

ANTIBIOTIC RESISTANCE PATTERN OF BACTERIAL ISOLATES ASSOCIATED WITH ASYMPTOMATIC URINARY TRACT INFECTIONS IN WUKARI METROPOLIS, TARABA STATE, NIGERIA.

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Abstract: Urinary tract infection is a significant disease in females due to their anatomical peculiarities. The study hence aimed to determine the prevalence of asymptomatic urinary tract infections (UTI) among sexually active undergraduate students within Wukari metropolis, Taraba State. One hundred and fifty urine specimens were collected and cultured aerobically. Bacterial colony count was carried out to determine UTI-positive and UTI-negative specimens. Bacterial identification was carried out using standard biochemical characterization of the isolates. Antibiotic resistance pattern of the isolates was determined using the modified Kirby-Bauer disc diffusion method and comparing observed inhibition zone diameters with standards. Of the 150 urine specimens collected, only 100 (66.67%) yielded significant bacteriuria and were hence considered asymptomatic UTI. *Escherichia coli* (41%) was the most prevalent bacterial uropathogen, followed by *Staphylococcus saprophyticus* (30%), *Staphylococcus aureus* (22%), *Pseudomonas aeruginosa* (4%), and *Proteus mirabilis* (3%). All the bacterial isolates were resistant to at least three (3) of the tested antibiotics. *S. saprophyticus* and *S. aureus* were most resistant to Amoxicillin and Chloramphenicol, although *S. saprophyticus* was significantly more resistant than *S. aureus*. *E. coli*, *P. aeruginosa*, and *P. mirabilis* were most resistant to Amoxicillin, Imipenem, and Ceftazidime, although *E. coli* was significantly more resistant than other Gram-negative bacterial isolates. High prevalence of asymptomatic UTI among undergraduate students in Wukari metropolis is of public health importance, and increasing antibiotic resistance rates do not help this challenge.

INTRODUCTION

Urinary tract infection (UTI) is a disease caused by microbial invasion and multiplication within

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the tissues of the urinary tract system [1]. It is one of the most common infections globally and is diagnosed using a combination of urinary symptoms plus significant bacteriuria of 10^5 CFU/mL [2]. Although UTIs have been associated with fungal and viral etiologies, it is most often caused by bacteria [3]. Sexual activity, physiological changes, anatomical position, poor personal hygiene, use of contraceptives, diabetes, obesity, and gender have been most reported as risk factors for the development of UTIs [4-5].

Although UTIs has been reported in all age groups and in both sexes, females are at a significantly higher risk due to their shorter urethra [6-7]. Also, young women have been reported to have a 60% chance of developing bacterial cystitis before the age of 24 years [8]. Hence, majority of women report with recurrent UTIs within one year of therapy [9]. Antibiotic resistance has remained a mainstay public health issue over the years. UTI is caused by different species of bacteria which display varying levels of resistance to conventional antibiotics. These resistances develop through varying mechanisms which ultimately complicate both prophylactic and therapeutic options for infection, extend hospital stay, and increase the cost of healthcare, as well as increase morbidity and mortality rates [10-12].

METHODOLOGY

Description of the study area: Wukari is a local government area in Taraba State, Nigeria and is located between longitude $7^{\circ}57'$ and latitude $9^{\circ}42'$ of the equator. Wukari is the local government capital and is bounded by Ibi LGA, Donga LGA, Gassol, LGA, and Ukum LGA to the north, east, northeast, and south respectively [13]. Wukari is a commercial town dominated by farmers, herdsman, fishermen, traders [14]. Wukari is home to Federal

University Wukari and Kwararafa University and other secondary and primary schools.

Ethical consideration: Ethical approval was sought and received from the student's affairs department of both institutions (Federal University Wukari and Kwararafa University Wukari). Participants were well informed about the aim and objectives as well as merits and demerits of the study before consenting to the study.

Collection of Urine Specimen: One hundred and fifty (150) clean-catch voided midstream urine specimens were collected in properly labelled sterile universal bottles. 20 – 30 mL of urine specimens were collected from undergraduate female students, within the age group of 21-30 years, attending either Kwararafa University and Federal University Wukari. Collected urine specimens were physically observed for turbidity and colour.

Urine Microscopy: 10 mL of each collected urine specimens were centrifuged at 3000 revolutions per minute (RPM) for five (5) minutes. Deposits of the sediment were then analysed under a compound microscope for the presence of pus cells, epithelial cells, yeast cells, red blood cells, white blood cells, crystals and casts in the urine.

Culture and Identification of bacterial isolates: 1 mL of urine was cultured aerobically on cysteine lactose electrolyte deficient (CLED) agar, blood agar, and MacConkey agar. Distinct colonies from the mixed culture were characterized based on their colonial morphologies and then Gram-stained. Biochemical characterization of bacterial isolates was carried out using catalase test, coagulase test, oxidase test, urease test, motility test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, and sugar fermentation test.

Determination of urinary tract infection (UTI) positive cases:

A UTI positive case was determined if there is significant bacteriuria ($\geq 10^5$ CFU/mL) of a single uropathogen found on a urine culture plate [1].

Antibiotic sensitivity testing: Antibiotic resistance pattern of bacterial isolates was carried out using the modified Kirby-Bauer disc diffusion method. Isolates were tested against Ciprofloxacin (5 µg), Norfloxacin (10 µg), Gentamycin (10 µg), Ampicillin (10 µg), Streptomycin (10 µg), Rifampicin (5 µg), Erythromycin (15 µg), Levofloxacin (5 µg), Chloramphenicol (30 µg), Cefepime (30 µg), Fosfomycin (200 µg), Aztreonam (30 µg), Imipenem (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Nalidixic acid (30 µg), and Cefuroxime (30 µg). Observed zone of inhibition diameter was measured and compared with standards as stated by Clinical Laboratories Standard Institute [15].

Data analysis: Descriptive statistics of collected data was done using tables constructed using Microsoft Excel (version 2016). P-value for inferential statistics was done using IBM statistical package for social sciences (SPSS version 21). Inferential statistics was done at 95% confidence interval.

RESULTS

From the one-hundred-and-fifty urine samples, only one hundred (66.67%) samples produced bacterial counts equivalent to significant bacteriuria (10^5 CFU/mL). Of these, thirty samples (30%) were collected from Kwararafa University students while seventy samples (70%) were collected from Federal

University Wukari students. In descending order of prevalence, the bacterial isolates associated with asymptomatic urinary tract infection in Wukari metropolis are: *Escherichia coli* (41.0%), *Staphylococcus saprophyticus* (30.0%), *Staphylococcus aureus* (22.0%), *Pseudomonas aeruginosa* (4.0%), and *Proteus mirabilis* (3.0%). However, there is no significant difference in the prevalence of the bacterial isolates from Federal University Wukari and Kwararafa University students (table 1).

The antibiotic resistance rates of *S. aureus* and *S. saprophyticus* isolates were Ciprofloxacin (36.36% and 40.0%), Norfloxacin (18.18% and 6.67%), Gentamycin (18.18% and 43.33%), Ampicillin (40.91% and 53.33%), Streptomycin (18.18% and 60.0%), Rifampicin (4.54% and 63.33%), Erythromycin (18.18% and 53.33%), Levofloxacin (27.27% and 63.33%), and Chloramphenicol (36.36% and 40.0%) respectively. *S. saprophyticus* isolates were statistically more resistant to the tested antibiotics than *S. aureus* (table 2). The resistance rates of *E. coli* isolates were Ciprofloxacin (14.63%), Norfloxacin (4.88%), Gentamicin (7.32%), Ampicillin (85.36%), Levofloxacin (24.39%), Cefepime (34.15%), Aztreonam (26.83%), Imipenem (82.93%), and Ceftazidime (34.15%). All isolates of *P. aeruginosa* and *Proteus mirabilis* were generally susceptible to Norfloxacin and Aztreonam while the highest resistances were for both organisms were Ampicillin (100% each), Gentamicin (75%, 66.67%), and Imipenem (25% and 66.67%) respectively (table 3).

Table 1: prevalence of bacterial isolates associated with asymptomatic UTI in Wukari metropolis

Bacterial isolate	FUW (%)	KUW (%)	Total (%)	p-value
<i>Escherichia coli</i>	29 (41.43)	12 (40.00)	41 (41.0)	0.101528
<i>Staphylococcus aureus</i>	15 (21.43)	7 (23.33)	22 (22.0)	
<i>Staphylococcus saprophyticus</i>	22 (31.43)	8 (26.67)	30 (30.0)	
<i>Pseudomonas aeruginosa</i>	1 (1.43)	3 (10.00)	4 (4.0)	
<i>Proteus mirabilis</i>	3 (4.29)	0 (0.0)	3 (3.0)	
Total	70 (70.0)	30 (30.0)	100 (100)	

Table 2: antibiotic resistance pattern of Gram-positive bacterial isolates associated with asymptomatic UTI

Antibiotic	<i>S. aureus</i> (%)	<i>S. saprophyticus</i> (%)	p-value
Ciprofloxacin	8 (36.36)	12 (40.0)	0.000408
Norfloxacin	4 (18.18)	2 (6.67)	
Gentamicin	4 (18.18)	13 (43.33)	
Amoxicillin	9 (40.91)	16 (53.33)	
Streptomycin	4 (18.18)	18 (60.0)	
Rifampicin	1 (4.54)	19 (63.33)	
Erythromycin	4 (18.18)	16 (53.33)	
Levofloxacin	6 (27.27)	19 (63.33)	
Chloramphenicol	8 (36.36)	12 (40.0)	

Table 3: antibiotic resistance pattern of Gram-negative bacterial isolates associated with asymptomatic UTI

Antibiotic	<i>E. coli</i> (%)	<i>P. aeruginosa</i> (%)	<i>P. mirabilis</i> (%)	p-value
Ciprofloxacin	6 (14.63)	1 (25.0)	1 (33.33)	0.000638
Norfloxacin	2 (4.88)	0 (0.0)	0 (0.0)	
Gentamicin	3 (7.32)	3 (75.0)	2 (66.67)	
Amoxicillin	35 (85.36)	4 (100.0)	3 (100.0)	
Levofloxacin	10 (24.39)	1 (25.0)	0 (0.0)	
Cefepime	14 (34.15)	0 (0.0)	2 (66.67)	
Aztreonam	11 (26.83)	0 (0.0)	0 (0.0)	
Imipenem	34 (82.93)	1 (25.0)	2 (66.67)	
Ceftazidime	14 (34.15)	1 (25.0)	1 (33.33)	

Discussion

UTI is most common among female individuals and up to 50% of all women experience UTI at

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some point in their lifetime [16]. Hence, the significance of symptomatic and asymptomatic UTI, especially among young sexually active females, cannot be overestimated. The 66.67% prevalence of asymptomatic UTI among undergraduate female students in Wukari metropolis is similar to 63.5% reported in Ugbokolo, Benue State [5]. However, the current prevalence is higher than those reported in Ahmadu Bello University, Kaduna State (52.9%) and 29% in Chukwuemeka Odumegwu Ojukwu University, Anambra State [17-18]. Similarly, research studies in other countries have reported lower prevalence of asymptomatic UTI, including 26.20% in Mekelle University, Ethiopia and 26.50% in Bangladesh, India [1, 19]. Higher prevalence of UTI in the study area can be attributed to poor personal hygiene and high sexual activity among the participants. Hence, UTI can be considered a significant public health challenge within the study area.

Escherichia coli was the most prevalent bacterial isolate. The 41% prevalence of *E. coli* reported in this study is similar to 38.6%, 46.8%, and 48.6% reported in Ahmadu Bello University, Kaduna State, Ugbokolo, Benue State, and Mekelle University, Ethiopia respectively [5, 18-19]. Hossain *et al.*, [1] reported a lower prevalence (32.08%) in Bangladesh, India, while Ezeadila *et al.*, [17] reported a higher prevalence (56.38%) in Chukwuemeka Odumegwu Ojukwu University, Anambra State. Low prevalence of *P. aeruginosa* (4%) and *P. mirabilis* (3%) have also been reported in similar studies. Hossain *et al.* [1] and Bishop and Shehu [18] reported 2.83% and 1.8% prevalence of *P. aeruginosa* in Bangladesh, India and Ahmadu Bello University, Kaduna State respectively while Adikwu *et al.*, [5], Ezeadila *et al.*, [17], and Gebremariam *et al.*, [19] reported 6.4%, 4.26%,

and 2.7% prevalence of *P. mirabilis* in Benue State, Anambra State, and Ethiopia respectively. The high prevalence of *E. coli* as reported in this study is similar with reports from other studies, hence, it can be concluded that *E. coli* is a significant pathogen in the development of UTI in the study area.

S. saprophyticus (30%), in this study, is more prevalent than *S. aureus* (22%). Ezeadila *et al.*, [17] also reported higher prevalence of *S. saprophyticus* (20.21%) than *S. aureus* (11.70%). In Mekelle University, Ethiopia, Gebremariam *et al.*, [19] also reported a higher prevalence of *S. saprophyticus* (23%) than *S. aureus* (13.5%). Also, Hossain *et al.*, [1] reported 24.53% prevalence of *S. saprophyticus* in Bangladesh, India while Adikwu *et al.*, [5] and Bishop and Shehu [18] reported 12.8% and 3.5% prevalence of *S. aureus* in Ugbokolo, Benue State and Ahmadu Bello University, Kaduna State respectively.

The bacterial uropathogens displayed varying resistance rates against different antibiotics. High resistance rates among *S. saprophyticus* and *S. aureus* is supported by similar reports in Ugbokolo, Benue State, Ahmadu Bello University, Kaduna State, Mekelle University, Ethiopia, and Bangladesh, India [1, 5, 18-19]. However, Gebremariam *et al.*, [19] reported 0% resistance rate for *S. saprophyticus* against Gentamicin and Ciprofloxacin in Mekelle University, Ethiopia.

High antibiotic resistance rate among *E. coli* isolates associated with UTIs have also been reported in Bangladesh, India and Ahmadu Bello University, Kaduna State [1, 18]. However, Adikwu *et al.*, [5] reported low resistance rates in Ugbokolo, Benue State except against Amoxicillin (95.5%) while Gebremariam *et al.*, [19] reported 8.3% resistance rate against both Ciprofloxacin and Gentamicin in Mekelle University, Ethiopia. High resistance to Gentamicin has also been

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reported in Ahmadu Bello University, Kaduna State (100%) and Bangladesh, India (66.6%) [1, 18]. However, lower rates of resistance against Ciprofloxacin and Imipenem, reported in Bangladesh negates those reported in this study. High resistance rates of *Proteus mirabilis* reported in this study contradicts low rates reported in Ugbokolo, Benue State and Mekelle University, Ethiopia [5, 19].

High antimicrobial resistance rates reported in this study can be associated with the extensive use and misuse of these antibiotics as they are readily available for on-the-counter purchase, except Gentamicin, an intravenous injectable antibiotic. However, high resistance rates against Gentamicin, as seen in isolates of both *P. aeruginosa* and *P. mirabilis*, is likely due to a significantly low number of tested isolates.

CONCLUSION

The prevalence of bacterial uropathogens associated with asymptomatic UTIs in the study area is not too different from those isolated from different study areas. However, elevated antibiotic resistance rates, especially among these uropathogens are causes of worry as these increases in antibiotic resistant strains complicate prevention and treatment options for UTIs within the study area.

CONFLICT OF INTEREST

We the author declared that there are no conflict of interest.

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