



Research Article

Prevalence and Susceptibility Pattern of *Escherichia coli* and *Staphylococcus saprophyticus* Isolated from Patients with Urinary Tract Infections in Selected Hospitals in Northern Kaduna, Nigeria

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ABSTRACT

Urinary tract infection (UTI) is the second most common bacterial infection affecting people of all ages worldwide. This study investigated the prevalence and susceptibility patterns of *Escherichia coli* and *Staphylococcus saprophyticus* among patients attending selected hospitals in Northern Kaduna, Nigeria. One hundred and ninety-five (195) midstream urine samples were obtained from the patients using standard procedures. Bacterial culture on Nutrient agar revealed 34.4% (67/195) of the samples with significant bacterial growth ($\geq 10^5$ CFU/ml). Among the positive cultures, *Escherichia coli* accounted for 50.7% (34/67), while *Staphylococcus saprophyticus* accounted for 31.3% (21/67). Female patients had higher occurrences of both *Escherichia coli* (19.5%) and *Staphylococcus saprophyticus* (17.7%). Patients aged 20-29 years had the highest occurrence of *Escherichia coli* (26.8%), while *Staphylococcus saprophyticus* accounted for 14.6% of the isolates. The highest occurrence of *Escherichia coli* was observed among patients with tertiary education (23.3%), employed individuals (22.0%), and single participants (25.0%). Patients with lower abdominal pain, urinary incontinence and foul-smelling urine were significantly infected with *Escherichia coli*. Patients with vaginal discharge, multiple sex partners, women at menopause and patients with pregnancy had significant infection with *Staphylococcus saprophyticus*. Antibiotic susceptibility was determined using Kirby-Bauer disk diffusion. Both isolates were 100% resistant to Ampicillin; *Escherichia coli* had 73.5% resistance to Trimethoprim-sulfamethoxazole, while *Staphylococcus saprophyticus* was 66.7% resistant to Trimethoprim-sulfamethoxazole. Both *Escherichia coli* and *Staphylococcus saprophyticus* showed the highest susceptibility to Ceftriaxone (55.9% and 41.2%) respectively. The observed resistance to commonly used antibiotics in the study highlights the need of regular and effective management of urinary tract infections.

Keywords: Antibiotic susceptibility; *Escherichia coli*; *Staphylococcus saprophyticus*; Urinary Tract Infection

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections affecting the urinary tract, including the kidneys, bladder, urethra, and prostate (Selim *et al.*, 2022). Most infections involve the lower urinary tract, the bladder and the urethra. Annually, millions of individuals are affected by UTIs, leading to substantial health issues (Mehrishi *et al.*, 2014). The clinical manifestations of UTIs vary

depending on the causative agent, severity of the bacterial infection and the patient's immune status (Olowe *et al.*, 2015; Odoki *et al.*, 2019). A wide range of microorganisms can colonize and infect the urinary tract and cause infection; however, members of the family *Enterobacteriaceae* are the predominant causative agents of UTIs. In healthy individuals, the urinary tract contains very low numbers of microorganisms, although clinically significant

bacteriuria is generally absent. *Escherichia coli* is the predominant uropathogen, accounting for approximately 80% to 90% of UTIs worldwide (Bishop and Shehu, 2016), whereas, *Staphylococcus saprophyticus* is the second most common uropathogen found among young sexually-active females. Over 40% of all young, sexually-active women have *Staphylococcus saprophyticus* as part of their normal genitourinary flora (Ehlers and Merrill, 2023).

MATERIALS AND METHODS

The study was conducted in Northern Kaduna, Nigeria. The target populations comprised male and female patients of different age groups suspected with urinary tract infections attending three general hospitals: Hajiya Gambo Sawaba General Hospital, Major Abdullahi Ibrahim Memorial Hospital and General Hospital Giwa. Figure 1 shows the location of the study area.

Ethical Approval and Informed Consent

Ethical approval for the study was obtained from the Kaduna State Ministry of Health Ethical Review Committee. Written informed consent was obtained from all participants before collection of samples.

Collection of Urine Samples

A total of 195 midstream urine samples were collected from patients attending three hospitals in northern Kaduna State, Nigeria: Hajiya Gambo Sawaba General Hospital Zaria, Major Ibrahim Bello Abdullahi General Hospital Sabon Gari and Giwa General Hospital Giwa. Sterile, wide-mouth, universal, urine containers were provided to the participants along with instructions on proper collection of midstream urine samples. The samples were transported in ice packs to the Department of Microbiology Ahmadu Bello University, Zaria, for further bacteriological analysis.

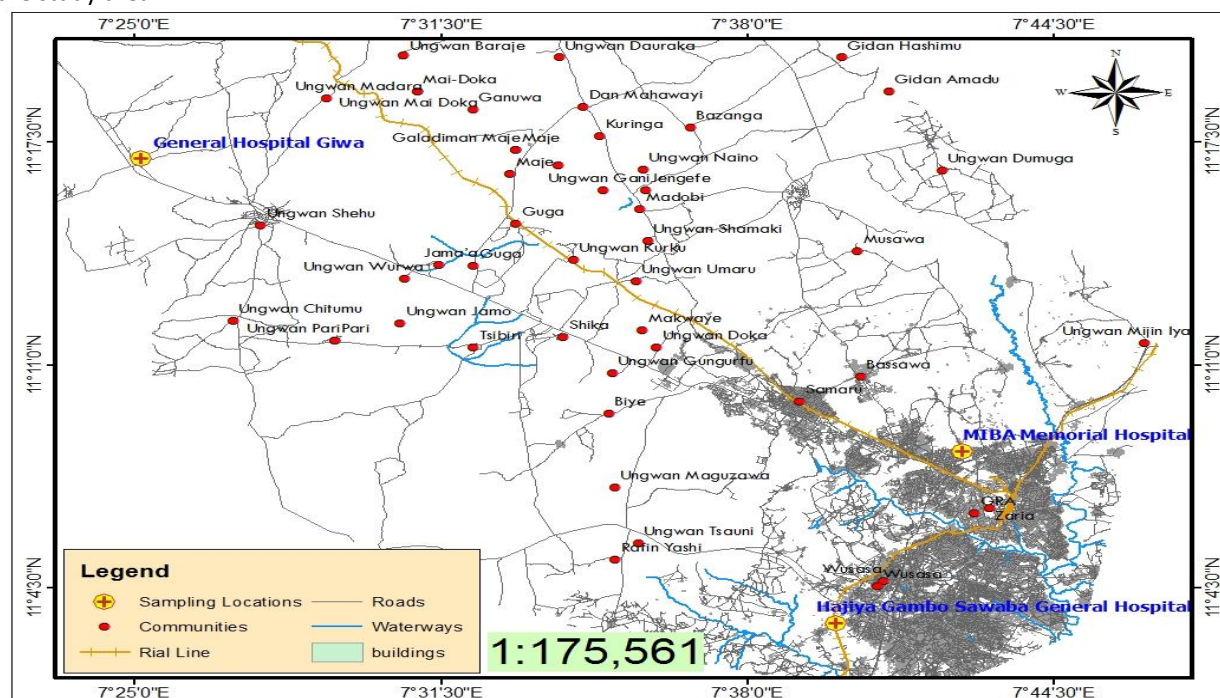


Figure1: Map of Study Area

Source: Modified From BBBIKE 2024 Using ArcGIS 10.3 Software

Determination of Significant Bacteriuria

A calibrated 0.01 ml wire loop was used to take a loopful of each urine sample and it was aseptically inoculated onto Nutrient agar (NA) and incubated at 37 °C for 24 hours. Colony counts were conducted at the end of the incubation period and samples yielding $\geq 10^5$ CFU/ml were considered to exhibit significant bacteriuria (Cheesebrough, 2006).

For isolation of *E. coli*, samples were inoculated onto Eosin Methylene Blue (EMB) agar at 37°C for 24 hours. After incubation period, colonies with metallic green sheen characteristics were suspected to be *E. coli* (Barwa *et al.*, 2022). Samples were inoculated onto Mannitol Salt Agar (MSA) at 37 °C for 24 hours for the isolation of *Staphylococcus saprophyticus*. After incubation period, colonies with a yellow characteristic were suspected to be *Staphylococcus*

saprophyticus (De Souza *et al.*, 2017). Pure cultures of the suspected isolates were maintained on nutrient agar (NA) slants at 4°C until further identification and susceptibility testing.

Gram Staining

Pure colonies obtained from the subcultures were Gram stained according to the standard procedure described by Cheesbrough (2006). The stained smears were examined microscopically for morphological characterization of the bacterial isolates.

Biochemical Tests

Based on the cultural characteristics, Gram reaction, and microscopic examination, the bacterial isolates were identified using standard biochemical tests as described by Cheesbrough (2006). *Escherichia coli* isolates were identified using the IMViC test series comprising Indole, Methyl red test, Voges-Proskauer, and Citrate utilization tests. *Staphylococcus saprophyticus* isolates were identified using catalase, coagulase, and urease tests followed by the novobiocin susceptibility test for confirmatory identification and differentiation from other coagulase-negative staphylococci.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Müller-Hinton agar according to CLSI (2020) guidelines. The antibiotics used were Ampicillin (30µg), Ciprofloxacin (5µg), Trimethoprim-sulfamethoxazole (25µg), Gentamicin (10µg), and Ceftriaxone (30µg). Fresh bacterial colonies were suspended in sterile normal saline and adjusted to 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the inoculum and excess medium was removed by passing the swab onto the wall of the tube. The surface of the plates was swabbed and allowed to dry for 3-5min before the antibiotic discs were aseptically placed on the agar surface. The plates were incubated at 37°C for 18-24 hours, after which the diameters of the zones of inhibition were measured in millimetres. The results were interpreted as susceptible or resistant according to CLSI (2020).

Data Analysis

Data generated from the study was analyzed using SPSS computer software version 20. Percentages were used for patients' socio-demographic characteristics. The Chi square test and Odds ratio were analyzed with 95% confidence interval. Factors with P values ≤ 0.05 were considered statistically significant.

RESULTS

A total of 67 (34.4%) out of the 195 urine samples showed significant bacteriuria. Among the positive isolates, *Escherichia coli* was the predominant uropathogen, accounting for 50.7% (34/67), followed by *Staphylococcus saprophyticus* with 31.3% (21/67) as shown in Figures 2 and 3.

Table 1 presents the distribution of *E. coli* and *S. saprophyticus* isolates according to socio-demographic factors of patients with urinary tract infections. Female patients had higher occurrence of both organisms with (19.5%) infection with *E. coli* and (17.7%) significant infection with *S. saprophyticus* (P=0.000). Patients aged 20-29 years had the highest occurrence of *E. coli* (26.8%), and *S. saprophyticus* (14.6%), followed by aged 40-49 years with 22.2% and 11.1% respectively. The occurrence of *E. coli* varied across educational levels, with the highest prevalence among patients with tertiary education (23.3%), and the lowest among those with informal education (5.9%). Infection with *S. saprophyticus* was highest among patients with secondary education (15.2%) and least among those who had attained tertiary education (7.0%), but the difference was not statistically significant (P>0.05). Based on marital status, occurrences of *E. coli* (25.0%) and *S. saprophyticus* (17.5%) were highest among single participants; however, the differences were not statistically significant (P>0.05). In terms of occupational status of the patients, infection with *E. coli* was higher among employed individuals (22.0%, P>0.05), with higher occurrence of infection with *S. saprophyticus* among unemployed patients (11.7%). Table 2a and 2b present the association between the signs and symptoms of urinary tract infections and the occurrence of *E. coli* and *S. saprophyticus* infections. Patients who presented with genital pruritus were more at risk of infections with *E. coli*. Vaginal discharge was significantly associated with infections with *S. saprophyticus*. Patients who experienced urinary urgency and urinary incontinence were more likely to be infected with *S. saprophyticus* (OR>1). Patients that had fatigue and flank pains were more likely to be infected with *E. coli* and *S. saprophyticus* respectively (OR>1). Foul-smelling urine was statistically associated with *E. coli* infection among the patients (P<0.05, OR>1). Association between the risk factors of UTIs and infection among the patients are presented in Table 3. Patients that had had previous history of UTIs had higher risk of infection with *S. saprophyticus*. Diabetic patients were more at risk of infection with *E. coli*. Those suffering from kidney diseases had higher

occurrences of both *E. coli* and *S. saprophyticus*, but it was not statistically significant ($P>0.05$). Postmenopausal women were significantly more likely to have *S. saprophyticus* ($P\leq 0.05$). Those with multiple sex partners were more at risk of infections with *E. coli*. Having multiple sexual partners was identified as a significant risk factor for infections with *S. saprophyticus*. Table 4 presents the antibiotic susceptibility patterns of *Escherichia coli* and *S. saprophyticus* isolates. *Escherichia coli* isolates

showed the highest susceptibility to ceftriaxone (55.9%), followed by gentamicin (41.2%). The isolates exhibited 73.5% resistance to trimethoprim-sulfamethoxazole and complete resistance (100%) to ampicillin. Similarly, *S. saprophyticus* isolates showed the highest susceptibility to ceftriaxone (76.2%), followed by gentamicin (42.9%) and trimethoprim-sulfamethoxazole (42.9%). Like *E. coli*, all isolates (100.0%) were resistant to ampicillin.

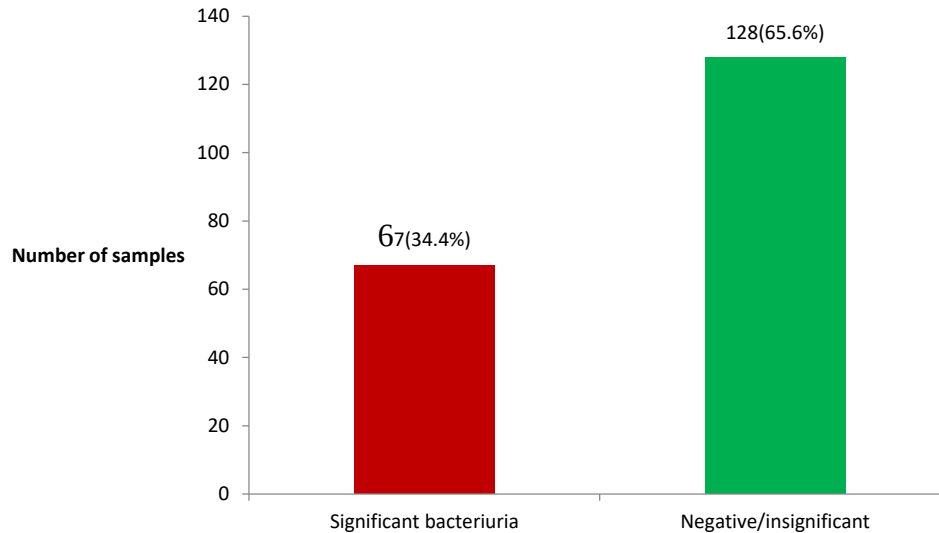


Figure 2: Prevalence of Urinary Tract Infection among Patients attending Selected Hospitals in Northern Kaduna, Nigeria

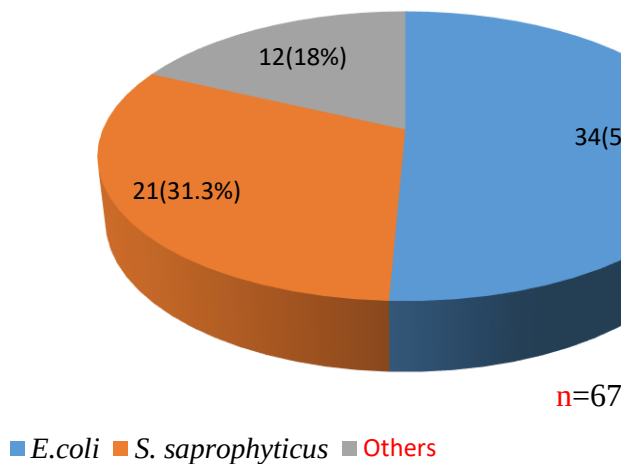


Figure 3: Occurrence of *Escherichia coli* and *Staphylococcus saprophyticus* among Patients with Urinary Tract Infection in Selected Hospitals in Northern Kaduna, Nigeria

Key: n= Number of UTI Positive Samples

Table 1: Distribution of *E. coli* and *S. saprophyticus* among Patients with Urinary Tract Infection based on Socio-Demographic Factors

Factor	No. examined	<i>E. coli</i> No. Positive (%)	<i>S. saprophyticus</i> No. Positive (%)
Age (Years)			
10-19	28	4(14.3)	4(14.3)
20-29	41	11(26.8)	6(14.6)
30-39	59	6(10.2)	5(8.5)
40-49	45	10(22.2)	5(11.1)
50-59	22	3(13.6)	1(4.5)
		$\chi^2=5.807$, df=4, P=0.214	$\chi^2=2.213$, df=4, P=0.697
Gender			
Female	113	22(19.5)	20(17.7)
Male	82	12(14.6)	1(1.2)
		$\chi^2=0.772$, df=1, P=0.380, OR=1.410	$\chi^2=13.429$, df=1, P=0.000, OR=17.419
Level of Education			
Informal	34	2(5.9)	3(8.8)
Primary	39	5(12.8)	3(7.7)
Secondary	79	17(21.5)	12(15.2)
Tertiary	43	10(23.3)	3(7.0)
		$\chi^2=5.656$, df=3, P=0.130	$\chi^2=2.768$, df=3, P=0.429
Marital Status			
Married	155	24(15.5)	14(9.0)
Single	40	10(25.0)	7(17.5)
		$\chi^2=2.00$, df=1, P=0.157, OR=1.819	$\chi^2=2.372$, df=1, P=0.123, OR=2.136
Occupational Status			
Employed	50	11(22.0)	4(8.0)
Unemployed	145	23(15.9)	17(11.7)
		$\chi^2=0.973$, df=1, P=0.324, OR=0.668	$\chi^2=0.537$, df=1, P=0.464, OR=1.527

Keys: No= Number, P= Probability, χ^2 =Chi square, df = Degrees of freedom, OR= Odds ratio

Table 2a: Signs and symptoms of *E. coli* and *S. saprophyticus* among Patients with Urinary Tract Infection

Sign/Symptom	No. examined	<i>E. coli</i> No. Positive (%)	<i>S. saprophyticus</i> No. Positive (%)
Genital Pruritus			
No	56	12(21.4)	4(7.1)
Yes	139	22(15.8)	17(12.2)
		$\chi^2=0.870, df=1$ P=0.351, OR=0.689	$\chi^2=1.075, df=1$ P=0.300, OR=1.811
Dysuria			
No	68	10(14.7)	5(7.4)
Yes	127	24(18.9)	16(12.6)
		$\chi^2=0.0=541, df=1$ P=0.462, OR=1.351	$\chi^2=1.268, df=1$ P=0.260, OR=1.816
Vaginal discharge			
No	49	11(22.4)	7(14.3)
Yes	64	11(17.2)	13(20.3)
Not applicable	82	12(14.6)	1(1.2)
		$\chi^2=1.305, df=2, P=0.521$	$\chi^2=14.478, df=2, P=0.001$
Haematuria			
No	168	31(18.5)	14(8.3)
Yes	27	3(11.1)	7(25.9)
		$\chi^2=0.871, df=1$ P=0.351, OR=0.552	$\chi^2=8.175, df=1$ P=0.006, OR=3.850
Lower abdominal pain			
No	71	12(16.9)	7(9.9)
Yes	124	22(17.7)	14(11.3)
		$\chi^2=0.022, df=1$ P=0.882, OR=1.060	$\chi^2=0.096, df=1$ P=0.765, OR=1.164
Fever and chills			
No	47	5(10.6)	3(6.4)
Yes	148	29(19.6)	18(12.2)
		$\chi^2=1.988, df=1$ P=0.159, OR=0.489	$\chi^2=1.240, df=1$ P=0.266, OR=0.492

Keys: No= Number, P= Probability, χ^2 =Chi square, df=Degrees of freedom, OR= Odds ratio

Table 2b: Signs and symptoms of *E. coli* and *S. saprophyticus* among Patients with Urinary Tract Infection (Cont'd)

Sign/ Symptom	No. examined	<i>E. coli</i> No. Positive (%)	<i>S. saprophyticus</i> No. Positive (%)
Urinary urgency			
No	52	9(17.3)	7(13.5)
Yes	143	25(17.5)	14(9.8)
		$\chi^2=0.001, df=1$ P=0.977, OR=0.988	$\chi^2=0.535, df=1$ P=0.465, OR=1.433
Fatigue			
No	84	15(17.9)	8(9.5)
Yes	111	19(17.1)	13(11.7)
		$\chi^2=0.018, df=1$ P=0.893, OR=1.053	$\chi^2=0.238, df=1$ P=0.626, OR=0.794
Flank pain			
No	81	12(14.8)	9(11.1)
Yes	114	22(19.3)	12(10.5)
		$\chi^2=0.661, df=1$ P=0.416, OR=0.727	$\chi^2=0.017, df=1$ P=0.897, OR=1.063
Urinary incontinence			
No	122	27(22.1)	13(10.7)
Yes	73	7(9.6)	8(11.0)
		$\chi^2=4.991, df=1$ P=0.025, OR=2.680	$\chi^2=0.004, df=1$ P=0.947, OR=0.969
Foul-smelling urine			
No	146	31(21.2)	14(9.6)
Yes	49	3(6.1)	7(14.3)
		$\chi^2=5.814, df=1$ P=0.016, OR=4.133	$\chi^2=0.842, df=1$ P=0.359, OR=0.636

Keys: No= Number, P= Probability, χ^2 =Chi square, df=Degrees of freedom, OR= Odds ratio

Table 3: Risk factors of *E. coli* and *S. saprophyticus* among Patients with Urinary Tract Infection

Risk factors	No. examined	<i>E. coli</i> No. Positive (%)	<i>S. saprophyticus</i> No. Positive (%)
Previous history of UTI			
No	141	24(17.0)	10(7.1)
Yes	54	10(18.5)	11(20.4)
		$\chi^2=0.061$, df=1 P =0.805 OR=0.903	$\chi^2=7.164$, df=1 P =0.007OR=0.298
Diabetes			
No	156	29(18.6)	17(10.9)
Yes	39	5(12.8)	4(10.3)
		$\chi^2=0.721$, df=1 P =0.396 OR=1.553	$\chi^2=0.013$ df=1 P =0.908 OR=0.298
Kidney Disease			
No	174	30(17.2)	18(10.3)
Yes	21	4(19.0)	4(19.0)
		$\chi^2=0.042$, df=1 P =0.837 OR=0.885	$\chi^2=0.303$ df=1 P =0.582 OR=0.692
Pregnancy			
No	97	19(19.6)	19(19.6)
Yes	16	3(18.8)	1(6.2)
Not applicable	82	12(14.6)	1(1.2)
		$\chi^2=0.778$, df=2, P=0.678	$\chi^2=15.972$, df=2, P=0.000
Menopause			
No	99	20(20.2)	19(19.2)
Yes	14	2(14.3)	1(7.1)
Not applicable	82	12(14.6)	1(1.2)
		$\chi^2=1.070$, df=2, P=0.586	$\chi^2=15.282$, df=2, P=0.000
Multiple sex partners			
No	141	27(19.1)	20(14.2)
Yes	54	7(13.0)	1(1.9)
		$\chi^2=1.070$,df=1 P =0.308, OR=1.590	$\chi^2=15.28$ df=1 P =0.013, OR=8.760

Keys: No= Number, P= Probability, χ^2 =Chi square, df=Degrees of freedom, OR= Odds ratio

Table 4: Antibiotic Susceptibility Patterns of *E. coli* and *S. saprophyticus* Isolated from UTI Patients

Antibiotic	<i>E. coli</i> (n=34)		<i>S. saprophyticus</i> (n=21)	
	S (%)	R (%)	S (%)	R (%)
Ampicillin (30µg)	0(0.0)	34(100.0)	0(0.0)	21(100.0)
Ciprofloxacin(5µg)	12(35.3)	22(64.7)	7(33.3)	14(66.7)
Ceftriaxone (30µg)	19(55.9)	15(44.1)	16(76.2)	5(23.8)
Gentamicin (10µg)	14(41.2)	20(58.8)	9(42.9)	12(57.1)
Trimethoprim-sulfamethoxazole (25µg)	9(26.5)	25(73.5)	9(42.9)	12(57.1)

Keys: S = Sensitive, R = Resistant

DISCUSSION

Urinary Tract Infection (UTI) is a complicated problem that continues to present new challenges. In the present study, significant bacteriuria was detected in

34.4% (67/195) of urine samples collected from patients attending selected hospitals in Northern Kaduna State. This prevalence is lower than the 41.0% reported by Mlugu *et al.* (2023) but higher than the

31.6% reported by Gebretensaie *et al.* (2023). The observed differences could be due to personal hygiene practices, healthcare access, study population, socio-economic status and immune status of the study participants.

Escherichia coli was the predominant uropathogen accounting for 50.7% (34/67) of all isolates. The predominance of *E. coli* in UTIs is consistent with previous reports. It has been the most frequently isolated pathogen in UTI cases because it rapidly adapts and colonizes the urinary tract than any other uropathogens (Bishop and Shehu, 2016). Similar findings 39.0% have been reported by Bishop and Shehu (2016), 41.9% by Odoki *et al.* (2019) and 2.0% by Barwa *et al.* (2022). However, despite many studies showing that *E. coli* is the predominant uropathogen, a study by Simba *et al.* (2022) reported it as the second most prevalent pathogen after *Staphylococcus aureus*. Gram-positive bacteria are generally less frequently isolated from UTI cases than Gram-negative bacteria. Besides *Escherichia coli*, which accounts for approximately 80% of UTIs, *S. saprophyticus* is considered the second most important uropathogen, accounting for approximately 10–15% of cases (Ronald, 2002; Widerstrom *et al.*, 2007; Alao *et al.*, 2020). In the present study, *S. saprophyticus* accounted for 31.3% (21/67) of all isolates. This prevalence is higher than the 25% reported by Olatunbosun (2023). The relatively high prevalence observed in this study may be attributed to the higher proportion of more female participants recruited, as *Staphylococcus saprophyticus* is predominantly found among young and sexually-active women (Bishop and Shehu, 2016). Patients aged 20-29 years had highest occurrence of *E. coli* and *S. saprophyticus*. This age group is more sexually active and could have been more exposed to the infections than younger and older age groups. Female patients were found with higher occurrences of both *E. coli* and *S. saprophyticus*. Many studies had consistently reported higher occurrences of bacterial uropathogens among female individuals than among the males (Odoki *et al.* 2019; Jalil and Al Atbee, 2022; Barwa *et al.*, 2022; Islam *et al.*, 2022). The anatomical structures and close proximity of the female urethra to the anus makes females more susceptible to bacterial contamination and subsequent urinary tract infections (Makeri *et al.*, 2023). Infection due to *S. saprophyticus* is more common among sexually-active young women (Raz *et al.*, 2005). Childbirth, use of contraceptives and menopause greatly predispose women to UTIs (Iregbu and Nwajiobi-Princewill, 2013; Bishop and Shehu, 2016; Lema and Lema, 2018). Also,

unhygienic use of the toilet could result in infections because female urine is ejected with force and creates great splashes which could re-introduce pathogenic organisms from the environment into their vulvae (Bishop and Shehu, 2016; Barwa *et al.* 2022).

It is expected that with improved education and awareness, individuals will adopt protective measures against infections; however, it was not the case observed in this study. The occurrence of *E. coli* was observed to increase with an increase in educational level. The higher occurrence of *E. coli* among individuals with tertiary education maybe related to lifestyle and behavioural factors, including increased sexual activity among some young adults, although this association requires further investigation. The lower occurrence of *E. coli* and *S. saprophyticus* among married participants may probably be due to limited number of sexual partners, compared to singles who are sexually active individuals who may not have restricted sexual contacts. Significant symptoms of the infection included vaginal discharge, haematuria and urinary incontinence. A study by Gebretensaie *et al.* (2023) reported several clinical symptoms, including increased urinary frequency, urgency, haematuria, abdominal discomfort, suprapubic pain, and fever/chills, which were significantly associated with UTIs. These symptoms had been similarly reported by Barwa *et al.* (2022). Patients with diabetes mellitus, a previous history of UTI, or self-medication were more likely to be infected with *E. coli*. Significant risk factors for *S. saprophyticus* included pregnancy, menopause, and multiple sex partners.

Selecting on an appropriate treatment against UTI is very crucial for the effective management of urinary tract infections. The sensitivity patterns of *Escherichia coli* in this study revealed that Ceftriaxone was highly effective followed by Gentamicin and completely resistant to Ampicillin. Treatment options for UTIs caused by *S. saprophyticus* are limited, as they remain highly resistant to most of the antibiotic tested in this study. These findings suggest that empirical antibiotic therapy may be ineffective against *S. saprophyticus* in the study area, highlighting the need for routine antimicrobial susceptibility testing before treatment.

CONCLUSION

The observed prevalence of urinary tract infection demonstrates that UTIs continue to constitute a considerable health burden in the study area. Also, the high level of resistance to commonly used antibiotics observed in the study highlights the need

for continuous antibacterial resistance surveillance and routine antibacterial susceptibility testing for improved patient management.

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