Full Length Research Paper

Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern

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The antimicrobial sensitivity pattern of bacterial isolates from suspected urinary tract infection (UTI) patients at Igbinedion University Teaching Hospital was carried out from November 2004 to November 2005 using the disc diffusion method. The subjects were made up of 330 (60%) males and 220 (40%) females. The commonest isolates were *Escherichia coli* (51.2%), *Staphylococcus aureus* (27.3%), and *Klebsiella pneumoniae* (12.8%) respectively. Both methicillin-resistant (MRSA) and methicillin sensitive (MSSA) *S. aureus* were isolated in the study. The isolates were highly sensitive to ofloxacin but low to moderately sensitive to gentimicin, tobramycin, nalidixic acid, ciprofloxacin, tetracycline, nitrofurantoin, and cefuroxine. The MSSA isolates were highly sensitive to ciprofloxaxin and ofloxacin while the MRSA were sensitive to ofloxacin. In addition, the isolates showed multi-drug resistance.

Key words: Bacterial resistance, β-lactamases, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin sensitive (MSSA) *S. aureus*.

INTRODUCTION

The incidence of microbial drug resistance is alarming and in view of its development pharmaceutical industries are shifting away from traditional strategies to newer approaches in order to cope with the problem (Westby et al., 2005; Huelsmann et al., 2006; Giang et al., 2006; Jabra-Rizk et al., 2006). In Africa the problem stems from factors such as indiscriminate use of antibiotics, inappro-priate advertisement, and erratic prescription by unqua-lified drug sellers (Al-Jabri, 2005; Chinedum, 2005).

Aminoglycosides are bactericidal and exhibit synergy with other antimicrobials, most notably β- lactams, with which they are often administered (Jones et al., 2003; Johnson et al., 2005). Therapeutic options are significan-

tly limited because methicillin-resistant *Staphylococcus aureus* (MRSA) for example are resistant to all β-lactam antibiotics (Diederen et al., 2005). Currently even more worrisome is the presence of extended-spectrum-β-lactamase-producing bacteria which are usually resistant to other antibiotics such as aminoglycosides, tetracyc-lines, chloramphenicol, trimethoprim, sulfonamides and quinolones, often due to the presence of different genes on transferable elements such as plasmids, transposons or integrons and/or genetic structures generated by combinational evolution of different interactive pieces ((Bonnet, 2004; Baquero, 2004; Poirel et al., 2005; Machado et al., 2005).

The incidence of bacterial resistance mediated by β-lactamases has been reported in several African coun-tries including Nigeria (Olayinka et al., 2003; Zeba, 2005;

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Table 1. Distribution of patients by age and gender.

Sex	Age group					
	< 18	18-30	31-43	44-56	>57	Total
Male	15	68	88	78	96	330
Female	8	60	76	48	36	220
Total	23	128	164	126	132	550

Zeba et al., 2005, Onanuga et al., 2005). According to Pankuch et al. (2006) agar dilution, micro-dilution, E-test and disk diffusion are satisfactory methods for susceptibility testing. The present study, however, aims to examine the susceptibility pattern of various bacteria in the urine samples of patients in Okada village of Edo State, Nigeria through the disk diffusion method.

MATERIALS AND METHODS

Subjects

Between November 2004 to November 2005, 550 patients in the Igbinedion Teaching Hospital with suspected urinary tract infection (UTI) were voluntarily recruited into the study. The subjects were made up of 330 males (60%) and 220 females (40%).

Sample collection

Clean catch urine samples were collected in sterile universal containers as described by Karlowsky et al. (2006) and Solberg et al. (2006). A calibrated loop delivering approximately 0.001 ml of urine was used for inoculation on agar plates made up of MacConkey and two mannitol salt (Difco, Sparks Maryland), one of which was supplemented with oxacillin (4 μg/ml) (Johnson et al., 2005). The plates were incubated at 37^{0}C for 48 h. The *Staphylococcus* strains were identified by colony morphology, Gram staining and catalase testing as described by Palazzo et al. (2005). Methicillin-susceptible *S. aureus* (MSSA) was primarily detected by its charac-teristic growth on mannitol salt agar and the absence of growth in the presence of oxacillin, while growth on both agar plates was presumed to indicate the presence of MRSA (Lu et al., 2005). All the other bacteria were isolated strictly on MacConkey agar using standard biochemical methods.

Antibiotic sensitivity test

The disc diffusion technique was used for antibiotic sensitivity testing using Mueller -Hinton agar as described by Pankuch et al. (2006) . Isolates were tested against the following antibiotics: cefuroxime (30 g), ciprofloxacin (10 g), methicillin (10 g), ofloxacin (5 g), tobramycin (10 g), tetracycline (10 g) and nitrofurantoin (50 g) supplied by Oxoid and gentamicin (15 g) and nalidixic acid (15 g) supplied by Bio Rad.

Statistical analysis

The values for the distribution of patients by age and gender were analyzed by a t-test and ANOVA while percentages and frequenc-

ies were computed for the other variables as described by Snedecor and Cochran (1987) and Ejembi et al. (2006).

RESULTS

Table 1 portrays the distribution of patients by age and gender. Over half of the patients were males, most of which belonged to the <57 age group (96) followed by 31-43 (88) and 44-56 (78) age groups respectively. Most of the female patients belong to the 31-43 age group (76) followed by 18-30 (60) and 44-56 (48) respectively. However, an independent sample t-test showed that there was no significant difference between the number of males and females (p>0.05) although there was a higher mean value for the males. Irrespective of gender, most of the patients belong to the 31- 43 age group (164 patients). However, the use of factorial ANOVA confirmed the fact that there was no significant difference between the number of patients in the various age groups (p>0.05).

Table 2 depicts the susceptibility pattern of UTI isolates to various antimicrobial agents. The highest number of isolates was *E. coli* (63), followed by *S. aureus* (36), *Klebsiella pneumoniae* (17), *Pseudomonas aeruginosa* (6) and *Proteus vulgaris* (5) or *Enterococcus faecalis* (5) respectively. All of the *P. vulgaris* isolates were highly sensitive to ofloxacin while some were highly to moderately sensitive to ciprofloxacin (80%) and nalidixic acid (60%) respectively. *K. pneumoniae* was highly sensitive to ofloxacin (94%) but moderately sensitive to ciprofloxacin (47%) and cefuroxime (53%). The *E. coli* isolates were highly sensitive to ofloxacin (81%) but moderately sensitive to gentamycin (54%) and cipro-floxacin (41%). Both methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) were isolated. The MRSA were highly sensitive to ofloxacin (79.2%) but showed low sensitivity to the remaining antibiotics while the MSSA isolates were highly sensitive to methicillin (100%), ofloxacin (91.7%), and ciprofloxacin (83.3%) but moderately sensitive to cefuroxime (66.7%), tobramicin (58.3%) and gentamicin (75%). *E. faecalis* was moderately and equally sensitive to gentamycin, tobramycin and ofloxacin (60%) while *P. aeruginosa* was moderately sensitive to ofloxacin (67%).

Table 3 depicts the multi-drug resistance pattern of the various isolates. The *E. coli* isolates had the highest value for multi-drug resistance (9) followed by *P. aerugi*

Table 2. Frequency/percentage of isolates susceptible to selected antimicrobial agents.

Organism (Number isolated)	Gentamycin	Tobramycin	Nalidixic acid	Ciprofloxacin	Ofloxacin	Tetracycline	Methicillin	Nitrofurantoin	Cefuroxime
Proteus vulgaris (5)	2(40)	1(20)	3(60)	4(80)	5(100)	1(20)	-	2(40)	1(20)
Klebsiella pneumoniae (17)	7(41)	4(24)	1(6)	8(47)	16(94)	3(18)	-	1(6)	9(53)
Escherichia coli (63)	34(54)	29(46)	13(21)	26(41)	51(81)	6(10)	-	14(22)	22(35)
MRSA (24)	4(16.7)	1(4.2)	1(4.2)	9(37.5)	19(79.2)	1(4.2)	0	0	1(4.2)
MSSA (12)	9(75)	7(58.3)	3(25)	10(83.3)	11(91.7)	3(25)	12(100)	5(41.7)	8(66.7)
Pseudomonas aeruginosa (6)	2(33)	1(17)	-	2(33)	4(67)	-	-	-	-
Enterococcus faecalis (5)	3(60)	3(60)	2(40)	1(20)	3(60)	1(20)	-	2(40)	2(40)

MRSA=Methicillin resistant *Staphylococcus aureus*.
MSSA=Methicillin susceptible *Staphylococcus aureus*.

Table 3. Frequency of bacterial isolates showing multi-drug resistance.

Organism	Resistance to antimicrobial agents			
	2 - 3	4 - 5	6 - 7	8 - 9
Proteus vulgaris	3	1	-	1
Klebsiella pneumoniae	23	16	15	9
Escherichia coli	4	6	5	2
S. aureus	17	10	8	1
Pseudomonas aeruginosa	-	-	2	4
Enterococcus faecalis	3	-	2	-

nosa (4), *K. pneumoniae* (2) and *P. vulgaris* (1) or *S. aureus* (1) respectively. In general, however, *E. coli* and *S. aureus* had the highest values for multi-drug resistance.

DISCUSSION

Bonferroni's multiple comparison test revealed that there was no significant difference between the number of patients in the various age groups (p>0.05) while the independent sample t-test showed that there was no significant difference between the number of males and females (p>0.05). Unlike the results reported by Ibadin (2002), the higher mean value for male patients could be explained by the fact that the selection of resistant bacteria which are more often present in complicated UTI's are in turn more common among males (Alhambra et al., 2004).

The *P. aeruginosa* and *P. vulgaris* isolates were resistant to aminoglycosides such as gentamycin and tobramycin as previously reported by Lyytikainen et al. (2001) Bouza et al. (2001) Jones et al. (2003) and Poole (2005). Of immense concern, however, is the fact that these organisms are inherently resistant to tigecycline, an ex-panded broad-spectrum antibiotic representing a new class called glycyclines (Ruzin et al., 2005) . Previous studies revealed the involvement of multi-drug efflux systems such as MexXy and AcrAB in the decreased tigecycline susceptibility of *P. aeruginosa*. These pumps belong to the resistance-nodulation-division (RND) family that combines bacterial transposons with a tripartite architecture and broad substrate specificity (Lomovskaya and Watkins, 2001). Due to the broad substrate specificity of RND pumps, their over expression usually results in the multi-drug resistance patterns observed in Table 3 (Ishida et al., 1995; Visalli et al., 2003; Poirel et al., 2005). In addition, the prevalence of quinolone-resistant *P. aeruginosa* concurs with the findings of Kaye et al. (2006).

The 17 *K. pneumoniae* isolates showed moderate to low sensitivity to the antibiotics used. Multiple-antibiotic-resistant *Klebsiella* spp (Table 3) are important nosocomial pathogens and commonly express extended-spectrum - lactamase (ESBL) enzymes belonging to the SHV family, encoded by bla_{SHV} genes (Jones et al., 2005). A number of ESBL-producing *Klebsiella* spp in Australia and Tunisia were also similarly reported to be resistant to gentamycin and tobramycin by virtue of an *aad*B gene cassette (Jones et al., 2005; Ktari et al., 2006).

Extended-spectrum -lactamases are increasingly prevalent worldwide among *E. coli* bacteria, mostly in community-acquired urinary tract infections (Naas et al., 2007; Pai et al., 2007). Genes encoding ESBL are usually located on conjugative plasmids (such as bla_{TEM} or bla_{SHV}), although many of the most recently described ESBL genes are frequently found within integron-like structures (such as bla_{CTX-M}, bla_{GES}, or bla_{VEB-1}) (Machado et al., 2005). These ESBL-producing isolates are usually resistant to antibiotics such as aminoglycosides, tetra-cyclines, chloramphenicol, trimethoprim and sulfona-mides as observed in Paris, Tunis, Bangui and in this present study (Lavollay et al., 2006; Lavigne et al., 2006; Karlowsky et al., 2006; Naas et al., 2007). However, unlike the other studies, the isolates were susceptible to quinolone (ofloxacin).

E. faecalis was basically resistant to the antibiotics used in this study. The organism is important because of its prominence in multi-drug resistant nosocomial infections that are difficult to treat or control, its propensity for incorporation of mobile elements and its ability to transfer resistance phenotypes to other pathogens, more especially the transfer of vancomycin resistance to methicillin-resistant *S. aureus* in humans (Weigel et al., 2003; Nalla-pareddy et al., 2005; LaPlante et al., 2006). The strains resist penicillin-aminoglycoside synergy by the production of plasmid mediated aminoglycoside-modifying enzymes such as aminoglycoside 3'-phospho-transferase which has a broad range of substrate specificity (Calderwood et al., 1981).

Both MRSA and MSSA were isolated in this study as previously reported by Olayinka et al. (2003) and Onanuga et al. (2005). Whereas the MRSA isolates of Onanuga et al. (2005) were susceptible to gentamicin and ciprofloxacin, our present findings and those of Baddour et al. (2006) in Riyadh and Akpaka et al. (2006) in Trinidad and Tobago revealed a low percentage of susceptible isolates to both antibiotics. However, the high level of MRSA susceptibility to ofloxacin observed by Onanuga et al. (2005) concurred with our findings. In addition, ofloxacin was also very effective against the MSSA isolates while nalidixic acid and tetracycline were poor as also reported by Ibadin (2002).

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