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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN URINE OF STUDENTS OF AHMADU BELLO UNIVERSITY

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ABSTRACT
When non-resident pathogenic microorganisms invade and multiply in the urinary tract, it leads to urinary tract infections (UTIs). UTIs that are not diagnosed and treated early enough can result into some complications. This research determined the prevalence of *Escherichia coli* and *Klebsiella pneumoniae* in asymptomatic students. Mid-stream urine samples were collected from 151 Microbiology students of Ahmadu Bello University, Zaria. Structured questionnaires were administered to ascertain some socio-demographic factors. The samples were inoculated onto sterile plates of MacConkey agar and Eosin Methylene Blue agar, incubated at 37^{o}C for 18-24hr. Pure isolates were identified by Gram staining and biochemical characterization. The isolates were tested against some selected antibiotics. The overall prevalence of *Escherichia coli* and *Klebsiella pneumoniae* was 3(2.0%) and 1(0.7%) respectively. Both *Escherichia coli* (3.9%) and *Klebsiella pneumoniae* (0.1%) were isolated only from the female students, and absent in the males. Students within the age group of 16-20 years old had 4.6% *Escherichia coli* and 1.5% *Klebsiella pneumoniae* infections, but absent in other age groups. Infections of 2.7% *Escherichia coli* and 0.9% *Klebsiella pneumoniae* were found only in students who resided within the school hostels. Only those who used public latrines had infections with *Escherichia coli* (2.3%) and *Klebsiella pneumoniae* (0.8%). Students that used water to clean up after using the toilet had 2.0% *Escherichia coli* and 0.7% *Klebsiella pneumoniae* infections, but no infection among those who used toilet roll. All the infections with the two pathogens were found in students who used backward direction for anal cleaning up. The *Escherichia coli* isolates were equally most susceptible to Ciprofloxacin (10µg), Pefloxacin (10µg) and Tarivid (10µg). But the *Klebsiella pneumoniae* isolate was susceptible to most of the antibiotics except Septrin (30µg) and Augmentin (30µg).

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, Urinary tract Infection, antibiotic susceptibility, students.

INTRODUCTION

Bacteriuria is the presence of bacteria in the urine. Asymptomatic bacteriuria is the presence and proliferation of bacteria in the urine without any clinical signs or symptoms (Obiogbolu *et al.*, 2009). Symptomatic UTI occurs when there is bacteriuria with urinary symptoms (Ekwealor *et al.*, 2016). It is a condition where actively multiplying bacteria persist in the urine at any point from the kidney to the urethral meatus (Osungunna and Adeyemi, 2016). Therefore, the presence or absence of symptoms is the determining factor of whether a urinary tract infection (UTI) is symptomatic or asymptomatic. Asymptomatic bacteriuria is more common among women than among men probably because of the shorter female urethra, which allows bacteria from the urethral meatus and the perineum to gain access into the bladder (Amali *et al.*, 2009). It is more common in older adults than in younger adults and the prevalence increases considerably with age in both men and women (Rowe and Juthani-Mehta, 2013). Infections of the urinary tract are second in frequency only to infections of the respiratory tract (Kass, 2002). UTI can occur in all age groups and usually calls for urgent treatment (Anago *et al.*, 2015). Several factors such as gender, age, race, circumcision, HIV, diabetes, urinary catheter, genitourinary tract abnormalities, pregnancy, infancy, elderly and hospitalization status and poor personal hygiene bear significant risks for recurrent UTIs (Ekwealor *et al.*, 2016; Odoki *et al.*, 2019). UTI can be caused by both Gram-negative and Gram-positive bacteria, in addition to certain fungi (Shanthi *et al.*, 2018). *Escherichia coli* is a significant causative agent as it accounts for 80% to 90% of UTIs, while *Klebsiella* species rank second in prevalence (Bishop and Shehu, 2016; Ayoyi *et al.*, 2017; Oluwafemi *et al.*, 2018). It can also be caused by *Pseudomonas aeruginosa, Staphylococcus* species, *Enterobacter* species, *Proteus mirabilis, Enterococcus, Serratia* species, and *Candida* species (Bishop and Shehu, 2016; Al-Mijalli, 2017). UTI is treated often by broad-spectrum antibiotics (Ahmed *et al.*, 2019), based on information derived from antimicrobial susceptibility results (Ekwealor *et al.*, 2016).

MATERIALS AND METHODS

Study population and area

The research included 151 apparently healthy Microbiology students of Ahmadu Bello University, Zaria between the ages of 16 and 30 years, who willingly consented. The University is located on latitude 11^{o}15'N to 11^{o}3'N and longitude 7^{o}30'E to 7^{o}45'E.

Collection of urine samples

Sterile (wide-mouth, screw-capped) urine sample bottles were given to the participants, with instruction on how to collect the urine. The samples were transported for analysis at the Bacteriology Laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria.

Cultural isolation of uropathogens

The urine samples were examined physically for turbidity. Aseptic inoculations were made on sterile MacConkey agar plates, followed by aerobic incubation at 37˚C for 18-24 hours. Sub-cultures on Eosin Methylene Blue (EMB) agar were made. Pure cultures of the suspected bacteria were Gram-stained and stored on Nutrient Agar slants at 4˚C for further laboratory investigations.

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Biochemical tests performed included sugar fermentation in Triple Sugar Iron agar, indole, Methyl red, Voges Proskaeur and citrate utilization tests. All media were prepared according to the manufacturers' instructions. The procedures for isolation and biochemical tests were carried out as suggested by Cheesbrough (2006).

Antibiotic susceptibility testing

All the isolates were subjected to the following antibiotics: Septrin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxacillin (30µg), Augmentin (30µg), Gentamycin (10µg), Pefloxacin (30µg), Tarivid (10µg), and Streptomycin (30µg). Inocula were prepared according to 0.5 McFarland standards. Agar disc diffusion method was used and the zones of inhibition of the isolates were measured to the nearest millimeter and compared with Clinical Laboratory Standard Institute standards to determine their susceptibility patterns (CLSI, 2014).

RESULTS

Out of 151 urine samples of apparently healthy students (which comprised of 75 males and 76 females), only 4 samples yielded the growth of Gram-negative bacteria. Prevalence of *Escherichia coli* was 3(2.0%), while *Klebsiella pneumoniae* was 1(0.7%) as shown in Table 1. The female students had higher occurrences of both organisms: 3(3.9%) for *Escherichia coli* and 1(0.1%) for *Klebsiella pneumoniae.* Age group of 16-20 years had the highest occurrences of the bacterial isolates (Table 2). All of the students from whom the pathogens were isolated resided in the hostels, used public latrines and water for anal cleaning up, using the backward direction (Table 3). The study subjects did not show any sign/symptom associated with UTI. However, the pathogens were mostly isolated from subjects who experienced frequent urination and passed out turbid urine (Table 4). *Escherichia coli* isolates were most susceptible to Ciprofloxacin (10µg), Pefloxacin (30µg) and Tarivid (10µg). On the other hand, the *Klebsiella pneumoniae* isolate was susceptible to all the antibiotics except Septrin (30µg) and Augmentin (30µg) as shown in Table 5.

Table 1: Prevalence of *Escherichia coli* and *Klebsiella pneumoniae* among asymptomatic Microbiology Students of Ahmadu Bello University, Zaria (n=151)

Pathogen	Number Positive	Prevalence (%)
Escherichia coli	3	2.0
Klebsiella pneumoniae	1	0.7
Total	4	2.7

Table 2: Gender and age distribution of *Escherichia coli* and *Klebsiella pneumoniae* among asymptomatic Microbiology Students

Factor	Category	Number examined	*Escherichia coli* (%)	*Klebsiella pneumoniae* (%)
Gender	Female	76	3 (3.9)	1 (0.1)
	Male	75	0(0.0)	0(0.0)
Age (years)	16-20	65	3 (4.6)	1 (1.5)
	21-25	78	0 (0.0)	0 (0.0)
	26-30	8	0 (0.0)	0 (0.0)

Table 3: Risk factors of UTI among asymptomatic Microbiology Students of Ahmadu Bello University, Zaria

Risk Factors	Category	Number Examined	*Escherichia coli* (%)	*Klebsiella pneumoniae* (%)
Residence	Hostel	113	3(2.7)	1(0.9)
	Home	7	0(0.0)	0(0.0)
	Off-Campus	31	0(0.0)	0(0.0)
Latrine	Private	21	0(0.0)	0(0.0)
	Public	130	3(2.3)	1(0.8)
Clean-up material used in the toilet	Toilet Roll	2	0(0.0)	0(0.0)
	Water	149	3(2.0)	1(0.7)
Direction of anal cleaning	Backward	80	3(3.8)	1(1.3)
	Forward	71	0(0.0)	0(0.0)

Table 4: Signs/symptoms of UTI among asymptomatic Microbiology Students of Ahmadu Bello University

Sign/Symptom	Category	Number examined	*Escherichia coli* Number positive (%)	*Klebsiella pneumoniae* Number Positive (%)
Abdominal pain	Yes	26	0(0.0)	0(0.0)
	No	125	3(2.4)	1(0.8)
Painful urination	Yes	6	0(0.0)	0(0.0)
	No	145	3(2.1)	1(0.7)
Frequent urination	Yes	47	1(2.1)	0(0.0)
	No	104	2(1.9)	1(1.0)
Turbidity	Clear	106	2(1.9)	0(0.0)
	Turbid	45	1(2.2)	1(2.2)

Table 5: Antibiotic susceptibility patterns of *Escherichia coli* and *Klebsiella pneumoniae* among asymptomatic Microbiology Students of Ahmadu Bello University

Isolate	Number	Percentage (%) susceptibility									
Antibiotic		SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
E. coli	3	0(0)	1(33)	1(33)	2(66)	1(33)	0(0)	1(33)	2(66)	2(66)	1(33)
K. pneumoniae	1	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)

Key:SXT=Septrin(30µg), CH=Chloramphenicol(30µg), SP=Sparfloxacin(30µg), CPX=Ciprofloxacin(10µg), AM=Amoxacillin(10µg), AU=Augmentin(30µg), CN=Gentamycin(10µg), PEF=Pefloxacin(10µg), OFX=Tarivid(10µg), S=Streptomycin(30µg)

DISCUSSION

Among the apparently healthy students that participated in this study, a very low prevalence of *Escherichia coli* (2.0%) and *Klebsiella pneumoniae* (0.7%) were isolated and identified from their urine samples. However, *Escherichia coli* was more common than *Klebsiella pneumoniae*. These two uropathogens are the most common causes of symptomatic UTIs (Bishop and Shehu, 2016; Ayoyi *et al.*, 2017; Oluwafemi *et al.*, 2018). A higher prevalence of 41.9% for *Escherichia coli* had been reported by Odoki *et al.* (2019).

All the cases of the asymptomatic UTI in this study were found among the female students of ages between 16-20 years. Females are more prone to UTIs than males, majorly due to the shortness of their urethra and ease of bacterial contamination (Cheesbrough, 2006; Bishop and Shehu, 2016). One-third of women are diagnosed with UTI before the age of 24 years unlike in men where it rarely occurs (Basseye *et al.*, 2016). Other studies had reported higher occurrence of uropathogens among female subjects than in the men (Odoki *et al.* (2019). The female urethra is structurally less effective in preventing bacterial entry. Their genital tracts and urethra are also too close. Certain factors like use of contraceptives, childbirth and menopause predispose them to UTIs (Iregbu and Nwajiobi-Princewill, 2013; Lema and Lema, 2018). Improper 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 urinary opening.

Students who resided in the school hostels and used public latrine had all the cases of the uropathogens but infection was absent in those who resided at home or off-campus. Overcrowding in hostels can cause general lack of adequate hygiene in latrines shared by all. Users of such latrines can easily pick up infections.
No case of asymptomatic UTI was found in subjects who used toilet roll for cleaning up after defecation. However, those who used water for cleaning up had asymptomatic UTI. It is easier to inoculate the urinary tract during washing the anus with water than during wiping with a toilet roll. Inadequate water supply or use of contaminated water in the latrines, poor personal hygiene and inadequate toilet facilities can promote the occurrence of UTIs among students living in crowded hostels (Amali *et al.*, 2009; Olabimtan *et al.*, 2018). No sign/symptom was associated with the occurrence of the bacterial uropathogens because the study subjects were all apparently healthy. However, the urine samples of majority of the positive cases were turbid, indicating possible bacteriuria or other infections of the urinary tract.

The *in vitro* sensitivity patterns of *Escherichia coli* in this study revealed that Ciprofloxacin, Pefloxacin and Tarivid were highly effective against it. They are relatively more expensive and less abused (Bishop and Shehu, 2016). Though *Klebsiella pneumoniae* was more susceptible to most of the antibiotics tested, it was rather resistant to two, namely Septrin (30µg) and Augmentin (30µg). It can be inferred that Septrin and Augmentin had no effect at all on both *Escherichia coli* and *Klebsiella pneumoniae* because they are commonly abused (Adekunle *et al.*, 2013).

Conclusion

Escherichia coli and *Klebsiella pneumoniae* were isolated and identified from the urine of apparently healthy Microbiology students of Ahmadu Bello University, Zaria with very low prevalence of 2.0% and 0.7% respectively. All the cases of the asymptomatic UTIs were found in the females of 16-20 years of age, but absent among the males.

The infections were recorded only in subjects who resided within the school hostels, used public latrines and water for cleaning up after defecation. All the infections were found only in those who cleaned up from the backward direction. It is important to maintain good personal hygiene when using public latrines among students. No sign/symptom was associated with the infections because the study subjects were all apparently healthy. However, most of the positive cases were from turbid urine samples. UTIs whether they are asymptomatic or not, require treatment to prevent complications that may arise.

Antibiotics to be used for treatment should be carefully selected and given in proper dosage to avoid antibiotic resistance. Both isolates were most susceptible to Ciprofloxacin (10µg), Pefloxacin (30µg) and Tarivid (10µg), but resistant to Septrin (30µg) and Augmentin (30µg).

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