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Full Length Research Paper

Antibiotic susceptibility of common bacterial pathogens in urinary tract infections in a Teaching hospital in Southwestern Nigeria

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Urinary tract infection (UTI) is one of the most common bacterial infections in humans and a major cause of morbidity. However, the aetiology of UTI and their antibiotic sensitivity patterns vary from time to time and across different areas. This study determines the frequently isolated UTI bacteria from in-patients and out-patients of a Teaching hospital in Southwestern Nigeria and their antibiotic susceptibility patterns. Sixty (60) bacterial isolates from urine of patients with significant bacteriuria in UTI were collected and evaluated for their antibiotic sensitivity using modified Kirby-Bauer method. UTI was frequently encountered in females (61.7%) and older patients (50%). 55% of the isolates were from in-patients. The most common etiological organisms of UTI were *Esherichia coli* (46.7%), *Pseudomonas aeruginosa* (18.3%), *Klebsiella* spp (13.3%), *Staphylococcus aureus* (13.3%) and *Proteus* spp (6.7%). *Pseudomonas aeruginosa* was found only among hospital isolates while *Staphylococcus aureus* was isolated from females only. Antibiotic susceptibility pattern revealed that the *Enterobacteriaceae* were highly susceptible to Ciprofloxacin (71.4 to 75%) while Nitrofurantoin demonstrated the best activity against *S. aureus* isolates (100%) in both community and hospital acquired UTI. All the isolates exhibited high degree of resistance to Augmentin and amoxicillin. High rate of multi-drug resistance was recorded among all isolates. In view of the increasing rate of resistance of UTI pathogens to commonly used antibiotics, rational prescription and use of antibiotics is advocated.

Key words: Urinary tract infections, bacterial pathogens, susceptibility.

INTRODUCTION

Urinary tract infection among the most common bacterial infection in humans both in the community and hospital settings. It occurs in all age groups and in both genders and usually require urgent medical treatment (Orret and Davis, 2006; Omoregie et al., 2008). Urinary tract infection is the major cause of morbidity in both the hospital and the community settings (Omigie et al., 2009). It poses a significant health risk because it can lead to urosepsis and or renal scarring, progressive kidney damage with associated high mortality, morbidity and economic loss (Saffar et al, 2008). For many years, pathogens associated with uncomplicated UTI have remained constant with *Escherichia coli* identified as aetiologic agent in about 75 to 90% of infections (Karlowsky, 2002; Nakhjavani et al., 2007; Omigie et al, 2009). The remaining Gram negative urinary pathogens are other Enterobacteria, especially, *Klebsiella* spp*,* *Proteus mirabilis* and *Pseudomonas aeruginosa*. Enterococci and coagulase negative *Staphylococci* (e.g. *Staphylococcus saprophyticus*) are the most frequently implicated Gram positive organisms (Shankel, 2007). In the community and hospital settings, the aetiology of urinary tract infections and the antimicrobial susceptibility of urinary pathogens have been changing over the years and from place to place (Gruneberg, 1980; Gales et al., 2000; Saffar et al., 2008). Despite the fact that antibiotics have revolutionized the management of many clinical syndromes caused by infections, their increasing use in many ways such as, indiscriminate prescribing,

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Table 1. Prevalence of the isolates according to Gender.

Bacteria	Total number isolated		Proportion			
			Male		Female	
	N	%	N	%	N	%
E. coli	28	46.7	12	52.2	16	43.3
P. aeruginosa	11	18.3	6	26.1	5	13.5
Klebsiella spp	8	13.3	2	8.7	6	16.2
S. aureus	8	13.3	0	0	8	21.6
Proteus spp	4	6.7	2	8.7	2	5.4
Enterococcus spp	1	1.7	1	4.3	0	0
Total	60	100	23	100	37	100

N= number; %=percentage

inappropriate dosing and duration of treatment, over the counter availability of antibiotics to the general public, use in animal husbandry, and use to control infections in horticulture has contributed to the rise of antibiotic resistance among various common pathogens (Kerr, 2005). The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation (Abubakar, 2009). Hence, the changing spectrum of microorganisms involved in urinary tract infections and emergence of resistance across institutions and geographical areas have made imperative the conduct of antibiotic susceptibility pattern study of UTI pathogens in various regions from time to time. This study determines prevalence of frequently isolated urinary pathogens and their antibiotic susceptibility profile in nosocomial and community-acquired infections in a Teaching hospital in Southwestern Nigeria.

METHODS

Collection and preparation of test clinical bacterial isolates

A total of bacterial UTI isolates collected from the Medical Laboratory of University College Hospital, Ibadan, Oyo state, Nigeria were used in the study. The isolates were from in-patients and out-patients who had been confirmed to have UTI by urine culture of colony count $>10^5$ cfu/ml. The bacterial isolates were collected on nutrient agar slants in bijou bottles and incubated at 37°C for 24 h. The isolates were subcultured periodically. The isolates were re-identified and confirmed using standard microbiological method which includes Gram staining, colonial morphology on media, and growth on selective media, lactose fermentation, catalase, oxidase, coagulase, indole, citrate utilization, litmus milk decolorization and Urease tests.

Antibiotic sensitivity testing

Antibiotic susceptibility testing was performed by the disc diffusion assay on Muller Hinton Agar by modified Kirby-Bauer method (WHO SEARO, 2006) using the following antibiotics disc, Amoxycillin 25 mcg, Co-trimoxazole 25 mcg, Nitrofurantoin 200 mcg, Gentamicin 10 mcg, Nalidixic 30 mcg acid Ofloxacin 5 mcg,ciprofloxacin 5 mcg, Augmentin 30 mcg, and Tetracycline 30 mcg. Interpretation of diameter of growth inhibition zone was done by using the standard interpretative chart. Organisms were scored as sensitive, intermediate or resistant. *S. aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 *and E. coli* ATCC 25922 were used as control strains. Diameters of growth inhibition zones were recorded to the nearest mm for an average of 2 readings in each case. The zone diameter with control strains were within the limits published by NCCLS.

RESULTS

Out of the 60 isolates used for this study, 37 (61.7%) were recovered from female patients while 23 (38.3%) were from male patients. Occurrence of bacterial UTI was the highest in the age group of 50 years and above (50%) and lowest in the age group of 20 years and below (13.8%). The female to male ratios within the age groups were: < 20 years (1:1), 20 to 50 years (4.5:1) and > 50 years(1:1). 51 (85%) of the isolated bacterial pathogens were Gram negative bacilli while 9(15%) were Gram positive cocci. The distribution of the bacterial species was as follows: *E. coli* (46.7%), *P. aeruginosa* (18.3%), *Klebsiella* spp (13.3%), *S. aureus* (13.3%), *Proteus* spp (6.7%) and *Enterococcus* spp (1.7%)Table 1.The prevalence of the UTI isolates in relation to gender is also shown in Table 1. *E. coli* were the most frequently isolated pathogen in both sexes while *S. aureus* was isolated from females only. More isolates were recovered from outpatients (55%) than from inpatients (45%). The distribution of the isolates among the inpatients and outpatients is shown in Table 2. *P. aeruginosa* and *Enterococcus* spp were isolated from in-patients only. The susceptibility pattern of the bacterial isolates to the selected antibiotics in this study is depicted in Table 3. All the isolates were 100% resistant to Amoxycillin and Augmentin. The only *Enterococcus* spp isolate was resistant to all the antibiotics tested. *S. aureus*

Table 2. Prevalence of bacteria isolates among inpatients and outpatients.

Bacteria	Inpatients		Outpatients	
	N	%	N	%
E. coli	12	36.5	16	59.3
P. aeruginosa	11	33.3	0	0
Klebsiella spp	4	12.1	4	14.8
S. aureus	4	12.1	4	14.8
Proteus spp	1	3.0	3	11.1
Enterococcus spp	1	3.0	0	0

Table 3. Proportion of isolates susceptible to the selected antibiotics.

Antibiotics	Susceptibility of the isolates to antibiotics						
	E. coli **(N = 28) %**	***P. aeruginosa*** **(N = 11) %**	***Klebsiella*** **spp (N = 8) %**	***S. aureus*** **(N = 8) %**	***Proteus*** **spp (N = 4) %**	***Enterococcus*** **spp (N = 1) %**	**Total (N = 60) %**
CF	71.4	45.5	75.0	75.0	75.0	0	66.7
OFL	50.4	45.5	50.0	50.0	25.0	0	46.7
GEN	46.4	54.5	62.5	50.0	0	0	46.7
NIT	42.9	36.4	50.0	100.0	25.0	0	48.3
NAL	39.3	36.4	12.5	25.0	25.0	0	31.7
COT	28.5	18.2	12.5	25.0	25.0	0	23.3
TET	17.9	18.2	25.0	25.0	25.0	0	20.0
AUG	0	0	0	0	0	0	0
AMX	0	0	0	0	0	0	0

CF – Ciprofloxacin , TET-Tetracycline, OFL-Ofloxacin, AUG-Augmentin, GEN-Gentamicin, AMX-Amoxycillin, NIT-Nitrofurantoin , COT-Co-trimoxazole, NAL-Nalidixic.

Table 4. Frequency of Isolates showing multidrug resistance.

Bacteria	Resistance to antibiotics			
	3 – 4	**5 – 6**	**7 – 8**	**9**
E. coli (N = 28)	2	11	9	3
P. aeruginosa (N = 11)	-	3	3	3
Klebsiella spp (N = 8)	2	3	2	1
Staphylococcus aureus (N = 8)	2	1	4	-
Proteus spp (N = 4)	1	-	2	1
Enterococcus spp (N = 1)	-	-	-	1

isolates were 100% susceptible to nitrofurantoin, 75% to ciprofloxacin and 50% to ofloxacin and gentamicin. Among the Enterobacteriaceae, susceptibility to the fluoroquinolones was high for the ciprofloxacin than for the ofloxacin except in *P. aeruginosa*. *P. aeruginosa* showed more resistance to all the antibiotics than *E. coli* except against gentamicin. Generally, the proportion of all the isolates that showed susceptibility to the various antibiotics tested are as follows: ciprofloxacin (66.7%), Nitrofurantoin (48.3%), Ofloxacin (46.7%), Gentamicin (46.7%), Nalidixic acid (31.7%), Cotrimoxazole (23.3%), Tetracycline (20.0%), Augmentin (0%) and Amoxicillin (0%). The frequency of isolates showing multidrug resistance is given in Table 4. A rate of multidrug resistance was recorded among all the isolates. 9 isolates were resistant to all the 9 antibiotics tested. Only a small proportion of all isolates: *Escherichia coli*, *S. aureus* and *P.* aeruginosa, were susceptible to 7 out of 9 antibiotics. All the *Klebsiella* spp and *Proteus* spp isolates were resistant to at least 4 antibiotics. None of the isolates was susceptible to all the 9 antibiotics used in this study.

DISCUSSION

This study observes that the incidence of UTI was high among the females (61.7%) than males (38.3%) and high among the age group of 50 years and above (50%) and low among the age group of 20 years and below (13.8%). However, the female to male ratio in terms of incidence of infection was highest for the age range of 20 to 50years. This result is in agreement with other reports which showed that UTI were more common in females than males during adolescence and adulthood (Orret and Shurland, 1998; Gales et al., 2000; Tambekar et al., 2006; Theodore, 2007; Adedeji and Abdulkadir, 2009; Kebira et al., 2009; Kolawole, 2009). Factors such as short urethra and its closeness to the anus as well as sexual activity have been reported to influence the higher prevalence of UTI in females (Adedeji and Abdulkadir, 2009). The female to male ratio among age groups of less than 20 years and age group of more than 50 years was equal (1:1) and higher for age group of 20 to 50 years (4.5:1). This may be due to increase sexual activity at this age range which predisposes the female to the possibility of contracting UTI. This is different from reports by Kebira et al. (2009) and Kalantar et al. (2008) where a lower female to male ratio was recorded among neonates and young children. Rates of UTI in boys been associated with the high incidence of congenital malformations and uncircumcised genitalia that often gets contaminated from the prepuce or introtal area that is not always clean (Kebira et al., 2009). Incidence of UTI recorded among the elderly (> 50years, 50%) than among young age patients(20 to 50 years,36.2%) and paediatrics or teenagers(< 20 years,13.8%) in this study, differs from the reports of Dimitrov et al.(2004) and Omigie et al. (2009) in Kuwait and Nigeria, respectively in which the highestincidence of UTI was recorded among the age group 20 to 50 years (63.4 and 74.7%, respectively) and lowest among the age group > 50 years (13.3 and 10.3%, respectively). It however agrees with a 20 year period study in Japan where a trend of increasing number of elderly patients with complicated UTI was reported (Shigemura et al., 2005). Increasing frequency of prostate disease in males is a factor responsible for increase in the incidence of UTI and decrease in female : male ratio in patients above 50 years (Shankel, 2007). In this study, the Gram negative bacilli constituted 85% of the bacterial isolates implicated in UTI cases while Gram positive cocci constituted 15%. The Enterobacteriaceae (66.7%) constituted the largest group of bacteria isolated of which *Escherichia coli* (46.7%) was the most prevalent bacterial agent causing UTI. This result is consistent with reports from other studies (Grunerberg, 1980; Orret and Shurland, 1998; Daza et al., 2001; Dimitrov et al., 2004; Inabo and Obanibi, 2006; Dash, 2008; Abubakar, 2009; Omogie, 2009) but differs from the reports of Ehinmidu (2003) and Aboderin et al. (2009) which recorded *P. aeruginosa* and *Klebsiella* spp, respectively as the predominant bacteria. Other isolated bacteria from UTI cases in this study were *P. aeruginosa* (18.3%), *Klebsiella* spp and *S. aureus* (13.3%) and Proteus spp (6.7%). *P. aeruginosa* was also reported as the second most common bacterial isolate in UTI from studies in India (Tambekar et al., 2006) and Lafia, Nigeria (Kolawole, 2009) However, *Klebsiella* spp was reported as the second most frequently implicated organism in UTI in some other studies(Gales et al., 2000; Al-Sweih et al., 2005; Uwaezuoke and Ogbulie, 2006; Haghi-Asteiani et al., 2007; Dash, 2008; Abubakar, 2009). The incidence of community acquired UTI due to *E. coli* is more in this study (59.3%) than hospital acquired infections (36.5%). *P. aeruginosa* was mostly responsible for UTI cases among in patients where it accounted for 33.3% of the infections. This is possibly due to the opportunistic nature of the organism and its versatility in causing nosocomial infections in hospitalized patients especially those fitted with catheters. It has been previously reported that *P. aeruginosa* is being increasingly isolated from both community and hospital acquired UTIs (Orret and Shurland, 1998; Shigemura et al., 2005; Tambekar et al., 2006; Kolawole, 2009; and Omigie et al., 2009). *S. aureus,* a normal flora of female perineum and vulva can easily be carried into the urethra either by massaging during sexual intercourse or alteration of vaginal flora due to insertion of a contraceptive device thereby resulting in an opportunistic UTI due to the organism. This might be responsible for the 21.6% prevalence of the organism limited only to the females in this study. Considering the fact that most of the infecting organisms are commensals of perianal and vaginal regions, increase in personal hygiene especially in females may be important in reducing the incidence of UTI. *E. coli*, the predominant aetiologic organism of UTI in this study showed moderate to high susceptibility to the fluoroquinolones (ofloxacin, 50% and ciprofloxacin, 71.4%) and varying degree of susceptibility to other commonly used antibiotics Table 3. Earlier studies conducted in Nigeria and other countries have reported good susceptibility of the bacteria to fluoroquinolones (Ehinmidu, 2003; Adeleke et al., 2005; Shigemura et al., 2005; Al Sweih, 2005; Mordi and Erah, 2006; Idowu and Odelola, 2007; Theodore, 2007; Saffar et al., 2008; Kolawole, 2009; Omigie et al, 2009; Tambekar et al., 2006). However, resistance to fluoroquinolones is on the increase in the locality of this study when the susceptibility data in this study (50, 71, and 4%) is compared with previous reports by Okesola and Oni (2009) and Idowu and Odelola (2007) of 100% and 97% susceptibility, respectively. The poor susceptibility of *E. coli* isolates in this report to nalidixic acid, (39.3%) nitrofurantoin (42.9%) and gentamicin (46.4%) is also showing a decreasing trend when compared to higher values recorded in previous works (Uwaezuoke and Ogbulie, 2006; Mordi and Erah, 2006; Tambekar et al., 2006; Saffar et al., 2008; Aboderin, 2009). The *P. aeruginosa* isolates showed reduced susceptibility to the fluoroquinolones (45.5% for Ciprofloxacin and Ofloxacin), gentamicin, 54.5% and resistance to other antibiotics tested. These results present a worrying pattern of resistance comparable to what obtained in other similar studies (Daza et al., 2001; Shigemura et al., 2005; Haghi-Asteiani et al., 2007; Okesola and Oni, 2009). *P. aeruginosa* is notorious for its intrinsic capability for resistance to antibiotics. The antibiotic susceptibility profile for all the UTI bacterial isolates in this study was ciprofloxacin (66.7%), nitrofurantoin (48.3%), ofloxacin (46.7%), Gentamicin (46.7%), Nalidixic acid (31.7%), Co-trimoxazole (23.3%), Tetracycline (20.0%), Augmentin and Amoxicillin (0%) Table 3. Ciprofloxacin was the most active drug in this study showing the highest activity against the Enterobacteriaceae and *S. aureus* isolates in both community and hospital acquired UTI. However, this result showed general increase in resistance of UTI bacterial isolates to fluoroquinolones with higher resistance to ofloxacin than ciprofloxacin. The superiority of ciprofloxacin in efficacy and potency against uropathogens to ofloxacin and nalidixic acid among the quinolones was reported by Omigie et al. (2009). Increase in widespread use of fluoroquinolones in medical centres is a possible cause of high-level resistance to fluoroquinolones in UTI patients (Nakhjavani et al., 2006). All the isolates were highly resistant to older drugs (Nalidixic acid, Co-trimoxazole, tetracycline, Augmentin and amoxicillin). Activity of gentamicin against all the isolates (46.7%) may be due to its widespread use in the hospital environment as a broad-spectrum antibiotic. Use of aminoglycosides, especially when only one type is employed, may lead to an increased level of resistance (Swartz, 1997). Resistance of UTI pathogens to commonly use antibiotics may not be unconnected with their frequent prescription in the hospital, their easy availability in the community without prescription and their low cost which make them subject to abuse (Abubakar, 2009). The total or complete resistance (100%) of all the isolates in this study to Augmentin and amoxycillin is worrisome as they may have lost their value in the treatment of UTI in this area of study. Rate of multi-drug resistance was recorded in both community and hospital isolates with highest degree recorded among *Enterococcus* spp*,* *Proteus* spp and *P. aeruginosa.* High rate of multidrug resistance among the isolates in this study is very alarming and should be of great concern. Hryniewicz et al. (2001) in a study conducted in Poland reported that multidrug resistance was usually related to production of Extended Spectrum Beta Lactamases (ESBL) among the Enterobacteriaceae in both community and hospital isolates.

Multi-drug resistant P*. aeruginosa*, *Klebsiella* spp, *S. aureus* and *Enterococcus* spp strains have been widely reported in some studies (Gale, 2000; Aiyegoro et al., 2007; Abubakar, 2009). This study was conducted in a tertiary hospital where patients with more complicated UTI that have failed previous therapy due to exposure to more resistant bacteria are referred and thus may have contributed to the high rate of multi-drug resistance recorded in this study. High prevalence of multiple antibiotic resistance strains is a possible indication that very large population of bacterial isolates has been exposed to several antibiotics.

Conclusion

The data obtained from this study suggest that while UTI pathogens are still susceptible to the fluoroquinolones such as ciprofloxacin and ofloxacin, resistance to these antibiotics is on the increase when compared with previous studies. Other commonly prescribed antibiotics in UTI such as nalidixic acid, co-trimoxazole, tetracycline, Augmentin and amoxicillin are rather ineffective and may have lost their value in the chemotherapy of UTI. Consequently, empirical antibiotic selection in treatment of UTI should be based on the knowledge of local prevalence of causative organisms and their antimicrobial sensitivities rather than on universal guidelines so as to reduce the incidence of resistance. Indiscriminate prescription and use of antibiotics should be discouraged in both community and hospital settings by continuous public enlightenment on rational antibiotic use as well as adoption of strict national antibiotic policy to regulate the prescription, sale and use of antibiotics.

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