(RESEARCH ARTICLE)

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Prevalence and antibiotic susceptibility pattern of Uro-pathogens isolated from urine in a tertiary care hospital in Nigeria

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Abstract

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting. Urinary tract infection (UTI) is the most common health care -associated group of bacterial infection affecting humans in Africa. This study was done to evaluate the frequency of uropathogens isolated from urine and their susceptibility pattern in Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) Bauchi. A total of 373 urine samples from out-patients and hospitalized patients were studied. Samples were inoculated on Cystine Lactose Electolyte-deficient (CLED) agar. 165 isolates were obtained which were further identified by standard Microbiological methods. Antimicrobial Susceptibility pattern was studied by Kirby-Bauer's disc diffusion method. UTIs were found more common in females 122(73.9%). Among the 165 uropathogens isolated from patients with UTI, the commonest isolate was *E. coli* (29.1%) followed by *Klebsiella pneumoniae* (15.8%) with the least *Citrobacter spp*. 1(0.6%). The overall prevalence of UTI in this study is 44.2%. Multidrug resistance was found to be significantly (P<0.05) more in uropathogens (77%). Monitoring of antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with UTI.

Keywords: Antibiotic; Uropathogens; Multidrug Resistance; Susceptibility; UTIs

1. Introduction

Urinary tract infections (UTIs) remain the common infections diagnosed in outpatients as well as hospitalized patients. Urinary tract infection is the most common bacterial infection with a high rate of morbidity and financial cost (1). It also includes the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital acquired infections (1,2). The practice of antibiotic prophylaxis against urinary tract infection (UTI), with hospitalization reserved for severe or complicated cases, has led to changes in the nature and culprit uropathogens of community-acquired (CA) and hospital-treated UTI leading to inappropriate use of antibiotics and treatment failure (3). As such, current knowledge on antimicrobial susceptibility pattern of uropathogens is mandatory for appropriate therapy.

Urinary tract infection (UTI) is the second most common infectious presentation in community (4,5). Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars (6).

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The extensive use of antimicrobial agents have invariably resulted in the development of antibiotic resistance which, in recent years, has become a major problem worldwide (7).

2. Methodology

2.1. Study Area

Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Bauchi State, a referral center in northeastern Nigeria.

2.2. Study Design

The study was a hospital based, descriptive and cross-sectional

2.3. Study Population

Both out-patients and in-patients in ATBUTH were considered for the study

2.4. Sampling Method

Convenient (non-probability) Sampling

2.5. Specimen Collection

First urine passed by the patient was targeted; usually the mid-stream urine (10-20ml) was collected in sterile, dry, wide-necked, leak-proof, screw- capped universal bottle and transported immediately to the hospital medical microbiology laboratory.

2.6. Bacterial Identification

The specimen was inoculated on CLED agar, Blood agar, and Mac conkey in a culture plates. The plates were incubated at 37^0C for 24 hours. Preliminary identification of isolates obtained was carried out based on morphological features (texture, size, edge, elevation, odor, hemolysis, color) and chemical reaction (gram reaction) in accordance to microbiological standards (17). In addition, biochemical tests (citrate test, urea test, catalase and coagulase tests, motility, and triple sugar ion were performed on gram negative isolates using the standard procedures described by (17). The isolates were further characterized based on the Bergey's manual of systemic bacteriology.

2.7. Antibiotic Susceptibility testing (agar diffusion method)

Kirby-Bauer disk diffusion method was employed. Discrete colonies were picked and emulsified in 3ml sterile aqueous normal saline. The suspension optical density is standardized to a McFarland density of 0.5 with the aid of a Densi ChekTM densitometer (bioMerieux, USA) apparatus. The suspensions were used within 15 minutes of standardization. Dry, sterile, absorbent cotton wool was dipped into the standardized suspension and excess moisture drained by pressing the wet cotton wool against the walls of the test tube. A second swab stick was then dipped into the suspension and then used to streak the surface of a Mueller-Hinton agar plate, which was earlier poured to a uniform depth of 5 mm and dried in the incubator for 15 minutes to reduce excess moisture. The inoculated plates were allowed to stand for 5 minutes, and the antibiotic susceptibility discs were placed on the inoculated Mueller-Hinton agar plate. The plates were then incubated aerobically at 37°C for 16 hours. After overnight incubation, the zone of inhibitions were measured with the aid of a meter rule in two directions across each inhibition zone and the results were averaged and recorded. CLSI 2024 guidelines for interpretative criteria for susceptibility to antibiotics were adopted. The antibiotic discs used in this study include Amoxicillin (30ug), Augmentin, (25ug), Chloramphenicol (30ug), (25ug), Cotrimoxazole (30ug), Gentamicin (10ug), Ciprofloxacin (10 μg), Pefloxacin (10μg), Streptomycin (30μg), Ampiclox (30μg), Zinnacef (20 μg), Cefotaxime (30μg), Ceftazidime (30μg).

2.8. Data Analysis

Data collected was recorded into a computer and analyzed using statistical package for social sciences version 17.0 (SPSS Chicago III, USA). Results were presented when necessary, as tables, figures and photographs.

2.9. Ethical Consideration

The study was reviewed and approved by the ethical review committee of ATBUTH.

3. Results

Of the 373 urine samples collected for the study, 99(60%) were from the outpatients while the inpatients constituted 66(40%) as shown in table 1. Table 2 shows the distribution of urine samples in relation to age and gender of the patients, with females having the highest frequency (64.9%) while males have the least with (35.1%). The age bracket of 25-30 had the highest number of samples collected followed by 17-24, with the least being 0-16 (6.7). Out of the 373 urine samples collected 165(44.2%) uropathogens were obtained. Most of the uropathogens were isolated from the female patients (73.9%), while the uropathogens isolated from the male patients constituted 26.1%. In addition the age group 25-32 has the highest distribution rate of 21.0% of the uropathogens, followed by 33-40 (20.0%), with 0-16 age group having the least occurrence rate of the uropathogens isolated. Of the 165 (44.2%) isolates were Escherichia coli ranked highest 48(29.1%), others were *Klebsiella pneumoniae* 26 (15.8%), *Klebsiella oxytoca* 25 (15.2%), *Proteus mirabilis* 19 (11.5%), *Proteus vulgaris* 5 (3.0%), *Pseudomonas aeruginisa* 4(2.4%), *Enterobacter spp* 2 (1.2%) and *Citrobacter spp* 1 (0.6%). Of the gram-positive bacterial isolates, Coagulase-negative Staphylococcus was 21 (12.7%), *Staphylococcus aureus* 10 (6.1%), and *Candida albicans* 4 (2.4%) (Table 4). Table 5 shows the antimicrobial susceptibility test of gram-negative isolates. It showed that most of the *E. coli* were resistant to amoxicilin and Ceftazidime (54.2%), pefloxacin (50.0%). In addition, 41.7% were sensitive to both Augmentin and gentamycin respectively. 37.5% of the *E. coli* were sensitive to Ciprofloxacin, streptomycin (35.5%) 15(31.3%), and cotrimoxazole. Of the *Klebsiella pneumoniae* isolates 68.8% resistance to cotrimoxazole and Augmentin respectively, 54.5% were resistant to cefotaxime, 45.5% to ceftazidime, 27.3% to amoxicillin and 72.2% to ciprofloxacin. However, 27.3% were sensitive to cefotaxime, pefloxacin, and amoxicillin respectively*. Proteus mirabilis* sensitive to ciprofloxacin, gentamycin amoxicillin, and ceftazidime were 3(15.8%), 5(26.3%), 8(42.1), and 4(21.1) respectively. Least susceptibility was to ciprofloxacin (57.9%), 42.1% to gentamycin, and 68.4% to third-generation cephalosporins (cefotaxime). *Pseudomonas aeruginosa* isolates were resistant to cotrimoxazole, amoxicillin, ceftotaxime and ceftazidime 4(100%), Chloramphenicol 2(50.0%), pefloxacin 3(75.0%), but 1(25.0%). *Citrobacter spp* was resistant to cotrimoxazole, chloramphenicol, ciprofloxacin, and augmentin 1 (100%) respectively. Likewise, it was observed that the isolates were sensitive to cefotaxime and ceftazidime 1(100%).

The gram-positive bacterial isolates showed varying susceptibility patterns 11(52.4%) Coagulase-negative Staphylococcus isolates were found to be resistant to cotrimoxazole, ciprofloxacin, and ampiclox respectively. 3 (14.3%) were sensitive to rocephin, amoxicillin and ciprofloxacin respectively. Of the 10 Staphylococcus aureus isolates 50.0% were resistant to rocephin, and Pefloxacin. 60.0% were resistant to augmentin with 90.0% of the isolate resistant to cotrimoxazole. The study observed reduced resistance to augmentin, amoxicillin and rocephin 4(40.0%).

Table 1 Out Patient and In-patient distribution of Patients

Source	No. Of Samples n=373	No.Positive (%)
Outpatient	224	99(60.0)
Inpatient	149	66(40.0)
Total	373	165

Table 2 Distribution of samples in relation to age and sex

Age group	Sex		Total
	Male	**Female**	
0-16	9(6.9)	16(6.6)	25(6.7)
17-24	25(19.1)	45(18.5)	70(18.8)
25-32	28(21.3)	47(19.4)	75(20.1)
33-40	18(13.7)	38(15.7)	56(15.0)
41-48	12(9.2)	27(11.2)	39(10.5)
49-56	20(15.3)	31(12.8)	51(13.7)

57-64	9(6.9)	19(7.9)	28(7.5)
>64	10(7.6)	19(7.9)	29(7.8)
Total	131(35.1)	242(64.9)	373

Table 3 Distribution of bacterial isolates in relation to patient demography

Demographic details	No. Of Samples collected (n=373)	No. Of bacterial isolate (n=165)	Percentage (%)
Gender			
Male	131	43	26.1
Female	242	122	73.9
Age			
0-16	25	08	5.0
17-24	70	32	19.0
25-32	62	35	21.0
33-40	56	33	20.0
41-48	45	17	10.0
49-56	40	19	12.0
57-64	36	9	6.0
>64	39	12	7.0

Table 4 Distribution of uropathogen isolates

Isolate	No.Isolated n =165	% Occurrence
Citrobacter spp	1	0.6
Escherichia coli	48	29.1
Enterobacter spp	2	1.2
Klebsiella oxytoca	25	15.2
Klebsiella pneumonae	26	15.8
Proteus mirabilis	19	11.5
Proteus vulgaris	5	3.0
Pseudomonas aeruginosa	4	2.4
Staphylococcus aureus	10	6.1
Coagulase negative (CON)Staph.	21	12.7
Candida albican	4	2.4

Table 5 Antimicrobial susceptibility pattern gram-negative bacteria isolated

Bacteria	Pattern	SXT no. (%)	CH no.(%)	CPX no. (%)	AU no. (%)	CN no.(%)	AM no. (%)	PEF no. (%)	S no. (%)	CTX no. (%)	CAZ no. (%)
E .coli (n=48)	S	15(31.3)	4 (8.3)	18(37.5)	20(41.7)	20(41.7)	16(33.3)	18(37.5)	17(35.4)	8(167)	9(18.8)
	I	3 (6.25)	20(41.7)	17(35.4)	8(16.7)	10(20.8)	6(12.5)	6(12.5)	11(22.9)	18(37.5)	11(22.9)
	R	30 (62.5)	24(50.0)	23(47.9)	20(41.7)	18(37.5)	26(54.2)	24(50.0)	20(41.7)	22(45.8)	26(54.2)
K.pneumoniae (n=22)	S	3 (13.6)	5(22.7)	2(9.1)	3(13.6)	8(36.3)	6(27.3)	2(90.1)	3(13.6)	4(18.2)	4(18.2)
	I	4 (18.2)	6(27.3)	4(18.2)	4(18.2)	7(31.8)	6(27.3)	6(27.3)	16(72.7)	6(27.3)	8(36.3)
	R	15(68.8)	11(50.0)	16(72.7)	15(688)	7(31.8)	10(45.5)	14(63.6)	3(13.6)	12(54.5)	10(45.5)
K.oxytoca (n=15)	S	2 (13.3)	4(18.2)	1(6.7)	2(13.3)	3(20.0)	2(13.3)	2(13.3)	1(6.7)	4(18.2)	3(20.0)
	I	4 (26.7)	5(33.3)	3(20.0)	2(13.3)	5(33.3)	4(18.2)	0(0.0)	3(20.0)	2(13.3)	4(18.2)
	R	9 (60.0)	6(40.0)	11(73.3)	11(73.3)	7(46.7)	9(60.0)	13(86.7)	11(73.3)	9(60.0)	8(53.3)
P.mirabilis (n=19)	S	10 (52.6)	6(31.6)	3(15.8)	5(26.3)	5(26.3)	8(42.1)	4(21.1)	7(36.8)	0(0.0)	4(21.1)
	I	7 (36.8)	4(21.1)	5(26.3)	4(21.1)	10(52.6)	3(15.8)	3(15.8)	3(15.8)	6(31.6)	3(15.8)
	R	2 (10.5)	9(47.4)	11(57.9)	10(52.6)	4(21.1)	8(42.1)	12(63.2)	9(47.4)	13(68.4)	12(63.2)
P.vulgaris (n=5)	S	2 (40.0)	3(60.0)	1 (20.0)	2(40.0)	1 (20.0)	1 (20.0)	0(0.0)	1 (20.0)	1 (20.0)	1 (20.0)
	I	1 (20.0)	0(0)	1 (20.0)	0(0.0)	2(40.0)	1 (20.0)	2(40.0)	0(0.0)	0(0.0)	0(0.0)
	R	2 (40.0)	2(40.0)	3(60.0)	3(60.0)	2(40.0)	3(60.0)	3(60.0)	4(80.0)	4(80.0)	4(80.0)
P.aeruginosa (n=4)	S	0(0.0)	1(25.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	I	0(0.0)	1(25.0)	1(25.0)	0(0.0)	1(25.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)
	R	4 (100)	2(50.0)	3(75.0)	3(75.0)	2(25.0)	4(100)	3(75.0)	3(75.0)	4(100)	4(100)
Citrobacter spp (n=1)	S	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1	1	1	1	0(0.0)	0(0.0)
	I	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100)	1(100)
	R	1 (100)	1(100)	1(100)	1(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Enterobacter spp (n=2)	S	0(0.0)	0(0.0)	1(100)	1(100)	1(100)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)
	I	1 (50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)
	R	1 (50.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	1(50.0)	2(100)	2(100)

SXT Cotrimoxazole; CH Chloramphenicol; CPX Ciprofloxacin; AM Amoxicillin; AU Augmentin; CN Gentamycin; PEF Pefloxacin; S Streptomycin; CTX Cefotaxime; CAZ Ceftazidime ; R= Resistant; S = Sensitive; I = Intermediate

Table 6 Antimicrobial susceptibility pattern of gram-positive organisms

Bacteria	Pattern	SXT no. (%)	CH no.(%)	CPX no. (%)	AU no. (%)	CN no.(%)	AM no. (%)	PEF no. (%)	S no. (%)	APX no. (%)	R no. (%)
CON Staph.(n=21)	S	0(0.0)	6(28.6)	3(14.3)	6(28.6)	4(19.4)	3(14.3)	4(19.0)	4(19.0)	5(23.8)	3(14.3)
	I	10(47.6)	5(23.8)	7(33.3)	8(38.1)	15(71.4)	6(28.6)	4(19.0)	3(14.3)	5(23.8)	3(14.3)
	R	11(52.4)	10(47.6)	11(52.4)	7(33.3)	3(14.3)	12(57.1)	13(61.9)	14(66.7)	11(52.4)	15(71.4)
S.aureus (n=10)	S	0(0.0)	7(70.0)	6(60.0)	0(0.0)	4(40.0)	2(20.0)	2(20.0)	0(0.0)	4(40.0)	1(10.0)
	I	1(10.0)	1(10.0)	0(0.0)	4(40.0)	3(30.0)	4(40.0)	3(30.0)	4(40.0)	2(20.0)	4(40.0)
	R	9(90.0)	2(20.0)	4(40.0)	6(60.0)	3(30.0)	4(40.0)	5(50.0)	6(60.0)	4(40.0)	5(50.0)

SXT Septrin; CH Chloramphenicol; CPX Ciprofloxacin; AM Amoxicillin; AU Augmentin; CN Gentamycin; PEF Pefloxacin; S Streptomycin; APX Ampiclox R Rocephin R= Resistant; S = Sensitive; I = Intermediate.

4. Discussion

This study revealed a prevalence of UTI of 44.2% in patients attending ATBUTH. This prevalence was found to be higher than the prevalence of the study carried out by Iregubu, (8) which showed 13%, but lower than the studies in Enugu (77.9%) and Yola (67.2). The variation in prevalence may be attributed to the differences in study populations and in the criteria used by centers in selecting urine samples for culture as most of the requests in this study came from the outpatient department which sees most of the cases coming in directly from the community. The study showed that UTI was more frequent in women (73.1%) than men (26.1) which agrees with previous studies (2,9,10). The higher frequency in females has been attributed to the shorter female urethra and the proximity of this to the gastrointestinal outlet, hence making it easier for enteric flora to colonize this area (11,12). Other contributory factors may include the use of contraceptives, childbirth, and menopause (3). The age group with the highest incidence was found to be among the sexually active age group. This finding is in line with that of, thus explaining the relatively high incidence rate within these age groups (9). The isolated pathogens in this study include both gram-positive and gram-negative bacteria. The gram-negative constituted the highest incidence (78.8%) as compared to the gram-positive (21.2%). This finding agrees with the reports of (2,9,10). *Escherichia coli* was the most frequently isolated common UTI pathogens (29.1%). This agrees with previous reports of (9,10) with incidences of 45.9% and 36.0%, respectively. The high prevalence of E. coli could be that it is the most common commensal organism. *K. pneumoniae* which was the second most common uropathogen isolated in this study is an indication that the organism is achieving more prominence as etiological agents of UTI than previously reported by (14). Coagulase-negative Staphylococcus with a prevalence rate of 12.4% constituted the highest incidence in gram-positive bacterial isolates. The frequency of antimicrobial resistance among microorganisms that cause UTI is increasing worldwide and is a major factor in selecting antibiotics for treatment. There are local variations in the antimicrobial susceptibility among urinary pathogens in different hospitals. The results of the Gram-negative antibiotic susceptibility test revealed varied susceptibility ranging from sensitive, intermediate, and resistant. All the Gram-negative organisms were variably resistant to Chloramphenicol, Ciprofloxacin, Augmentin, and Amoxicillin. The gram-positive drug susceptibility pattern showed high resistance to quinolones, aminoglycoside, and Cephalosporins. This finding is similar to that of (1,15,16). The resistance to these antibiotics may be attributed to the purchase of drugs over the counter, administration of inappropriate drugs in treating cases when no prior test is carried out, and misuse of drugs. Effective management of patients suffering from UTIs commonly relies on the accurate identification of etiological agents and the selection of an appropriate antimicrobial agent. The study showed that UTI is caused by both gram-positive and gram-negative bacteria, and they showed resistance to more than two classes of antibiotics, thereby termed multidrug resistance. This is a public health concern as the choice of drug for the treatment of UTI will be limited. The multidrug-resistant status of these isolates in this study indicates the possible production of resistance enzymes like extended spectrum beta lactamases (ESBLs) and carbapenemases. Therefore efforts should be made to screen of ESBL and Carbapenemase in ATBUTH.

5. Conclusion

The results of this study showed high rates of uropathogens isolated from urine with *E. coli* having the highest occurrence. In addition each of these isolates were showing differences in their rates of antibiotic susceptibility. The study also showed that the uropathogens isolated were resistant to different classes of antibiotics thereby confirming them as multi-drug resistant. This study hereby suggested that an antibiotic prescription formular should be developed in ATBUTH that will guide the usage of antibiotics, especially the third-generation cephalosporin for therapeutic purposes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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