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Prevalence and antibiotic susceptibility patterns of bacterial etiologies of urinary tract infections among students attending Sick-Bay of Ahmadu Bello University, Nigeria

Henry Gabriel Bishop, Fahad Shehu

ABSTRACT

Aims: Urinary tract infections (UTIs) affect people of all ages, genders and races. Urinary tract infections result from the invasion and colonization of tissues of parts of the urinary tract, with inflammatory responses. Asymptomatic cases occur but complicated cases are often discomforting. Treatment is necessary, but relies largely on appropriate diagnoses and antibiotic susceptibility testing. Hence, this research determined the bacterial etiologies of UTIs, their distribution and susceptibility to antibiotics amongst students presenting with UTI symptoms at Ahmadu Bello University Sick-bay, Nigeria. Methods: Mid-stream urine (MSU) samples were collected from 100 students with UTI symptoms and inoculated onto sterile plates of Cystine Lactos-Electrolyte-Deficient (CLED) agar and Mannitol Salt Agar (MSA). They were incubated at 37oC for 24–48 hours. The structured questionnaires were administered to the patients to gather some demographic data. Pure isolates were identified by Gram staining and biochemical characterization. The isolates were challenged with selected antibiotics. Results were statistically analyzed at p = 0.05. Results: Bacterial growths occurred from half (50) of the samples. The isolates comprised of 43(86.0%) and 7(14.0%) Gram-negative and Gram-positive bacteria respectively. The most prevalent uropathogen was *E. coli* (39%). Klebsiella pneumoniae (3%), *Pseudomonas aeruginosa* (1%), *Staphylococcus aureus* (7%) were also isolated. *E. coli* had higher occurrence among the females (22%). Age-group 26–30 years had the highest occurrences of *E. coli* and *S. aureus*, but *P. aeruginosa* occurred only in age-group 36–40 years. Bacterial UTI cases increased with increase in age, peaked at 26–30 years, and then gradually decreased in like fashion. All the isolates were most susceptible to ciprofloxacin (10 µg), oflaxacin (10 µg) followed by pefloxacin (30 µg) and chloramphenicol (30 µg). Gentamycin (10 µg) had the least activity against the isolates. Conclusion: Bacterial UTI cases increased with age, but after peaking at 26–30 years age group, they decreased continuously. *Escherichia coli* remains the predominant cause of UTIs. The most potent antibiotics against bacterial UTI-etiologies were ciprofloxacin (10 µg), oflaxacin (10 µg) followed by pefloxacin (30 µg) and chloramphenicol (30 µg). Gentamycin (10 µg) was the least effective against the isolates.

Keywords: Antibiotic, *E. coli*, *Escherichia coli*, Urinary tract infections (UTIs), UTI-etiology, Zaria

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INTRODUCTION

When bacteria invade any part of the urinary tract, they are capable of causing UTIs [1]. Among myriads of bacterial infections, UTIs are the most common [2–5]. Urinary tract infections affect all ages but its prevalence varies with age and sex [6]. Successful diagnosis depends on presence of symptoms and cultural isolation of the etiologies [2]. Women are generally more prone to UTIs than men [2] as well as patients with immunodeficiencies, urologic abnormalities and extremes of ages [6].

Escherichia coli (*E. coli*) is the most common cause of (80–90%) UTIs [5–7]. It can be caused by *Proteus mirabilis*, *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterobacter* spp., *Serratia* spp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus saprophyticus* and *Candida* spp. [6–8]. Where the anterior of the urinary tract was infected (causing urethritis), *Neisseria gonorrhoeae* (with predominance in men), staphylococci, streptococci and chlamydiae have been implicated [7]. *Salmonella typhi* and *Salmonella paratyphi* can be recovered from urine, especially in typhoid carriers who shed the bacteria for many years, even commonly in endemic regions of schistosomiasis [7].

The occurrence of $>10^5$ significant bacteriuria in fresh or boric acid-preserved urine [7] is not a necessary indication of serious UTI in an individual [9]. Hence, such an individual may be asymptomatic with no overt pains or inflammation [10].

Antimicrobial resistance among these uropathogens is a threat for increasing economic burden [8], which has been estimated at $1.6 billion [2] to $6 billion [11] annual cost of treatment. However, catheters-associated UTIs are the most prevalent cases of nosocomial infections [2] of 40% due to formation of biofilm [11]. Urinary tract infections are divided into two: lower UTIs (including cystitis, urethritis, prostatitis), and upper UTI (including pyelonephritis, intra-renal abscess, perinephric abscess) [5, 6].

UTIs can either be complicated or uncomplicated [6]. Men <50 years have rare cases of bacteriuria but UTIs increase in men above that age. Women are more frequently affected by UTIs [5, 7] because of the shortness of their urethras; they remain prone to both asymptomatic and symptomatic UTIs during pregnancy [7]. About one in every three women by the age of 24 will have developed UTI [2] and many of the uncomplicated cases result in long-term sequalae or renal damage [12]. Over 150 million global cases of UTIs occur annually [12]. Generally, half of all women will suffer from at least one symptomatic UTI in their lifetime [2,12] but only 20% of all cases occur in men [12]. Uropathogens get into the urinary tract via hematogenous or ascending routes [5, 6]. A greater threat can be posed by undetected/untreated persistent or recurrent asymptomatic UTIs, which can lead to pyelonephritis and renal failures [7, 12] but they are not common [13]. Other bacteria are becoming emerging etiologies of UTIs, for example *Mycoplasma genitalium* [11].

In view of complications, community-acquired UTIs are often uncomplicated but almost all nosocomial UTIs are not only complicated but serve as the largest hospital reservoir of nosocomial antibiotic-resistant uropathogens [14]. It is important to correctly diagnose UTI and make appropriate choice of antibiotics for treatment.

MATERIALS AND METHODS

Study area

The research was conducted in Ahmadu Bello University (A.B.U.), Samaru Campus Zaria, Nigeria. The subjects were students (between 15–45 years) of the University that presented at the University Health Services (Sick-Bay) with urinary tract complaints. The University is located on latitude 11°15'N to 11°3'N and longitude 7°30'E to 7°45'E [15].

Urine sample collection

All students with urinary tract complaints were asked and guided on how submit 5 ml early morning MSU in sterile wide-mouth, screw-capped plastic sampling bottles as suggested by Cheesbrough [7]. The samples were transferred immediately for analysis at Bacteriology Laboratory in the Department of Microbiology, A.B.U. Zaria, Nigeria.

Cultural isolation of etiologies

Each of the samples was mixed by gently shaking before opening the cap. Aseptically, inoculations were made on prepared sterile plates of CLED agar [7] and MSA. All inoculated plates were incubated aerobically at 37°C for 24–48 hr and observations were taken thereafter. Distinct colonies were Gram-stained. Pure isolates were maintained on Nutrient Agar (NA) slants at 4°C for further laboratory investigations. Biochemical tests (including catalase, coagulase, indole, Methyl red (MR), Voges Proskauer (VP), citrate utilization and sugar fermentation in Triple sugar Iron (TSI) agar) were performed. All media were prepared using instruction manuals of their manufacturers, with isolation and biochemical tests procedures as suggested by Cheesbrough [7].

Antibiotic susceptibility testing

All the isolates were subjected to the following antibiotics: ciprofloxacin (10 µg), amoxicillin (30 µg), gentamycin (10 µg), pefloxacin (30 µg), and streptomycin (30 µg), chloramphenicol (30 µg) and oflaxacin (10 µg). Inocula were prepared according to 0.5 McFarland standards. Agar disc diffusion method was used and the zones of inhibition of the UTI isolates were measured to the nearest millimeter and compared with Clinical Laboratory Standard Institute standards [16] to determine their susceptibility patterns.

Statistical analysis

Demographic data collected during MSU samples collection together with laboratory findings were subjected to chi square ($\chi 2$) and lLikelihood ratio analyses with the IBM SPSS Statistics Version 21 at p=0.05. Results were simplified in tables and charts.

RESULTS

Out of 100 students (comprising of 57 females and 43 males) who presented with urinary tract complaints and submitted MSU samples, only 50 samples yielded bacterial growths after a 24–48 hr aerobic incubation at 37°C. Out of these positive-culture samples, 43 (86.0%) were Gram-negative bacteria and the remaining 7 (14.0%) were Gram-positive bacteria (Figure 1).

No bacterial co-infection was found. The most prevalent bacteria were *E. coli* (39.0%), followed by *Staphylococcus aureus* (*S. aureus*) (7%). *P. aeruginosa* (1.0%) was the least occurring UTI-etiology (Table 1). In both sexes, *E. coli* was the most occurring UTI-etiology. About 47.4% and 53.5% of female and male MSU samples respectively were culture positive, yielding higher occurrence of *E. coli* and *K. pneumoniae* amongst the females. There was no occurrence of *P. aeruginosa* as UTI-etiology in males. The male students had higher occurrence of *S. aureus* in their MSU samples (Table 2).

Age-group 26–30 years had the highest culture-positive MSU samples (Figure 2). In the same age-group, *K. pneumoniae* and *P. aeruginosa* were absent. Also, age-group 41–45 years coincided with the highest occurrences of *E. coli* and *S. aureus*. The occurrence of *E. coli* increased and fell continuously with successive age-groups (Table 3). Generally, the prevalence of bacterial UTI cases gradually increased with age of the students, peaked again at age-group 26–30 years, then decreased continuously in similar fashion (Figure 2).

E. coli showed highest susceptibility to ciproflaxicin (10 µg) followed by streptomycin (30 µg). Only about half of the isolates were susceptible to amoxicillin (30 µg) and gentamycin (10 µg). Out of the three *K. pneumoniae* isolates, none was susceptible to gentamycin (10 µg) but all were susceptible to ciprofloxacin (10 µg). The single *P. aeruginosa* isolate was susceptible to chloramphenicol (30 µg), ciprofloxacin (10 µg), pefloxacin (30 µg) and oflaxacin (10 µg), but it was not susceptible to amoxicillin (30 µg) and gentamycin (10 µg). Generally, all the isolates were most susceptible to ciprofloxacin (10 µg), oflaxacin (10 µg) followed by pefloxacin (30 µg) and chloramphenicol (30 µg). Gentamycin (10 µg) had the least activity against the isolates (Table 4).

DISCUSSION

Gram-negative bacteria are among the most predominant uropathogens of UTIs. This research uncovered a frequency of 43 (86.0%) of UTI cases caused by Gram-negative bacteria. Although in 90% of reported UTI cases from Nigeria, *E. coli*, *Proteus* spp. and *Klebsiella* spp. are most incriminated [17]. This research also incriminated *S. aureus* and *P. aeruginosa* with exception of *Proteus* spp. Of these Gram-negative bacteria, *E. coli* was the most predominant UTI-etiology with a prevalence of 39.0%. *E. coli* has been implicated by many studies to be the most common cause of bacterial UTIs [6]. *E. coli* has a rapid adaptation and colonization of the urinary tract. There had been a reported prevalence of 40.7% of *E. coli*, which had occurred less than *P. aeruginosa* and *S. aureus* [17]. Gram-positive bacteria occur in UTI cases but less frequently. *Staphylococcus saprophyticus* (*S. saprophyticus*) is found among young and sexually active women [6]. Although, *S. saprophyticus* was not isolated

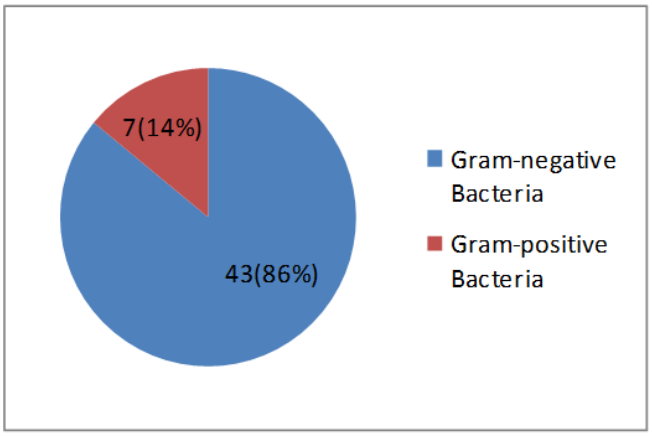

Figure 1: Relative frequencies of Gram-positive and Gram-negative bacteria as etiologies of Urinary tract infections.

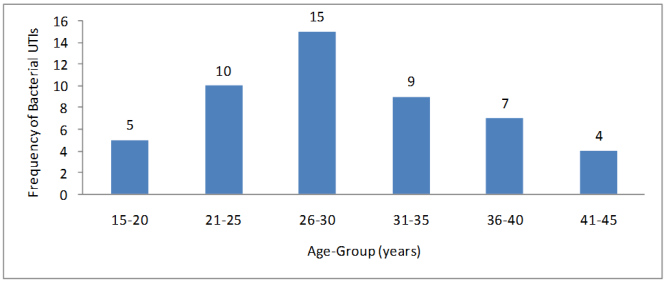

Figure 2: Bacterial Urinary tract infections cases distribution by age of students with Urinary complaints.

Table 1: Prevalence of bacterial uropathogens among students with urinary complaints (n=100)

Isolated Etiologies	Number of Positive Samples	Prevalence (%)
Escherichia coli	39	39.0
Staphylococcus aureus	7	7.0
Klebsiella pneumoniae	3	6.0
Pseudomonas aeruginosa	1	1.0
Total	50	50

Table 2: Gender distribution of Urinary tract infections etiologies among students presenting with urinary complaints

Gender	No. of Samples Examined	*Escherichia coli* Number (%)	*Klebsiella* pneumoniae Number (%)	Pseudomonas aeruginosa Number (%)	*Staphylococcus aureus* Number (%)	Culture-negative Samples Number (%)
Female	57	22 (38.6)	2 (3.5)	1 (1.8)	2 (3.5)	30 (52.6)
Male	43	17 (39.5)	1 (2.3)	0 (0.0)	5 (11.6)	20 (46.5)
Total	100	39 (39.0)	3 (3.0)	1 (1.0)	7 (7.0)	50 (50.0)

χ^2=3.366; df = 4; P = 0.499, LR = 3.744; df = 4; P = 0.442

Table 3: Distributions of Urinary tract infections etiologies by age of students with urinary complaints

Age Group (Years)	No. of Samples examined	*E. coli* Number (%)	*Klebsiella pneumoniae* Number (%)	*Pseudomonas aeruginosa* Number (%)	*Staphylococcus aureus* Number (%)	Culture-negative Samples Number (%)
15–20	11	4 (36.4)	0 (0.0)	0 (0.0)	1 (9.1)	6 (54.5)
21–25	22	8 (36.4)	1 (4.5)	0 (0.0)	1 (4.5)	12 (54.5)
26–30	35	12 (34.3)	0 (0.0)	0 (0.0)	3 (8.6)	20 (57.1)
31–35	17	8 (47.1)	1 (5.9)	0 (0.0)	1 (5.9)	7 (41.2)
36–40	9	4 (44.4)	1 (11.1)	1 (11.1)	0 (0.0)	3 (33.3)
41–45	6	3 (50.0)	0 (0.0)	0 (0.0)	1 (16.7)	2 (33.3)
Total	100	39 (39.0)	3 (3.0)	1 (1.0)	7 (7.0)	50 (50.0)

χ^2=18.521; df = 20; P = 0.553, LR = 14.344; df = 20; P = 0.813

Table 4: Antibiotic susceptibility patterns of bacterial isolates from Urinary tract infections cases among students of Ahmadu Bello University Zaria

Uropathogen	No. of Isolates	Percentage (%) susceptibility						
		CH (30 µg)	CIP (10 µg)	AM (30 µg)	GN (10 µg)	PEF (30 µg)	STR (30 µg)	OFL (10 µg)
E. coli	39	20 (51.3)	30 (76.9)	15 (38.5)	16 (41.0)	28 (71.8)	29 (74.4)	24 (61.5)
K. pneumoniae	3	2 (66.7)	3 (100.0)	1 (33.3)	0 (0.0)	2 (66.7)	1 (33.3)	3 (100.0)
P. aeruginosa	1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.)
S. aureus	7	5 (71.4)	7 (100.0)	5 (71.4)	5 (71.4)	6 (85.7)	5 (71.4)	6 (85.7)
Total	50	28 (56.0)	41 (82.0)	21 (42.0)	21 (42.0)	37 (74.0)	35 (70.0)	28 (68.0)

Key: CH Chloramphenicol, CIP Ciprofloxacin, AM Amoxicillin, GN Gentamycin, PEF Pefloxacin, STR Streptomycin, OFL Oflaxacin. Antibiotics applied Using Clinical Laboratory Standard Institute [16]

in this research, *S. aureus* tend to occur higher in the males than females and in the age-group 41–45 years. This age group is among the sexually active groups.

Urinary tract infections are causable by many different types of uropathogens. Bacterial cases examined in this research revealed a gradual pattern of increase with increase in age of the students. It peaked in age-group 26–30 years, which is among the sexually active individuals (though information of sexual activities was not captured). Among this age group, *E. coli* appeared to be the most prevalent, signifying it is important in the causation of UTIs. The implication of bacterial cases of UTI among these students could indicate the possible persistence of poor personal hygiene [17].

With the exception of *S. aureus* (as the only Gram-positive bacterial pathogen in this research), the Gram-negative bacterial isolates were more predominant in the female students. Invariably, females are general more prone to bacterial invasion and colonization of their vaginal and urethral openings, resulting in high frequency in urinary complaints and bacteria isolation in their MSU samples. The location and structure of the female urethral and vaginal openings are far less effective in the prevention of bacterial entry [18]. Unhygienic manner of wiping the anus from back to front helps in inoculating the vulva and vagina with fecal-borne pathogens. Pregnancy and sexual intercourse have been emphasized by [19] to increase risk of UTIs in females as bacteria are pushed into the vagina during sex. Glycosuria due to increased plasma volume and decreased urine concentration favors bacterial growth in urine [19]. During vaginal childbirth, bacteria can also be push up into the bladder [20]. Hence, females are at continuous risk of ascending UTIs with further possible complications [21, 22] like renal damage [12].

All the isolates were most susceptible to ciprofloxacin (10 µg) and pefloxacin (30 µg). These antibiotics are relatively expensive and hence less abused as outlined by [17]. However, gentamycin (10 µg) which is intravenously administered (and relatively cheaper) showed lesser activities against the isolates; hence, opposed the findings of [17].

CONCLUSION

Urinary tract infections, whether complicated or uncomplicated require immediate treatment. Treatment success is a measure of adequate diagnosis and selection of recommended antibiotics. Half of the MSU samples indicated that urinary complaints among the university students were due to bacterial uropathogens. Gram-negative bacteria (86.0%) are the most prevalent bacterial uropathogens. The frequency of the bacterial uropathogens increased with increase in age, peaked at 26–30 years age-group, and then began to decrease continuously. *E. coli* (39.0%) remains one of the leading UTI-etiologies, which was more prevalent among the male students and in people within age-group 41–45 years. *S. aureus* was frequently isolated among the males. All the demographic distributions of the bacterial uropathogens were statistically insignificant (as $p>0.05$). All the isolated UTI-etiologies were most susceptible to ciprofloxacin (10 µg) and pefloxacin (30 µg) but gentamycin (10 µg) was ineffective.

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Conflict of Interest
Authors declare no conflict of interest.

REFERENCES

1. Urinary tract infections. Bend urology. Assessed on 22nd January, 2016. [Available at: http://www.bendurology.com/urological-conditions/urinary-tract-infections]
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002 Jul 8;113 Suppl 1A:5S–13S.
3. Platt FW, Keating KN. Differences in physician and patient perceptions of uncomplicated UTI symptom severity: understanding the communication gap. Int J Clin Pract 2007 Feb;61(2):303–8.
4. August SL, De Rosa MJ. Evaluation of the prevalence of urinary tract infection in rural Panamanian women. PLoS One 2012;7(10):e47752.

5. Kattan JN, Gordon S. Acute uncomplicated Urinary Tract Infections. 2013. [Available at: http://www.clevelandclinicmeded.com/medicalpubs/diseasemanagement/infectious-disease/urinary-tract-infection/]
6. Sobieszczyk ME. Urinary Tract Infections. Division of Infectious Diseases, Columbia University. Assessed on 21th January, 2016. [Available at: http://www.columbia.edu/itc/hs/medical/pathophys/id/2009/utiNotes.pdf]
7. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 1, 2ed. Cambridge, UK: Cambridge University Press: 2006. p. 97–115.
8. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015 May;13(5):269–84.
9. Hanson LA, Kajser LL, Jodal Lidin U, Jonson Robine JB. Frequency of E. coli K antigens in UTI in Children. Lancent 1977;11:100–3.
10. Johnson C. Definition, classification and clinical presentation of UTI. The Medicine, Clinics of North America,1991;75(2):241–54.
11. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis 2001 Mar 1;183 Suppl 1:S1–4.
12. Grandy S, Fox KM, Hardy E. Prevalence and recurrence of urinary tract and genital infections among adults with and without type 2 diabetes mellitus in the general population: a longitudinal cohort study. Journal of Diabetes Research and Clinical Metabolism 2013;2(1):5.
13. Orskov I, Orskov F. Escherichia coli in extra-intestinal infections. J Hyg (Lond) 1985 Dec;95(3):551–75.
14. Wagenlehner FM, Naber KG. Current challenges in the treatment of complicated urinary tract infections and prostatitis. Clin Microbiol Infect 2006 May;12 Suppl 3:67–80.
15. Abbas II, Arigbede YA. Green area mapping of Ahmadu Bello University Main Campus, Zaria, Nigeria using remote sensing (Rs) and geographic information system (Gis) techniques. Journal of Geography and Regional Planning 2012;5(10):287–92.
16. Clinical Laboratory Standard Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24 2014;34(1).
17. Ehinmidu JO. Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. Tropical Journal of Pharmaceutical Research 2003;2(2):223–8.
18. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis 1999 Oct;29(4):745–58.
19. Arul KC, Prakasam KG, Kumar D, Vijayan M. A cross sectional study on distribution of urinary tract infection and their antibiotic utilization pattern in Kerala. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012;3(3):1125–30.
20. Lucas MJ, Cunningham FG. Urinary infection in pregnancy. Clin Obstet Gynecol 1993 Dec;36(4):855–68.
21. Ebie MY, Kandaki-Olukemi YT, Ayanbadejo J, Tanyigna KB. UTI Infections in a Nigerian Military Hospital. Nigerian Journal of Microbiology 2001;15(1):31–7.
22. Kolawole AM, Kolawole SO, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. International Journal of Medicinal and Medical Sciences 2009;5(1):163–7.