

Original Research Article

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Antibiotic Profile of Urinary Pathogens in a Tertiary Hospital, in Ekiti State, Nigeria

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ABSTRACT

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Urinary tract infections (UTI) have the potential for serious and life-threatening sequelae if left untreated or poorly treated. Empirical antibiotics treatment may be warranted in the face of symptomatic infections pending availability of laboratory results, hence the need to study the prevalent pathogen in a particular location and its susceptibility pattern to enable healthcare providers make informed decision on their choice of drug treatment for patients. We review 1,658 suspected cases of Urinary Tract Infections analysed between January 2014 and September 2015 (20 months) for bacterial isolates and their antibiotic susceptibility pattern. The results showed that there were 856 females and 802 males. Majority of the males were >40 years of age (55.49%) while majority of the females were between 21-40 years age group(62.74%). Organisms isolated include *E.coli* (54.42%), *Pseudomonas* spp (6.19%), *Klebsiella* spp (20.13%), *Proteus* spp (14.38%), *Enterococcus faecalis* (4.87%) All isolates had some level of resistance to each antibiotic tested. Ceftriaxone (65%) showed highest level of sensitivity to *E. coli*, followed by ofloxacin (58.5%), ciprofloxacin (56.5%), and levofloxacin (51.2%). Lowest level of sensitivity to erythromycin (8.9%) and cotrimoxazole (1.6%) was seen among isolates. All isolates were however found to have appreciable sensitivity to Ofloxacin. *E.feacalis* isolate had 100% sensitivity to Ofloxacin, levonofloxacin, and cotrimoxazole. in contrast to 50% sensitivity to Augmentin. The low level of sensitivity seen in this study is not unconnected to the poor regulation of antibiotic usage in our environment. This is a heavy challenge to the Ministry of Health and other stakeholders in our health sector.

Introduction

Urinary tract infection (UTI) is an inflammatory disease of any part of the urinary system namely: kidneys, ureters,

bladder and urethra, secondary to microorganism invasion. UTI can be classified into lower or upper, depending on

the site and organs involved; complicated and uncomplicated depending on the anatomy of the urinary tract; symptomatic and asymptomatic depending on the presence or absence of clinical manifestations, acute or chronic depending on the duration. UTI could also be recurrent or persistent (Fadeyi, 2010). UTI is a broad term that encompasses asymptomatic bacteriuria and symptomatic infection with microbial invasion and inflammation of the urinary tract. It is one of the most common clinical syndromes encountered in general and gynecological practices (IramShaifali *et al.*, 2012). They are among the most common infections described in outpatients setting and hospital patients (IramShaifali *et al.*, 2012). Adult women (40–50%) have a history of at least one episode of UTI in their lifetime (Urinary tract infection in women, 2016).

The pathogenesis of urinary tract infection involves ascending infection with bacteria colonizing the perineum in susceptible women. The normal urinary tract with the exception of urethral tract is resistant to colonization by bacteria and rapidly eliminates pathogenic and non-pathogenic micro-organisms that get access to the bladder. Uropathogenic clones of bacteria agents that cause infection in normal tract however have certain attributes which help them to invade the urinary tract and this include increased adherence to vaginal and uroepithelial cells, resistance to serum antibacterial activity, higher K antigen quantity, presence of aerobacin, presence of cytotoxic factor type 1 and hemolysin production (Walter, 2006).

Typical symptoms associated with UTI include the triad of dysuria, urgency and frequency, especially in adults. This might not be classically seen in the extremes of age. Neonates for instance might present

with failure to thrive, vomiting and fever while elderly patients are majorly asymptomatic and some presents with shock. The symptoms a patient presents with are functions of the site involved in the infection with upper UTI presenting with fever and loin pains and lower UTI with lower abdominal pain or heaviness. Up to 90% of the patients with UTIs complain of urinary tract symptoms, one third or more of the patients with these symptoms however do not have bacteriuria (Medina-Bombardó *et al.*, 2003). The most common symptoms for which most patients seek treatment are dysuria and frequency. Dysuria and frequency together raise the probability of UTI to more than 90%, effectively ruling in the diagnosis by history alone. Presumptive diagnostic criteria of UTI include presence of leukocytes of >5/hpf in microscopic examination of centrifuged urine, presence of bacteria in uncentrifuged urine, presence of red blood cells on urine microscopy, positive urine nitrite on dipstick, etc Isolation of the offending organism(s) via culture is however confirmatory.

There is public health issues associated with UTI with respect to quality of life. While not generally considered a cause of significant mortality, UTI do represent an important cause of morbidity. This is because UTI has the potential for serious and life-threatening sequelae if untreated or poorly treated. This is more likely to be the case where access to or availability of timely and appropriate medical intervention is limited due to inadequate numbers of health care providers, use of substandard drugs, missed diagnosis and lack of laboratory confirmation. Possible sequelae include ascending infection up to the level of the kidney (pyelonephritis), renal scarring and sepsis. UTI can be particularly dangerous in pregnant women in whom up to 50% of those with asymptomatic bacteriuria go on

to develop pyelonephritis. In addition, these women experience higher rates of intrauterine growth retardation and low birth-weight infants. UTI has also been shown to increase the risk of preterm labour, premature delivery, preeclampsia, amnionitis and anemia in pregnancy (Okonko *et al.*, 2010).

UTI occurs in all class and age groups of patients. It may be community acquired or nosocomial. The incidence increases with increasing age and patients on admission in hospitals have higher incidence than out-patients. A wide spectrum of microorganisms could be associated with UTI. Most are caused by bacterial agents. Majority of UTIs are caused by a single infecting agent especially in the first acute episode. Presence of more than 2 isolates in the sample suggests contamination. Prevalent organisms vary depending on geographical location and the antibiotic susceptibility of isolates varies. Many individuals with symptoms suggestive of UTI presenting to hospitals are likely to be started on empirical antibiotics pending availability of laboratory results, hence the need to study the prevalent organisms implicated in a particular location and the antibiotic susceptibility pattern.

Methodology

Study design and population

This is a retrospective study of 1,658 urine samples from patients with suspected UTI, submitted to Medical Microbiology laboratory, Ekiti State University Teaching Hospital (EKSUTH), Ado Ekiti between January 2014 and September, 2015.

Study Location

EKSUTH is located in Ado Ekiti, Ekiti state, south western, Nigeria. It is one of the 36

states of the federal republic of Nigeria. The state was created on the 1st of October, 1996 from Ondo state which itself was originally a part of the old western state. Ekiti state is located between latitudes 7°25' and 80°5'N and longitudes 4°45' and 5°46' east (Local government areas in Ekiti state, 2014). It is a state bound in the north by Kwara and Kogi States, east by Edo state, west by Osun state and south by Ondo state. Agriculture is the main source of the state economy apart from the regular allocation from the federation account. The landscape is undulating with surrounding hills in a tropical climate with two seasons- rainy season (April to October) and dry season (November to March). Temperature ranges between 21°C to 28°C. According to the national 2006 population census figure, Ekiti state has a population of 2,384,212 people constituting 1.703% of Nigerian population of 140,003,542 (Ekiti state population; Ekiti state population).

Laboratory procedure

Specimen: About 15mls of clean catch, mid-stream urine collected in a sterile universal bottle was sent to the laboratory within 2 hours of collection for microscopy, culture and sensitivity testing.

Processing: Macroscopic examination of the sample was done, observing the colour, presence of blood clots, turbidity and presence of foam. With a sterile calibrated wire loop, sample was inoculated on to CLED and blood agar plate, streaked and incubated aerobically overnight at 35-37°C and examined for evidence of growth after overnight incubation. Urine was centrifuged and loop-full of well mixed sediment was placed on a glass slide for microscopic examination. Presence of pus cells, urinary casts, red cells and yeast cells were noted. Antibiotic susceptibility testing was done

following the modified Kirby Bauer method. This was done by emulsifying 1-2 colonies of isolates in normal saline at a turbidity compared to 0.5 Mac Farland's standard. Surface of Mueller - Hinton agar was swabbed with sterile cotton-tip swab soaked in emulsified isolate. Single antibiotic impregnated discs were placed on the agar surface at 25mm apart and 20mm from the plate edge and incubated at 35-37°C for 18-24 hrs.¹² Antibiotic discs tested were Augmentin (20/10µg), Gentamycin (10µg), Ciprofloxacin (5µg), Erythromycin (15µg), Ceftriazone (30µg), Ofloxacin (5µg), Cotrimoxazole (1.25/23.75µg), Levofloxacin (5µg) and Cefuroxime (30µg).

Control strains of *Staphylococcus aureus* (ATCC 25923) and *Escherichiacoli* (ATCC 25922) were used as gram-positive and gram-negative control, respectively. All procedures were done as recommended by the Clinical Laboratory Standard Institute (CLSI).

Interpretation: Growth limited to the primary inoculum was regarded as insignificant. Evidence of growth must be seen atleast on the primary streak line to be regarded as significant. Each significant isolate was identified by colonial morphology, gram staining and biochemical reactions. Samples from which more than 2 isolates were considered contaminated. Interpretation of the susceptibility testing was done by comparing the diameter of zone of inhibition with those of a standard table in three grades of susceptibility which are sensitive, intermediate and resistant.

Finding were analysed using the SPSS version 19 software package for data analysis.

Results and Discussion

Out of the 1,658 cases reviewed, 856(51.6%) were females while 802(48.4%)

were males. Majority of the males were above 50 years in age (55.49%), while majority (62.74%) of the females were between 21 and 40 years old (Table 1).

Table 2 showed the outcome of culture of samples tested. Seven hundred and seventy-eight (46.9%) of sampled specimen had no organism isolated, the growth in another 316 specimens (19.1 %) was not significant while a few (0.6%) had mixed growth. Mixed growth was mostly seen among samples from female patients (80%). *S.aureus* was isolated from 102 (6.1%) patients while other pathogenic organisms were isolated from 452 specimens (27.3 %). The pathogenic organisms isolated were *E.coli*, which was in the majority (54.4%), *Klebsiella* spp (20.1%), *Proteus* spp (14.4%), *Pseudomonas* spp (6.2%) and the least frequent isolate was *E.faecalis* (4.9%) (Figure. I).

Review of antibiotic susceptibility pattern (Table 3) showed that none of the *E.feacalis* was sensitive to gentamycin, ceftazidine and cefuroxime; sensitivity of *Pseudomonas* to antibiotics tested were very poor as none of the *Pseudomonas* spp was sensitive to erythromycin, cotrimoxazole, levofloxacin and cefuroxime while sensitivity to augmentin (7.1 %), sensitivity to gentamycin (28.6 %) and ciprofloxacin (35.7 %) were low, however a fair sensitivities were seen to ofloxacin (50%) and ceftriazone (42.9 %). Ceftriazone (65%) showed highest level of action against *E. coli*, followed by ofloxacin (58.5%), ciprofloxacin (56.5%), and levofloxacin (51.2%) while lowest level of sensitivity was seen to erythromycin (8.9%) and cotrimoxazole (1.6%) among *E coli* isolates.

All isolates were found to have appreciable sensitivity to Ofloxacin. Most had appreciable sensitivity to ceftriazone.

E.feacalis isolate had 100% sensitivity to ofloxacin, levonofloxacin, and cotrimoxazole in contrast to 50% sensitivity to Augmentin. While none of the *Proteus* spp isolated was sensitive to erythromycin and cotrimoxazole, they were sensitive to ceftriaxone and levofloxacin (78.5% each). *Klebsiella* spp were most sensitive to gentamycin (64.8%). (Table 2)

UTI is a common disease in the tropics, especially among the female sex and at extremes of age. As reported in this study, female subjects (51.63%) were more prone to UTI than males. This observation can be explained by the anatomy of the women urethra and its proximity to the anus which favour the faecal and skin flora easy access to the urethra. In this study, the incidence of UTI peaks between age 20years and 40years in females and above 49years of age in males. Similar to other findings, generally, the females are reportedly more prone to UTI than their male counterpart and the likelihood of developing UTI increases with age. Definitive diagnosis of UTI is dependent on the demonstration of the presence of an isolate although in some cases the causative organisms are not cultivable and in some cases the organisms are not recovered. The presumptive diagnosis of UTI in practice is mostly clinical, confirmation of which is done in the laboratory but in most cases, the organism are not recovered. As seen in this study, 778(46.9 %; $p<0.001$) of all samples examined yielded no isolate. This is probably due to misuse of antibiotics due to availability of antibiotics as over the counter drugs in Nigeria thereby encouraging indiscriminate antibiotic use before patients' presentation at the hospital for clinical assessment, sample collection and treatment. In most part of the country, today, antimicrobial drugs are widely used with or

without prescription by appropriately qualified personnel resulting in inappropriate use of the various available antibiotics. Unfortunately, the overuse and misuse of these drugs resulted in the adaptation and resistance of these bacterial to the inhibitory effects of antibiotics (Okwori *et al.*, 2011). Likewise, many clinicians will start a patient on empirical antibiotics once signs and symptoms are suggestive of UTI without recourse to the laboratory only to request much later for laboratory diagnosis when patient failed to respond to treatment.

Most UTIs are ascending infections of bacteria that colonize the vaginal wall and eventually the urethra. UTIs have been reported to be majorly caused by Gram-negative enterobacteriaceae with *E. coli* being the most prevalent. However, *S. aureus* as etiologic agent of UTI raises a lot of questions. The occurrence of *Staphylococcus aureus* isolation was significant, reflecting a deficiency in the knowledge of how to properly collect urine samples in a way that is void of contamination.

The significance of *S.aureus* isolate can be queried being a normal flora of the urinary tract. Specimen not properly collected can be contaminated by the flora of the urethra; commonest of such contaminant is the *Staph aureus*. Most users of the clinical microbiology laboratory do not instruct patients on how to properly and appropriately collect urine samples for analysis, making specimen submitted to the laboratory prone to contamination and giving room for confusion in internal quality control and reporting of results by pathologists. The isolate may be a true pathogen, however, in this study, most of the samples with *S aureus* growth have no corresponding inflammatory response

suggestive of UTI during microscopic examination of the urine specimens (less than 4-5 WBCs per high power field)

making the isolation of *S. aureus* in this samples of doubtful significance.

Table.1 Demographic characteristics of the study population

AGE GROUP	MALE (%)	FEMALE (%)	TOTAL (%)	χ^2 (p value)
0 – 5	47 (5.86)	48 (5.61)	95 (5.73)	
6 – 10	57 (7.11)	55 (6.43)	112 (6.76)	
11 – 15	49 (6.11)	45 (5.26)	94 (5.67)	
16 – 20	25 (3.12)	30 (3.5)	55 (3.32)	
21 – 30	91 (11.35)	306 (35.75)	397 (23.94)	
31 – 40	88 (10.97)	231 (26.99)	319 (19.24)	
>40	445 (55.49)	141 (16.47)	586 (35.34)	
TOTAL	802 (100%)	856 (100%)	1658 (100%)	337.516 (<0.0001*)

χ^2 : Chi square; *: p value <0.05

Table.2 Outcomes of culture of Urine samples of patients with suspected UTI

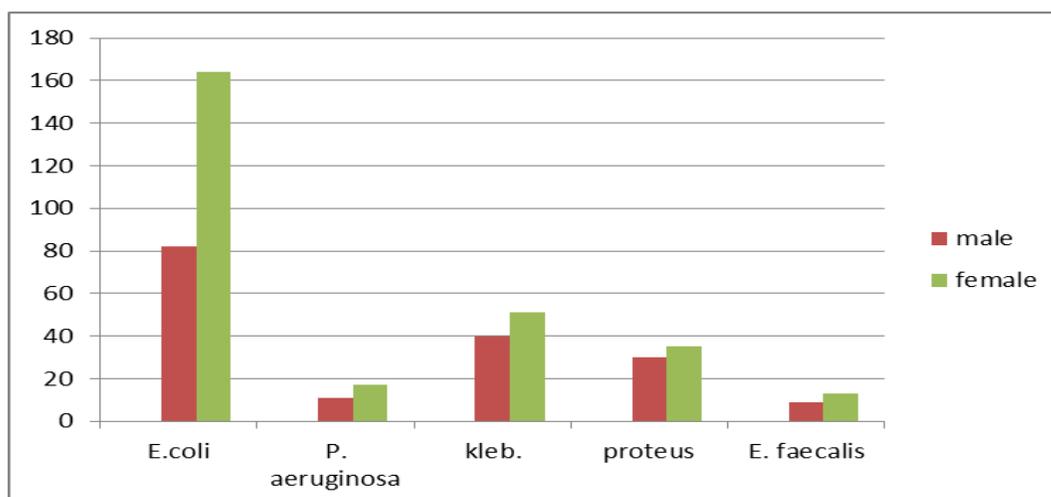
TYPES OF GROWTH	MALES n(%)	FEMALE n(%)	TOTAL n(%)	χ^2 (p value)
NO GROWTH	450 (56.1)	328 (38.3)	778 (46.9)	58.904 (<0.001*)
NO SIGNIFICANT GROWTH	142 (17.7)	174 (20.3)	316 (19.1)	
MIXED GROWTH	2 (0.2)	8 (0.9)	10 (0.6)	
<i>S. aureus</i>	36 (4.5)	66 (7.8)	102 (6.1)	
PATHOGENIC ISOLATES	172 (21.5)	280 (32.7)	452 (27.3)	
TOTAL	802 (100.0)	856 (100.0)	1,658 (100.0)	

χ^2 : Chi square; *: p value <0.05

Table.3 Sensitivity pattern of significant bacteria isolates

BACTERIA	ANTIBIOTICS								
	Aug(%)	Gent(%)	Ery(%)	Ceft(%)	Oflox(%)	Cipro(%)	Cotri(%)	Levo(%)	Cefu(%)
<i>E.coli</i> N=246	6(34.9)	139 (56.5)	22(8.9)	160(65)	44(58.5)	135(56.5)	4(13.8)	126(51.2)	110(45)
<i>Kleb spp</i> N=91	26(28.6)	59(64.8)	6(6.6)	55(60.4)	50(54.9)	47(51.7)	7(7.7)	49(53.8)	27(29.7)
<i>E. feacalis</i> N=22	11(50)	0(0.0)	–	0(0.0)	22(100)	–	22(100)	22(100)	0(0.0)
<i>Pseudomonas spp</i> N=28	2(7.1)	8(28.6)	0(0.0)	12(42.9)	14(50)	10(35.7)	0(0.0)	0(0.0)	0(0.0)
<i>Proteus spp</i> N=65	18(27.7)	36(55.4)	0(0.0)	51(78.5)	36(55.4)	33(13.4)	0(0.0)	51(78.5)	22(33.9)

Fig.1 Pathogenic isolates from samples



A significant relationship (p value <0.001) was found between the types of growth and the gender in this study. Pathogenic isolates was found in about 33% of females as compared to 22% of males. However, cultures with no growth were higher in males (56%). The other pathogenic organisms implicated in UTI in this study are GNB enterobacteriaceae family especially *E.coli* which was significantly higher in urine samples of both males (47.7%; p <0.001) and females (58.6%; p<0.001) compared with other isolates.

This is similar to findings in previous studies in which Gram-negative bacteria, particularly *E. coli* was the most implicated pathogen isolated in patients with UTIs (Burbige *et al.*, 1984; Akinyemi *et al.*, 1997; Okonofua *et al.*, 1989). Findings contradict the report of Omonigho *et al.*, (2001) who found *Klebsiella* spp. to be more prevalent than *E. coli* in UTIs. This result compares favourably with the findings of other workers who found *E. coli* more predominant over *Klebsiella* spp. in similar studies in UTIs (Delzell, 2000; De-Mouy *et al.*, 1988; Farooqui *et al.*, 1989). GI commensals, *E.coli* being one of them are implicated in UTI due to the anatomic

proximity of the GI tract to the urinary tract. In view of this, poor personal hygiene has been pencilled as a risk factor in UTI.

Other pathogens isolated in order of prevalence include *Klebsiella* spp., *P. mirabilis*, *P. aeruginosa*, and *E. faecalis*. This is in keeping with the findings of Anyamene *et al.*, (2002) that the dominant etiologic agents accounting for more than 85% of cases of UTIs are the Gram-negative bacilli which are normal flora of the intestinal tract. The prevalence of UTI varies with the gender and age of the patient. In neonates and infants, UTIs are more common in males but by school age, all through to adulthood, higher prevalence is seen in the females. By far the most common population at risk for UTI is the sexually active female. The pathogens causing however does not vary with gender or age except in 'honeymoon cystitis' caused majorly by *Staphylococcus saprophyticus* (Washington *et al.*, 1997).

The high rate of resistance to antibiotics which was also noted among common urinary isolates in this study is similar to what was reported in a study done in Benue where a general resistance was recorded in

urinary isolates especially to Ampicillin, Tetracycline and Co-trimoxazole both AIDS and non-AIDS patients. This is probably due to the indiscriminate use of antibiotics which can be easily procured over the counter without a clinician's authorised prescription.

In conclusion, there is a high level suspected UTI which could not be confirmed by culture most likely due to indiscriminate use of antibiotics in our environment. *E.coli* is still the most common bacterial pathogen seen in UTI in this environment. The peak incidence of UTI was seen between age 20-40 years in females and above 40 years in males in this study. There is a high level of antibiotic resistance by isolated pathogens.

Recommendation

With the emerging trend of antibiotic resistance noted in this study, judicious and rational use of antibiotics is a must if we must avert the impending disaster of massive resistance to antimicrobial agents.

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