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Detection and Antibiogram of Urinary Tract Infection among Out-patients Attending a Tertiary Health Facility in Port-Harcourt, Southern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author PIO designed the study, wrote the protocol and the first draft of the manuscript. Authors GMI, UAO and UEE managed the sample collection and analyses of the study. Authors CLI and PCM managed the literature searches and performed statistical analysis. All the authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to explore microbial pathogens implicated in urinary tract infection (UTI), their antibiotic susceptibility and multi-drug resistant patterns as prevalent in UTI symptomatic and asymptomatic outpatients attending clinic at University of Port-Harcourt teaching hospital. One hundred mid-stream urine samples were collected from consented patients and transported to the microbiology laboratory of University of Port-Harcourt where it was analyzed using standard microbiological methods. A total of 27(27.0%) UTI isolates were obtained with *E. coli* 7(7.0%), *K. pneumoniae* 5(5.0%), *C. albicans* 4(4.0%), *S. aureus* 3(3.0%), *P. aeruginosa, H. alvei* and *P. mirabilis* 2(2.0%) respectively and *Klebsiella oxytoca* and *Enterococcus faecalis* 1(1.0%)

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respectively. There was a preponderance of UTI cases in female patients than male patients and people within the reproductive age of 25-34 years constituted the most risk group. Of the eight antimicrobials tested, high susceptibility pattern was observed for Imipenem 19(82.6%), Gentamycin 14(60.9), Ampicillin and Erythromycin 12(52.2%) respectively, while the least susceptibility pattern was observed in Ciprofloxacin 9(39.1%). All but one of the isolates from *E. coli* and *K. pneumoniae* respectively, were multi-drug resistant, while all the *S. aureus* and *P. mirabilis* isolates were multi-drug resistant. One isolate from *E. faecalis* and *H. alvei* respectively were multi-drug resistant while no multi-drug resistance was obtained from *K. oxytoca* and *P. aeruginosa* isolates. Prevention of UTI in the study area can be achieved through good personal hygiene, while its empirical treatment can be achieved with the use of Imipenem, Gentamycin, Ampicillin and Erythromycin as revealed in this study.

Keywords: Urinary tract infection; susceptibility testing; multi-drug resistance; out-patients.

1. INTRODUCTION

Urinary tract infections (UTI) are caused by the multiplication, presence and growth of microorganisms in the urinary tract of an individual. They are seen as the single most common bacterial infections affecting humans [1]. The urinary tract of humans is composed of organs that collect, store and discharge urine. These organs include kidneys, bladder, ureter and urethra and are found within the urine flow line path [2]. The urinary tract infection is a common clinical infection encountered in so many established hospitals in the world, with millions of people getting infected yearly [3].

Urinary tract infection (UTI) is characterized by bacterial invasion and multiplication involving the kidneys and urinary tract pathways, after their strong natural defenses have been compromised [4]. Urinary tract infection has emerged over the years as one of the most common nosocomial infections, accounting for about 35% of hospital-acquired infections and second most common cause of bacteremia in hospitalized patients [5]. Severe infections among patients can lead to irreversible damage to the kidney, which can result to renal hypertension and renal failure depending on how challenging the case is [6].

Urinary tract infection is the most prevalent bacterial infection in women and occurs three times or more frequently in women than their male counterparts [7]. They occur mostly between the ages of 16-35 years in women, with over 10% of them being infected yearly and 60% having the infection at one stage of their lives [8]. The short urethra in females and its proximity to the vagina and anus places it at high risk of getting infected [9]. Other factors include their inability to empty their bladder completely, the physiological increase in plasma volume and decrease in urine concentration during pregnancy which leads to glycosuria in about 70% of women, encouraging bacterial growth in urine [10].

The pathogenesis of UTI starts with urethral colonization by uropathogens from the anus. This is accompanied by an upward movement (ascension) through the urethra into the bladder. When they get to the kidney through the ureter, pyelonephritis occurs. Most common pathogens usually isolated from UTI patients include *Escherichia coli, Staphylococcus aureus, Klebsiella* spp, *Pseudomonas aeruginosa, Proteus* spp, *Streptococcus faecalis, Enterococcus* sp, *Enterobacter* spp, among others [4].

The degree of occurrence and prevalence of these organisms are dependent on the environment of the patients and personal hygiene [1]. Thus, this study reports the degree of occurrence of urinary tract infection, their antibiogram and multi-drug resistance among out patients in a tertiary health facility in Port Harcourt and by extension, inhabitants of Port Harcourt city.

2. MATERIALS AND METHODS

2.1 Study Area

Port Harcourt is the capital and largest city of Rivers state of Nigeria. It lies along the Bonny River and is located in the Niger Delta. It has an estimated population of 1,865,000 inhabitants (Census, 2006). It features a tropical wet climate with lengthy and heavy rainy seasons and very short dry seasons. Only the months of December and January truly qualifies as dry season months in the city. The average temperature of the city is 25℃-28℃. It has a longitude and latitude of

4.8156℃N and 7.0498℃E, with an elevation of 5 meter or 16.4 feet.

2.2 Study Population

A total of 100 urine samples were collected from asymptomatic and symptomatic out-patients aged 15-60 years visiting the clinic.

2.3 Ethical Clearance

An Ethical approval letter was obtained from the Ethical review board of the hospital before carrying out the study, while informed consent was obtained from the patients before they were enlisted into the study.

2.4 Sample Collection

Sterile containers were used to collect mid-stream urine from patients, which was labelled appropriately and transported in an ice pack to the Microbiology Laboratory of University of Port-Harcourt for Analysis.

2.5 Sample Analysis

The obtained urine samples were subjected to urine Microscopy test to detect presence of ten or more pus cells per high power focus using X40 objective lens. All samples with significant bacterial count and ten pus cells and above were taken to the culture bench for sample culturing.

Ten-fold serial dilution was made by transferring 1.0 ml of the sample into a 9.0 ml sterile physiological saline. One ml of the diluent was then inoculated onto a Nutrient agar and swirled gently to allow for even homogenization. This was later incubated at 37℃ for 24 hours. Growth colonies on the Nutrient agar were enumerated to determine urine samples with significant bacteriuria after incubation.

A calibrated wireloop method was used for culture and isolation of uropathogens. A sterile wire loop that holds 0.002 ml of urine sample (1/500 ml per mid-stream urine sample) was inoculated onto a Sabouraud Dextrose agar, Cysteine Lactose Electrolyte Deficient Agar (CLED) and Blood Agar, which was incubated at 37℃ for 24 hours. Plates with growth colonies were selected; the number of bacterial colonies were counted and multiplied by 500. This is to achieve an estimate number of bacteria per milliliter of the urine sample. A significant bacteriuria was taken as any count ≥ 10^5 per milliliter. The selected colonies were isolated and stored in agar slants, which was refrigerated until when required for further analysis.

2.6 Bacterial Identification

Twenty-four hours growth culture of the isolated stored colonies was subjected to Gram reaction. The Gram negative bacteria were further identified using a Microbact 24 E (Oxoid UK) system, while the Gram positive bacteria were further subjected to Catalase, Coagulase and DNase test for identification. Isolates suspected to be *Enterococcus* sp were sub cultured on Bile Esculin agar and further identified.

2.7 Antimicrobial Susceptibility Testing

Isolates were subjected to susceptibility testing by the conventional Kirby-Bauer disc diffusion method using Mueller-Hinton Agar plates prepared following standard procedures. The results were interpreted according to the CLSI guidelines (2014). The antibiotics used were Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Erythromycin (5 µg), Gentamycin (10 µg), Cotrimoxazole (1.25 µg/23.75 µg), Ampicillin (10 µg), Cefuroxime (30 µg) and Imipenem (10 µg).

2.8 Multi-drug Resistance Detection

UTI isolates which were resistant to three or more classes of the antimicrobial agents used were regarded as multi-drug resistant isolates.

2.9 Data Analysis

Demographic variables and frequency of UTI occurrence was analysed using SPSS version 17.0.

3. RESULTS

The age and sex distribution of patients is shown on Table 1. The age group 25-34 years had the highest number of patients recruited into the study, amounting to about 37% of the total participants. While the age group ≥45 years had the least number of subjects recruited into the study, which was about 14% of the total participants.

The frequency of UTI among patients by age and sex is represented on Table 2. Of the 27(27.0%) of patients identified with UTI, the age group 25-34 years had the highest number of infected patients 11(32.4%), closely followed by the age

group 35-44 years with a prevalence of 32.0%. The age group ≥45 years had a prevalence of 28.6% while the age group 15-24 years had the least occurrence of UTI with 14.8%. *Escherichia coli* 7(7.0%) was the most prevalent organism, followed by *Klebsiella pneumoniae* 5(5.0%), while *Klebsiella oxytoca* and *Enterococcus faecalis* were the least prevalent organisms with 1(1.0%) respectively. The prevalence of *C. albicans* was 4(4.0%), *S. aureus* 3(3.0), while *P. aeruginosa, H. alvei* and *P. mirabilis* were 2(2.0%) respectively. The female patients had a higher prevalence of 21(42.0%) when compared to their male counterpart with 6(12.0%).

The antimicrobial susceptibility pattern of the bacteria isolates is shown on Table 3. Of the eight antimicrobials tested on the 27 isolates, high susceptibility pattern was observed for Imipenem 19(82.6%), Gentamycin 14(60.9), Ampicillin and Erythromycin 12(52.2%) respectively. while a low susceptibility pattern was observed in Cefuroxime 11(47.8%), Cotrimoxazole and Ceftriaxone 10(43.5%) respectively and Ciprofloxacin 9(39.1%).

Table 1. Age and sex distribution of patients

Age group (years)	Sex		Total (%)
	Male (%)	Female (%)	
15-24	15(30.0)	12(24.0)	27
25-34	13(26.0)	21(42.0)	34
35-44	14(28.0)	11(22.0)	25
≥45	8(16.0)	6(12.0)	14
Total	50	50	100

Table 2. Frequency of UTI among patients by age and sex

Item	No. tested (n=100)	UTI *(%)* (n=27)	A	B	C	D	E	F	G	H	I
Age group (years)											
15-24	27	4(14.8)	2(7.4)	-	-	-	-	1(3.7)	-	-	1(3.7)
25-34	34	11(32.4)	2(5.9)	1(2.9)	1(2.9)	1(2.9)	1(2.9)	2(5.9)	1(2.9)	2(5.9)	-
35-44	25	8(32.0)	2(8.0)	1(4.0)	1(4.0)	-	-	1(4.0)	1(4.0)	2(8.0)	-
≥45	14	4(28.6)	1(7.1)	1(7.1)	-	1(7.1)	-	1(7.1)	-	-	-
Total	100	27(27.0)	7(7.0)	3(3.0)	2(2.0)	2(2.0)	1(1.0)	5(5.0)	2(2.0)	4(4.0)	1(1.0)
Sex											
Male	50	6(12.0)	2(4.0)	2(4.0)	-	-	-	1(2.0)	1(2.0)	-	-
Female	50	21(42.0)	5(10.0)	1(6.0)	2(4.0)	2(4.0)	1(2.0)	4(8.0)	1(2.0)	4(8.0)	1(2.0)
Total	100	27(27.0)	7(7.0)	3(3.0)	2(2.0)	2(2.0)	1(1.0)	5(5.0)	2(2.0)	4(4.0)	1(1.0)

Key: A = E. coli, B = S. aureus, C = P. mirabilis, D = H. alvei, E = K. oxytoca, F = K. pneumoniae, G = P. aeruginosa, H = C. albicans, I = E. faecalis

Table 3. Antimicrobial susceptibility pattern of the bacterial isolates (N=23)

Organisms isolated	No. isolated	CIP	CN	E	CEX	CEF	SXT	AMP	IMP
E. coli	7	2(28.6)	4(57.1)	4(57.1)	3(42.9)	4(57.1)	1(14.3)	3(42.9)	6(85.7)
S. aureus	3	1(33.3)	2(66.7)	2(66.7)	2(66.7)	-	2(66.7)	-	1(3.33)
P. mirabilis	2	2(100.0)	1(50.0)	-	1(50.0)	-	1(50.0)	1(50.0)	2(100.0)
H. alvei	2	1(50.0)	2(100.0)	2(100.0)	2(100.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)
K. oxytoca	1	1(100.0)	1(100.0)	1(100.0)	-	1(100.0)	-	1(100.0)	1(100.0)
K. pneumoniae	5	1(20.0)	1(20.0)	2(40.0)	1(20.0)	2(40.0)	2(40.0)	3(60.0)	4(80.0)
P. aeruginosa	2	1(50.0)	2(100.0)	1(50.0)	1(50.0)	2(100.0)	2(100.0)	2(100.0)	2(100.0)
E. faecalis	1	-	1(100.0)	-	1(100.0)	-	1(100.0)	1(100.0)	1(100.0)
Total	23	9(39.1)	14(60.9)	12(52.2)	11(47.8)	10(43.5)	10(43.5)	12(52.2)	19(82.6)

Key: CEF = Ceftriaxone, CIP = Ciprofloxacin, E = Erythromycin, CN = Gentamycin, SXT = Cotrimoxazole, AMP = Ampicilin, CEX = Cefuroxime, IMP = Imipenem

The multi-drug resistant profile of the UTI isolates is represented on Table 4. Of the seven *E. coli* isolates, all but one was multi-drug resistant. The highest resistance was seen in isolates E, F and G, being resistant to 6 antimicrobial agents. All the *S. aureus* and *P. mirabilis* isolates were multi-drug resistant. None of the *K. oxytoca* and *P. aeruginosa* isolates was multi-drug resistant. Only one isolate of *E. faecalis* and *H. alvei* was multi-drug resistant while four out of the five isolates of *K. pneumoniae* were multi-drug resistant.

4. DISCUSSION

This study was carried out to determine the microbial pathogens implicated in urinary tract infection with their individual antibiotic susceptibility patterns and multi-drug resistance as prevalent in UTI asymptomatic and symptomatic outpatients. Of the 100 samples analysed for bacteriuria in this study, 27(27.0%) had significant bacteriuria and fungi growth. About 73 of the samples had no significant growth. The reason for such result could be that some of the subjects were already on some antimicrobial therapy before coming to the clinic and as such the antimicrobials must have inhibited the growth and multiplication of the organisms [11].

The UTI prevalence of 27.0% as obtained in this study is similar to that obtained in three health facilities in Iran by Saffer et al. [12], but low when compared with 35.3% obtained in a study by Ebie et al. [13] in a rural community in South Eastern Nigeria and a 39.7% obtained in a military hospital by Oladeinde et al. [14] in western Nigeria. This variation could be as a result of the level of sanitary measures put up by the individuals in the study, and the sensitization level of UTI in the various study areas. Of the 27 isolated uropathogens, 21(42.0%) were obtained from female subjects, while 6(12.0) were obtained from their male counterpart. This is because asymptomatic Bacteriuria is very uncommon in young men, except in older men over the age of 65. In females, the shortness of their urethra which is about 1.5 inches compared to 8 inches in males, and its proximity to the anus coupled with decrease in estrogen level at menopause make them vulnerable to urinary tract infections more than their male counterpart [15].

The uropathogens obtained in this study include *Escherichia coli* 7(7.0%), *Klebsiella pneumoniae* 5(5.0%), *C. albicans* 4(4.0%), *S. aureus* (3.0%), *P. aeruginosa, H. alvei* and *P. mirabilis* were 2(2.0%) respectively, *Klebsiella oxytoca* and *E. faecalis* 1(1.0%) respectively. The isolation of *H. alvei* and *Klebsiella oxytoca* in this study is of special interest, as they have not been reported in previous related studies in the hospital. This could be as a result of the method of isolation and identification employed by previous researchers. *Escherichia coli* was the prominent uropathogen obtained in this study, followed by *Klebsiella pneumoniae.* This is similar to the result obtained from a previous study by Wariso et al. [16]. The high occurrence of *Escherichia coli* could be as a result of its large number in the human bowel and the possession of fimbrae (adhensins) used for adherence to the mucosal surfaces of the urinary tract [17]. *Staphylococcus aureus* was more in male subjects 2(4.0%) than in female subjects 1(2.0%). The reason for this result is not clear, but lack of circumcision, HIV infection and other individual life styles can predispose males to UTI [18].

In this study, UTI was highest within the age group 25-34 years 32.4%, followed by the age group 35-44 years 32.0%. The age group ≥45 years had a 28.6% occurrence while the age group 15-24 years had the least occurrence with 14.8%. These age brackets consist of teenagers, young people and elderly people. The high occurrence of UTI may be due to increased sexual activity and poor sanitary measures and hormonal changes (women) which predispose the subjects to the infections. This result is similar to that obtained from previous studies with high prevalence of UTI across the same age groups [19,13].

The classes of antimicrobials used in this study include Macrolides, Trimethroprim-Sulfonamides, Aminoglycosides, Carbapenems, Aminopenicillins, Cephalosporins and Fluoroquinolones. High susceptibility was observed in Imipenem 19(82.6%), Gentamycin 14(60.9%), Ampicillin and Erythromycin 12(52.2%) respectively. Low susceptibility was observed in Cefuroxime 11(47.8%), Cotrimoxazole and Ceftriaxone 10(43.5%) respectively, and Ciprofloxacin 9(39.1%). The low susceptibility observed in some of these antimicrobials can be attributed to frequent abuse and subsequent development of resistance to these antimicrobials; these drugs have been employed for the treatment of UTI for quite a while now and happened to be the most commonly used antibiotics in this environment.

Table 4. Multi-drug resistant profile of the UTI isolates

Isolates	No. of resistant drugs	Resistant drug combinations (N=8)
***Escherichia coli* (N=7)**		
A	2	CIP,E
B	3	CIP,CN,SXT
C	3	CIP,CEX,SXT
D	4	CIP,CEF,SXT,AMP
E	5	E,CEX,CEF,SXT,AMP
F	6	CN,E,CEX,CEF,SXT,AMP
G	6	CIP,CN,CEX,SXT,AMP,IMP
Staphylococcus aureus (N=3)		
H	4	CIP,CEF,AMP,IMP
I	5	CN, E, CEF, AMP, IMP
J	6	CIP,CEX,SXT,CEF,AMP,IMP
***Proteus mirabilis* (N=2)**		
K	4	CN,E,CEF,SXT
L	5	E,CEX,CEF,AMP,IMP
***Hafnia alvei* (N=2)**		
M	1	CIP
N	3	SXT,IMP,CEF
***Klebsiella oxytoca* (N=1)**		
O	2	CEX,SXT
***Klebsiella pneumonia* (N=5)**		
P	2	CN,CEX
Q	3	CIP,E,AMP
R	6	CIP,CN,E,CEX,CEF,SXT
S	6	CIP,CN,CEX,CEF,SXT,IMP
T	7	CIP,CN,E,CEX,CEF,SXT,AMP
***Pseudomonas aeruginosa* (N=2)**		
U	1	E
V	2	CIP,CEX
***Enterococcus faecalis* (N=1)**		
W	1	CIP,E,CEF

Key: A-W = Isolates from different subjects, CEF = Ceftriaxone, CIP = Ciprofloxacin, E = Erythromycin, CN = Gentamycin, SXT = Cotrimoxazole, AMP = Ampicilin, CEX = Cefuroxime, IMP = Imipenem

In sub-Saharan African countries, high resistance to commonly prescribed and administered agents is a common norm, because these agents can easily be purchased over the counter, administered for variety of clinical conditions and taken by individuals without prescription by a physician, while some are substandard antimicrobials; this may have influenced the low susceptibility rate observed in this study. Worthy of note, is the low susceptibility pattern to Ciprofloxacin 39.1% observed in this study. This agent has been abused by individuals in our community, as it forms better synergy with antimalarials in combating *Plasmodium* infections, which is endemic in this part of the world [20].

The UTI isolates obtained in this study exhibited a high level of multi-drug resistance, being resistant to three or more classes of the antimicrobials used [21]. All the *S. aureus* and *P. mirabilis* isolates were multi-drug resistant, while all but one isolate from *E. coli* and *K. pneumoniae* respectively, were multi-drug resistant. Only one isolate from *E. faecalis* and *H. alvei* were multi-drug resistant. High prevalence of multi-drug resistant strains is an indication that large populations of bacterial isolates have been exposed to several routine antimicrobials used in this environment. Multi-drug resistant *S. aureus* and *E. faecalis* from UTI isolates have been reported in previous studies with results similar to that obtained in this study [22,23,24].

5. CONCLUSION

In conclusion, to reduce incident of community acquired UTI and antimicrobial resistance, individuals should be sensitized on the possible causes and effects of UTI, through jingles, health talk shows or extensive health education

programs by Healthcare delivery agencies. Use of drug combinations for synergistic effects should be encouraged, as this will reduce the emergence of resistant strains within the population and provide for effective and holistic management of patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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