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Research article

Multidrug-resistant *Klebsiella pneumoniae* isolated from an infected wound in Iraq

Mohammed Talab Mohammed[1,2], Ayaid Khadem Zgair[1]*

ABSTRACT

The infected wound with multidrug-resistant (MDR) bacteria represents one of the big issues for public health. *Klebsiella pneumoniae,* especially MDR, is responsible for infecting different human organs, particularly wound infections. This current study aims to investigate the antibiotic susceptibility patterns of *K. pneumoniae* isolates that are responsible for wound infection. In the present study, the samples were collected from Al-Fallujah General Hospital, Al-Anbar, Iraq. A total of 89 wound swabs were collected from patients aged 15 to 65 years, following ethical standards. The microbiological methods were followed for isolation and identification of *K. pneumoniae*, e.g., culture on selective media, Gram staining, and biochemical tests. The Kirby–Bauer disk diffusion method was used to evaluate susceptibility against eight antibiotics, and results were interpreted using CLSI 2024 guidelines. From 89 wound swabs, 12 isolates of *K. pneumoniae* were identified, with an infection incidence rate of 13.48%. The antibiotic susceptibility analysis showed a high prevalence of MDR in the isolates. The highest resistance rate was observed against ceftriaxone, followed by cefepime and aminoglycosides (gentamicin, tobramycin, and amikacin). Imipenem showed high antibacterial efficacy, with four isolates showing susceptibility and one intermediate response. Levofloxacin also showed high effectiveness, with five sensitive isolates. Several isolates exhibited extensive drug resistance, with susceptibility to only one or two antibiotics. The study is highlighting the alarming emergence of MDR *K. pneumoniae* in wound infections, emphasizing the need for continuous surveillance and rational antibiotic use. Imipenem and levofloxacin remain among the most effective agents that need gene molecular characterization.

Keywords: Antibiotics, *Klebsiella pneumoniae*, MDR, Wound.

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1. INTRODUCTION

Wound infections are a big problem in healthcare, especially in areas affected by war, lacking healthcare infrastructure, and with high patient numbers [1]. *Klebsiella pneumoniae* has become a powerful opportunistic organism among the bacteria that can cause wound infections. This Gram-negative, encapsulated bacterium is common in the environment and in the normal flora of the human gastrointestinal tract. However, it can cause serious infections when it gets into places that are usually sterile, like open wounds. It's scary that *K. pneumoniae* is becoming more common in wound infections, especially since it is becoming more resistant to many types of antibiotics [2,3].

In Iraq, the problems with wound infections are made worse by ongoing security problems, injuries from trauma, and inconsistent access to healthcare [4]. These conditions make it easy for multidrug-resistant (MDR) pathogens to spread and stay alive. Recent research has found that Iraqi healthcare settings have a lot of MDR Gram-negative bacteria, and *K. pneumoniae* is one of the most common pathogens found there [5]. The situation is especially bad because the bacterium can get and spread resistance genes through mobile genetic elements. This makes it resistant to beta-lactams, aminoglycosides, fluoroquinolones, and even last-resort drugs like carbapenems [5].

* Correspondence: **Dr. Ayaid Khadem Zgair**, Ph.D. E-mail: ayaid.zgair@sc.uobaghdad.edu.iq
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Full list of author information is available at the end of the article.

Infections with multidrug-resistant *K. pneumoniae* make treatment much harder, lengthen hospital stays, raise healthcare costs, and raise the rates of illness and death [6]. The bacterium's ability to survive in harsh conditions and resist standard treatments is also helped by its resistance mechanisms, which include making extended-spectrum beta-lactamases (ESBLs), carbapenemases, efflux pumps, and biofilm [7]. These traits highlight the importance of constant vigilance, identifying resistant strains promptly, and employing effective antimicrobial stewardship to prevent the spread of resistant strains and facilitate optimal clinical management.

There isn't much information about MDR *K. pneumoniae* in wound infections in Iraq, even though it has serious effects on public health. It is very important to describe local isolates, learn about their resistance profiles, and find out how they affect patients. The goal of this study is to find and identify *K. pneumoniae* in an infected wound on an Iraqi patient and test its antibiotic susceptibility profile to see how resistant it is to multiple drugs. This study aims to contribute to the regional database on MDR pathogens, thereby informing local treatment guidelines, supporting infection control policies, and highlighting the need for new treatment options as antimicrobial resistance increases.

2. MATERIALS and METHODS

2.1. Clinical cases and sample collection

The current study involved 89 infected wound swab isolates from patients admitted to Fallujah General Hospital, Al-Anbar, Iraq. The Helsinki guideline was followed to manage clinical presentations and collect samples included in this study. The patients ranged in age from 15 to 65 years, with an average age of 46.7 ± 13.1 years. There were 53 men and 46 women among the participants. All patients agreed to participate in the study. The patients had stopped using antibiotics for two to three days prior to sample collection. The infected wounds ranged from mild to moderate severity. Pathological samples were collected with sterile swabs, which were then transferred directly to the laboratory for culture on MacConkey, blood, and nutrient agar. Mucoid Lactose-fermenting colonies were isolated and purified, and various biochemical tests (oxidase, catalase, Indole, and Simon citrate test) were performed, along with bacterial morphology studies after Gram staining. The purified isolates were stored for a short period by inoculating them on nutrient agar and kept at 4°C for 2 weeks. The isolates were stored for the long term by inoculating them into nutrient broth containing 20% glycerol (incubated previously at 37 °C for 18 h) and then stored at -20 °C for a year [8, 9].

2.2. Antibiotic susceptibility

In this method, the Kirby–Bauer technique was implemented for antimicrobial susceptibility testing. Briefly, standard inocula of bacterial isolates of *K. pneumoniae* (10^8 CFU/ml) were spread onto Mueller-Hinton agar (MHA, Himedia) plates. Standard commercial antibiotic discs (six discs were put on each plate). The standard antibiotic discs imipenem (IMP 10 µg), tobramycin (TOB 10 µg), tetracycline (TE 10 µg), vancomycin (VA 30 µg), Levofloxacin (LEV, 5 µg), Amikacin (AK 10 µg), gentamycin (GM, 10 µg), Ceftriaxone (CRO 10 µg), cefepime (FEP 30 µg) were checked against all isolates of *K. pneumoniea*. The plate was then incubated for 18 h at 37°C. The scale was used to measure the inhibition zones. The diameters were compared with the measured diameters of the Clinical and Laboratory Standards Institute (CLSI) breakpoint charts to determine the Sensitive (S), Intermediate (I), and Resistant (R) bacteria to the antibiotics [10].

2.3. Statistical analyses

The statistical analysis was conducted, and the graphs were generated utilizing Origin v. 8.6 software (OriginLab, Northampton, USA). The data were presented as means ± standard error (M ± SE).

3. RESULTS

3.1. Rate of infection incidence

In the present study from 89 infected wound swabs that collected from different patients, 12 isolates of *K. pneumoniea* were isolated. The number of K. pneumoniea isolates that isolated from 89 clinical samples were 12 isolates. Thus, the moderate incidence rate of wound infection with K. pneumoniea was 13.48 %. The bacterial isolates obtained from infected burn wounds were identified using biochemical tests and confirmed by the VITIK 2 system. The number of male infected wound was higher than the number of female infected wounds.

3.2. Antibiotic susceptibility pattern

Table 1 shows the diameter of the inhibitory zones around the ten antibiotic disks. The CLSI breakpoints were used to interpret the results. Figure 1 shows the number of K. pneumoniea that responded to the ten antibiotics.

Table 1. Antibiotic susceptibility profiles of *K. pneumoniae* isolates (Kp1–Kp12) based on disk diffusion zone diameters (mm) and CLSI 2024 interpretations. IMP: Imipenem; TOB: Tobramycin; TE: Tetracycline; LEV: Levofloxacin; AK: Amikacin; GM: Gentamicin; CRO: Ceftriaxone; FEP: Cefepime. Zone diameters (mm) are shown with the corresponding interpretation: S (Susceptible), I (Intermediate), and R (Resistant) according to CLSI M100 guidelines. A value of 0 indicates no zone of inhibition, representing resistance.

	IMP	TOB	TE	LEV	AK	GM	CRO	FEP
Kp1	30 (S)	9 (R)	0 (R)	16 (I)	12 (R)	11 (R)	0 (R)	12 (R)
Kp2	0 (R)	10 (R)	9 (R)	22 (S)	9 (R)	10 (R)	0 (R)	10 (R)
Kp3	14 (S)	18 (S)	20 (S)	23 (S)	17 (S)	19 (S)	0 (R)	10 (R)
Kp4	22 (I)	17 (S)	24 (S)	36 (S)	20 (S)	23 (S)	30 (S)	30 (S)
Kp5	26 (S)	16 (S)	0 (R)	11 (R)	0 (R)	15 (S)	14 (R)	11 (R)
Kp6	12 (R)	0 (R)	0 (R)	25 (S)	14 (R)	0 (R)	13 (R)	14 (R)
Kp7	0 (R)	0 (R)	18 (S)	0 (R)	0 (R)	11 (R)	0 (R)	0 (R)
Kp8	18 (R)	14 (I)	0 (R)	24 (S)	19 (S)	0 (R)	0 (R)	23 (S)
Kp9	9 (R)	0 (R)	10 (R)	0 (R)	0 (R)	0 (R)	9 (R)	0 (R)
Kp10	0 (R)	0 (R)	0 (R)	0 (R)	17 (S)	12 (R)	9 (R)	0 (R)
Kp11	23 (S)	10 (R)	0 (R)	10 (R)	14 (R)	16 (S)	0 (R)	0 (R)
Kp12	17 (R)	18 (S)	17 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)

The present study reported that four isolates were sensitive to IMP, one isolate responded in intermediate (Kp4), while seven isolates were resistant to IMP (Kp2, Kp5, Kp6, Kp7, Kp8, Kp9, Kp10, and Kp12). It can be suggested that carbapenem resistance is present but not universal. Four isolates were sensitive to Tobramycin (TOB) (Kp3, Kp4, Kp5, Kp12). One isolate was intermediately resistant to the antibiotic, and seven isolates were resistant to TOB. Four isolates were sensitive to tetracycline, and

eight isolates were resistant to tetracycline. Five isolates were sensitive to levofloxacin, one intermediate, and six were resistant to the antibiotic. Four isolates were sensitive to amikacin, and eight were resistant to amikacin. Four isolates were sensitive to gentamycin and eight were resisted to it. Only one isolate was sensitive to Ceftriaxone, while two were only sensitive to cefepime. The present study showed that the isolates show multidrug resistance (MDR), especially to cephalosporins (ceftriaxone and cefepime). IPM is still the most effective agent for many isolates, but carbapenem resistance is evident in several (half of the isolates are resistant or intermediate). Aminoglycosides (amikacin, gentamicin, tobramycin) show variable susceptibility, with a tendency towards resistance. Fluoroquinolone (levofloxacin) susceptibility is moderate but promising in many isolates. Some isolates are extensively drug resistant (e.g., Kp7, Kp9, Kp10), showing susceptibility to very few or only one antibiotic. High cephalosporin resistance suggests possible ESBL or carbapenemase producers. The results showed that the highest number of bacterial isolates were resistant to Ceftriaxone, while the lowest resistance was to levofloxacin.

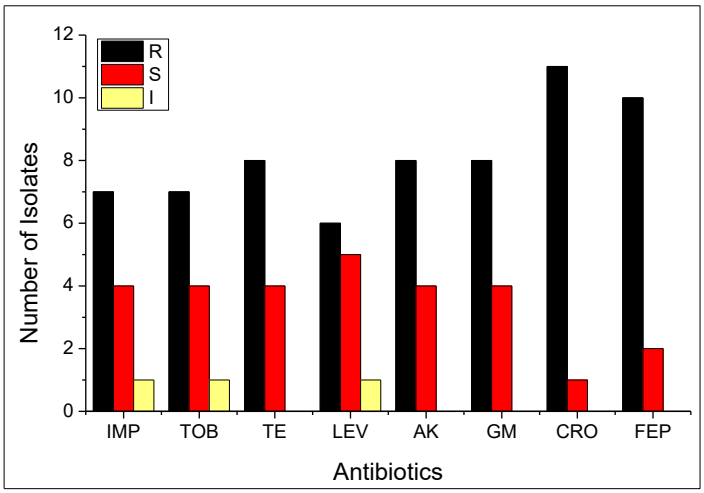

Fig 1. Distribution of *K. pneumoniae* isolates according to their susceptibility to different antibiotics. The number of isolates exhibiting resistance (R), susceptibility (S), and intermediate resistance (I) to each antibiotic is shown. Antibiotics tested include imipenem (IMP), tobramycin (TOB), tetracycline (TE), levofloxacin (LEV), amikacin (AK), gentamicin (GM), ceftriaxone (CRO), and cefepime (FEP). Data are presented as bar charts with resistance (black), susceptibility (red), and intermediate (yellow) categories.

4. DISCUSSION

The infected wound with MDR *K. pneumoniae* represents a big issue for clinicians [12]. The number of MDR *K. pneumoniae* increased dramatically over time for several reasons, one of which is the misuse of antibiotics [13]. The study highlights the emergence and prevalence of *MDR K. pneumoniae* in infected wound cases from Al-Fallujah General Hospital, Al-Anbar, Iraq. The identification of bacteria was 13.48% of the total 89 wound swab samples. This is in line with a previous study that showed *K. pneumoniae* as a frequent cause of healthcare-associated infections, especially in immunocompromised or hospitalized patients with open wounds [14].

The antibiotic susceptibility profile showed a worrying pattern of resistance, with most isolates being resistant to more than one commonly used antibiotic. Ceftriaxone resistance was significantly the highest, followed by cefepime and aminoglycosides (gentamicin, tobramycin, and amikacin). This pattern suggests the possible presence of extended-spectrum β-lactamases (ESBLs) and/or carbapenemase-producing strains, both of which are recognized mechanisms of resistance in *K. pneumoniae* [15]. The elevated incidence of cephalosporin resistance undermines the effectiveness of these agents in empirical treatment regimens for wound infections [16].

Imipenem and levofloxacin exhibited the most dependable antibacterial efficacy [17], with 4 and 5 isolates, respectively, displaying sensitivity. However, finding imipenem-intermediate and resistant isolates makes me worry about the spread of carbapenem-resistant *K. pneumoniae* (CRKP), which is much harder to treat [18]. It is a big public health issue that there are isolates that are resistant to almost all tested antibiotics, including those that are classified as extensively drug-resistant (XDR), and this has increased over the last decade [19].

The differences in how susceptible different isolates are suggest that they have different genes, and this shows how important it is to do molecular studies to find the specific resistance genes and plasmids that are involved. This kind of molecular characterization would also help figure out how the disease spreads and how resistance spreads in healthcare settings [20].

This study emphasizes the imperative for ongoing antibiotic resistance monitoring, enhanced infection control measures, and the prudent application of antibiotics. It also emphasizes the necessity of customizing antibiotic therapy according to local susceptibility data instead of depending exclusively on standard empirical regimens. Future studies should incorporate molecular diagnostics to identify resistance determinants and facilitate the formulation of targeted interventions to mitigate the dissemination of MDR *K. pneumoniae* in Iraqi healthcare institutions.

5. CONCUSION

Wound infections caused by antibiotic-resistant bacteria are one of the most common hospital-associated infections, especially among immunocompromised individuals. This study presents the presence of MDR *K. pneumoniae* in wound infections, which was 13.48% of wound swabs. The antibiotic susceptibility investigation showed a high resistance rate to commonly used antibiotics, especially cephalosporins and aminoglycosides. Imipenem and levofloxacin were the most effective antibacterial agents. These findings underscore the urgent need for local and national antimicrobial resistance monitoring, strict infection control practices, and rational antibiotic prescribing policies, and further molecular investigations to the genes that responsible for resistance mechanisms and responsible for rising prevalence of MDR *K. pneumoniae* in wound infections.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

This study was approved by the Ethics Committee of the University of Baghdad (CSEC/1124/0113; November 27, 2024). Since it was a retrospective analysis of routinely collected clinical data, individual patient consent was waived in accordance with national ethical guidelines.

Author contributions

Mohammed MT: Investigation; Methodology; Resources.

Zgair AK: Resources; Methodology, Supervision; Validation; Roles/Writing, Writing–review, Investigation; Project administration; Roles/Writing - original draft; and Writing-review & editing.

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Author affiliation

1. Department of Biology, College of Science, Baghdad, Iraq.
2. Fallujah General Hospital, Al-Anbar, Iraq.

ORCID:
Ayaid K. Zgair: https://orcid.org/0000-0002-2356-3338.
Mohammed T Mohammed: https://orcid.org/0009-0005-0549-0234