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Prevalence and Risk Factors of Klebsiella pneumoniae Infection Among Urine of Patients Attending Federal Teaching Hospital Katsina, Katsina State

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Abstract

Urinary tract infection (UTI) is a common infectious disease worldwide that affects people of all genders and ages, with women being more frequently affected due to anatomical and physiological factors. Escherichia coli is the most prevalent causative agent of UTIs, followed by Klebsiella pneumoniae, both of which belong to the family Enterobacteriaceae. While K. pneumoniae is less commonly isolated than E. coli, it is often associated with higher levels of antibiotic resistance, which can make treatment more difficult and outcomes potentially more severe in healthcare-associated infections. This study aimed to determine the prevalence and risk factors of Klebsiella pneumoniae in the urine of patients attending Federal Teaching Hospital Katsina, Katsina State, Nigeria. Mid-stream urine samples were collected from 200 patients. Structured questionnaires were administered to gather some socio-demographic and risk factors. The samples were inoculated onto sterile plates of MacConkey agar, incubated at 37 ˚C for 24 hours, and lactose-fermenting mucoid colonies were suspected to be K. pneumoniae. Gram staining, biochemical tests, and molecular characterisation identified pure isolates. In this study, sixteen (16) isolates, representing a prevalence of 8.0%, were presumptively identified as Klebsiella pneumoniae, while polymerase chain reaction (PCR) confirmed eleven (11) of these isolates, resulting in a confirmed prevalence of 5.5%. Socio-demographic factor that was significantly associated with the prevalence of urinary Klebsiella pneumoniae infection among the patients was the female gender. However, none of the risk factors considered in this study were significantly associated with the prevalence of infection among the study population.

Keywords: UTI, K. pneumoniae, PCR, Prevalence, Risk factors

INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated bacterium that functions as a facultative anaerobe (Martins *et al.*, 2023). It belongs to the family Enterobacteriaceae *K. pneumoniae*, often known as Friedlander's bacillus, it was initially identified by Friedlander in 1882 from the pulmonary tissue of a patient who died of pneumonia (Wyres *et al.*, 2024). Gastrointestinal colonisation often precedes *K. pneumoniae* infections, and the gastrointestinal system is considered the primary reservoir for bacterial transmission (Tian *et al.*, 2024). *Klebsiella pneumoniae* exhibits several virulence characteristics, including a large polysaccharide capsule found in most clinical isolates, several adhesions, lipopolysaccharide (LPS), and iron-scavenging proteins (Khan *et al.*, 2023).

Klebsiella pneumoniae is an opportunistic microorganism which causes serious disease such as septicemia, pneumonia, urinary tract infection (UTI), chronic lung disorders and nosocomial infection in immunocompromised patients (Russo *et al.*, 2023) *Klebsiella pneumoniae* has emerged as a significant pathogen in hospitals causing infections that are acquired during hospital stays and are associated with outbreaks affecting 20% of the patient s respiratory tracts being the most commonly affected. The main factors contributing to its pathogenicity are capsular polysaccharide (CPS), lipopolysaccharide (LPS), and fimbriae (Russo *et al.*, 2019).

Urinary tract infections (UTIs) are common bacterial infections that affect a large number of individuals worldwide (Mancuso *et al.*, 2023). The increasing incidence and antibiotic resistance of *K. pneumoniae* have made it a significant concern in the context of urinary tract infections (UTIs). *Klebsiella pneumoniae* is a Gram-negative bacterium that possesses the capacity to inhibit and proliferate in the urinary system, resulting in various infections, ranging from uncomplicated bladder irritation to

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pyelonephritis (Pitout *et al.*, 2015). *Klebsiella pneumoniae*, belonging to the family Enterobacteriaceae, is a natural inhabitant of the gastrointestinal tract of healthy humans and animals (Martin and Michael 2018). The worldwide occurrence of *K. pneumoniae* in urinary tract infections (UTIs) has been on the rise, leading to a substantial burden on healthcare systems and highlighting the need for the implementation of efficient management strategies (Trecarichi *et al.*, 2022). Furthermore, the appearance of *K. pneumoniae* strains that are resistant to many drugs has made the treatment of UTIs more difficult, highlighting the need for understanding the patterns of antibiotic resistance in this disease (Beyene *et al.*, 2023). The objective of this study is to assess socio-demographic and risk factors associated with *K. pneumoniae*-induced urinary tract infections (UTIs) among patients at the Federal Teaching Hospital, Katsina.

MATERIALS AND METHODS

Study Area

This study was conducted at Federal Teaching Hospital Katsina, located in Katsina State, Nigeria. Katsina is a Local Government Area (LGA) established during Nigeria's 1976 administrative reforms (National Bureau of Statistics, 2023; Katsina State Government, 2021).

The study population consisted of patients who presented with symptoms of urinary tract infection attending the Federal Teaching Hospital, Katsina State, Nigeria. The study was a hospital-based cross-sectional study. All patients referred to the Microbiology Laboratory at Federal Teaching Hospital Katsina with suspected UTIs and who consented to the study were included. Patients who did not present with any symptoms of UTIs or those with symptoms but did not consent to the study were excluded. Ethical approval was sought from the Management of Federal Teaching Hospital Katsina, Katsina State, Nigeria. Consent of each patient was sought before enrollment into the study.

The sample size of the study was determined by using the formula below and a prevalence of 14.78% (Abdelfattai *et al.*, 2023).

$N = [Z^2pq]/e^2$

where:
n = Number of samples
p = Prevalence rate
Z = Standard normal deviate at 95% confidence interval P =14.78%, = 0.147
q = 1-0.147 = 0.853
e = Allowable error for 5% (0.05)
$n = [1.962\times 0.147\times 0.853] / 0.05^2$
$n = [3.8416\times 0.147\times 0.853] / 0.0025$
= 191

However, 200 samples were collected for quality and precision.

Questionnaire Administration

A structured questionnaire was administered to consenting patients participating in the study. The questionnaire was designed to capture data on socio-demography, risk factors, and symptoms of UTIs associated with *K. pneumoniae* infection.

Samples collection

Ten millilitres of urine were collected from each participant using sterile containers and a convenient sampling technique (Adeyemi *et al.*, 2021). Samples collected were processed following standard procedures at the Federal Teaching Hospital, Katsina, for the isolation of *K. pneumoniae*.

Urine Culture

Urine samples were cultured onto MacConkey Agar and incubated at 37°C overnight. Mucoid, lactose-fermenting colonies were sub-cultured to obtain pure isolates, which were then inoculated into slant bottles, incubated for 24 hours, and stored at 4°C for preservation (Brown & Smith, 2023).

Gram staining

A thin smear was prepared from a pure bacterial isolate using a wire loop and normal saline on a clean glass slide. After air-drying and heat-fixing, the smear underwent Gram staining: it was stained with crystal violet, treated with Gram's iodine, decolourized with 95% alcohol, and counterstained with safranin. The slide was then air-dried and examined under a microscope using the oil immersion objective (100×) (Brown & Smith, 2023).

Biochemical Characterization of *Klebsiella pneumoniae*

Indole test

The test organism was grown in 5 mL Peptone water and incubated at 37°C for 24 hours. After

incubation, 0.5 mL of Kovac's reagent was added and was gently shaken. A red colour formation in the agent layer above the broth within 1minute indicates a positive test, while the absence of the red layer indicates a negative test (Wright *et al.*, 2017).

Methyl red

The isolates were inoculated in MR-VP broth at 37°C for 48 hours. After incubation, the broth was split into two portions for the Methyl Red (MR) and Voges-Proskauer (VP) tests. Two drops of methyl red indicator were added to one portion; a red color indicated a positive result, while a yellow color indicated a negative result (Bindu and Krishnaiah, 2010).

Voges-Proskauertests

The Voges-Proskauer (VP) test was performed by adding 0.6 mL of 5% α-naphthol and 0.2 mL of 40% KOH to the second half of the MR-VP broth, followed by vigorous shaking. A red color within 15 minutes indicated a positive result, while no color change signified a negative result (Bindu and Krishnaiah, 2010).

Citrate utilization test

Each isolate was first streaked on Nutrient Agar and incubated at 37°C for 24 hours. A colony was then transferred to Simmon Citrate medium and incubated under the same conditions. A color change from green to blue indicated a positive result (citrate utilization), while no color change indicated a negative result (MacWilliams, 2015).

Molecular detection of *Klebsiella pneumoniae*

Molecular detection of *Klebsiella pneumoniae* was done by conventional PCR using *K. pneumoniae* specific primers (Amani *et al.*, 2016). Bacterial DNA was amplified in a 20 µL reaction mix containing Taq buffer, dNTPs, primers (249bp), and lysate, using a thermal cycler (25 cycles: 94°C denaturation, 58°C annealing, 60°C extension). Products were electrophoresed, stained with ethidium bromide, and visualized under UV (Yusuf *et al.*, 2018).

Extraction of genomic DNA using the Conventional Method

Extraction of DNA from *K. pneumoniae* A single colony was suspended in 200 µL distilled water, colony was emulsified to create a homogenous suspension heat-lysed at 100°C for 10 min, cooled on ice, and centrifuged (10,000 rpm, 10 min) The resulting supernatant, containing crude genomic DNA, was carefully collected and used as the template for downstream PCR applications (Brown and Smith, 2023; Khan *et al.*, 2023; Munoz-Price *et al.*, 2023).

Agarose Gel Electrophoresis of PCR Products

DNA fragments and their primers were analyzed using agarose gel electrophoresis (1.5%). Ethidium bromide (0.5 µg/mL) was used to stain the DNA for visualization under UV light. The gel was prepared with TAE buffer, and DNA samples were loaded into wells. Electrophoresis was run at 80–150 V for 1 hour. Afterward, the gel was viewed under UV light, and a DNA ladder in the first lane served as a reference for interpreting the bands (Magdeldin, 2012).

Primer sequences for the detection of *Klebsiella pneumoniae*

Gene	Primers Sequence	Amplicon Size	Reference
yhal	F- ATTTGAGCGGCTGGAAAGAG R- AGCGGCCGATATCATGCAT	249bp	Poirier *et al.*, 2022

Statistical Analysis

Odds ratio (OR) analysis was conducted to assess the association between *Klebsiella pneumoniae* infection and various socio-demographic and risk factors using IBM SPSS Version 24. The final results were presented in simplified form using tables and charts.

RESULTS

Out of the 200 urine samples analyzed, eleven (11) isolates were confirmed as *Klebsiella pneumoniae* by molecular technique (PCR), indicating a prevalence of 5.5%, as shown in Figure 1 and Plate 1.

The study demonstrated in Table 1, a notably higher prevalence of *Klebsiella pneumoniae* infection among female patients (7.8%) compared to their male counterparts (1.4%), despite males showing a greater likelihood of infection (OR = 6.017). The highest infection rate was recorded among participants with informal education (7.3%), though the variations across educational levels were not statistically

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significant. In terms of age distribution, individuals aged 32-45 years had the highest prevalence (8.0%), followed by those aged 18-31 years (7.1%) and 46-59 years (2.9%).

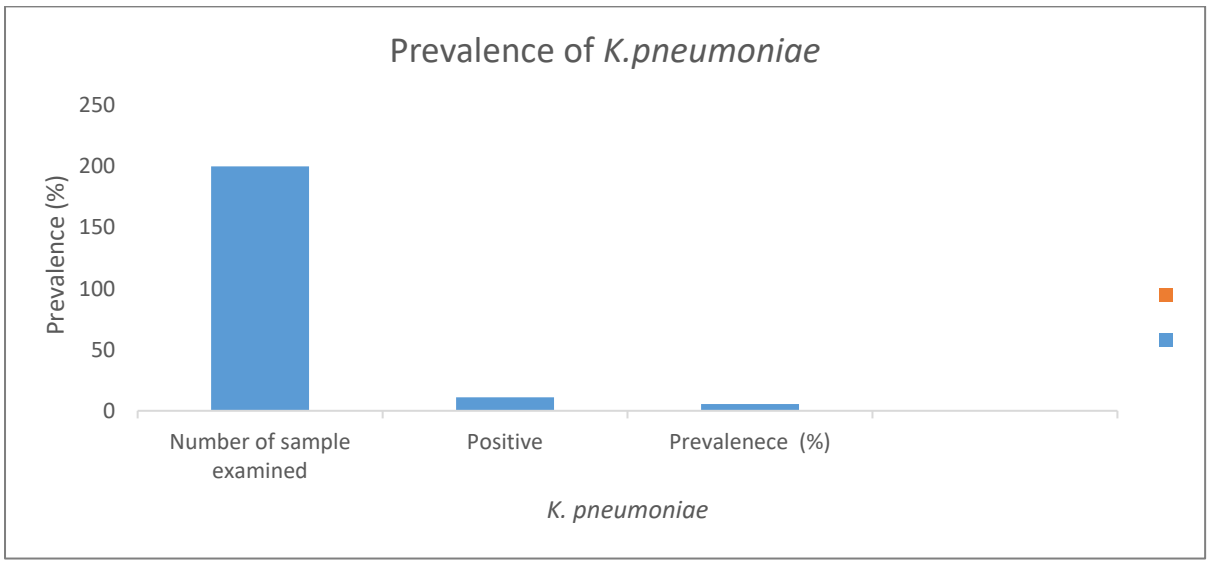

Figure 1: Prevalence of *K. pneumoniae*

Plate I: Amplified fragment of *yhaI of Klebsiella pneumoniae*
Expected amplicon size (249bp)
M = Molecular marker size of (100bp)
NC = Nuclease-free water as negative control
Lane:- 2, 3, 4, 5, 6, 7, 8, 12, 13, 15, and 16: Positive
Lane: - 1, 9, 10, 11, and 14 : Negative

The study found higher occurrences of *Klebsiella pneumoniae* infection among diabetic patients (6.7%) compared to non-diabetics (5.4%), and among those with prolonged antibiotic use (7.7%) versus those without (2.4%). Pregnant patients (9.1%) and those with a history of catheter use (11.1%) also showed higher infection rates compared to their counterparts. However, none of these factors—diabetes, prolonged antibiotic use, pregnancy, or

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catheterization were statistically significant risk factors for infection, as all had $p > 0.05$ and Odds Ratios (OR<1) Table 2.

DISCUSSION

The study recorded a 5.5% prevalence of *Klebsiella pneumoniae*-associated urinary tract infections (UTIs). This relatively low prevalence may be attributed to effective antibiotic stewardship programs that limit the spread of resistant strains, as well as strong host immune responses that help eliminate the infection. (Bolton *et al.*, 2019). Compared to this study's findings, higher prevalence rates of *Klebsiella pneumoniae* infection have been reported in other parts of Nigeria. A similar study conducted in Kano State by Hamza and Abdulhadi (2016) recorded a 7.0% prevalence.

Table 1: Socio-demographic Distribution of *Klebsiella pneumoniae* in Urine of Patients Attending Federal Teaching Hospital Katsina

Demographic factors	Number examined	Number Positive (%)	x^2	Df	P- value
Gender					
Female	128	10 (7.8)			
Male	72	1 (1.4)	3.658	1	0.050
Level of education					
Informal	82	6 (7.3)			
Primary	5	0 (0.0)			
Secondary	66	4 (6.1)			
Tertiary	47	1 (2.1)	1.880	3	0.598
Age group (years)					
4 - 17	15	0(0)			
18 - 31	42	3(7.1)			
32 - 45	75	6(8.0)			
46 - 59	68	2(2.9)	2.850	3	0.415

[a]OR = 0.166 (0.021 - 1.326); [b]OR = 6.017

Table 2: Risk Factors of urinary *Klebsiella pneumoniae* infection among patients attending Federal Teaching Hospital Katsina

Risk Factors	Number Examined	Number Positive (%)	Odd-ratio	x^2	df	p-value
Diabetes						
No	185	10 (5.4)	1.250	0.042	1	0.837
Yes	15	1 (6.7)	0.800			
Prolonged antibiotic use						
No	83	2 (2.4)	3.375	2.607	1	0.106
Yes	117	9 (7.7)	0.296			
Pregnancy						
No	156	7 (4.5)	2.129	1.388	1	0.237
Yes	44	4 (9.1)	0.470			
History of Catheterization						
No	182	9 (4.9)	2.403	1.198	1	0.274
Yes	18	2 (11.1	0.416			

The prevalence in this study is consistent with the one carried out in Jimma University Specialist Hospital, Jimma in Ethiopia, which reported a prevalence of 5.3% (Ragas *et al.*, 2015). A higher prevalence of 18.2% was also reported in different research studies performed in Anambra, Nigeria (Ogbuka *et al.*, 2016). A study carried out in the Federal Capital Territory in Abuja showed a prevalence of 21.0% (Ajobiew *et al.*, 2020). Also, Abdulfatai *et al.* (2023) conducted research in Kaduna and reported a prevalence of 14.78%. Differences in *Klebsiella pneumoniae* prevalence across studies may be due to varying environmental conditions and risk factors specific to each region where the research was conducted. This study found a higher prevalence of *Klebsiella pneumoniae* infection in females (7.8%) compared to males (1.4%), aligning with findings by Hamza and Abdulhadi (2016), but contrasting with Chang-Phone *et al.* (2012), who reported a higher prevalence in males (57.6%).

The increased prevalence in females is commonly linked to anatomical factors, such as a shorter urethra and closer proximity of the

urethral opening to the anus, which facilitate microbial colonization. The study observed a higher prevalence of *Klebsiella pneumoniae* infection among individuals with informal education, followed by those with secondary and tertiary education, while no cases were found among those with primary education. This suggests that educational background may influence awareness and adoption of protective measures, with individuals having formal education more likely to practice effective UTI prevention. The study recorded the highest prevalence of *Klebsiella pneumoniae* infection among individuals aged 32–45 years, consistent with findings by Aneke *et al.* (2022). This was followed by those aged 18–31 years and then by patients aged 46–59 years. No infections were observed in the youngest group (4–17 years). The results support existing evidence that UTIs are more common among young and middle-aged adults, as also noted by Akoachere *et al.* (2012). However, Loveth *et al.* (2023) reported a peak prevalence in the 21–30 age group, highlighting some variation across studies. *Klebsiella pneumoniae* is increasingly recognized as a common cause of urinary tract infections (UTIs), particularly among hospitalized patients with a history of urinary catheter use in Nigeria. Catheter-associated urinary tract infections (CAUTIs) remain a significant issue in Nigerian healthcare facilities, largely due to the widespread use of indwelling catheters in surgical wards, intensive care units, and long-term care settings (Onuoha and Fatokun, 2014). Research from various regions of Nigeria has consistently shown a high prevalence of *Klebsiella pneumoniae* among catheterized patients. In a study conducted in Southwestern Nigeria, Oluwasemowo *et al.* (2023) identified *K. pneumoniae* as the second most common isolate, exhibiting significant resistance to commonly used antibiotics. The high rates of colonization and infection have been linked to prolonged catheter use and inadequate adherence to aseptic techniques (Iregbu *et al.*, 2013). Similarly, a study in Jos, North Central Nigeria, reported that *K. pneumoniae* made up over 20% of isolates from catheterized individuals (Aliyu *et al.*, 2017).

Recent research in Nigeria underscores the prominent role of *Klebsiella pneumoniae* as a uropathogen among diabetic patients. In a study conducted at a university medical center in Awka, Anambra State, *K. pneumoniae* was isolated in 11.4% of diabetic UTI cases, compared to 10.71% in non-diabetics. Overall, diabetics had a higher UTI prevalence (61.1%) than non-diabetics (38.9%), suggesting diabetes is a notable risk factor for UTIs (Ezebialu *et al.*, 2024). Similarly, a study in Enugu reported *Klebsiella* species accounted for 16.9% of uropathogens in diabetic patients, significantly exceeding the 6% found in non-diabetics (Okwume *et al.*, 2021*)*. Overall, the evidence highlights *Klebsiella pneumoniae* as a major uropathogen among diabetic patients in Nigeria. Its increased prevalence is likely linked to diabetes-related factors such as weakened immune function, glycosuria, and other complications that heighten susceptibility to infection (Okwume *et al.*, 2021). Additionally, frequent use of broad-spectrum antibiotics in the country has contributed to the emergence of multidrug-resistant (MDR) *K. pneumoniae* strains, which are more challenging to treat and often associated with recurrent or complicated urinary tract infections.

Recent studies in Nigeria have established a strong link between prior antibiotic use and the emergence of multidrug-resistant (*MDR*) *Klebsiella pneumoniae* in UTI patients. Ashefo *et al.* (2023) found that *K. pneumoniae* isolates were highly resistant to beta-lactam antibiotics and frequently carried extended-spectrum beta-lactamase (ESBL) genes, commonly associated with previous antibiotic exposure. Similarly, Ayeni *et al.* (2024) in Keffi reported that most isolates came from patients with recent antibiotic use and showed resistance to multiple drug classes, including cephalosporins, fluoroquinolones, and aminoglycosides. These findings underscore the impact of antibiotic overuse in driving resistance. The World Health Organization (WHO) has also identified antibiotic misuse - especially common in low- and middle-income countries like Nigeria, where antibiotics are often obtained without prescriptions—as a major contributor to antimicrobial resistance. Studies, including Nwobodo *et al.* (2025), highlight the urgent need for improved antimicrobial stewardship across Nigerian healthcare settings.

Pregnant women were found to have a higher rate of *Klebsiella pneumoniae* infections compared to non-pregnant individuals. This increased susceptibility is likely due to reduced immune function during pregnancy, along with a combination of immunological, physiological, and hormonal changes (Samuel *et al.*, 2016; Bishop and Shehu, 2016). Anatomical factors, such as the shorter female urethra and the potential for fecal contamination from improper hygiene, further elevate the risk of UTIs in women. During pregnancy, additional factors - such as haemodilution, urinary stasis, decreased

urine concentration, and glycosuria - create a favorable environment for bacterial colonization in the urinary tract (Smaill and Vazquez, 2023; Almehda *et al.*, 2017).

CONCLUSION

The prevalence of *K. pneumoniae* in the Urine of patients attending Federal Teaching Hospital Katsina, Nigeria was 5.5%, A socio-demographic factor that was significantly associated with urinary *Klebsiella pneumoniae* infection among the patients was the female gender (P < 0.05). However, none of the risk factors considered in this study were significantly associated with the prevalence of *K. pneumoniae* infection among the study population

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