multidrug resistance K. pneumonia isolated form UTI.

Materials and Methods

Patient and Sampling

A total of 280 urine samples were collected from patients at Medical City Hospital in Baghdad, Iraq, over a period extending from November 2023 to May 2024. The samples were obtained from both male and female patients, aged 18 to 65 years, who presented with symptoms indicative of urinary tract infections (UTIs), such as dysuria, burning sensation, or hematuria, and were examined by a physician prior to sample collection. For microbiological analysis, the urine samples were cultured on MacConkey agar, nutrient agar, blood agar, and chromogenic UTI medium, and incubated at 37°C for 24 hours to allow for bacterial growth.

Ethical approval for the study was obtained from the Ethical Committee of the College of Medicine, Al-Iraqia University (Approval No. 328, dated 11/12/2023).

Inclusion criteria: Patients with UTI symptoms

Exclusion criteria: Patient treated with antibiotics for UTIs for less than 1 month.

Isolation and Identification of bacteria

Urine sample from patients with TCC-BC and patients with negative cystoscope were cultured on MacConkey agar, nutrient agar, blood agar, and UTI medium for bacterial growth (incubated at 37°C for 24 hours). The turbidity was adjusted to 0.5 MacFarland turbidity range and measured using visible spectrophotometer DensiChekTM Plus. The bacterial suspension was used to inoculate the VITEK 2 system (bioMérieux/France).

Interpretation of results was performed according to VITEK 2 compact system special software to identify bacterial species and strains**.**

Determination of antibiotic susceptibility

Susceptibility to the following antimicrobial agents (depending on bacterial genus) was determined using VITEK2 compact system: Ticarcillin, Ticarcillin/clavunic acid, Piperacillin, Piperacillin/Tazobactam, Imipenem, Meropenem, Amikacin, Trimethoprim/sulfamethoxazole, Gentamicin, Tobramycin, Ciprofloxacin, Ceftazidime, Minocycline, Cefepime and Aztreonam.
The break point for each antimicrobial used was determined according to (CLSI, 2019).

String test

The string test was used to identify hvKp from cKp whenever an inoculation loop was able to stretch bacterial colonies on a blood agar plate and form a sticky string of 5 mm long, the result was considered positive. Once the length of the string was ≤ 5 mm or no string was visible, the result was considered negative

Genomic DNA extraction

Extraction of DNA was done using using the purification Wizard genomic DNA kit. following manufacturer instructions. PCR was performed using a specific primer set for the detection of K. pneumoniae *serotype* in bacterial extracted DNA (table 1). PCR products were electrophoresed in 1.5% agarose gel. The appearance of a band with detected size referred to the amplification of *specific genes.*

Table 1. PCR primers used for detection of resistance genes.

Primer Name	Seq.	Size	Tm. (°C)
MagAF1 MagAR1	5' GGTGCTCTTTACATCATTGC 3' 5' GCAATGGCCATTTGCGTTAG 3'	1,283	62
K2wzy-F1 K2wzy-R1	5'GACCCGATATTCATACTTGACAGAG3' 5'CCTGAAGTAAAATCGTAAATAGATGGC3'	641	60

Results

Bacterial Isolation

A total of 250 urine samples were collected from patients presenting with symptoms of urinary tract infection (UTI), including itching, dysuria, and a burning sensation. Culture analysis revealed that 201 out of 250 samples (84.6%) were positive for bacterial growth, encompassing a range of bacterial species. The bacterial isolates were cultivated using standard culture media, including blood agar, nutrient agar, MacConkey agar, and chromogenic UTI agar. The majority of isolates were Gram-negative bacteria, accounting for 183 out of 201 (91.1%), while Gram-positive bacteria represented 18 out of 201 (8.9%). Females were more frequently affected by UTIs than males, with a mean patient age of 54 ± 12 years. Preliminary bacterial identification was based on colony morphology, hemolysis patterns on blood agar, lactose fermentation, and Gram staining. Further identification involved a series of biochemical tests, including catalase, oxidase,

and IMViC tests. Final confirmation and species-level identification were performed using the VITEK 2 Compact System, which evaluates 49 biochemical characteristics. *Escherichia coli* was the most frequently isolated pathogen, followed by *Klebsiella pneumoniae*. Less frequently encountered species included *Acinetobacter baumannii* , Figure 1.

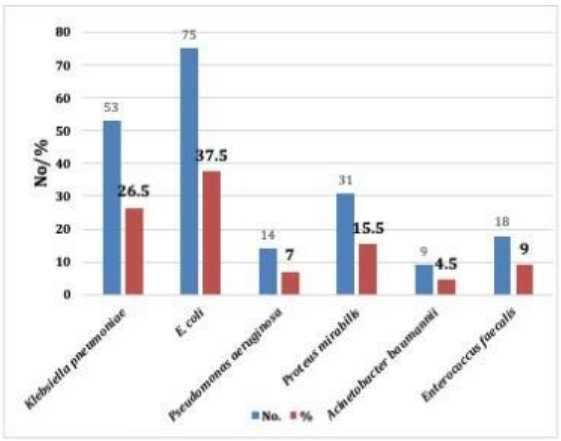

Figure 1: Bacterial species frequency from urine samples

Serotyping

A total of 62 isolates of identified *K. pneumoniae* were identified from which 83.8% female, the male percentage was 17.2%. Based on the results of the modified loop test, hypervirulent phenotypes were found in 21 (33.9%) of the 62 isolates. A total of 66.1% of cKp strains were isolated. The proportion of patients with cKp was much higher. Gender and specimen type did not associate with the positive string test (both P >0.05).

Detection for virulence genes

The virulence gene of K. pneumoniae was detects using PCR, cKp stains only 19/41 belong to serotype K1, K2 and the other 22/41 may related to other serotype isolates while hvKp strains showed 16/21 isolates belong to K1, K2 and 5/21 belong to another serotype, as in table 2 and figure 2, 3.

Figure 2. PCR amplification products for the K1 capsular gene. Lane M represents the DNA ladder (100 bp–1500 bp) used as a molecular size marker. Lanes 1 and 2 show positive amplification for the K1 gene, with visible bands at the 1283 bp position. Lanes 3–10 show K1-negative.

Table 2. Phenotyping identification of *K. pneumoniae*

Tested serotypes	k1 (%)	k2 (%)
C N (%)	11(57.9)	8(42.1)
H N (%)	7(39)	9(50)

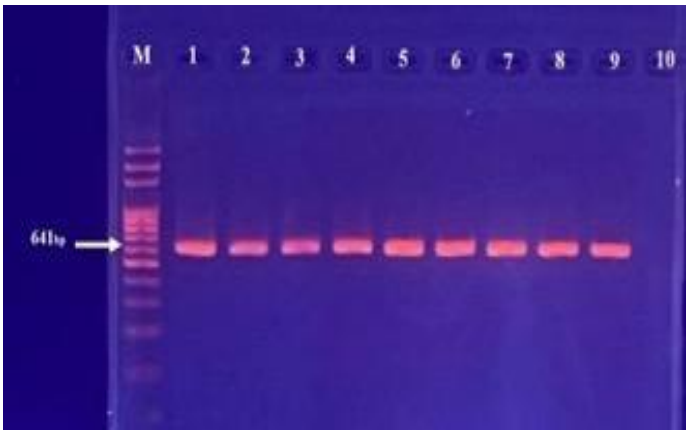

Figure 3. DNA samples were separated on a 2% agarose gel and visualized under UV light after ethidium bromide staining. Lane M represents the DNA ladder. Lanes 1–10 exhibited positive amplification, indicated by distinct bands at the 641 bp position.

Antibiotic susceptibility

In the present study, the isolates of *Klebsiella pneumoniae* exhibited varying degrees of resistance to the antibiotics tested. Among the classical *K. pneumoniae* (cKp) isolates, 34% were classified as multidrug-resistant (MDR), meaning they were resistant to at least one agent in three or more antimicrobial categories. The highest resistance rate was recorded for ampicillin (100%), a β-lactam antibiotic targeting cell wall synthesis. Other β-lactam antibiotics, such as piperacillin and ceftazidime, also showed considerable resistance levels of 12% and 48.7%, respectively. In contrast, the most effective antibiotics against cKp isolates were Ertapenem and Imipenem, both carbapenems, showing a high sensitivity rate of 95.2%. Amikacin (92.8%) and Gentamycin (80.6%), which inhibit protein synthesis, also exhibited strong antibacterial activity. The complete resistance pattern for cKp isolates is illustrated in Table 3. The situation was more concerning in hypervirulent *K. pneumoniae* (hvKp) isolates, where 90.4% were found to be MDR. These isolates demonstrated a notably higher resistance to most antibiotics, particularly those inhibiting cell wall and folic acid synthesis. Ampicillin resistance remained at 100%, while resistance to Trimethoprim/Sulfamethoxazole reached 85.8%, and both Ceftazidime and Ceftriaxone showed resistance levels of 81%. Only Ertapenem (62%) and Imipenem (57.3%) showed relatively better effectiveness against hvKp strains, yet still with significant resistance rates, indicating a narrowing spectrum of effective therapeutic options. These results align with growing concerns over the spread of MDR hvKp strains globally. The complete resistance profile for hvKp is presented in Table 4.

Table 3. Antibiotic Resistance Pattern for cKp

Antibiotic class according to mode of action	Antibiotic	Percentage		
		S	I	R
Inhibit cell wall synthesis	Ampicillin	-	-	100%
	Piperacillin	76%	12%	12%
	Ceftazidime	51.3%	-	48.7%
	Ceftriaxone	78.1%	2.4%	19.5%
	Cefepime	78.1%	2.4%	19.5%
	Etrapenem	95.2%	-	4.8%
	Imipenem	95.2%	-	4.8%
Inhibit protein synthesis	Amikacin	92.8%	2.4%	4.8%
	Gentamycin	80.6%	2.4%	17%
Inhibit DNA synthesis	Ciprofloxacin	48.7%	2.4%	48.9%
	Levofloxacin	48.9%	2.4%	48.7%
Inhibit folic acid synthesis	Trimethoprim/Sulfamethoxazole	56%	-	44%

S: sensitive, R: resistant, I: intermediate

Table 4. Antibiotic Resistance Pattern for hvKp

Antibiotic class according to mode of action	Antibiotic	Percentage		
		S	I	R
Inhibit cell wall synthesis	Ampicillin	-	-	100%
	Piperacillin	28.5%	14.2%	57.3%
	Ceftazidime	19%	-	81%
	Ceftriaxone	19%	-	81%
	Cefepime	23.8%	14.2%	62%
	Etrapenem	62%	4.7%	33.3%
	Imipenem	57.3%	4.7%	38%
Inhibit protein synthesis	Amikacin	28.5%	2.5%	71.5%
	Gentamycin	28.5%	9.5%	62%
Inhibit DNA synthesis	Ciprofloxacin	14.2%	19%	66.8%
	Levofloxacin	14.2%	14.2%	71.6%
Inhibit folic acid synthesis	Trimethoprim/Sulfamethoxazole	14.2%	-	85.8%

S: sensitive, R: resistant, I: intermediate

The comparing antibiotic resistance in cKp and hvKp illustrated in figure 4.

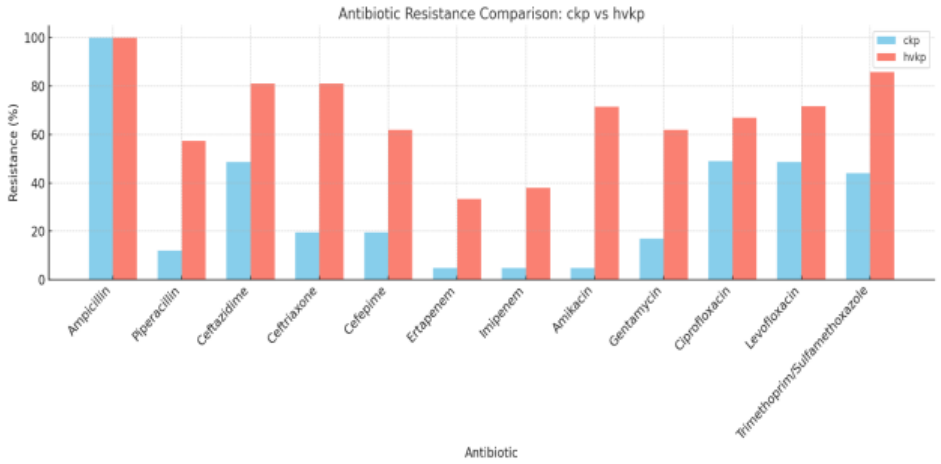

Figure 4. Comparing antibiotic resistance percentages between classical *K. pneumoniae* (cKp) and hypervirulent K. pneumoniae (hvKp) isolates

A hierarchical clustering dendrogram comparing the antibiotic resistance profiles of cKp and hvKp in figure 5. It shows how closely related the two resistance patterns are based on their overall profiles.

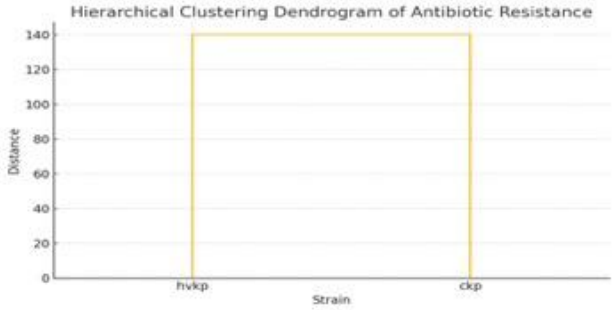

Figure 5. Similarity histogram among *K. pneumoniae* and hypervirulent *K. pneumoniae*

The dendrogram is a hierarchical clustering tree that illustrates the similarity between the antibiotic resistance profiles of cKp and hvKp Each leaf (end point) stands for either the "cKp" or the "hvKp" bacterial group. Their resistance profiles differ in how high the branch (vertical line) is where they combine. Because of its very high branching point, hvKp resistance profile differs significantly from cKp. his is expected, as hvKp showed significantly higher resistance across most antibiotics. The Percentage of Isolates by Antibiotic Class reveals hvKp shows consistently higher resistance across almost all antibiotic classes. While cKp strains are generally more sensitive, especially to carbapenems (Ertapenem, Imipenem) and aminoglycosides (Amikacin, Gentamycin). Resistance to last-resort drugs like carbapenems and aminoglycosides is significantly rising in hvKp, figure 6.

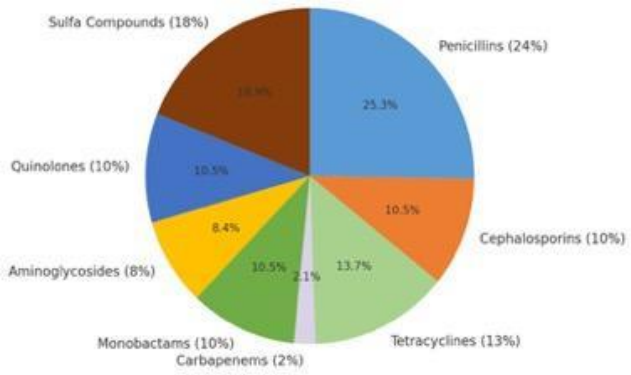

Figure 6. Percentage of Isolates by Antibiotic Class

Discussion

The findings of this study highlight the growing clinical concern posed by Klebsiella pneumoniae, particularly hypervirulent and multidrug-resistant strains, in urinary tract infections (UTIs). The high positivity rate (84.6%) for bacterial growth among symptomatic patients reinforces the prevalence of UTIs in the studied population, with *E. coli* and *K. pneumoniae* being the leading pathogens. The predominance of Gram-negative bacteria (91.1%) aligns with global epidemiological trends, where uropathogens like *K. pneumoniae* are increasingly reported in both community and hospital settings. Notably, the significant proportion of hypervirulent K. pneumoniae (33.9%) among isolates is alarming due to its strong association with severe infections and its enhanced ability to evade host immune responses. The PCR-based identification of K1 and K2 capsular serotypes further confirms the presence of hypervirulent strains, as these serotypes are well-documented markers of increased pathogenicity. Moreover, the high percentage of multidrug resistance (90.4%) observed among hvKp isolates, compared to 34% in cKp, underscores the urgent need for enhanced surveillance and stewardship measures. The resistance of hvKp to commonly used antibiotics, including β-lactams, fluoroquinolones, and even last-line agents such as carbapenems and aminoglycosides, significantly narrows therapeutic options. This trend mirrors the global rise in antimicrobial resistance and supports WHO's categorization of carbapenem-resistant K. pneumoniae as a critical priority pathogen. The clustering analysis further emphasized the distinct resistance profiles between hvKp and cKp, with hvKp showing more diverse and extensive resistance patterns. These findings call for routine screening for virulence and resistance markers, especially in healthcare settings, to facilitate early detection and control of hvKp outbreaks. For instance, a well-documented outbreak in China involved hvKp strains causing severe liver abscesses and metastatic infections in otherwise healthy individuals, while a separate hospital outbreak in France reported the rapid spread of carbapenem-resistant hv (9). Targeted antibiotic policies and molecular epidemiological surveillance are essential to mitigate the spread of these formidable pathogens.

The percentage was accepted as proved by previous study such as a local study in Baghdad included 62 patients with UTI concluded the most prevalence bacteria was *E. coli (10)*.

The hypervirulent with an increasing propensity for multidrug resistance, *Klebsiella pneumoniae* is a pathogen that is more virulent than cKp and quickly turns into a clinical adversary. Clinical signs typically include liver abscesses, pneumonia, osteomyelitis,

endophthalmitis, and meningitis, as well as infections at several locations or eventual metastatic progression (6).

The magA gene, which is a crucial virulence gene for K. pneumoniae strains that cause liver abscesses and may be utilized as a diagnostic tool, was present in K. pneumoniae isolates from liver abscesses. Only liver abscesses have the magA gene. As international research on Klebsiella isolates has expanded to various nations, including North America, Singapore, and Korea, they have demonstrated that the magA gene has been isolated from other instances, including acquired bacteremia, sepsis, meningitis, and endophthalmitis (11). A study from Najaf in 2021 investigated virulence genes rmpA and magA in K. pneumoniae isolates from diabetic foot ulcer patients. The magA gene, linked to the K1 serotype and hypermucoviscosity, was detected in 21 / 36 clinical isolates (58%) (12). Serotyping of isolates for both classical isolates and hyper virulence isolates and the results revealed serotype K1 was predominant. A study in Iran investigated the prevalence of magA gene of Klebsiella spp. isolated from clinical samples, showed that only (3.8%) of K. pneumoniae isolates harbor magA gene (11).

The isolates showed a wide and varying range of resistance to each antibiotic. The percentage of MDR isolates in cKp was 34%. The antibiotics targeting cell wall synthesis were the most resisted by the bacterial isolates under study. The highest resistance was observed for Ampicillin (100%). On the other hand, the following antibiotics were more effective against the bacteria, showing sensitivity rates of 95.2 % for Ertapenem and Imipenem, 92.8% for Amikacin, 80.6% for Gentamycin. By its very nature, hvKp has increased the danger of the associated infections for immunocompromised people and made healthy people more vulnerable to them. Furthermore, the hvKp may become the lethal CR-hvKp if drug-resistant genes are acquired. Because of this, there are now more serious infections and less effective therapies available (13).

HvKp showed more resistance for antibiotic than classical strain as the percentage of MDR was 90.4%. The antibiotics targeting cell wall and folic acid synthesis were the most resisted. The highest resistance was observed for Ampicillin (100%), Trimethoprim/Sulfamethoxazole (85.8%), and (81%) for Ceftazidime and Ceftriaxone, and (80%). While only two antibiotics were more effective against the hvKp, showing sensitivity rates of (62%) and (57.3%) for Ertapenem and Imipenem respectively. In an earlier study conducted in China that involved 86 K. pneumoniae infection cases, the resistance profiles of hypervirulent Klebsiella pneumoniae (hvKp) and classical Klebsiella pneumoniae (cKp) were compared in an effort to clarify their potential clinical implications. They discovered different patterns of resistance between hvKp and cKp, emphasizing how chromosomal mutations and plasmid-mediated gene transfer contribute to antibiotic resistance. The development of carbapenemases and extended-spectrum β-lactamases (ESBLs) was one of the notable resistance tendencies that hvKp strains displayed that were different from those of cKp ((14).

Our findings demonstrated that the hvKp strains exhibited a considerably greater rate of resistance to common antibiotics than the cKp strins, and that the hvKp strains produced ESBLs more frequently than the cKp strains. Which may relate to the mechanisms underlying antibiotic resistance may differ between hvKp and cKp, or might be brought on by hvKp's capacity to acquire plasmids containing antimicrobial resistance or by the capsule's overexpression, which could operate as a physical barrier to prevent antibiotics from entering the bacterium. But according to an Egyptian investigation, the antimicrobial resistance pattern of hvKp and cKp strains did not differ significantly (15). Treatment for hvKp must be more vigorous and individualized due of its complicated resistance patterns and enhanced virulence.Priority should be given to the early detection

and separation of hvKp from cKp in clinical specimens in order to enable suitable therapeutic approaches. These draw attention to the pressing need for innovative treatment approaches and medicines, especially those that address the processes of antibiotic resistance and biofilm formation in hvKp (14,16).

Conclusion

hvKp strains exhibited greater antibiotic resistance and higher ESBL rates in comparison to standard Kp strains. To improve clinical recognition and management of hvKp infections, it is crucial to address the recent increase in hvKp, particularly ESBL-hvKp and MDR-hvKp, which are hard to treat.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available.

Declarations

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Competing interest statement

None, the authors declare that they have no conflicts of interest.

Ethics statement

The authors declare that the author approved that this research follows the journal's Attach Ethic Approval guidelines as appeared on the journal's author guidelines page.

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