



The Antimicrobial Resistance Pattern of *Klebsiella pneumoniae* Isolated from the Clinical Specimens in Duhok City in Kurdistan Region of Iraq

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Abstract

Background: *Klebsiella pneumoniae* is an important opportunistic enteric bacterial strain, which is a major cause of pneumonia and urinary tract infection. Antimicrobial resistance is an increasingly severe threat to the Global Public Health, requiring immediate action across governmental sectors and communities.

Objectives: The present study aimed to determine the sensitivity pattern of *K. pneumoniae* isolated from various clinical specimens for common antibiotics in Duhok City, Iraq.

Methods: This study was conducted in Duhok City during January 2017-February 2019 on 130 clinical samples of urine, blood, sputum, wound swabs, central venous lines, and oral swabs. *K. pneumoniae* strains were confirmed and tested in terms of susceptibility to various antimicrobial drugs using the VITEK-2 compact system.

Results: In total, 130 positive *K. pneumoniae* cultures from various clinical samples were examined. The isolates were more predominant in the females (n = 99; 76.2%) compared to males (n = 31; 23.8%). The antibiotic resistance rate of *K. pneumoniae* varied among different isolate clinical sample sources. Overall, high resistance rates were recorded for ampicillin (96.9%), ceftriaxone (65.8%), and cefepime (60.8%). However, ertapenem (93.8%) and imipenem (82.3%) showed the highest susceptibility rate against the isolates.

Conclusions: According to the results, *K. pneumoniae* isolated from various clinical specimens varied in terms of the antibiotic susceptibility pattern with high resistance to common antibiotics, particularly ampicillin. Ertapenem and imipenem were the most effective antibiotics against the isolates. Our findings could help physicians and clinicians to select appropriate antimicrobial therapies in the region.

Keywords: *K. pneumoniae*, Clinical Samples, Antimicrobial Resistance, Duhok City

1. Background

Klebsiella pneumoniae is an important opportunistic pathogen that causes a wide range of clinical diseases, including hospital acquired pneumonia, urinary tract, digestive, and blood infections, skin and soft tissue infections, and septicemia in immunocompromised patients (1-3). *K. pneumoniae* is a Gram-negative, rod-shaped, aerobic, non-motile bacillus with a large polysaccharide capsule. It is one of the most important enteric bacteria accountable for up to 10% of nosocomial infections associated with high morbidity and mortality rates (4).

Antimicrobial resistance (AMR) is a major health concern worldwide (5, 6), as well as a major cause of treatment failure in infectious diseases, which is associated with increased morbidity, mortality, and financial burden on the healthcare system (7). It is estimated that approximately

700,000 deaths occur each year due to AMR. Furthermore, it is predicted that if appropriate control and prevention measures are not taken, AMR could become a leading cause of mortality in hospitalized and non-hospitalized patients in developing and developed countries (8).

The emergence of multidrug-resistant *K. pneumoniae* is considered to be a global public health issue (9). The carriage of *K. pneumoniae* and development of multidrug-resistant bacteria have increased due to the frequent use of broad-spectrum antibiotics for hospitalized patients (10). In several countries, *K. pneumoniae* has developed resistance to the most common antibiotics and even the higher classes of antibiotics, such as third-generation cephalosporins (11). In addition, *K. pneumoniae* has been reported to be the most common pathogenic bacteria to develop resistance to broad-spectrum beta-lactam anti-

otics via extended-spectrum beta-lactamase (ESBL) (5).

Previous studies in Duhok City, Iraq have denoted the alarming rates of antibiotic resistance to several bacteria (12-15). While the continuous monitoring of antibiotic sensitivity patterns is essential to the treatment of various infections, the current data is scarce on *K. pneumoniae* in Duhok City.

2. Objectives

The present study aimed to determine the antimicrobial susceptibility pattern of *K. pneumoniae* isolated from various clinical specimens in Duhok City, located in the Kurdistan Region of Iraq.

3. Methods

3.1. Sample Collection and Processing

This cross-sectional study was conducted at a private clinical health center in Duhok City during January 2017-February 2019 on 130 isolates that were obtained from various clinical specimens, including 86 urine specimens, 16 blood specimens, nine sputum specimens, 13 wound swabs, three central venous (CV) lines, and three oral swabs. The patients were within the age range of 10 - 65 years from both genders (31 males and 99 females). All the patients were clinically positive for *K. pneumoniae* and examined in accordance with the standard operation procedure.

Clean-catch midstream urine specimens were collected using sterile disposable glass containers (5 mL) from the patients in order to avoid contamination. Blood samples were obtained from the patients aseptically in blood culture tubes, and sputum was collected from each patient using sputum containers with screw caps. In addition, the clinical wound and oral samples were collected using sterile cotton swabs.

The inclusion criteria of the study were as follows: (1) both male and female patients; (2) age of more than 10 years; (3) positive microbiological evidence of *K. pneumoniae* and (4) consent to participate in the study. The patients who did not consent to participate were excluded.

3.2. Identification and Antimicrobial Sensitivity

The clinical isolates of *K. pneumoniae* were identified based on the morphological characteristics on the MacConkey agar media. The large mucoid colonies obtained from the MacConkey agar plate were classified based on the gram stain according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). In the gram staining, all the isolates were confirmed as gram-negative

and rod-shaped. The identification of *K. pneumoniae* and antimicrobial susceptibility testing were performed using the VITEK-2 compact bacterial identification and monitoring system (bioMerieux, US) in accordance with the instructions of the manufacturer.

3.3. Ethical Considerations

The study protocol was approved by the Ethics committee of the College of Medicine at the University of Zakho in Kurdistan Region, Iraq. Informed consent was obtained from all the patients prior to participation.

4. Results

In total, 130 positive *K. pneumoniae* cultures of various clinical samples were evaluated. The highest number of the *K. pneumoniae* isolates was obtained from the urine samples (n = 86; 66.2%), followed by the blood samples (n = 16; 12.3%), and wound swabs (n = 13; 10%) (Table 1). In terms of gender, the female patients were more affected by *K. pneumoniae* (n = 99; 76.2%) compared to the males (n = 31; 23.8%) (Table 1). Moreover, the distribution of *K. pneumoniae* was higher in the females compared to the males in the urine, blood, wound, and oral samples. However, the distribution of the isolates was higher in the males compared to the females in the CV line and sputum samples (Table 1).

Table 1. Distribution of *K. pneumoniae* Isolated From Various Clinical Specimens in Terms of Gender^a

Source of Isolation	Gender		Total
	Male	Female	
Urine	14 (16.3)	72 (83.7)	86 (66.2)
Blood	2 (12.5)	14 (87.5)	16 (12.3)
Wound Swab	4 (30.8)	9 (69.23)	13 (10)
Sputum	8 (88.9)	1 (11.1)	9 (6.9)
CV Line	2 (66.7)	1 (33.3)	3 (2.3)
Oral Swab	1 (33.3)	2 (66.7)	3 (2.3)
Total	31 (23.8)	99 (76.2)	130 (100)

Abbreviation: CV Line, central venous line.

^aValues are expressed as No. (%).

Table 2 shows the resistance rate of the isolated *K. pneumoniae* from various clinical samples to common antibiotics. In the urine samples, *K. pneumoniae* was observed to be highly resistant to ampicillin (97.7%), ceftriaxone (62.8%), and cefepime (60.5%), while also highly sensitive to ertapenem (97.7%) and imipenem (96.5%). *K. pneumoniae* isolated from the blood samples was extremely effective against ciprofloxacin (100%) and levofloxacin (100%),

while the *K. pneumoniae* isolates were resistant to ampicillin (93.8%) and cefazolin (87.5%), and approximately 81% were resistant to ceftiofloxacin, ceftriaxone, cefepime, and gentamicin (Table 2).

According to the findings, 100% of the isolated *K. pneumoniae* from the sputum samples were resistant to ampicillin, and 77.8% were sensitive to gentamicin (Table 2). The sensitivity of the *K. pneumoniae* isolated from the wound swabs to ertapenem was estimated at 92.3%, while these isolates were highly resistant to ampicillin (92.3%). The strains isolated from the CV line exhibited the highest resistance rates against all the antibiotics, with the exception of ertapenem (Table 2). In addition, 100% of the isolated *K. pneumoniae* from the oral swabs were highly sensitive to all the common antibiotics, with the exception of ampicillin (Table 3).

Table 3 shows the overall susceptibility profile of the *K. pneumoniae* isolates from various clinical specimens. Accordingly, 96.9%, 65.8%, and 60.8% of the isolated *K. pneumoniae* strains were resistant to ampicillin, ceftriaxone, and cefepime, respectively. However, the highest sensitivity rate was observed against ertapenem (93.8%) and imipenem (82.3%) (Table 3).

5. Discussion

K. pneumoniae is an important causative agent of several clinical diseases, including urinary tract infection, pneumonia, skin and soft tissue infections, and bacteremia septicemia, which are associated with high morbidity and mortality (2, 3). Currently, drug resistance to human pathogenic bacteria such as *K. pneumoniae* is frequently reported worldwide (9). This is considered to be an alarming issue in both developing and developed countries due to the wide usage of the antibiotics used in medical practice (5). To investigate the potential difference in drug resistance, we analyzed the *K. pneumoniae* isolated from various clinical specimens in Duhok City (Iraq) in the present study.

In the present study, 130 *K. pneumoniae* isolates were collected from various clinical sample sources. Among the patients, *K. pneumoniae* infection was the predominant organism recovered from the urine samples (66.2%), followed by the blood samples (12.3%) and wound swabs (10%). Several studies in Iraq have reported that urine is a most common source of urinary tract infection (14, 16, 17). For instance, Riaz et al. (3) and Akter et al. (6) reported that urine is the principle source of *Klebsiella* species.

Regarding gender, the findings of the current research indicated that the frequency of the *K. pneumoniae* isolated from various clinical samples was higher in the female patients (76.2%) compared to the males (23.8%). In the case

of the urine, blood, wound, and oral samples, the female patients were observed to be more affected by *K. pneumoniae* compared to the males, while in the case of the CV line and sputum samples, the frequency of the isolates was higher in the males compared to the females. This is consistent with the studies reporting that *K. pneumoniae* was the more predominant pathogen isolated from female patients compared to male patients (8), while in contrast to a study conducted in Bangladesh, the results of which indicated that male patients were more susceptible to *Klebsiella* infection isolated from urine and wound samples compared to females (6). This discrepancy is rather difficult to explain and could be due to the variations in the sample collection, study design, sample population, inclusion criteria of patients, environmental factors, and personal hygiene.

K. pneumoniae was observed to be the most prevalent causative agent isolated from the female patients with urinary tract infection in the present study, which could be due to several factors, such as the shortness of the urethra and its proximity to the anus in women as a major cause of urinary tract infection. In addition, sexual activities could also increase the inoculation of the pathogen into the bladder in women (18). In a study conducted in Iran, *K. pneumoniae* was reported to be the most prevalent infection in female patients to cause urinary tract infection (4). The other studies conducted in Iran have also denoted that women tend to be affected more frequently by *K. pneumoniae* and experience urinary tract infection more commonly than men (2).

The effectiveness of currently available antibiotics is declining due to the increasing number of the resistant strains that cause infections (11). The available therapeutic options for antibiotic-resistant pathogens are severely limited as these pathogens frequently exhibit a multidrug-resistant phenotype (19, 20). In the present study, *K. pneumoniae* isolated from various clinical samples demonstrated variable degrees of sensitivity to the common antibiotics. In addition, *K. pneumoniae* isolated from the urine samples was highly resistant to ampicillin (97.7%), while highly effective against ertapenem (97.7%) and imipenem (96.5%).

According to a research performed in Iraq, 100% of isolated *Klebsiella* strains were resistant to meropenem and amikacin (17), which is in line with the results of another study in Bangladesh, showing that all *Klebsiella* isolates were highly resistant to ampicillin (100%) (10). In the same study, *Klebsiella* was reported to be moderately sensitive to ceftriaxone, ciprofloxacin, and gentamicin (10). However, another study in Pakistan indicated that *Klebsiella* isolates from urine samples were sensitive against gentamicin (21). The discrepancy between our findings and the

Table 2. Antibiotic Resistance Pattern of *K. pneumoniae* Strains Isolated From Clinical Samples^a

Antibiotic	Resistance of Isolates From Clinical Samples					
	Urine (N = 86)	Blood (N = 16)	Sputum (N = 9)	Wound Swab (N = 13)	CV Line (N = 3)	Oral Swab (N = 3)
Ampicillin	84 (97.7)	15 (93.8)	9 (100)	8 (92.3)	3 (100)	3 (100)
Amoxicillin/clavulanic acid	36 (41.9)	9 (56.3)	5 (55.6)	10 (76.9)	3 (100)	1 (33.3)
Piperacillin/tazobactam	20 (23.3)	8 (50)	5 (55.6)	7 (53.8)	3 (100)	0 (0)
Cefazolin	41 (47.7)	14 (87.5)	6 (66.7)	11 (84.6)	3 (100)	2 (66.7)
Cefoxitin	31 (36.1)	13 (81.3)	6 (66.7)	5 (38.5)	3 (100)	0 (0)
Ceftriaxone	54 (62.8)	13 (81.3)	6 (66.7)	8 (61.5)	3 (100)	1 (33.3)
Cefepime	52 (60.5)	13 (81.3)	4 (44.4)	6 (46.2)	3 (100)	1 (33.3)
Ertapenem	2 (2.3)	2 (12.5)	4 (44.4)	1 (7.7)	0 (0)	0 (0)
Imipenem	3 (3.5)	8 (50)	4 (44.4)	5 (38.5)	3 (100)	0 (0)
Gentamicin	12 (13.9)	13 (81.3)	2 (22.2)	6 (46.2)	3 (100)	0 (0)
Tobramycin	17 (19.8)	12 (75)	6 (66.7)	4 (30.8)	3 (100)	0 (0)
Ciprofloxacin	20 (23.3)	0 (0)	3 (33.3)	5 (38.5)	1 (33.3)	0 (0)
Levofloxacin	19 (22.1)	0 (0)	3 (33.3)	5 (38.5)	1 (33.3)	0 (0)
Nitrofurantoin	26 (30.2)	6 (37.5)	4 (44.4)	8 (61.5)	1 (33.3)	2 (66.7)

^aValues are expressed as No. (%).

Table 3. Overall Antimicrobial Susceptibility Pattern of *K. pneumoniae* Isolated From Various Clinical Specimens^a

Antibiotic	Susceptibility Patterns (N = 130)	
	Resistance	Sensitivity
Ampicillin	126 (96.9)	4 (3.1)
Amoxicillin/clavulanic acid	63 (48.5)	67 (51.5)
Piperacillin/tazobactam	43 (33.1)	87 (66.9)
Cefazolin	77 (59.2)	53 (40.8)
Cefoxitin	58 (44.6)	72 (55.4)
Ceftriaxone	85 (65.4)	45 (34.6)
Cefepime	79 (60.8)	51 (39.2)
Ertapenem	8 (6.2)	122 (93.8)
Imipenem	23 (17.7)	107 (82.3)
Gentamicin	34 (26.2)	96 (73.8)
Tobramycin	40 (30.8)	90 (69.2)
Ciprofloxacin	29 (22.3)	101 (77.7)
Levofloxacin	29 (22.3)	101 (77.7)
Nitrofurantoin	40 (30.8)	90 (69.2)

^aValues are expressed as No. (%).

mentioned studies regarding the higher sensitivity against gentamicin may be due to selective pressure in various regions.

According to the current research, *K. pneumoniae* iso-

lated from the blood samples was highly sensitive to levofloxacin (100%) and ciprofloxacin (100%). However, the *K. pneumoniae* isolates showed maximum resistance to ampicillin (93.8%) and cefazolin (87.5%), approximately 81% of which were also resistant to cefoxitin, ceftriaxone, cefepime, and gentamicin. This finding is comparable with the studies conducted in developing countries, reporting that *Klebsiella* isolates showed 100% resistance to ampicillin (22, 23), which could be due to the widespread use of this antibiotic owing to the low cost and easy administration.

In the present study, 100% of the isolated *K. pneumoniae* from the sputum samples were resistant to ampicillin, and approximately 78% of the isolates were sensitive to gentamicin. This is in line with the results of a study conducted in India, which indicated that 92% of the *Klebsiella* isolates from sputum showed resistance to ampicillin (8). However, this is inconsistent with the other studies that indicated that the majority of the strains isolated from sputum were sensitive to amikacin (24). In the current research, the sensitivity of the *K. pneumoniae* isolated from the wound swabs to ertapenem and levofloxacin was estimated at 87.5% and 75%, respectively, while 100% resistance was recorded to ampicillin. In addition, ertapenem (100%) was observed to be the most potential drug against the *K. pneumoniae* isolated from the digestive system, followed by imipenem, gentamicin, and tobramycin, while it was highly resistant to ampicillin, amoxicillin/clavulanic acid,

cefazolin and ceftriaxone. In a study conducted in Iraq, the *K. pneumoniae* bacterium was mostly sensitive to amikacin and imipenem, while mostly resistant to ceftriaxone and tetracycline (24).

Unfortunately, the strains isolated from the CV line in the present study showed high resistance rates to all the common antibiotics, with the exception of ertapenem, which could be dispensed without prescription. Therefore, patients and general public education are crucially required in this regard. Widespread, excessive dispensing and irresponsible use of antibiotics have led to the development of multi-resistant strains. On the other hand, 100% of the isolated *K. pneumoniae* strains from the oral swabs in the current research were highly sensitive to all the common antibiotics, with the exception of ampicillin. These antibiotics should be the preferred drugs for the treatment of the *K. pneumoniae* infections isolated from oral swabs. Penicillin resistance is due to the ability of *K. pneumoniae* to carry the plasmid-producing beta-lactamase variants (21), and beta-lactamase production is considered to be the most common mechanism of gram-negative pathogens (22).

In terms of the overall susceptibility pattern of the *K. pneumoniae* isolates of various clinical specimens, it was observed that the *K. pneumoniae* isolates were highly resistant to ampicillin, ceftriaxone, and cefepime, while the highest susceptibility rate was observed against ertapenem and imipenem. These antibiotics have been proposed as alternatives for the treatment of *K. pneumoniae* infections in the studied region, which is in contrast to another study conducted in Iraq, the results of which indicated that amikacin, ciprofloxacin, and gentamicin were the most effective antimicrobial agents against *K. pneumoniae* (23). According to another research, *K. pneumoniae* isolates obtained from clinical samples exhibited good sensitivity to amikacin and meropenem, while they were only moderately sensitive to levofloxacin, ceftazidime, and ciprofloxacin. Therefore, it could be inferred that the resistance rate of the isolates from various sample sources in the current research have numerous differences in comparison with the aforementioned studies in all the antibiotics, which could be due to the significant differences in the sample size, sampling methods, and geographical/environment variations. In addition, the high resistance rate to ampicillin, ceftriaxone, and cefepime antibiotics in the present study compared to other countries is rather alarming in terms of the spread of antibiotic resistance among the clinical isolates in our region. This shows the limited possibility of using these antibiotics in the empirical treatment of the patients with *K. pneumoniae* infections. Therefore, an urgent measure is required to control the threatening development of such resistance in the

country.

The main limitation of the present study was the small sample size (n=130). In addition, the study was mainly conducted in Duhok City, and the patients aged less than 10 years were not evaluated. Therefore, further investigations are required in this regard using advanced molecular tools to diagnose and evaluate the sensitivity of bacteria in order to overcome these limitations and obtained more accurate results.

5.1. Conclusions

According to the results, *K. pneumoniae* infection was the predominant bacteria isolated from the urine samples in the female patients. Ertapenem and imipenem were observed to be the most effective antibiotics against the *K. pneumoniae* isolates from various clinical samples. However, the isolates were extremely resistant to ampicillin, ceftriaxone, and cefepime. The emergence of resistant bacteria to various antibiotics has urged the periodic assessment of empirical, first-line antibiotic treatments at a regional level. Our findings could help physicians and clinicians to select appropriate antimicrobial therapies in this regard.

Footnotes

Authors' Contribution: We confirm that the manuscript has been contributed, reviewed and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of Interests: There is no conflicts of interest associated with this publication.

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