



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.edu/>

Original article

Assessment of healthcare-associated infection of *Klebsiella pneumoniae* isolated from patients attended private clinics in Basrah governorate

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ARTICLE INFO

Article history:

Received 1 August 2025

Received in revised form 19 September 2025

Accepted 20 September 2025

Keywords:

Healthcare-associated infections
private clinics environment
MDR *Klebsiella pneumoniae*
ESBL
Carbapenemase
blaCTX-M

ABSTRACT

Background: Occurrence of *Klebsiella pneumoniae* in patients who attended private clinics poses a threat to increase antibiotics resistance and sporadic transmission; it has acquired a significance similar to that of infections in hospitals and other healthcare facilities. **Aim:** to highlight *Klebsiella pneumoniae*, its antibiotics susceptibility pattern, type of resistance, prevalence and frequency in private medical clinics. **Methods:** 180 clinical samples obtained from urine, blood, sputum, and surgical wounds that subjected to identification and susceptibility analysis by biochemical means, VITEK® 2 and PCR for gene identification. **Results:** Out of 180 specimen, 118 isolates of *Klebsiella pneumoniae* showed sensitivity to colistin and tigecycline, with high resistance to third-generation of cephalosporins and fluoroquinolones. (77.9%) of multidrug-resistant isolates exhibited production of extended spectrum beta-lactamases (ESBLs), (66%) exhibited carbapenemase activity. The antibiotic resistance genes blaCTX-M was the most commonly observed among other detected resistance genes. **Conclusion:** Based on the frequency of infection of patients attending private medical clinics with *Klebsiella pneumoniae*, attention must be paid to this pathogen demonstrates resistance to beta-lactams along with carbapenems and additional antibiotic groups. .

Introduction

Bacterial infections with multidrug resistance (MDR) or extensively drug-resistant (XDR) are currently the biggest health threat facing global public health in the twenty-first century [1,2]. *K. pneumoniae* stands out among many significant pathogens due to its increasing role in healthcare-associated infections [3,4], appear alongside pneumonia, bloodstream infection (septicemia) and surgical site infections as well as urinary tract infections when the targeted population consists of immunocompromised patients or those receiving hospital care [5,6]. *K. pneumoniae* presents a

serious infection threat to hospitalized patients because it survives hospital conditions and successfully infects medical equipment and circumvents immune responses within the body [7,8]. The quick development of high resistant strains of *K. pneumoniae* reduced doctor's ability to provide suitable treatments which resulted in reduced patient treatment success [9]. Strains which produce the extended-spectrum beta-lactamases (ESBLs) and carbapenemases enzymes are most concerning because they effectively destroy a vast spectrum of beta-lactam antibiotics including carbapenems which are the final therapeutic option

[10,11]. *K. pneumoniae* resistance creates more patient fatalities while lengthening hospital admissions and increasing medical expenses and straining healthcare infrastructure [12]. The World Health Organization established carbapenem-resistant *K. pneumoniae* as one of the urgent antibiotic-resistant pathogens requiring new medical treatments [13,14]. Several genetics-related and biochemical processes enable *K. pneumoniae* to become antimicrobial-resistant. ESBLs together with carbapenemases including KPC (*K. pneumoniae* carbapenemase), NDM (New Delhi metallo-beta-lactamase) and OXA-48-like enzymes represent the main resistance mechanisms exhibited by this bacterium [15,16]. Such genetic resistance factors enable quick movement between *K. pneumoniae* populations and diverse bacterial species for both strains [18,19]. As a consequence, **aim** of the current study is to assess the prevalence, antibiogram pattern of *K. pneumonia* isolated from patients attended in private medical clinics in Basrah governorate.

Method

Study Design

The research study took place from July to December 2024 at 20 private clinics in different places from Basrah governorate (south of Iraq). Samples were collected from patients who visit private medical clinics and their affiliated facilities for medical examination and consultations. Some patients contracted a bacterial infections after (1-2) weeks of visiting these private medical clinics. Disease-causing bacteria are transmitted through several means and methods, such as contact with contaminated surfaces, when dealing with sample collection laboratories, or even through contact with other patients who visit private medical clinics and are infected with bacteria. Also, usage and exchange of urinary catheters, changing medical bandages, and even injecting needles were means of transmission of infection. The current investigation focusing on *K. pneumonia* infections due to seriousness and high resistance to antibiotics.

Samples Collection

Samples were collected from 180 patient's. Various clinical samples were collected such as urine, blood, sputum and wounds swabs.

The inclusion criteria

- Patients visited private clinics > 4 times
- Patients aged >18 years.

- Patients whom visit private clinics for specific medical conditions, then get bacterial infection during that time.
- Availability of complete clinical history.
- Positive culture for *K. pneumoniae*.

The exclusion criteria

- Patients visited private clinics < 4 times
- Patients who refused to participate in the study.
- Negative culture for *K. pneumoniae*.

A structured data collection form recorded patient demographic in combination with clinical data which included age, sex with additional information on presence of comorbidities like diabetes mellitus and cancer along with previous antibiotic use and private clinics admission status and final clinical outcomes.

Bacterial Identification

Initial identification of *K. pneumoniae* isolates was performed using conventional biochemical tests, including the IMViC series (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization tests), urease test, and Triple Sugar Iron (TSI) agar test [20]. Further confirmation and species-level identification were conducted using the automated VITEK® 2 Compact system (bioMérieux, France).

Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility of the isolates was assessed by using VITEK® 2 Compact system, following Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines [21]. The types of antibiotics used in antibiotics susceptibility test are as follows with their concentrations: (Ampicillin 10 µg, Cefotaxime 30µg, Ceftazidime 30µg, Ciprofloxacin 5µg, Imipenem 10µg, Meropenem 10µg, Amikacin 30µg, Colistin 10 mcg, Tigecycline 15µg).

Detection of Resistance Mechanisms

Phenotypic detection

ESBL production was evaluated through the Double Disk Synergy Test (DDST) which followed CLSI guidelines [21]. The Modified Hodge Test evaluated Carbapenemase productivity whereas the Carba NP test affirmed the results according to CLSI standards.

Molecular detection of *Klebsiella pneumoniae* resistance genes

Bacterial genomic DNA extraction and purification

A total of six resistance genes were included in the molecular characterization studies; these consisted of an extraction kit enabled DNA extraction and specific primers amplified DNA through PCR while following conditions established by [22]. In order to retrieve genomic DNA, the procedure was done by using commercially available DNA extraction and purification kit, QIAamp DNA Mini Kit (Qiagen, Germany) with the manufacturer's protocol applied.

Detection of the whole DNA

The purified DNA was detected by electrophoresis (70V) in 1% agarose gel with ethidium bromide, for 1hr. Methylene blue stain added to the DNA samples and visualizes the DNA by the U.V. light.

Amplification of *blaCTX-M*, *blaSHV*, *blaTEM*, *blaNDM*, *blaOXA-48*, and *blaKPC* genes by conventional PCR

The *blaCTX-M*, *blaSHV*, *blaTEM*, *blaNDM*, *blaOXA-48*, and *blaKPC* genes is used as the standard for identification of *Klebsiella pneumoniae* resistance markers, eighty (80) DNA templates for it were subjected to PCR application with specific *blaCTX-M*, *blaSHV*, *blaTEM*, *blaNDM*, *blaOXA-48*, and *blaKPC* gene primers.

These primers were employed to increase the probability of detection *K. pneumoniae* and estimated by using designed primers. The primers sequences are presented in Table 1.

Table 1. Primer sequences and expected PCR product sizes for resistance genes

Target gene	Primer Sequence (5'–3')	Amplicon size (bp)
<i>blaCTX-M</i>	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	593
<i>blaSHV</i>	F: ATGCGTTATATTCGCCTGTG R: TGCTTTGTTATTCGGGCCAA	867
<i>blaTEM</i>	F: ATGAGTATTCAACATTTCCGTG R: TTACCAATGCTTAATCAGTGAG	867
<i>blaNDM</i>	F: GGTTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621
<i>blaOXA-48</i>	F: GCGTGGTTAAGGATGAACAC R: CATCAAGTTCAACCCAACCG	438
<i>blaKPC</i>	F: CATTCAAGGGCTTTCTTGCTGC R: ACGACGGCATAGTCATTTCG	798

Conventional PCR assays were carried out in mixture contents as follows: Master Mix (12.5 µl), F primer (2.5 µl), R primer (2.5 µl), template DNA (4 µl), nuclease-free water (3.5 µl). Total mixture volume 25 µl.

Cycling conditions were: first denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds, followed by annealing at 60°C for *blaCTX-M*, 56°C for *blaSHV* and *blaTEM*, 52°C for *blaNDM*, 55°C for *blaOXA-48*, and 58°C for *blaKPC* for 30 seconds; extension at 72°C for 1 minute; final extension at 72°C for 7 minutes.

Clinical Data Collection

The retrospective analysis of patient information drew data from private clinics records which included demographic data as well as underlying health conditions together with previous antibiotic use and ICU stay time and clinical outcomes of hospital discharge or mortality.

Statistical Analysis

Research data entry and analysis took place through SPSS software version 26.0 (IBM Corp., Armonk, NY, USA) allowed for analysis of categorical variables using the Chi-square test. The investigators conducted multivariate logistic regression tests to detect separate factors correlating to MDR *K. pneumoniae* infections. Research found statistical significance at a p-value less than 0.05.

Results

A summary of patients demographic data described the 180 patients whom visit the private clinics to follow up on their health and medical conditions with their doctors, all of them provided *K. pneumoniae* isolates. Out of 180 substantial number of patients treated at the study facility were males 105(58.3%) and females 75(41.7%). The biggest comorbidities among them consisted of 65(36.1%) of the patients suffer from diabetes, 38(21%) patients with chronic kidney disease_CKD, 30(16.6%) patients with hypertension, 30(16.6%) patients suffer from various types of cancer, 17(9.4%) patients with ischemic heart disease_IHD. The patient's group averaged 54 years of age.

About the obtained 180 *K. pneumoniae* clinical isolates described in Table 2, contains the distribution data of tested isolates that came from multiple clinical specimens. The highest proportion of samples (36.1%) came from urine while blood samples made up (26.7%) , sputum (20.6%) and wounds swabs (16.6%) completed the dataset.

There was no statistically significant correlation between patient sex and *K. pneumoniae* infection, as indicated by a chi-squared test ($p = 0.21$). Conversely, patients aged ≥ 50 exhibited a significantly higher prevalence of *K. pneumoniae*

infections compared to younger patients ($p = 0.04$). The study shows that people over the age of 50 seem to be a more vulnerable group for infections. Also, the incidence of *K. pneumoniae* is generally higher in males than in females.

Table 2. Distribution of clinical specimens.

Sample type	Number of isolates	Percentage (%)
Urine	65	36.10%
Blood	48	26.70%
Sputum	37	20.60%
Wound swab	30	16.60%
Total	180	100%

Lab analysis showed that 118 out of 180 displayed *K. pneumoniae* isolates with resistant characteristics for a percentage of (65.5%). The spreadsheet in Table 3., shows the resistance levels of different antibiotics against the isolates. Test results showed (100%) resistance for ampicillin, (96.6%) for cefotaxime, (93.2%) ceftazidime and (85.6%) for ciprofloxacin. A significant portion of the tested carbapenem antibiotics showed resistance with imipenem at (67.8%) and meropenem at (64.4%). The resistance rates for colistin and tigecycline were (18.6%) and (12.7%) respectively, so these antibiotics may considered effectively treat *K. pneumoniae* strains.

Table 3. Resistance profile of *K. pneumoniae* (n=118).

Antibiotic types	Resistance (%)
Ampicillin	100
Cefotaxime	96.6
Ceftazidime	93.2
Ciprofloxacin	85.6
Imipenem	67.8
Meropenem	64.4
Amikacin	55.1
Colistin	18.6
Tigecycline	12.7

Results confirmed that 92 (77.9%) out of 118 isolates of *K. pneumoniae* were ESBL producers based on Double Disk Synergy Test results. Results also, documented that 66 isolate were positive results for carbapenemase production through validation by both MHT and Carba NP test methods.

According to the results of molecular diagnosis, analysis through PCR exhibited that 80 (67.7%) isolates out of 118 *K. pneumoniae* had

important resistance genes. A majority of resistance genes were blaCTX-M genes (26%) , blaSHV genes appeared in (21%) of isolates and blaTEM genes in (16%) of cases. The analysis demonstrated the presence of three carbapenemase-related genes among *K. pneumoniae* isolates with blaNDM detected in (15%), blaOXA-48 observed in (12.5%) and blaKPC found in (8.75%) of cases as indicated in Table 4. Also, results indicated that most of the isolates carried more than one resistance gene at the same time.

Table 4. Prevalence of resistance genes (n=80).

Resistance gene	Positive isolates	Percentage (%)
blaCTX-M	21	26
blaSHV	17	21
blaTEM	13	16
blaNDM	12	15
blaOXA-48	10	12.5
blaKPC	7	8.75

Discussion

The rapid spread of *K. pneumoniae* infections is not limited to infections in hospitals and other healthcare facilities, but has also spread to patients who visit private clinics. The research data demonstrates the severe difficulty that *K. pneumoniae* creates not within hospital environments only, but in healthcare facilities, including private medical clinics, where patients can contact their doctors to request medical advice and assistance. Disease-causing bacteria are transmitted through several means and methods, such as contact with contaminated surfaces, when dealing with sample collection laboratories, or even through contact with other patients who visit private medical clinics and are infected with bacteria [23]. The high resistance levels to carbapenems and third-generation cephalosporins limits available treatment options therefore requiring improved infection control measures [24,25]. The essential measures for controlling organisms spread and enhancing patient results consist of continuous antimicrobial resistance monitoring together with rigorous antimicrobial stewardship protocols [26].

The frequency of *K. pneumoniae* in this study is comparable to reports from Middle Eastern countries like Egypt (60%), Iran (62%), Turkey (58%) underlining prevalence of strains in the area. Total and high resistance to ampicillin (68.9%) and

third-generation cephalosporins cefotaxime (96.6%) and ceftazidime (93.2%), significantly documented earlier in India and Saudi Arabia [27,28] suggests extensive β -lactamase production. The high rate of detection of blaCTX-M among ESBL genes (26%) is consistent with global results highlighting its widespread commonality among Enterobacteriaceae [29]. Detection of carbapenemase genes such as blaNDM (15%), blaOXA-48 (12.5%), and blaKPC (8.75%) reflects poor management of infections and over prescription of antibiotics, conditions which promote gene transfer in Iraq [30]. MDR infection patients were hospitalized longer (14.7 vs. 8.2 days) and had an increased probability of dying (22.5% vs. 9.4%), matching previous worldwide research. Such findings underscore the frightening clinical and financial burden of multidrug-resistant and extensive drug-resistant infections [31,32,33]. The analysis showed that advanced age ($p = 0.04$) significantly contributed to *K. pneumoniae* infection, which is consistent with earlier research in which older population was more susceptible due to comorbidities and impaired immunity [34, 35]. The study did not establish any significant relationship between sex and *K. pneumoniae* infection ($p = 0.21$), similar with previous multicenter studies [36].

These findings provide a direct call for the formulation of antimicrobial stewardship, advanced molecular diagnostic tools, and improved infection prevention measures in Iraqi healthcare facilities in consonance with the WHO Global Action Plan on Antimicrobial Resistance.

Conclusion

High prevalence of resistant *K. pneumonia* isolates in healthcare facilities represented by private medical clinics is noticeable warning, leaving limited therapeutic options for managing its infections. Based on this and similar studies, the current study concluded that *K. pneumonia* utilizes various mechanisms to evade therapeutic molecules and other antibiotics particles even in non-hospital environments.

K. pneumoniae has many resistance mechanisms in addition to its production of enzymes that destroy the effect of antibiotics, in addition to the resistance genes that it carries innately in its chromosome. Therefore, the resistance pattern in this bacterium must be monitored continuously by following a antibiotics sensitivity test.

Also, avoiding random prescription of antibiotics or empirical treatment without resorting

to bacterial culture of pathological samples taken from patients, as the bacteria causing the infection can be diagnosed and then the most appropriate treatment for it can be determined through antibiotics susceptibility.

Limitation

This investigation draw attention to the fact that bacterial infections can be transmitted between patients in private medical clinics as well as in hospitals, focusing on *K. pneumoniae* due to their medical importance and role in antibiotic resistance.

Conflict of interest

The author (s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article. This manuscript has not been previously published and is not under consideration in another journal.

Financial disclosure

Authors did not receive any grants from funding agencies.

Data availability

The data generated or analyzed during the current study are included in this article.

Authors' contribution

All authors contributed to the study conception and design, EAS designed the study, provided us with the documents, collected samples and the data. AMH and SNK carried out the study analysis. QAQ prepared the statistical analysis of the study. EAS revised and edited the final version of the article. All authors read and approved the submitted final corrected version.

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