



Antimicrobial susceptibility of microorganisms isolated from patients with urinary tract infections in Ibadan, Southwestern, Nigeria

^aAkinleye Oludele Mathew, ^aDeji-Agboola A. Mopelola, ^aAlaka-Coker A. Aderonke, ^bAdebisi T. Ramoni, ^cSalako Oluremi Risikat, ^dAnorue Michael Chimaori and ^aOkoye A. Clement

^aDepartment of Medical Microbiology & Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria

^bDepartment of Community Health and Primary Health Care, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria

^cDepartment of Public Health Ministry of Health; Abeokuta-Ogun-State, Nigeria

^dFederal Teaching Hospital, Abakaliki Ebonyi State, Nigeria

ABSTRACT

Urinary tract infection (UTI) is one of the most common microorganisms infections encountered by clinicians in developing countries. Therefore, the present research was carried out to determine the antimicrobial susceptibility pattern of microorganisms causing urinary tract infections. This cross sectional study was carried out between January 2013 and June, 2014 in Oyo State, Nigeria. Out of 840 urine samples collected using the mid-stream "clean catch" method. Antimicrobial susceptibility test was performed for the isolated pathogens using Kirby-Bauer disk diffusion method according to clinical and laboratory standards institute guidelines. Of these samples 584 (69.5%) were reported to be positive with gram negative, gram positive and yeast with the following 414 (70.9%), 7122 (20.8%) and 48 (8.2%) respectively. Uropathogens most frequently isolated were *E.coli* (29.6%), *K.pneumoniae* (17.5%), *P.mirabilis* (14.4%), *P.vulgaris* (5.8%), *P.aeruginosa* (3.3%), *Staphylococcus aureus* (5.0%), *Enterococcus faecalis* (9.4%), *Coagulase-negative staphylococcus aureus* (6.5%) while yeasts were (8.2%). *E.coli* and *K.pneumoniae* showed the highest percentage of resistance to ampicillin and Gentamycin (98%) however, *S.aureus* and *coagulase negative staphylococcus* were resistant to ampicillin (100%) while all the gram positive organisms were susceptible to vancomycin and Teicoplanin. As drug resistance among microorganisms pathogens is an evolving process, routine surveillance to provide physicians knowledge on the updated and most effective diagnosis treatment of UTIs.

Key words: Urinary tract infection, Antimicrobial agents, Uropathogenic, sensitivity, Nigeria

INTRODUCTION

Among the most common infectious diseases, urinary tract infections (UTIs) are most commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million [1-2]. UTIs refers to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:- bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria) and also can asymptomatic or symptomatic UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated" whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities. Including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic [3-4]. It has been estimated that globally symptomatic UTIs result in as many as 7 million visit to out patient clinics, 1 million visit to emergency departments, and 100,000 hospitalizations annually [5].

Urinary tract infections are one of the most common types of bacterial infections in humans occurring both in the community and in health care settings and ranks high amongst the most common reason that compel an individual to seek medical attention[6-9]. UTIs encompass a spectrum of clinical entities ranging in severity from asymptomatic infections to acute cystitis, prostatitis, pyelonephritis and urethritis[10-11]. It represents one of the most common diseases encountered in medical practice today, affecting people of all ages, from the neonate to the geriatric age group[12]. Worldwide, about 150 million people are diagnosed each year with UTIs. Costing in excess of 6 billion dollars.[13].

Many different microorganisms can cause UTIs through the most common pathogens causing simple one in the community are *Escherichia coli* and other Enterobacteriaceae, which accounts approximately 75% of the isolates. In complicated urinary tract infections and hospitalized patients, organisms such as *Enterococcus faecalis* and highly resistant Gram-negative rods including *Pseudomonas* spp. are comparatively more common. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization[14].

There are urinary pathogen virulence factors that promote adherence to mucosal surface and subsequent infections[15]. Host factors such as the epithelial cell receptivity are also important in the infection process. Although, fungi and viruses are occasional etiological agents, UTIs are predominantly caused by bacteria. The most common bacteria implicated as intestine and include but not limited to *E. coli*, *Pseudomonas* spp, *Staphylococcus* spp, *Proteus* spp, *Klebsiella* spp, *Staphylococcus* spp, *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Candida* spp, *Mycoplasma*. Extremes of age, female gender, pregnancy, instrumentation, urinary tract (infection, 'neurologic dysfunctions, renal disease and expression of A, B and, H, blood group oligosaccharides on the surface of epithelial cell" are predisposing factors for the development of UTIs[8].

Treatment of UTIs cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens[5]. However, a large proportion of uncontrolled antibiotic usage has contributed to emergence of resistance bacterial infections[16-19]. As a result, the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide. Associated resistance i.e. the fact that a bacterium resistant to one antibiotic is often much more likely to be resistant to other antibiotics, drastically decreases our chance of getting a second empirical attempt right[20]. Resistance rates to the most common prescribed drugs used in treatment of UTIs vary considerably in different areas world wide. Therefore, the present research was carried out to determine the antimicrobial susceptibility of microorganisms isolated from patients with urinary tract infections over a period of 18 months between January 2013 and June, 2014 in Oyo South-Western Nigeria.

MATERIALS AND METHODS

1. Specimen Collection: The study was performed on UTI cases attending University College Hospital from January 2013 to May, 2014. A total of 840 clean catch midstream urine samples were collected in a wide mouth sterile container from the study subjects who have not received antimicrobials within previous fifteen days. Then the bacterial uropathogens were isolated and tested for antimicrobial drug resistance pattern.

2.2.1 Bacterial isolation and identification procedures: Isolation of Uropathogens was performed by a surface streak procedure on McConkery agar (Oxoid), Blood agar (Oxoid) and Cysteine Lactose Electrolyte Deficient agar (CLED International Diagnostic Group). Using calibrated wire loops for semi-quantitative method and incubated aerobically at 37°C for 24 hours, and those cultures which have been considered negative at the end of 24 hrs incubations were further incubated for 48 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/ml. Bacterial identification was made using biochemical tests, namely indole, citrate, oxidase, H₂S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, mannitol, fermentation and novobiocin susceptibility test (Bonadio et al; 2001). The Fungi isolated were identified using standard cultural and microscopic methods, and their susceptibility was measured on solid medium using the following disks: 5-Fluorocytosine (10ug), Fluconazole (15ug), itraconazole (10ug) and amphotericin B (10ug) (Rosco, Denmark).

2.2 Antimicrobial susceptibility testing :Antimicrobial susceptibility testing of isolates was tested for all 13 microorganisms uropathogens by the disk diffusion according to clinical laboratory standards Institute [17]. The antibiotic discs and their concentrations were: Amoxicillin (AML, 10ug), Ampicillin (AMP, 10ug), Ciprofloxacin (CIP, 5NG), Trimethoprim, sulphamethoxazole (SXT, 25Ng) Gentamicin (CN, 10ug), Ceftriaxone (CRO, 30ug), Nalidixic acid (NA, 30Ng), Ofloxacin (OFL, 10ug) and Nitrofurantoin (F, 300ug). All the antimicrobials used for the study were obtained from Oxoid Ltd.

Bashing store Hampshire UK A standard inoculums adjusted to 0.5McFarland was swabbed on to Muller-Hinton agar (Oxoid Ltd. Bashingstore- Hampshire, UK): antibiotic disc were dispensed after drying the plate for 3-5 min and incubated at 37°C for 24 hours. The reference strains used as control were *E.coli* (ATCC 25922), *S. aureus* (ATCC 25923) *P.aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212).

RESULTS

The demographic characteristic of the study showed that, the majority of them were (66.2%) living in urban area. Significant proportions were (65.2%) and (37.8%), female and male respectively. In the age categories of 19 to 39 years, 446 (53.1%), 517 (61.5%) married and 329 (39.2%) were either illiterate or read and write (Table 1). Out of 584 cultured urine specimens, Gram negative bacteria has the highest value 414 (70.89%) while Gram positive organisms were 124 (21.23%) and yeasts has lowest value 46 (7.88%) (table 2-4). The bacteria and isolated yeasts were *E.Coli* (29.6%), *K.pneumoniae* (17.5%), *P.mirabilis* (14.4%), *Enterococcus faecalis* (9.2%), Coagulase negative staph. (6.5%), *P.vulgaris* (5.8%), *S.aureus* (5.1%), *C.albicans* (4.8%), *P.aeruginosa* (3.3%), *C.glabrata* (1.9%), *candida spp* (0.9%), *C.tropicalis* and *Pseudomonas spp* were (0.3%) each respectively. The antimicrobial agents with the highest levels of activity against Gram-negative bacilli (Table 2), were Amikacin and Cefepime all of which are restricted to hospital use. Cefuroxime, Ciproflaxcin, Fosfomycin, Vancomycin, Teicoplanin, Nitrofurantoin and Ofloxacin, showed acceptable level of Nitrofurantoin was active against all strain of *S.aureus* (Table 3) and Fluconazole was active against the yeasts isolated (Table 4)

Table 1 Characteristics of study participants in university college hospital, Ibadan

Variables	Number	Percentage
Location	-	-
Urban	556	66.2
Rural	284	33.8
Sex	-	-
Male	292	37.8
Female	548	65.2
Age-categories		
Less or equal to 18 yrs	132	15.7
19 to 39 yrs	446	53.1
40 to 59 yrs	198	23.6
Greater than or equal to 60 yrs	64	7.6
Educational level		
Illiterate	202	24.1
Write and read only	127	15.1
Secondary	456	54.3
University level	55	6.3
Marital status		
Single	273	32.5
Married	517	61.5
Divorced	26	3.1
Widowed	24	2.9

Table 2: Percentage of Gram-negative Bacilli susceptible to various antimicrobial Agents

	E.coli	Kpneumoniae	P.mirabilis	P.vulgaris	P.aeruginosa	Pseudomonas
	(173) n(%)	(102) n(%)	(84) n (%)	(34) n(%)	(19) n(%)	(2) n (%) spp.
Ampicilin	18(3.5)	0(0)	52(62)	17(50)	-	-
Amo-clavulanate	105(61)	80(78)	79(94)	15(44)	-	-
Cefuroxime	145(84)	85(83)	82(98)	13(38)	-	-
Cefotaxime	161(93)	102(100)	81(96)	22(65)	1(5)	0(0)
Trimethoprim	60(35)	51(50)	40(48)	11(32)	1(5)	0(0)
Fosfomycin	171(99)	73(72)	65(77)	16(47)	-	-
N.acid	120(69)	95(93)	60(71)	15(44)	-	-
Nitrofurantoin	161(93)	65(64)	0(0)	2(6)	-	-
Ciprofloxacin	173(100)	102(100)	75(89)	25(74)	15(79)	2(100)
Amikacin	173(100)	102(100)	81(96)	33(97)	19(100)	2(100)
Ceftazidime	-	-	-	-	16(84)	2(100)
Ofloxacin	115(66)	60(59)	55(65)	25(74)	13(68)	1(50)
Gentamyan	45(26)	40(39)	41(49)	14(41)	6(32)	0(0)
Tobramycin	-	-	-	-	18(95)	2(100)
Cefepime	168(97)	102(100)	84(100)	32(94)	15(79)	2(100)

Table 3 Percentage of Gram-positive isolates susceptible to various antimicrobial agents.

	S.aureus (30)n (%)	Coagulase negative staphylococcus (38)n%	Enterococcus faecalis (54)n%
Ciprofloxacin	23(77)	23 (61)	-
Penicillin-G	2(7)	1(3)	46(85)
Oxacillin	26(87)	10(26)	-
Nitrofurantoin	30(100)	36(95)	-
Amoxicillin-clavulante	22(73)	9(24)	-
Trimethoprim sulphamethoxazole	29(97)	22(58)	-
Fosfomycin	19(63)	21(55)	-
Teicoplanin	30(100)	37(97)	52(96)
Ampicillin	-	-	50(93)
Gentamicin	22(73)	15(39)	46(85)
Rifampicin	29(97)	34(89)	-
Vancomycin	30(100)	37(97)	52(96)
Amikacin	25(83)	18(47)	-

Table 4 Percentage of yeast isolated in urine susceptible to various antifungal agents

	Calbicans (28)n(%)	C.glabrata (11)n(%)	C.tropicalis (2)n(%)	Candida spp. (7)n(%)
Fluorocytosine	28(100)	0(0)	2(100)	0(0)
Fluconazole	28(100)	11(100)	2(100)	7(100)
Amphotericin B	28(100)	11(100)	2(100)	7(100)
Itraconazole	28(100)	6(50)	2(100)	7(100)

DISCUSSION

Urinary tract infections (UTIs) have been a very major problem in the tropics. It is a common problem seen in community practice as well as in hospitals. In this study, researchers found a high prevalence of significant uropathogen microorganism in 584(69.5%) amongst the (UTI) patients attending U.C.H. Ibadan.

The gram-negative bacteria constituted the largest group with a prevalence of 414 (70.9%) followed by gram-positive bacteria constituted only 122 (20.8%) while yeasts has 48 (8.2%) of the total isolates. This value was much higher than the 60% reported for Lafia[9], 22% for Ibadan[21], 38.6% for Lagos[22], 35.5% for Jos [23] but lower than 77.9% for Enugu Mbata TI[24] and 74.7% for Yola El-Mahood;[25] all in Nigeria. Coincidentally, these are all large cities, being state capitals with high population densities. The high prevalence may be due to such factors like promiscuity, peer group influence, pregnancy, lower socio-economic status and non-hygienic environment which are common among Nigeria young men and women living in urban centres[9].

The various bacteria isolated from the urine samples were E.coli (29.6%), K.pneumonia (17.5%), P.mirabilis (14.4%), E.faecalis (9.2%), Coagulase negative staph. (6.5%), P.vulgeris (5.8%), S.aures (5.1%), C.albicans (4.8%), P.aeruginosa (3.3%), C.glabrata (1.9%), Candida spp (1.2%), CAropicals and Feudomonas spp.were (0.3%) respectively. These isolates clearly represented clinically significant pathogens, and are similar to the data obtained by El-Astal Z[26], in Palestine, EI-Sweih[8], in two large teaching hospitals in Kuwait, Mordi R.M and Erah P.O[27], in the university of Benin Teaching Hospital, Benin, Nigeria as well as Rai[28] in Nepal. The isolation frequency of the bacterial and fungi species reported in this study, falls within the range of frequencies reported in other countries such as Egypt,[29], China, Wang[30], Israel Turner D and Dagan R[31], Belgium, Goosen[32], Norway, Grude [33] and the United kingdom,(Hosein and Farrell[34-35]. E. coli has the highest prevalence (29.6%), closely followed by K.prenmoniae (17.5%) and this is similar to the reports of, Mordi RM and Erah PO[27] but differs with that of, Okesola AO and Oni AA[21,36-37] reported the prevalence of E. coli (52.3%), Enterococcus spp. (12.5%), Klebsiella spp. (7%), Proteus spp. (6.8%), P. aeruginosa (6.3%), S. aureus (1.3%), Citrobacter spp. (1.6%), Acionobacter spp. (1.3%), Serratia spp. (1.6%)and M.morgani (1.0%).

Although E.coli was the most common uropathogen in this study, there is a difference in its prevalence rates when compared with other reports, which gave a higher prevalence rate of 60-90% of E. coli, than other isolates[26,37-38]. Results from several studies have shown that the proportion of E.coli as a principal causative agent of UTIs is slowly declining, being replaced by other members of Enterobacteriaceae and Enterococcus[39-40]. Winstanley [41] reported a higher incidence of Proteus spp. Klebsiella spp. Enterobacter spp; citrobacter spp; Acinetobacter spp; Serratia spp. Enterococi and Pseudomonas in the isolates along sides with E.coli. This changing spectrum of microorganisms causing UTIs and the emerging resistance to many of the older and cheaper antimicrobial agents require continuous monitoring E.coli was observed to be a sensitive to almost all the antibiotics tested, though to varying degrees as shown in Table 2. E.coli was the most prevalent bacteria with a susceptibility of 61% Amo-

clavulanate), 93% (Cefotaxime), 84% (cefuroxime), 351% (trimethoprim) 99% (Fosfomycin), 69% (N.acid), 93% (Nitrofuratoin); 100% (ciprofloxacin); 100% (Amikacin); 66% (Ofloxacin) 45% (Gentamycin) and 97% (Cefepime).

The high rate of resistance to ampicillin; gentamicin and Trimethoprim observed in this study may reflect the fact that these are the most commonly prescribed antibiotics in the hospital and also the most easily available in the community without prescription.

The susceptibility profile of *K.pneumoniae* was 78% (Amo-clavulanate), 83% (cefuroxime), 51% (Trimethoprim), 73% (Fostomycin), 93%, (N.acid), 59% (ofloxacin), 39% (Gentamycin and resistance (Ampicillin), similar to the reports of Kumari and El-Astal Z[26&42]. The results of this study showed that most of the gram positive pathogens isolated were susceptible to almost antibiotics except only penicillin G (table 3). Researcher found that the percentage of yeasts isolated in urine susceptible to various antifungal agent.

CONCLUSION

It is necessary to continue monitoring the; resistance of strains of pathogens isolated from patients with UTI. As drug resistance among microorganisms pathogens is an evolving process, routine surveillance to provide physicians knowledge on the updated and most effective diagnosis treatment of UTIs.

REFERENCES

- [1] Ronald AR, Nicole Le, Stamm E, et al. *Int J Antimicrob Agent.* **20019** (17):343-348 (Pub Med).
- [2] Fowler, JE .**1986**. *Urol.Chin North Am.*(13):673-676.
- [3] Glusier MPC .**1991** . Urinary tract infection and pyelonephritis. In Medical Microbiology and infection diseases, Brande AIP, Sander, WB (ed) WB Comp.phihadelphia pp 1172-1183.
- [4] Kunin CM .**1994**. *clin.Infect. Dis.*(.18):1-12.
- [5] Gupta K .**2001** . *Int.J. Antimicrob. Agents.*(135):41-50.
- [6] Selfton Am.. *Int J, Antimicrob Agents.* **2000**;(16):489-491 (Pub.Meb).
- [7] Ofek I, Beachey Eh .**1980** , General concepts and principles of bacterial adherence, receptors and recognition. Bacterial adherence, Beachey Eh, Ed.Chapman and Hall, London PP.3-29.
- [8] Bonadio M. Meini M.Spetaleri P' Gilgi C. *Eur J Urol.***2001**;(40):439-445. (Pub Med).
- [9] Clinical and Laboratory Standards Institute, author. Supplemental tables. Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement CLSI publication M100-S15, M2-A8 and M7-A6. Pennsylvania: CLSI;**2005**.
- [10] Grude N. Tveten, Y, Kristianzen BE **2001**. *clin microbial. Infection* (7) .543-547.
- [11] Kripe C. *Am Fam physician.***2005**; (7)2215 :2219 (Pub Med)
- [12] Baris ICZ, Babic-Erceg A. Borzic El et al. *Int.J.Antimicrob Agent.***2003** (22):561-564.
- [13] .Sundyvist M. Kahlmeter G. *J Antimicrob Chemother.***2009**;(6) 4:227-228. (pub Med).
- [14] .Okesola AO, Oni AA **2009**. *American Eurasian J.Agric. Environ.Sci.* 5(3):327-330.
- [15] Akinyemi KO, Alabi SA, Taiwo MA, Omonigbehin EA **1997** *Niger, QTR.J.Hosp.Med*(1.):7-11.
- [16] Ebie M, Kandki-Olukemi YT. Ayanbadejo J, Tanyigana KB **2001**. *Niger .J. Microbiol* 15(1):31-37.
- [17] 17.Mbata TI **2007**. *Int.J.Microbiol.* 3(2):6-12.
- [18] 18.El-Mahood **2009** . *J of clinic Medicine and Research* Vol. 1(1)pp. 001-008.
- [19] El-Astal Z **2005**. *Pakistan J. Med.* 20 (4):365-370.
- [20] Mordi RM, Erah Po **2006** . *Afr J. Biotechnol.* 5(11):1067-1071.
- [21] Rai GK, Upreti HC, Rai Sk, Shan KP, Shrestha RM **2008**. *Nepal Med.Coll.J* .10(2):86-90.
- [22] El-Kholy A. Baseem H, Hall GS, Procop GW, Longworth DL **2003**. *J. Antimicrob Chemother* 51(3): 625-630.
- [23] Gonzalez (M, -Schaeffer AJ, *World J Urol.* **1999**:17:372-382 (pubmeh)
- [24] . Wang F, Zhu DM, HU PP, Zhang YY **2001**. *J. Infect. Chemother.* 7(2): 117-120.
- [25] Turner D, Dagan R **2001**. *Harefuah.*140 (10):923-929.
- [26] Goosens H **2000**. *Geneeskde Belgium* 62 (5):439-469.
- [27] Grude N. Tveten Y, Kristiansan BE **2001**. *Clin. Microbial. Infect.*(7):543-547.
- [28] Hosein IK, Hill DW, Jenkins LE, Magee JT **2002** . *The society of Applied Microbiology Symposium supplement*(92) :90-97.
- [29] Farrell DJ, Morrissey I, Robies D, Robbins M, Felmingham D **2003** . *A. J. Infect.*(46):94-100.
- [30] Akerele J, Ahonkhai I, Isah A **2000** . *J.Med.Lab.Sci* (9.) :47-52.
- [31] Fluit AC, Jones ME, Scharitz FJ, Acar J, Gupta R, Verhoef J **2000** ., 1997. Antonie Von leeuinhock(77):147-152.
- [32] Shaikh D, Ashfaq S, Shaikh K, Shaikh M, Naqvi Bs, Mahmood ZA, Majid R **2005**. *Med.J.Islamic Acad. Sci.*15 (4): 129-133.

- [33] Gruneberg RN **1994**. *J. Antimicrob. Chemother (suppl.A)* 33:1-6.
- [34] Stamm WE, Hooton TM. Management of urinary tract infection in adult. *N Eng J Med.***1993** (329) 1328-1334. (Pub Med).
- [35] Randrianirina F, Soares JL, carod JF, Ratsimas E, Thonnier V, Combe P, Grosjean P, Talarmin A **2006** . *J. Antimicrob. chemother* 14:401-407.
- [36] Winstanley TG, Limb DI, Eggington R, Hancoc KF **1999**. *J. Antimicrob. Chemother.* 40:591-594.
- [37] Kumari N, Ghimire G, Magar Jk, Mahapatra TM, Rai A **2005**.. *Nepal Med.J.* 7(2):116-118.
- [38] Wilson ML. Gaido L. *Clin infect Dis.***2004**;(38) 1150-1158. (Pubmed)
- [39] Susman M **1998** . Urinary tract infections. In Topley & Wilson Microbiology and Microbial infections 91h edition, (Collier L, Balows A, Sussman M. Eda.pp.601-621).
- [40] Tice AD **1999** . *J. Antimicrob chemother.*43:85-93.
- [41] El-Sweih N, Jamal W, Rotimi VO **2008** . *Med. Principle pract.* 14:401-407.
- [42] Kolawole As, Kolawole OM, Kandaki. Olukemi Y.T, Babatunde SK, Durowade KA, Kolawole CF **2009**. *Int. J Med. Sci.* 1(5); 163-167.