

Incidence and Antibiotic Sensitivity of *Klebsiella pneumoniae* Isolated from Urinary Tract Infection Patients in Zakho Emergency Hospital/Iraq

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Abstract:

Uropathogenic *Klebsiella pneumoniae* is considered the most important causes of urinary tract infection, and recently the antibiotic resistance of this pathogen has increased dramatically. The study aims at investigating the prevalence and antibiotic sensitivity of uropathogenic *K. pneumoniae* among different ages of groups at both genders. 1737 urine specimens were collected from individuals admitted to Zakho emergency hospital from January 2016 until December 2018. *K. pneumoniae* was identified based on Gram stain, colony characteristics and biochemical tests, and then it was tested for their antibiotic sensitivity. Out of all samples, 1076 (61.9%) showed significant bacterial yield. The UTIs among females (64.3%) were statistically higher than males (46.5%) ($P < 0.001$). The age group of 20-29 years was highly susceptible to UTIs (43%). Among the total UTIs, *K. pneumoniae* represented 16.2%. *K. pneumoniae* in males (23.4%) was significantly higher than females (15.4%) ($P=0.03$). The age group 40-49 years (32.2%) was the highest group that at risk for *K. pneumoniae* infection. *K. pneumoniae* was highly susceptible to imipenem (100%) and was highly resistant against cephalothin (90.8%). A high prevalence of UTIs were observed among the age group of 20-29 years and the females had more risk of having UTIs. The males were more susceptible to *K. pneumoniae* with higher prevalence in the age group of 40-49 years. *K. pneumoniae* showed high sensitivity toward imipenem and high resistance to cephalothin. More awareness is required in all healthcare sectors for regular surveillance of the use of antimicrobial agents to reduce the resistance rates and to eradicate the pathogens.

Keywords: Uropathogens, *K. pneumoniae*, Antibiotic resistance, Zakho

مدى الإصابة والحساسية للمضادات الحيوية لجراثومة الكليبسيلا الرئوية المعزولة من المرضى المصابين بأمراض الجهاز البولي في مستشفى طوارئ زاخو / العراق

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الخلاصة

يعتبر التهاب المجارى البولية بالكليبسيلا الرئوية السبب الرئيسي لعدوى المسالك البولية، وقد شهد مؤخراً زيادة كبيرة في مقاومتها للمضادات الحيوية. كان الهدف من الدراسة هو التحقق من مدى انتشار الكليبسيلا الرئوية المسببة لالتهاب المجارى البولية ومدى حساسيتها للمضادات الحيوية بين مختلف الفئات العمرية لدى كلا الجنسين. تم جمع 1737 عينة ادرار من الأفراد الذين تم إدخالهم إلى مستشفى طوارئ زاخو من كانون الثاني 2016 حتى كانون الاول 2018. تم تشخيص الكليبسيلا الرئوية بناءً على صبغة غرام، وخصائص المستعمرة واختبارات البيوكيميائية ثم تم اختبارها للتأكد من حساسيتها للمضادات الحيوية. من بين جميع العينات، أظهرت 1076 (61.9%) نمو بكتيري كثيف. كانت عدوى المسالك البولية بين الإناث (64.3%) أعلى إحصائياً من الذكور (46.5%) ($P < 0.001$). كانت الفئة العمرية 20-29 سنة الأكثر عرضة للإصابة بعدوى المسالك البولية (43%). من بين إجمالى عدوى المسالك البولية، مثلت الكليبسيلا الرئوية 16.2%. الكليبسيلا الرئوية كانت معنوياً أعلى لدى الذكور (23.4%) من الإناث (15.4%) ($P = 0.03$). كانت الفئة العمرية 40-49 سنة (32.2%) أعلى فئة معرضة لخطر الإصابة بالكليبسيلا الرئوية. كانت الكليبسيلا الرئوية أكثر حساسية تجاه المضاد الحيوي Imipenem (100%) وظهرت مقاومة عالية ضد المضاد الحيوي cephalothin (90.8%). لوحظ ارتفاع معدل انتشار عدوى المسالك البولية بين الفئة العمرية 20-29 سنة وكانت الإناث أكثر عرضة لخطر عدوى المسالك البولية. كان الذكور أكثر عرضة للإصابة بالكليبسيلا الرئوية مع انتشار أعلى في الفئة العمرية 40-49 سنة. أظهرت الكليبسيلا الرئوية حساسية عالية تجاه Imipenem ومقاومة عالية تجاه cephalothin. مطلوب المزيد من الوعي في جميع قطاعات الرعاية الصحية للمراقبة المنتظمة لاستخدام مضادات الميكروبات من أجل تقليل معدلات المقاومة والقضاء على مسببات الأمراض.

الكلمات المفتاحية: التهاب المجارى البولية، الكليبسيلا الرئوية، مقاومة المضادات الحيوية، زاخو

1. Introduction

Urinary tract infections (UTIs) can be considered as the most serious and occurring types of bacterial infections in the world [1]. In spite of being treatable, UTIs are now becoming increasingly hard to control because of the extensive antimicrobial resistance among uropathogens especially those belong to *Enterobacteriaceae* family [2-4]. *Klebsiella pneumoniae* is a prominent opportunistic pathogen belong to *Enterobacteriaceae* family; it causes upper respiratory tract infection, diarrhea, pneumonia, urinary tract infection (UTI), and septicemia [5]. As a general rule, *K. pneumoniae* is

problematic and responsible for severe infection mostly in people with a weakened immune system; the increasing spread of the strains with high virulent lead to an increasing number of individuals susceptible to infections such as healthy people [6]. Indeed, this pathogen is considered, with *E. coli*, as the main cause of UTIs. Though, in many reported cases, it was obvious that the pathogenicity of Klebsiella UTI was higher than *E. coli* [7]. *K. pneumoniae* pathogenicity is attributed to several virulence factors [8]. Over the past several decades, the antibiotic resistance of *K. pneumoniae* has increased due to its ability to acquire some more genetic characters to convert to more virulent or decrease its sensitivity to antibiotics [9]. Recently, the complete genomic analysis revealed that most of the strains of *K. pneumoniae* have genes, on plasmids, that are the code for antimicrobial resistance and virulence factors. This leads to a decrease in the effectiveness of these agents toward these bacteria [10]. This study conducted to isolate and investigate the antibiotic resistance of *K. pneumoniae* isolates from urine samples over a 3-year period (2016-2018) at the Zakho emergency hospital, Zakho, Kurdistan Region, Iraq.

2. Patients and Methods

2.1 Specimen collection

The cross-sectional study was performed from January 2016 until the end of December 2018 in the Zakho emergency hospital/Iraq. The urine specimens collected from 1737 patients (230 males and 1507 females) with clinical symptoms of UTI and sent to the microbiology laboratory. The patients with positive samples were detected; some information related to these patients, such as sex, age, was obtained.

2.2 Isolation, identification, and antibiotic sensitivity test

Urine specimens were directly inoculated aseptically on MacConkey and Brain heart infusion agar (BHI) plates and incubated for overnight at 37°C. In the next step, the isolated bacteria were identified by Gram staining of bacterial colonies, colony character and biochemical tests, based on standard procedures and international guidelines, which were urease, catalase, oxidase, and IMViC tests (which is the abbreviation for the Indole, Methyl-red, Voges-Proskauer and Simon's Citrate test) [11, 12]. Susceptibility to a range of antibiotics (Table 1) that were available and routinely used in hospital, was evaluated utilizing the method of disk diffusion according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) 2015 [13].

Table 1: Antimicrobials utilized in the current study

Antimicrobial group	Antimicrobials (Abbreviation)/(Disk content)
Beta-Lactams	Penicillins Ampicillin (AM) (10 µg) Amoxicillin + Clavulanic acid (AMC) (20/10 µg) Amoxicillin (AMX) (20 µg)
	Cephalosporins Cefixime (CFM) (5 µg) Cephalothin (KF) (30 µg) Ceftriaxone (CRO) (30 µg) Cefotaxime (CTX) (30 µg)
Tetracycline	Doxycycline (DOX) (30 µg) Tetracycline (TE) (30 µg)
Nitrofurans	Nitrofurantoin (NIT) (300 µg)
Quinolone	Nalidixic acid (NA) (30 µg)
Fluoroquinolone	Ciprofloxacin (CIP) (5 µg) Norfloxacin (NOR) (10 µg)
	Imipenem (IMP) (10 µg) Meropenem (MEM) (10 µg)
Carbapenems	
Macrolides	Azithromycin (AZM) (15 µg)
Aminoglycoside	Amikacin (AK) (30 µg) Gentamicin (CN) (10 µg)
	Trimethoprim-Sulfamethoxazole (SXT) (1.25/ 23.75 µg)
Sulfonamides	
Lincosamides	Clindamycin (DA) (2 µg)

2.3 Ethics Statement

The patients recruited were notified and the permission for the research design and methodology was approved by the scientific committee of the department of basic sciences, Hawler Medical University/ College of Dentistry, Kurdistan, Iraq.

2.4 Data Analysis

SPSS 16 software was used to the statistical analysis of all data. The differences were considered significant at P value <0.05 .

3. Results

The study showed that between 2016-2018, a total of 1737 patients recruited. Out of all the recruited patients, only 1076 (61.9%) samples showed significant bacterial yield. The total prevalence of UTIs among females population was 64.3% (969/1507) and in the males population, it was 46.5% (107/230). The UTIs in females were significantly higher than males ($P < 0.001$). The highest susceptible age group of UTIs individuals irrespective of gender was found to be in the age range 20-29 years (43%) followed by age range 30-39 years (30%), and then between 40-49 years (14%), while the least susceptible age group of UTIs was in the 1-9 years (2%).

Among the total patients, the *K. pneumoniae* represented 16.17% (174/1076) of all the urinary bacteria isolated. Among the male patients through 3 years, the *K. pneumoniae* rate was 23.4% (25/107). While among females, the percentage of *K. pneumoniae* isolates was 15.4% (149/969) (Table 2). In the males, the UTI caused by *K. pneumoniae* was higher and statistically significant compared to the females ($P=0.03$). During the three years of study, the highest rate of *K. pneumoniae* was recorded in 2016, but the difference was statistically not significant compared to other years.

Table 2: Distribution of uropathogenic *K. pneumoniae* according to the gender during 2016-2018.

Gender	2016 N (%)	2017 N (%)	2018 N (%)	Total N (%)
Male	9/42 (21.4)	12/29 (41.4)	4/36 (11.1)	25/107 (23.4)
Female	53/321 (16.5)	46/353 (13)	50/295 (16.9)	149/969 (15.4)
Total	62/363 (17.1)	58/382 (15.2)	54/331 (16.3)	174/1076 (16.2)

The highest percentage of *K. pneumoniae* isolates were from the individuals in the age range 40-49 years (32.2%) followed by age range 30-39 years (25.9%), and then between 20-29 years (14.9%), while the least susceptible age group of UTIs was in the 1-9 years (2.9%) (Table 3).

Table 3: Distribution of uropathogenic *K. pneumoniae* according to age groups during 2016-2018

Age group (Years)	2016 No (%)	2017 No (%)	2018 No (%)	3 years No (%)
1-9	3 (4.8)	1 (1.7)	1 (1.9)	5 (2.9)
10-19	7 (11.3)	11 (18.9)	2 (3.7)	20 (11.5)
20-29	7 (11.3)	15 (25.8)	4 (7.4)	26 (14.9)
30-39	18 (29)	14 (24.1)	13 (24)	45 (25.9)
40-49	20 (32.3)	10 (17.2)	26 (48.1)	56 (32.2)
50-59	6 (9.6)	3 (5.2)	7 (13)	16 (9.19)
≥60	1 (1.6)	4 (6.9)	1 (1.9)	6 (3.4)
Total	62	58	54	174

All the 174 *K. pneumoniae* isolates in this study were screened for their antimicrobial resistance toward different classes of antibiotics. The sensitivity of *K. pneumoniae* was 100% toward imipenem & meropenem, 63.2% to nitrofurantoin, 56.9% to ciprofloxacin and 54.6% to doxycycline. While the other tested antibiotics showed less than 50%. On the other hand, the *K. pneumoniae* isolates showed high resistance against cephalothin (90.8%), ampicillin (83.3%), cefixime (82.2%), azithromycin (81.6%), amoxicillin and nalidixic acid (78.2%) (Table 4).

Table 4: Susceptibility of the *K. pneumoniae* isolates against different antibiotics during 2016-2018

Antibiotic agents (Disk content)	Total isolates (n= 174)	
	Resistant No. (%)	Sensitive No. (%)
AM (10 µg)	145 (83.3)	29 (16.7)
AMC (20/10 µg)	68 (39)	106 (61)
AMX (20 µg)	136 (78.2)	38 (21.8)
CFM (5 µg)	143 (82.2)	31 (17.8)
KF (30 µg)	158 (90.8)	16 (9.2)
CRO (30 µg)	88 (50.6)	86 (49.4)
CTX (30 µg)	104 (59.8)	70 (40.2)
DOX (30 µg)	79 (45.4)	95 (54.6)
TE (30 µg)	88 (50.6)	86 (49.4)
NIT (300 µg)	64 (36.8)	110 (63.2)
NA (30 µg)	136 (78.2)	38 (21.8)
CIP (5 µg)	75 (43.1)	99 (56.9)
NOR (10 µg)	88 (50.6)	86 (49.4)
IMP (10 µg)	0 (0)	174 (100)
MEM (10 µg)	0 (0)	174 (100)
AZM (15 µg)	142 (81.6)	32 (18.4)
AK (30 µg)	97 (55.7)	77 (44.3)
CN (10 µg)	92 (52.9)	82 (47.1)
SXT (1.25/ 23.75 µg)	93 (53.4)	81 (46.6)
DA (2 µg)	101 (58)	73 (42)

4. Discussion

Urinary tract infection caused by bacteria is one of the most common infections in human beings. In the current study, 61.9% of the urine specimens were positive for bacterial growth. Females showed a significant rate (64.3%) of UTIs compared to males (46.5%). Several studies, locally and worldwide, showed similar results [14, 15]. Although this infection affects both genders, women are especially more prone to developing UTI compared to the men this may be due to their anatomy and reproductive physiology that allows bacterial quick access to the bladder [16]. One potential reason that could enable the auto-transmission and increasing the rate of UTIs in women could be related to the closeness of the genital tract and the urethra and anus [17]. The highest rate of UTIs was observed among people with age group 20-29 years (43%) and 30-39 years (30%). This might be due to the UTIs prevalence that increases with catheterization, sexual intercourse and hormonal changes such as menopause [18].

Indeed, *K. pneumoniae* comes after *E. coli* as the main cause of UTIs. Here, the *K. pneumoniae* rate among the UTIs found to be 16.2%. Studies revealed varied rates for *K. pneumoniae* such as 8.8% in Italy [16], 17.1% in Egypt [19], 23.5% in Saudi Arabia [20]. There are different pathogens associated with UTI, but the pathogenicity of *K. pneumoniae* was the highest. *K. pneumoniae* pathogenicity is attributed to several virulence factors that give bacteria the ability to invade the host and prevent bacteria phagocytosis as well as forming a biofilm [21].

The present study demonstrates that *K. pneumoniae* was prevalent among 23.4% of men and it was 15.4% among women. These results come in accordance to a study reported by Varughese and Beniwal [22], in India, who found that uropathogenic *K. pneumoniae* in males (31.6%) was more dominant than females (7.3%). Also, it was reported by Sattar *et al.*, [23], in Pakistan, that men were more susceptible to uropathogenic *K. pneumoniae*. Additionally, Lee *et al.*, [24], Korea, demonstrated that the rate of *K. pneumoniae* in UTIs was present in 7.7% of males and 5.3 in females. However, other studies revealed that female gender was more infected with uropathogenic *Klebsiella spp* than males [16, 25].

This study revealed that patients within the age range of 40-49 years have the highest prevalence rate with *K. pneumoniae* infection of 32.2%, followed by 30-39 years (25.9%), 20-29 years (14.9%), while below 10 years of age showed the lowest (2.9%). This result agrees with a study by Nirwati *et al.*, [26] and Yang and Zhang [27] who revealed that most *K. pneumoniae* were abundant among people in the age group of 18-65 and 40-65 years old respectively. Yang and Zhang [27] found that the age group above 70 years was with a high rate of this pathogen. It was also found that the maximum isolates were isolated from the age group of above 50 years (85.33%) followed by age group of age 21-50 years, no *K. Pneumoniae* was isolated from the age group of 0-5 years shown in [28]. On the other hand, Magliano *et al.*, [16] revealed no significant differences in isolation rates within the age groups. The age-related differences in people could be due to the response of the immune system. Adult patients (under 40 years) are likely to have stronger immune systems. Also, elderly people with an increased incidence of comorbid illness will be at higher risk of *K. pneumoniae* infection [29].

The increases of multidrug-resistant (MDR) pathogens have endangered the antibiotics treatment effectiveness [30-32]. *K. pneumoniae* is gaining renewed interest because of the emergence of MDR among *Klebsiella* associated UTI infections [33]. To guide empirical and pathogen-specific treatment for any region, it is important to know about the patterns of local antimicrobial susceptibility [34, 35]. In the present study, the pattern of antibiotic sensitivity of *K. pneumoniae* revealed that all isolates were multidrug-resistant to at least some commonly prescribed antibiotics. The *K. pneumoniae* susceptibility was 100% (174/174) to imipenem and meropenem which both belonged to carbapenem group of antibiotics making them optimal options for the empiric treatment of UTIs. Researchers found 100% susceptibility to carbapenems (imipenem and meropenem) (34). However, the overuse of these drugs will inevitably lead to selection pressure [36]. For the nitrofurantoin group, nitrofurantoin also found to be effective against *K. pneumoniae* isolates with the efficacy of 63.2% (110/174).

A comparable result was shown in several numbers of other studies in terms of resistance trends of *Klebsiella species* in urinary isolates. *K. pneumoniae* isolates showed varied susceptibility toward the penicillin group, for example, both ampicillin and amoxicillin showed a high resistance rate (83.3% and 78.2% respectively) for *K. pneumoniae*; while the combinations of amoxicillin + clavulanic acid remarkably increased the susceptibility of the isolated bacteria. Although amoxicillin alone is a broad spectrum and semisynthetic antibiotic, producing of the *beta*-lactamase and confirming the natural resistance to aminopenicillins by *K. pneumoniae* renders ineffective for treatment. On the other hand, clavulanic acid is effective against the clinically important plasmid-mediated β -lactamase; so the formulation of amoxicillin and clavulanic acid have the characteristic of β -lactamase inhibitor and broad-spectrum antibiotic.

The current study revealed that *K. pneumoniae* demonstrated an increased rate of resistance to first generation cephalosporins including cefixime 82.2% (143/174) and cephalothin 90.8% (158/174). This result was consistent with the data previously reported by [37] on UTIs. This could be explained by the most misuse of this antibiotic class and also by the fact that, with time, some strains have developed enzyme enabling them to resist to cephalosporins.

On the other hand, the susceptibility of the *K. pneumoniae* against third generation cephalosporin was found to be moderate with 49.4% (86/174) and 40.2% (70/174) for ceftriaxone and cefotaxime respectively. This result was similar to that reported by Moini and coworkers [38] who found that the resistance rate of the *K. pneumoniae* towards ampicillin and third-generation cephalosporins was 46.6%. The resistance against penicillins and cephalosporins (including third-generation cephalosporins) found to be mediated by Extended-spectrum β -lactamases (ESBL) [39]. Studies revealed variable rates of *K. pneumoniae* isolate that produces ESBL, and they have been found that higher rates were in the Arabian region. Two separate studies conducted by Huang *et al.*, [40] and Aljanaby and Alhasnawi [41] in Iraq showed that these proportions were 62.5% and 50%, respectively.

The antimicrobial susceptibility for *K. pneumoniae* in the current study was 21.8% and 56.9% for nalidixic acid and ciprofloxacin respectively. This variation might be explained by the fact that nalidixic acid can be considered as the first generation of quinolones and the overuse of these antibiotics in UTIs confer the resistance to bacteria [42]. Subsequently, ciprofloxacin was introduced into the clinical market as the second generation of quinolones after modifications in their molecular structures to improve their antimicrobial properties and pharmacokinetic profiles [43]. Expressing of *SHV* genes enables bacteria to resist different antimicrobial agents including aminoglycosides. In addition to that, the three antibiotics: Gentamicin, Norfloxacin, and Trimethoprim- Sulfamethoxazole which belong to three the different groups of antibiotic showed approximately similar rates of susceptibilities.

To resist the antibiotics, several mechanisms were observed in *K. pneumoniae* [44]. In general, the β -lactamases in the bacteria hydrolyze the antibiotic β -lactam ring [45]. The resistance to some β -lactams in *K. pneumoniae* species is intrinsic because some of the resistance genes exist in the chromosome. For instance, the *SHV* gene is regularly existed in the chromosome and conferring resistance against ampicillin [46, 47]. Other β -lactamases could be a part of the accessory genome [48]. In addition to chromosomal resistance, *K. pneumoniae* harbor both β -lactamase and Extended-spectrum β -lactamases (ESBL)-encoding plasmids that confer resistance to most penicillin antibiotics and third-generation cephalosporins respectively [49].

The hypervirulent *K. pneumoniae* is carbapenems-susceptible [50]. However, the carbapenem-resistant *K. pneumoniae* strains can convert to hypervirulent by getting a pLVPK-like virulence plasmid [51]. Carbapenem-resistant Hypervirulent *K. pneumoniae* decrease the choices of antimicrobial agents. For this reason, this considered an urgent challenge for public health [52]. Besides, carbapenem resistance in *K. pneumoniae* can also be mediated by the accessory genome. Also, carbapenem resistance in *K. pneumoniae* could be by the mutations in the chromosomal genome [53].

Conclusions

Conclusively, an overall prevalence of 61.9% UTIs was observed in the current study. Females had more risk of having UTIs than males. The UTIs in women were significantly higher than men. The UTIs were more dominant among the age group of 20-29 years patients. *K. pneumoniae* represented 16.2% of the isolated urinary bacteria. Males were more susceptible to uropathogenic *K. pneumoniae* than females. A high prevalence of *K. pneumoniae* was seen in the age group 40-49 years. The study revealed that the imipenem and meropenem as the most promising antimicrobial therapy for the treatment of UTI. *K. pneumoniae* isolates exhibited high resistance toward the most commonly used antibiotics. For this reason, clinicians and laboratories must be aware and more accurate testing is required to decrease the wrong therapy. Also, the current data shed light on the necessity for surveillance of microbial resistance for most of the uropathogenic bacteria to reduce the failure therapy and to enhance the methods of infection control and improve the guide of the use of antibiotics.

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