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Demographic Factors, Antibiotic Resistance, and Resistance Gene Prevalence in Klebsiella Pneumoniae-Associated Urinary Tract Infections

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Annotation: This study investigated the prevalence of Klebsiella pneumoniae in urinary tract infections, its associated antibiotic resistance patterns, and resistance genes in a specific population. Klebsiella. pneumoniae was found to be a significant pathogen, particularly among older adults (52.5% in the 50-69 years age group), females (64.4%), and individuals residing in rural areas 59.3%). The isolates exhibited high resistance to multiple antibiotics, including carbapenems, and notably, the highest resistance rate was Amikacin (84.7%), followed by Imipenem (79.7%), Ceftriaxone (76.3%), and Cefotaxime (74.6%). Moderate resistance was found against Meropenem (69.5%), Lincomycin (64.4%), Trimethoprim (57.6%), and Tobramycin (40.7%). The lowest resistance rates were seen against Chloramphenicol (35.6%), Erythromycin (30.5%), Oxacillin (25.4%), and Azithromycin (12%). Molecular analysis revealed the presence of resistance genes, such as bla-OXA-1 (72.9%), bla-CTX-M (49.2%), rmtC (54.2%), and lower prevalence was observed with armA (28.8%), contributing to the observed resistance. These findings highlight

the urgent need for effective strategies to combat the rising threat of antibiotic-resistant K. pneumoniae infections.

Keywords: Klebsiella pneumoniae, Antibiotic resistance, resistance genes, Urinary tract infections.

Introduction

The emergence of antibiotic resistance represents a critical challenge to global health, as multidrug-resistant (MDR) bacteria are responsible for severe infections and rising mortality rates. *Klebsiella pneumoniae*, a Gram-negative bacterium, is a prominent contributor to urinary tract infections (UTIs) and has acquired resistance to multiple antibiotics, including carbapenems, regarded as last-resort medications (Mancuso et al., 2021). The emergence of resistance genes, including *bla-CTM-MI*, *armA*, and *rmtC*, has played a significant role in disseminating MDR *K. pneumoniae* (Zhou et al., 2022). The genes in question are responsible for encoding enzymes that deactivate antibiotics, thereby making them ineffective. The *bla-CTM-MI* provides resistance against beta-lactam antibiotics, such as penicillins and cephalosporins (S. Zhang et al., 2024), *armA* and *rmtC* encode 16S rRNA methylases that modify the bacterial ribosome, thereby preventing the binding of aminoglycosides and conferring resistance to these antibiotics (Wachino et al., 2020). The rising occurrence of these resistance genes in *K. pneumoniae* obtained from UTIs is being observed globally, underscoring the critical necessity for robust surveillance and control strategies. Grasping the mechanisms of resistance and the propagation of these genes is essential for formulating innovative therapeutic approaches and curbing the further spread of MDR K. pneumoniae (Ferreira et al., 2019). *K. pneumoniae* represents a rising threat, contributing to considerable illness and death, especially in urinary tract infections. It is essential to comprehend the occurrence of *K. pneumoniae* UTIs across various demographic groups and to identify resistance profiles and genes to develop effective prevention and treatment strategies (Paczosa & Mecsas, 2016). This study examines the occurrence of *K. pneumoniae* UTIs within a defined population, emphasizing the impact of age, sex, and residency concerning antibiotic resistance and the prevalence of resistant genes. This information will enhance the understanding of the epidemiology and pathology of *K. pneumoniae* UTIs and guide targeted public health interventions.

Methodology

➢ *Population and Study Design*

This prospective cross-sectional study included 250 midstream urine samples from patients with urinary tract infections admitted to Baghdad's hospitals and healthcare centers. All demographic data (including age, sex, and residence) was documented directly from each patient. Regarding ethical approval, this work was carried out following the Helsinki Declaration on Human Rights and the instructions of the Iraqi Ministry of Health and the health departments in Baghdad, where verbal approval was obtained through direct interviews with each participant.

➢ *Urine Samples Processing*

All urine samples were collected using a sterile plastic container, examined under a Gram stain microscope, and cultured on blood agar and MacConkey agar under sterile environmental conditions. Diagnosis and analysis of antibiotic resistance profiles were done using the VITEK-2 compact system. After that, all cultures positive for K. pneumonia were preserved using brain-heart infusion broth media.

➢ *Molecular Aspects*

DNA extraction was performed using a DNA extraction kit (Geneaid- Taiwan) following the manufacturers' instructions. Regarding the detection of the gene, the primer set used for *bla-OXA-1, bla-CTM-MI, armA,* and *rmtC* genes were as follows [**F**:" GGCACCAGATTCAACTTTCAAG" **R**: "GACCCCAAGTTTCCTGTAAGTG" (564bp)] [**F**: "TTAGGAAGTGTGCCGCTGTA." **R**: "CGGTTTTATCCCCCACAAC" (655bp)]. [**F**:" ATTCTGCCTATCCTAATTGG". **R**: "ACCTATACTTTATCGTCGTC" (315bp)]. [**F**: "CGAAGAAGTAACAGCCAAAG." **R**: "ATCCCAACATCTCTCCCACT" (711bp], respectively. Regarding the components and final polymerase chain reaction volume was 25 µL stratified as follows (bacterial DNA template (5µL), primers (2 µL), nuclease-free water (3.5µL), and master mix (12.5µL). The protocol of molecular gene detection is performed as follows: the initiation denaturation at 95℃ for 5 minutes in 1 cycle, followed by 94℃ (58, 59.7, and 59.8℃), and 72 ℃ for denaturation, annealing, and extension for 45 seconds, 90 seconds, and one minute, respectively, in 35 cycles, and 72℃ for seven minutes in 1 cycle for final extension for bla-OXA-1, *bla*-$_{CTX-M1}$, and *rmtC* genes, respectively. At the same time, the protocol was repeated except for annealing (58.5 ℃) and extension for 90 seconds regarding the *armA* gene. All gene bands were detected using 1.5 g agarose gel after adding 3 µL of diamond nucleic acid dye, 5 µL of PCR products, and a DNA ladder for 1.5 hours under 90 volts.

➢ *Statistical analysis*

All data was statistically analyzed using SPSS for Windows version 22 "(SPSS Inc. Chicago, Illinois, United States)". The categorical data were portrayed through the utilization of frequency and percentage. The chi-square test was used to compare the percentages.

Results and Discussion

➢ *Bacterial Prevalence*

A total of 250 midstream urine samples were cultured. Bacterial growth was detected in 177 (70.8%) samples. The most prevalent bacterial isolate was *Escherichia coli* (41.8%), followed by *Klebsiella pneumoniae* (33.3%), *Proteus mirabilis* (8.5%), *Staphylococcus epidermidis* (6.8%), *Pseudomonas aeruginosa* (6.2%), and *Staphylococcus aureus* (3.4%) **(Table 1).** Gram-negative bacteria represent the leading cause of urinary tract infections, with E. coli identified as the primary pathogen, followed by *K. pneumonia* and *P. mirabilis* in secondary and tertiary positions, respectively (Al Yousef et al., 2016). Other Gram-positive bacteria, including Staphylococcus species, have the potential to lead to urinary tract infections, as supported by multiple studies (Gupta et al., 2011:Al Yousef et al., 2016:Regmi et al., 2018). *K. pneumoniae* represents a bacterial species capable of inducing a range of infections, such as urinary tract infections, and is frequently encountered in diverse environmental settings, including soil, water, and vegetation. It is also present in the human gut, which typically poses no harm. Nonetheless, *K. pneumoniae* has the potential to lead to infection upon entering the urinary tract (Abbas et al., 2024). The increased occurrence of *K. pneumonia* in urinary tract infections can be linked to various factors, including notable virulence traits that lead to infection, such as capsules that shield it from the host's immune response and adhesion proteins that enable the bacteria to adhere to the urinary tract (Paczosa & Mecsas, 2016).

Table (1) Prevalence of Bacterial Pathogens in Clinical Urine Specimens

Bacterial Pathogens species	Count	(%)
Escherichia coli	74	41.8
Proteus mirabilis	15	8.5
Klebsiella pneumonia	59	33.33
Staphylococcus epidermidis	12	6.78
Pseudomonas aeruginosa	11	6.21
Staphylococcus aureus	6	3.38
Total positive urine sample	177	100.00

➢ *Demographic and K. pneumonia Distribution*

This study showed that the age group 50-69 years was associated with higher bacterial infection 31 (52.5%) than age groups 30-49 years 18 (30.5%) and age group 18-29 years 10 (17%) **(Table 2)**. The findings suggest that *K. pneumoniae* is associated with a greater prevalence of UTIs in adults than other age groups, aligning with numerous studies (Miftode et al., 2021:Polse et al., 2020). In this regard, individuals of advanced age experience a decline in their immune system strength, rendering them more vulnerable to infections (Santoro et al., 2021). Furthermore, individuals link alterations in urinary tract function to aging, complicating bladder emptying, which may result in urine retention and foster conditions conducive to bacterial growth and proliferation. Additionally, older individuals tend to possess significant experience with antibiotic resistance and comorbidities compared to younger populations and other age demographics (Rowe & Juthani-Mehta, 2013).

Furthermore, this study found that the highest bacterial infection was in the female group, 38 (64.4%), compared to the male group, 21 (35.6%), consistent with (PAUL & TANIMU, 2024) **(Table 2)**. Various anatomical and behavioral variables lead to increased UTIs caused by *K. pneumoniae* in females relative to males. These factors include the shorter urethra and its proximity to the anus, sexual activity, hygiene practices, the use of spermicides, and hormonal fluctuations (Behzadi et al., 2010). Moreover, the population from rural regions exhibits more significant bacterial infection 35 (59.3%) than urban 24 (40.7%), in line with a previous Iraqi study (Alfetlawi & Jasim, 2022) **(Table 2)**. The greater incidence of UTIs caused by *K. pneumoniae* in rural regions compared to urban areas can be attributed to several factors, including inadequate sanitation and hygiene, excessive use of antibiotics, poverty, and insufficient education.

Table (2) Demographic Correlates of *Klebsiella pneumoniae* Infection

Age group (year)	**Frequency**	**(%)**
18-29	10	17
30-49	18	30.5
50-69	31	52.5
Sex group	**Frequency**	**(%)**
Male	21	35.6
Female	38	64.4
Residence	**Frequency**	**(%)**
Rural	35	59.3
Urban	24	40.7

➢ *Antibiotic Resistance Profiles*

Table (3) shows the resistance patterns of *K. pneumoniae* bacteria to various antibiotics. The highest resistance rates were observed against Amikacin (84.7%), followed by Imipenem (79.7%), Ceftriaxone (76.3%), and Cefotaxime (74.6%). Moderate resistance was found against Meropenem (69.5%), Lincomycin (64.4%), Trimethoprim (57.6%), and Tobramycin (40.7%). The lowest resistance rates were seen against Chloramphenicol (35.6%), Erythromycin (30.5%), Oxacillin (25.4%), and Azithromycin (12%). These findings indicated that K. pneumonia isolates predominantly exhibit different resistance rates against several antibiotic types supported by previous studies (Lin et al., 2022:Zhang et al., 2022). *K. pneumoniae* is a bacterium that exhibits a growing antibiotic resistance, presenting a considerable challenge due to its potential to cause severe infections. *K. pneumoniae* is increasingly exhibiting resistance to antibiotics for a variety of reasons. One reason is that bacteria are frequently present in hospitals, where they may encounter elevated levels of antibiotics, resulting in antibiotic-resistant strains. Growing resistance can also be attributed to the excessive use of antibiotics (Ndlovu et al., 2023). It is crucial to recognize that the resistance rates of *K. pneumoniae* may differ based on geographical location and the particular strain of the bacteria. It is essential to recognize that these resistance rates stem from laboratory testing, indicating that the efficacy of these antibiotics in addressing *K. pneumoniae* infections could vary.

Table (3) Prevalence of Antibiotic Resistance Among *Klebsiella pneumoniae* Isolate

Antibiotic profile	Sensitive isolates	%	Resistant isolates	%
Chloramphenicol	38	64.4	21	35.6
Ceftriaxone	14	23.7	45	76.3
Lincomycin	21	35.6	38	64.4
Erythromycin	41	69.5	18	30.5
Tobramycin	35	59.3	24	40.7
Amikacin	9	15.3	50	84.7
Oxacillin	44	74.6	15	25.4
Imipenem	12	20.3	47	79.7
Cefotaxime	15	25.4	44	74.6
Trimethoprim	25	42.4	34	57.6
Azithromycin	46	88	13	12
Meropenem	18	30.5	41	69.5

- ***Genes Prevalence***

A diverse array of gram-negative organisms has been documented to possess copies of the *blaOXA-1* gene in both plasmid and integron sites. A study has revealed that this gene is frequently associated with other genes responsible for coding extended-spectrum β–lactamases (Madhan Sugumar et al., 2014). The *bla-OXA-1* genes were transferred to *K. pneumoniae* through horizontal gene transfer (Mairi et al., 2018). The findings of this study indicate that *bla-OXA-1* was the most prevalent gene, occurring at a rate of 72.9% **(Table 4).** These findings are lower than that reported by (Ferreira et al., 2019) and higher than (Ogutu et al., 2015). The findings indicated that isolates of *K. pneumoniae* carrying *bla-OXA-1* exhibited significant resistance to cephalosporins. This resistance was linked to the strong binding ability of *blaOXA-1* to ceftazidime, leading to the cleavage of the antibiotic; this suggests that *blaOXA-1* is particularly effective in cleaving various β-lactam antimicrobials (Sugumar et al., 2014). Simultaneously, the prevalence of the *bla-CTX-M* gene was observed to be 49.2%, consistent with the findings presented in reference (Park et al., 2019) **(Table 4)**. Prior studies suggest that the uptake of transposons and plasmids carrying *blaCTX-M*-type ESBLs led to the dominance of *bla-CTX-M*-producing isolates, resulting in a shift in the types of ESBLs found in *K. pneumoniae*, which in turn triggered hospital epidemics. The widespread distribution of CTX-M enzymes leads to two or more -lactamases within the identical isolates. This potential is emerging as a common strategy bacterium employ to enhance drug resistance (Calbo & Garau, 2015).

The prevalence of the *rmtC* gene was 54.2% among bacterial isolates in this study **(Table 4),** which is lower than (Ahmadian Alashti & Ghane, 2020) and in agreement with (Ashrafian et al., 2015). The *armA* gene encodes enzymes that prevent aminoglycosides from binding to their 16S rRNA target, belonging to the 16S rRNA methylase gene family, carried in plasmids in *K. pneumonia* (Doi & Arakawa, 2007). Additionally, the gene with the lowest prevalence in this study was *armA* at 28.8% **(Table 4),** which is higher than (Wu et al., 2009) and lower than (Ashrafian et al., 2015). Research revealed that *K. pneumoniae* strains capable of capsule production possess a plasmid containing the *armA* gene, which imparts resistance to most aminoglycosides and is predominantly found in Asia (Saadatian Farivar et al., 2018). The variations in gene prevalence observed across various studies could be ascribed to the inherent genetic diversity in different countries, methodological errors, and optimization.

Table (4) Prevalence of Resistance Genes in *Klebsiella pneumoniae*

Resistance genes	Positive		Negative	
	Count	%	Count	%
bla-oxa-1	43	72.9	16	27.1
RmtC	32	54.2	27	45.8

ArmA	17	28.8	42	71.2
bla-ctx-m	29	49.2	30	50.8

Conclusion

This research examined the incidence of *K. pneumoniae*, in clinical urine samples and their related demographic characteristics and drug resistance profiles. The results indicated a notable predominance of *K. pneumoniae* among the bacterial isolates, underscoring its status as a primary causative agent of urinary tract infections. The demographic study revealed that older age cohorts and females were more susceptible to *K. pneumoniae* infections. Furthermore, persons living in rural regions had a greater incidence of these illnesses than those in urban areas. These data highlight the significance of focused preventative strategies and prompt diagnosis in these at-risk groups. A troubling trend has arisen in the antibiotic resistance profiles of *K. pneumoniae* isolates. Elevated resistance levels were detected against many antibiotic classes, including aminoglycosides, cephalosporins, and carbapenems. Resistance genes, including *bla-OXA-1, bla-CTX-M, rmtC,* and *armA,* further substantiated the rising antibiotic resistance in *K. pneumoniae* strains. These results underscore the pressing want for efficacious solutions to address antibiotic resistance and avert the spread of multidrug-resistant *K. pneumoniae*. Rigorous compliance with antibiotic stewardship protocols, timely identification, and the creation of innovative treatment strategies are crucial to alleviating the public health risk presented by this opportunistic infection.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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