



Isolation of Antibiotic Resistant *Klebsiella pneumoniae* from Students with Urinary Infections at a Tertiary Institution, Southwest Nigeria

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ABSTRACT

The antibiotic resistance of urinary pathogens has been varying over the period of years, in healthcare-associated infections. The study provided the current isolation of antibiotic resistant *Klebsiella pneumoniae* from students with UTI at a Tertiary Institution, Southwest Nigeria. In the study, 200 clean midstream urine samples were collected and studied using standard microbiological techniques. Information for the factors associated with UTIs was obtained using questionnaires. An agar disc diffusion technique was used to test for antibiotic susceptibility. The chi-square test was used to express associations among descriptive variables of UTI. The study revealed that *Klebsiella pneumoniae* strains showed a low prevalence of UTI with 47(23.5%). The study showed females are more susceptible to UTIs than males. The age-wise distribution shows that the incidence is more common in age bracket 21 to 30 years. The *Klebsiella pneumoniae* strains were most susceptible to imipenem 40(85.1%), meropenem 33(70.2%), ceftazidime 32(68.1%), and cefexime 25(53.2%) as compare to amoxicillin-clavulanate 43(91.5%), ampiclox 30(68.8%), cotrimoxazole 37(78.7%) and tetracycline 24(51.1%) with high resistance rate. *K. pneumoniae* shown resistance to 9 different antibiotics with multiple antibiotic-resistance index of 0.6. Appropriate diagnosis and management of UTI are aimed at treating the acute occurrence as well as preventing recurrences of this infection.

Keywords: Urinary tract infection (UTI); *Klebsiella pneumoniae*; Isolation; Antibiotic-Resistant; Student

INTRODUCTION

UTIs involve bacterial invasion and increase of the pathogen in the organs of the urinary tract system. The *Klebsiella pneumoniae* has also been recognized for its ability to initiate and cause a wide array of diseases in humans (Fatima *et al.*, 2021). The ability of organism to cause diseases has also been attributed to a wide range of virulence factors present in the organism (Choby *et al.*, 2020). Nosocomial *Klebsiella* infections are commonly involve in the urinary and respiratory tracts.

Since these two body sites differ considerably concerning the host defense mechanisms, it should be predicted that the pattern of virulence factors found in UTI-causing strains of *Klebsiella* will differ from that observed in strains isolated from pulmonary sources of patients with pneumonia (Abayneh *et al.*, 2018). Some *Klebsiella sp* have become highly resistant to antibiotics. *Klebsiella pneumoniae* carbapenemase (KPC) are enzyme that degrades penicillin, cephalosporin and broad-spectrum beta-lactams such as the carbapenems. Antibiotic resistance is a disturbing occurrence in the

today world, and the widespread menace necessitates applying antibiotic control and management strategies in the health-care centers (Ozgen and Eyupoglu, 2020).

Klebsiella pneumoniae is an important bacterium that causes serious infections in humans, and its symptoms differ depending on the body part affected by the bacteria. Over the previous years, *Klebsiella* with multiple antibiotic resistances is responsible for clinical-acquired and community-acquired infections (Azimi *et al.*, 2019), has developed to public health risk and continues to cause multi-drug resistance (MDR) infections round the globe. However, the pathogen is known as an "urgent threat to human health" (Bengoechea and Sa Pessoa, 2019).

Klebsiella beta-lactamases are typically prone to carbapenems, such as imipenem, and to beta-lactamase inhibitors, such as clavulanic acid, sulbactam and tazobactam (Jasim *et al.*, 2020). The rise of antibiotic resistant *K. pneumoniae* to commonly used antimicrobial agents is rapidly increasing. However, surveillance studies of bacterial resistance are among the important measures in controlling the spread of these resistant organisms in communities. Hence, the study investigated the antibiotic susceptibility profile of *Klebsiella pneumoniae* from students with Urinary infections at a Tertiary Institution, Southwest Nigeria.

MATERIALS AND METHODS

Study Design

In the cross-sectional study performed from April to August 2023, to determine the occurrence of antimicrobial-resistant *Klebsiella pneumoniae* isolated from students with Urinary infections at a Tertiary Institution, Southwest Nigeria. Standardized questionnaires were collected for relevant

information such as their age, sex, level of education and medical data.

Sample Collection

Two hundred midstream urine samples were collected into a clean universal bottles and sent to the research laboratory for analysis. The Federal University Oye-Ekiti (FUOYE) were the Institutions used in this study. Students who gave their informed consent were used and students without given consent were not included in the study.

Isolation and Identification of Bacterial Isolates

A loopful of the mid-stream urine sample were picked and streaked in Cystine Lactose Electrolyte Deficient Agar (CLED), incubated at 37°C in aerobic condition for 24 hours in confirmation for significant bacterial number. The urinary tract infection was defined as positive urine culture with the significant growth of $\geq 10^5$ CFU/ml or more (Bilsen *et al.*, 2023). After incubation, the cultures were sub-cultured on MacConkey agar for isolation. *K. pneumoniae* isolates were identified with the use of microscopic appearance, gram reaction, and biochemical testing as previously described by Cheesborough (2009).

Antimicrobial Susceptibility Testing

The tests were carried out using the Kirby Bauer disk diffusion technique as described by (Anosike *et al.*, 2020). The bacteria were cultured overnight on sterile Mueller Hinton agar plates. Few colonies were inoculated onto sterile normal saline adjusted to match the 0.5 McFarland turbidity standards, with the help of a sterile swab stick, test strains were streaked as a lawn on the MHA. The desired antibiotic was aseptically pressed gently down on the surface of the media, ensuring proper lap with the media. The plates were incubated at 37 °C for 24 hours. The

zones of inhibition were then measured and recorded (CLSI, 2020).

The antimicrobial agents tested were: cefexime (5µg), ceftazidime (30µg), cefotaxime (25µg), imipenem (10µg), ofloxacin (5µg), tetracycline (30µg), amikacin (30µg), Levofloxacin (5µg), ciprofloxacin (5µg), ampiclox (µg), amoxicillin clavulanate (20/10µg), nitrofurantoin (300ug), meropenem (10ug), cotrimoxazole (µg) and gentamycin (10ug) (Oxoid, England). Multidrug resistant bacteria were analyzed according to Jalil and Naji Al-Atebee (2012). The multiple antibiotic resistance index were calculated according to the method described by Osundiya *et al.*, (2013).

Data Analysis

Data from the findings were evaluated using SPSS software version 20. Descriptive statistics were used and the Chi-squared test with 95% level of confidence.

RESULTS

From 200 urine samples analyzed, only 47(23.5%) samples were identified as *Klebsiella pneumoniae*. Identification of *Klebsiella pneumoniae* by cultural and morphological characteristics appeared mucoid lactose fermenter with pink colour on MaConkey agar. Biochemically, only those isolates that expressed positive reactions to citrate utilization, voges-proskauer, catalase, urease and negative reactions to H₂S, oxidase and indole test were considered authentic isolates of *Klebsiella pneumoniae* for this work. The presumptive isolates that react to the biochemical test were stored at -70°C in 40% glycerol broth medium for further analysis.

The distribution of the incidence according to gender showed that out of 78 samples collected from male, 14 were positive with a prevalence of 17.9% (figure 1). Similarly, the 122 samples collected from the female, 33 were positive with a prevalence of 27.0%. Male= 78 (n= 14; 17.9%), Female =122 (n= 33; 27%).

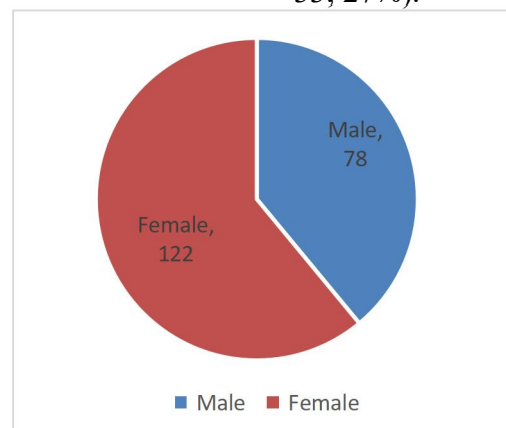


Figure 1: The prevalence Of UTI among the Gender

There was an increase in the number of *K. pneumoniae* strains across the age groups (Table 1). In the age bracket of 11 – 20 years, 12 were positive with a prevalence of 25.5% (n = 76). The age bracket of 21 – 30 years, the

prevalence was 66.0% as 31 were positive (n = 109). The age group >31- 40 years, the prevalence was 4.5% as 4 were positive among the 13 samples that were collected.

Table 1: The prevalence of UTI among the age groups

AGE GROUPS	Number of the samples (%)	Positive UTI (%)
11-20	76(38.0%)	12(25.5%)
21-30	109(54.5%)	31(66.0%)
31-40	13(6.5%)	4(4.5%)
Total	200(100%)	47(100%)

Table 2 shows risk factors of the respondents to *K. pneumoniae*. The result showed that those with personal hygiene were 183(91.5%). Those with regular sexual activity were 48(24.0%). The respondents with the history

of UTI were 21(10.5%) while those with catheter usage were 7(3.5%). The least samples were seen in those with diabetes were 1(0.5%) and those that had surgery were 1(0.5%).

Table 2: Risk factors associated with UTI

Medical History	Yes	No
History of UTI	21(10.5%)	179(89.5%)
Catheter Usage	7(3.5%)	193(96.5%)
Undergone Surgery	1(0.5%)	199(99.5%)
Suffered From Kidney Disease/ Diabetes	1(0.5%)	99(49.5%)
Personal hygiene	183(91.5%)	17(8.5%)
Regular sexual activity	48(24.0%)	152(76.0%)

In Table 3, *Klebsiella pneumoniae* isolates were most resistant to 43(91.5%) amoxicillin-clavulanate, 37(78.7%) to cotrimoxazole and 30(63.8%) to ampiclox as against having maximum sensitivity to impenem 40(85.1%), meropenem 33(70.2%), ceftazidime 32(68.1%)

and cefexime 25(53.2%). *Klebsiella pneumoniae* strains recorded high resistance to penicillin class of antibiotic followed by macrolides, tetracycline, cotrimoxazole, fluoroquinolones, cephalosporin and carbapenem in descending order.

Table 3: Antibiotic resistant and susceptibility profile of the *Klebsiella pneumoniae* isolates

Antibiotics	Types of Antibiotic	Resistant (%) n= 47	Intermediate (%) n= 47	Sensitive (%) n= 47
Carbapenem	Meropenem	10(21.3)	4(8.5)	33(70.2)
	Imipenem	4(8.5)	3(6.4)	40 (85.1)
Cephalosporin	Cefexime	3(6.4)	19 (40.4)	25(53.2)
	Ceftazidime	3(6.4)	12 (25.5)	32(68.1)
	Cefotaxime	10(21.3)	17 (36.2)	20(42.6)
Fluoroquinolones	Ofloxacin	12(25.5)	23(48.9)	12(25.5)
	Ciprofloxacin	17(36.2)	22(46.8)	8(17.0)
	Levofloxacin	22(46.8)	6(12.8)	19(40.2)
	Amikacin	15(31.9)	9(19.1)	23(48.9)
Aminoglycoside	Gentamycin	17(36.2)	10(21.3)	20(46.6)
Penicillin	Amoxicillin clavulanate	43(91.5)	0(0)	4(8.5)
	Ampiclox	30(63.8)	11(23.4)	6(12.8)
Tetracycline	Tetracycline	24(51.1)	10(21.3)	13(27.7)
Macrolide	Nitrofurantoin	19(40.2)	9(19.1)	19(40.4)
Cotrimoxazole	Cotrimoxazole	37(78.7)	3(6.4)	7(14.9)

Three isolates (6.4%) were found to be resistant to 2 and 3 antibiotics while twelve

isolates (25.5%) were resistant to 4 antibiotics. Eight isolates (17.0%) are resistant to five

antibiotics while nine isolates (19.1%) showed resistant to six antibiotics. Four out of 47 isolates (10.6%) were resistant to 8

antibiotics and One isolates were resistant to 9 antibiotics with the highest MARI index of 0.6 (Table 4).

Table 4: Multiple resistance pattern of *K. pneumoniae* isolates

Number of Antibiotic	Resistance Pattern	Number of Isolates n= 47 (%)	MARI			
Two	Tet, Acx	3(6.4)	0.13			
	Acx, Cot					
Three	Tet, Gen, Acx	3(6.4)	0.20			
	Cip, Acx, Cot					
	Cip, Aug, Amk					
Four	Cpz, Aug, Acx, Cot	12(25.5)	0.26			
	Tet, Cip, Nf, Cot					
	Cip, Ofx, Lbc, Cot					
	Gen, Acx, Lbc, Cot					
	Mem, Tet, Acx, Lbc					
	Cip, Nf, Acx, Amk					
	Tet, Gen, Ofx, Lbc					
	Mem, Tet, Acx, Cot					
	Mem, Gen, Nf, Cot					
	Cip, Aug, Acx, Cot					
	Mem, Ctx, Acx, Cot					
	Tet, Gen, Amk, Cot					
	Five			Tet, Ofx, Aug, Lbc, Cot	8(17.0)	0.33
				Tet, Aug, Ctx, Lbc, Cot		
Zem, Aug, Nf, Acx, Cot						
Ofx, Aug, Acx, Lbc, Amk						
Tet, Gen, Nf, Acx, Cot						
Mem, Gen, Cip, Acx, Cot						
Six	Tet, Cip, Ofx, Nf, Lbc	9(19.1)	0.40			
	Tet, Zem, Aug, Acx, Lbc, Amk					
	Mem, Cip, Aug, Nf, Lbc, Cot					
	Imp, Nf, Ctx, Acx, Lbc, Cot					
	Mem, Tet, Aug, Nf, Acx, Cot					
	Gen, Ctx, Acx, Lbc, Amk, Cot					
	Tet, Cip, Aug, Cpz, Lbc, Cot					
	Gen, Ofx, Aug, Ctx, Acx, Cot					
	Tet, Aug, Nf, Ctx, Acx, Lbc					
	Tet, Ofx, Aug, Acx, Amk, Cot					
Seven	Tet, Cip, Ofx, Ctx, Acx, Lbc, Cot	7(14.9)	0.46			
	Tet, Gen, Ofx, Imp, Aug, Lbc, Cot					
	Gen, Ofx, Aug, Acx, Lbc, Amk, Cot					
	Gen, Zem, Ofx, Aug, Nf, Acx, Cot					
	Tet, Aug, Nf, Acx, Lbc, Amk, Cot					
	Gen, Cip, Imp, Aug, Nf, Lbc, Cot					
	Tet, Cip, Nf, Ctx, Acx, Amk, Cot					
	Eight			Mem, Tet, Cip, Ofx, Aug, Nf, Acx, Amk	4(10.6)	0.53
Tet, Gen, Cip, Aug, Acx, Lbc, Amk, Cot						
Mem, Tet, Cip, Nf, Acx, Lbc, Amk, Cot						
Zem, Cip, Cpz, Nf, Aug, Acx, Amk, Cot						
Nine	Mem, Tet, Gen, Aug, Nf, Ctx, Acx, Amk, Cot	1(2.1)	0.6			

Mem-Meropenem, Gen-Gentamycin, Tet-Tetracyclin, Zem-Cefexime, Cip-Ciprofloxacin, Ofx-Ofloxacin, Imp-Imipenem, Aug-Amoxicillin-Clavulanate, Cpz-Ceftazidime, Nf-Nitrofurantoin, Ctx-Cefotaxime, Acx-Ampiclox, Lbc-Levofloxacin, Amk-Amikacin, Cot-Cotrimoxazole.

DISCUSSION

This study revealed the prevalence of *Klebsiella pneumoniae* among the student with UTI was low 47(23.5%) which could be contributed to student hygiene practices in Federal University Oye- Ekiti. In a previous findings conducted among students in Federal Polytechnic, Bida, Niger State *Klebsiella pneumoniae* was found in 11(19.29%) of UTI linking the low rate to level of personal hygiene or state of toilet amenities in the hostels (Alfa *et al.*, 2022). Likewise, similar studies in laboratory presented a lower prevalence of 16.2%, 18.4%, in Gabon and Nigeria respectively (Ndzime *et al.*, 2021; EL-Mahmood, 2021). The study showed a low prevalence of UTI as a result of population disparity and significant changes in the sample sizes. It can be due to good community hygiene and great progresses in management of UTIs.

In this study, the majority of *K. pneumoniae* isolates were found to be from female students, with 61%. This is because of the closeness of anus to the warm urethral duct. Likewise, the urethral duct of the women is short, and this cuts the space moved by the bacteria to the bladder. The direction of cleaning of perineum area from front to back is poorly practiced among female students and could be responsible for high occurrence of *K. pneumoniae* strains in female urine sample. The previous studies also acknowledged high prevalence of *K. pneumoniae* in UTI in females compared with males (Jalil *et al.*, 2022; Anosike *et al.*, 2020; Islam *et al.*, 2022). The risk factors of UTI are enhanced by inadequate funds, and poor sanitation. The change in the vaginal microflora encourages

colonization with coliforms bacteria in the vagina.

The prevalence of *K. pneumoniae* UTI was high in the age bracket of ≤ 21 to 31 years (66.0%), unlike the study of Al-Bshabshe *et al.* (2020) who reported that 42% of *K. pneumoniae* infections were in patients >60 years old. This is possibly due to the weakening of the immune system with aging, which allows the bacteria to grow and cause infection. This present study is related to the study reported in Bida and Akure, (Alfa *et al.*, 2022; Erinle *et al.*, 2022). Sexual intercourse signaled UTI in most of sexually active teenagers and recurring infections are seen predominately in the young adult group. Poor menstrual hygiene and sexual hygiene contributed to UTI.

Regular sexual activity is one of the most common lifestyle risk factors for UTIs, among University students these days, particularly female students. Students with a family history of UTI had a risk of developing UTI compared to those without. Likewise, students with the poor personal hygiene attitude have a chance of developing UTI. People with a weak immune system are more vulnerable to *Klebsiella pneumoniae* other risk factors common at young adult ages such as urinary retention, bladder dysfunction and catheter usage contributed to urinary tract infections.

The results of this study also revealed the rise of antimicrobial resistance, where *K. pneumoniae* is resistant to the most commonly used antibiotics. Throughout the study period, amoxicillin-clavunate showed the highest resistance rate among the tested antibiotics, with a 91.5% follow by cotrimoxazole (78.7%) and ampiclox (63.8%). *K. pneumoniae* was

found to be less resistant for imipenem (85.1%), meropenem (70.2%), and ceftazidime (68.1%). Amoxicillin-clavunate is a combination penicillin-type antibiotic that is active against many bacteria and has been used to treat a wide variety of bacterial infections for a long time.

It is a less expensive drug and is being abused by young adult who can accessed the drug easily without prescription from various pharmaceutical stores in Nigeria. Students who had cough/catarrh were most users of antibiotics. As a result, the resistance of most bacteria to antibiotic developed and has been attributed to the production of beta-lactamases capable of degrading these antibiotics. This could explain why *K. pneumoniae* strains in this study (91.5%) were resistant to amoxicillin-clavunate.

Cotrimoxazole is another combination of two antibiotics trimethoprim and sulfamethoxazole subjected to antibiotics self-medication. The absence of strict laws to control the sale of these drugs in Nigeria may have contributed to the increased use among the young adults. Similar resistant percentages were reported in other part of Nigeria and Iraq (Anosike *et al.*, 2020; Jalil and Al Atebee, 2022). The findings differed in the resistance rates of bacteria that cause UTIs, which could be ascribed to the unselective use of antibiotics by the patient without any healthcare assistance; thus leading to the development of bacterial resistance to antibiotics. These support the results of other previous findings (Jasim *et al.*, 2020; Anosike *et al.*, 2020).

In this study, resistance was defined as the reduced susceptibility to minimum of three antibiotics of different groups. *K. pneumoniae* showed resistance pattern to 9 different antibiotics with multiple antibiotic-resistance index of 0.6. This observation has serious

consequence to healthcare and public health. The rise of resistant-*K. pneumoniae* in community setting showed that resistance to multiple antibiotics of different classes compromises the efficacy of the antibiotics and reduces the therapeutic options available for treatment of infectious diseases. However, considering the high level of resistant observed in this study, measures and awareness of antibiotic resistance threat must be imparted among the young adults in tertiary Institutions to inhibit the spreading of these resistant bacteria.

CONCLUSION

The high resistance of *Klebsiella pneumoniae* strains to commonly used antibiotics is the key cause of prolonged infection, increased hospitalization, treatment cost, diseases and death. Routine investigation of the antibiotic susceptibility profile may aid in overcoming the unselective use of drugs, which is a key cause for the rise of drug resistance among bacteria and for increasing antibiotic policies. The data of this study can be used to determine trends in antimicrobial stewardship and to support clinicians in making the balanced optimal of antibiotic therapy.

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