Multi-Drug Resistant (MDR) Urinopathogens among Patients Attending a Tertiary Hospital in Lagos, Nigeria

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Abstract: Bacterial agents are the cause of urinary tract infection. This study was carried out to investigate the aetiology of urinary tract infection in patient attending out-patient clinic in a tertiary hospital in Lagos, Nigeria. Two hundred and fifty (250) mid-stream urine samples were examined for significant bacteriuria, of which 58(23.2%) yielded significant bacteriuria while 192(76.8%) showed no growth. Organisms were identified based on their morphological characteristics, Gram reaction and biochemical reactions. Eight different pathogens were isolated and identified with *Escherichia coli* having the highest prevalence of 29.31%. This was followed by *Enterococcus sp* 11(19.0%), *Klebsiella pneumoniae* 9(15.5%), *Staphylococccus aureus* 7(12.1%), *Staphylococcus saprophyticus* 6(10.3%), *Pseudomonas aeruginosa* 4(6.9%), *Proteus species* 2(3.5%) and *Candida albicans* 2(3.5%). Most of the strains were sensitive to Ceftraxone, Gentamycin, Ciprofloxacin and Tetracycline and majority of the isolates showed multi-drug resistant (MDR) pattern to most of the antibiotics used. The study showed the presence of multi-drug resistant (MDR) urinopathogens among patient attending out-patient clinic in a tertiary hospital in Lagos, Nigeria and this calls for particular public health attention.

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1. INTRODUCTION

The urinary system is a sterile unit, which consists of the kidneys, ureter, bladder and the urethra. The key elements in the systems are the kidneys which are pair of purplish brown organ located below the ribs towards the middle of the back (Gilernwater *et al*, 2002). Urine is stored in the bladder and emptied through the urethra (Gillenwater *et al*, 2002). About 90% of all episodes of bacterial urethrocystitis have been found to be caused by Gram negative bacilli of the family enterobacteriaceae. The Entero-bacteriaceae is Gram negative bacilli 2um-3um long by 0.6um in transverse diameter. They are aerobic and facultatively anaerobic, growing well on artificial culture mediums. All species ferment glucose (Allan *et al*, 2003). However, *E. coli* was isolated from two thirds of the urban children while *S. aureus* was the commonest organism among rural children (Akinkugbe et al., 1973). *Staphylococcus aureus* was also reported to be an important cause of UTI (10.0%) among pregnant women in Nairobi (Mati, 1994; Allan *et al*., 2003). *Serratia sp* are almost always hospital acquired by patients with in dwelling urethral catheters who are under treatment with antimicrobials.

Pseudomonas aeruginosa are motile Gram negative bacilli that cause urethrocystits, almost always secondary to urinary instrumentation. Among the Gram positive or genus enterococcal, *Streptococci sp* (Lancefield Groups B) each causes about 2% of episode of urethrocystitis. *Staphylococcus saprophyticus* (Coagulase negative) is the second most common cause of urethrocystitis in sexually active young female, others are *Candida albicans* and other species may cause urinary infection without evidence of renal involvement, a situation most often encountered by patients with diabetes mellitus or in dwelling urinary catheters. Although morphology changes have been observed in the urinary tract with infections caused by cytomegalovirus (intranuclear inclusion in renal tubular cells); virus in Bowman's capsules tubular, epithelium and urethral and bladder-mucosa (Hovelius and Mardh, 1984).

Emergence of multiple resistances to antibiotics by organisms has also been documented (Cheesebrough, 2006; Chikere *et al.,* 2008; Okonko *et al.,* 2009, 2010). According to Suchitra and Lakshmidevi (2009), intensive medical therapies and frequent use of antimicrobial drugs are capable of selection of resistant microbial flora. This also points to the fact that the prevalence of such multi-drug resistant (MDR) organisms should be checkmated since their economic implication cannot be over emphasized (Okonko *et al.,* 2010). A prominent reason for concern with regard to these MDR isolates is the recognized emergence of antimicrobial resistance among key species. However, a number of studies in the literature indicated a gradual increase in the emergence of antibiotic-resistant microorganisms especially in hospitals (Suchitra and Lakshmidevi, 2009). Many factors apart from antibiotic exposure can contribute to the development of antibiotic resistance in bacterial isolates.

This study was carried out to investigate the aetiology of urinary tract infection in patient attending out-patient clinic in a tertiary hospital in Lagos, Nigeria.

2. MATERIALS AND METHODS

2.1. Study population

A total of 250 urine samples of patients attending outpatient clinic in a tertiary hospital in Lagos State were involved in the study between April-June 2009. The subjects were made up of 125 females and 98 males.

2.2. Collection of Sample

A total of 250 mid-stream clean catch urine samples were collected in sterile containers from patients suspected of having urinary tract infection and were attending Out-patients Clinic of the hospital between April-June 2009. All the collected urine samples were examined immediately and those which were not immediately examined were stored in the refrigerator at 4^0C but were cultured within 6 hours of collection.

2.3. Culturing, Isolation and Identification

A modified semi-quantitative method was employed by inoculating non-centrifuged fresh urine samples using a standard inoculating loop, which delivered a fixed amount of urine (0.002ml) on agar plate. This was achieved by immersing sterile wire loop vertically in undiluted urine samples. This is then inoculated on to the surface of well-dried media. Plates were incubated aerobically at 37^0c for 24 hours, and the average number of colony is obtained by counting the viable growth. Significant bacteria was however, determine by counting the number and different types of bacteria isolated from a urine sample. A count of 10^5 colony unit (CFU/ml) was regarded as significant. A culture was considered to have significant growth, if (200) two hundred or more colonies of a single species were isolated on the culture plate. All the pathogens that produced significant growth were subjected to further identification and confirmatory tests by standard procedure (Cheesbrough, 2000). The cultural characteristics of the isolates were observed and were useful in identifying the organism. These include the colour, size, shape, elevation, margin, and surface texture of the colonies; and the production of heamolysis on blood agar plates. A single colony was picked from either MacConkey or blood agar plate and sub-culture on to a MacConkey plate. Each plate was incubated at 37^0c, for 24 hours to obtain pure colonies. From this purity plate, a single colony was picked and sub-cultured onto blood base agar slope and incubated at 37^0c, for 24 hours. All bacterial isolated were identified on the basis of the cultural characteristics, Gram stain and biochemical reactions (Jolt et al., 1994; Cheesbrough, 2006).

2.4. Antibiotic susceptibility Testing

Antibiotic susceptibility was determined by the agar diffusion technique as described by Bauer et al. (1966). Isolates were considered as sensitive or resistant to an antibiotic according to the diameter of inhibition zone size interpretative chart (Clinical and Laboratory Standard Institute, 2006).

3. RESULT ANALYSIS

The result from bacteriological examination of the 250 urine sampled, shows that 58(23.2%) had significant bacterial growth of $\leq 10^5$ colony unit (CFU/ml), and 192(76.8%) had no growth. Age group 21-30 had the highest bacterial isolates while the least was found in age groups ≤ 10, 51-60, 61- 70 and 71-80 years (Table 1).

Table 1: Age Distribution of the subjects and pattern of growth of Urinopathogens among Patients Attending a Tertiary Hospital in Lagos, Nigeria

Age groups (years)	No. Tested	Significant Growth (%)	No growth (%)
≤ 10	19	2 (10.5)	17(89.5)
11-20	32	5 (15.6)	27(84.4)
21-30	61	22 (36.1)	39 (63.9)
31-40	76	16 (21.1)	60 (78.9)
41-50	42	8 (19.1)	34 (80.9)
51-60	10	2 (20.0)	8 (80.0)
61-70	5	2 (40.0)	3 (60.0)
71-80	5	2 (33.3)	3 (66.7)
Total	**250**	**58 (23.2)**	**192(76.8)**

Table 2 shows the frequency of occurrence of the urinopathogens. *Escherichia coli* formed the bulk of bacteria isolated from the urine culture of the patients accounting 17(29.3%). This was followed by *Enterococcus species* 11(19.0%), *Klebsiella pneumoniae* 9(15.5%), *Staphylococcus aureus* 7(12.1%), *Staphylococcus saprohyticus* 6(10.3%), *Pseudomonas aeruginosa* 4(7.0%), *Candida albicans* 2(3.5%), and *Proteus species* 2(3.5%) as shown in Table 2. Out of the 58 samples with significant growth, 41(70.7%) were from females while 17(29.3%) were from males. *Escherichia coli* were found to be most prevalent organism causing urinary tract infection among the sexes. *Candida albicans* were found among the females only.

Table 2: Frequency of occurrence of Urinopathogens from Patients Attending a Tertiary Hospital in Lagos, Nigeria

Urinopathogens	No. (%)	No. Males (%)	No. Females (%)
Escherichia coli	17(29.3)	6 (35.3)	11 (64.7)
K. pnuemoniae	9(15.5)	4 (44.4)	5 (55.6)
P. aeruginosa	4(7.0)	2 (50.0)	2 (50.0)
Proteus species	2(3.5)	1(50.0)	1 (50.0)
S. aureus	7(12.1)	0(0.0)	7 (100.0)
Enterococcus spp	11(19.0)	4 (36.4)	7 (63.6)
Candida albicans	2(3.5)	0(0.0)	2 (100.0)
S. saprohyticus	6(10.3)	0(0.0)	6 (100.0)
Total	**58(100.0)**	**17(29.3)**	**41(70.7)**

Table 3 shows the antibiotics sensitivity patterns of the urinopathogens. *Escherichia coli* had highest sensitivity rate of 70.6% to Ceftriaxone and 64.7% to Ceftazidime. *Pseudomonas aeruginosa* had highest sensitivity rate to Ciprofloxacin (75.0%) and recorded 100.0% resistant to Ampicillin and Ceftraxone while *Staphylococcus* sp had the highest sensitivity to Ciprofloxacin. *Proteus* sp. showed resistant to Ampicillin, Colistin, Gentamycin, Cefuroxime, Ceftrazone and Tetracycline (Table 3). The overall percentage resistance to antibiotics ranged from 41.4% to

77.6%. The urinopathogens showed a multi-drug resistant pattern to all the antibiotics drugs used (Table 3).

Table 3: Antibiotics Susceptibility and Resistant pattern of the Urinopathogens from Patients Attending a Tertiary Hospital in Lagos, Nigeria

Urinopathogens	No.	Antibiotics Sensitivity and Resistant Pattern (%)															
		Colistin		Gentamycin		Cefuroxine		Ceftazidime		Ampicillin		Ceftraxone		Ciprofloxacin		Tetracycline	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. coli	17	7(41.2)	10(58.8)	11(64.7)	6(35.3)	0(0.0)	17(100.0)	11(64.7)	6(35.3)	5(29.4)	12(70.6)	12(70.6)	5(29.4)	2(11.8)	15(88.2)	4(23.5)	13(76.5)
S. aureus	7	4(57.1)	3(42.9)	3(42.9)	4(57.1)	2(28.6)	5(71.4)	2(28.6)	5(71.4)	1(14.3)	6(85.7)	4(57.1)	3(42.9)	6(85.7)	1(14.3)	4(57.1)	3(42.9)
K. pneumoniae	9	7(77.8)	2(22.2)	0(0.0)	9(100.0)	4(44.4)	5(55.6)	5(55.6)	4(44.4)	0(0.0)	9(100.0)	7(77.8)	2(22.2)	5(55.6)	4(44.4)	5(55.6)	4(44.4)
Proteus sp	2	1(50.0)	1(50.0)	0(0.0)	2(100.0)	0(0.0)	2(100.0)	1(50.0)	1(50.0)	0(0.0)	2(100.0)	0(0.0)	2(100.0)	2(100.0)	0(0.0)	0(0.0)	2(100.0)
P. aeruginosa	4	0(0.0)	4(100.0)	1(25.0)	3(75.0)	2(50.0)	2(50.0)	1(25.0)	3(75.0)	0(0.0)	4(100.0)	0(0.0)	4(100.0)	3(75.0)	1(25.0)	1(25.0)	3(75.0)
Enterococcus sp	11	7(63.6)	4(36.4)	3(27.3)	8(72.7)	2(18.2)	9(81.8)	5(45.5)	6(54.5)	4(36.4)	7(63.6)	7(63.6)	4(36.4)	9(81.8)	2(18.2)	7(63.6)	4(36.4)
S. saprophyticus	6	3(50.0)	3(50.0)	4(66.7)	2(33.3)	3(50.0)	3(50.0)	1(16.7)	5(83.3)	3(50.0)	3(50.0)	4(66.7)	2(33.3)	5(83.3)	1(16.7)	4(66.7)	2(33.3)
Total	58	29(50.0)	29(50.0)	22(37.9)	36(62.1)	13(22.4)	45(77.6)	26(44.8)	32(55.2)	13(22.4)	45(77.6)	34(58.6)	24(41.4)	32(55.2)	26(44.8)	25(43.1)	33(56.9)

Keys: S= Sensitive, R= Resistant, % = Percentage

4. DISCUSSION

The aim of this study was to investigate the aetiology of urinary tract infection in patient attending out-patient clinic in a tertiary hospital in Lagos, Nigeria. From the result of this study carried out, eight genera species of microbial isolates were gotten from the urine samples examined. The isolates which are mainly gram negative bacilli of the family, enterobactericeae are *Escherichia coli*, *Klebsiella sp*, *Pseudomonas sp*, *Proteus sp*, other organisms are coagulase positive staphylococcus, *Candida albicans*, coagulase negative staphylococcus, *Enterococcus sp*. Out of the 250 urine samples examined, 58 (23.2%) samples showed signs of bacteriuria with high incidence of bacteriuria caused by single organisms relatively found in females than in males while 192 showed no growth. Factors such as poor hygiene during menses, coitus, pregnancy and vaginitis this coupled with poor toilet habits and proximity of the urethra to the anus are possible reasons for the increased infection among females (Kunin, 1997). This calls for increase in personal hygiene among females like washing the external genitalia after defecating, they should be taught to clean from front backwards and not the other way after defaecating as this may frequently lead to infecting the urethra with faecal commensals and pathogens.

In this study, *Escherichia coli* had the highest prevalence 17 (29.31%), followed by *Enterococcus sp*. (19.0%). This showed the prevalence of *E. coli* over the other pathogens giving credence to the work done by Akinkugbe et al. (1973) where *E. coli* was isolated from two third of the urban children giving an incidence of 67.0% and also agrees with Cheesbrough (2000). *Klebsiella pneumoniae* was found in 9(15.52%) samples. *Pseudomonas aeruginosa* was found in 4(6.89%) samples, *Candida albicans* was found in 2(3.45%) samples and *Proteus sp* was found in 2(3.45%) samples. These pathogens may also have resulted from contamination of the urinary tract due to unhygienic practices.

Out of the 250 patients of various age groups screened for UTI, the 21-30 years age group had the highest number of infection 22(36.07%). The in vitro antibiotic susceptibility pattern of the Gram negative organisms showed high resistance to commonly used antibiotics such as Ampicillin, Gentamycin, Tetracycline, Cefruroxime, Colistin and Ciprofloxacin. The urinopathogens showed a multi-drug resistant pattern to all the antibiotics drugs used. The overall percentage resistance to antibiotics ranged from 41.4% to 77.6%. *Klebsiella* sp showed resistance ranging from 22.2% to 100.0% in this study. *Proteus* sp also showed resistance ranging from 50.0% to 100.0% to the antibiotics tested. *E. coli* showed resistance ranging from 29.4% to 100.0% of the antibiotics tested. In this study, tetracycline inhibited *S. saprophyticus* (66.7%), *Enterococcus* sp. (63.6%), *S. aureus* (57.1%), *K. pneumoniae* (55.6%), *P. aeruginosa* (25.0%) and *Escherichia coli* (23.5%) and was resisted by *Proteus* sp. (100.0%). *Proteus* sp. was 100.0% susceptible to ciprofloxacin. This is in agreement with what was reported by Mordi and Momoh (2009) and Okonko *et al.* (2010), who reported *Proteus* sp to be susceptible to ofloxacin and ciprofloxacin. Resistance of *Proteus* sp to tetracycline reported by Mordi and Momoh (2009) and Okonko *et al.* (2010) is similar to the findings of this present study. Resistance of *Proteus* sp to gentamicin reported in this study is at variance to the findings of Mordi and Momoh (2009) and Okonko *et al.* (2010). According to Mordi and Momoh (2009) and Okonko *et al.* (2010), literature reports indicated that most strains of all strains of *Proteus* are susceptible to gentamicin.

Escherichia coli, the most common pathogen producing UTI, had a sensitivity rate of 70.6% to Ceftriaxone. *Pseudomonas aeruginosa* showed the highest sensitivity to Ciprofloxacin. *Staphylococcus* sp. had the highest sensitivity to Ciprofloxacin while *Proteus sp* showed resistant to Ampicillin, Colistin, Gentamycin, Cefuroxime, Ceftrazone and Tetracycline. This agrees with the studies of Okonko et al. (2010), Ibiene et al.

(2012) and Adebayo-Tayo et al. (2012), which states that the in-vitro antibiotic susceptibility pattern of the negative organisms showed high resistant to commonly used antibiotic. Also, in this study, high percentage resistance rate of 29.4% to 100.0% was observed for *E. coli* and other urinopathogens. This has satisfied multidrug resistant (MDR) pattern of resistance to >3 antibiotics. This agrees with the findings of Okonko *et al.* (2010), who reported *E. coli* resistance to gentamycin. The MDR pattern reported on *E. coli* in this study is comparable to previous studies (Dolejska *et al*., 2007; Sjölund *et al.,* 2008; Ibiene et al., 2012). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Karlowsky *et al.,* 2004; Okonko *et al.,* 2010; Ibiene et al., 2012). This finding on *E. coli* showed close resemblance to those of a recent study of ciprofloxacin-resistant *E. coli* from humans and chickens in the late 1990s in Barcelona, Spain reported by Johnson et al. (2007) as ciprofloxacin-resistant *E. coli* was reported in this study. The resistances obtained with these test antibiotics were comparable with those reported in other studies (Abdellah *et al.,* 2009; Okonko *et al.,* 2010; Ibiene et al., 2012; Adebayo-Tayo et al., 2012).

5. CONCLUSION

The most common multi-drug resistance (>3 drugs) patterns included resistance to all the antibiotics tested in this study. The presence of multidrug resistant pathogens such as *Escherichia coli*, *Enterococcus species*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus saprohyticus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Proteus species* encountered in these subjects is alarming. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Alim *et al.,* 2009; Okonko *et al*., 2010). It is so obvious from the results of this study that bacteria agents are the cause of urinary tract infection. It also showed Ceftrazone and Ceftazidime had high sensitivity to the most incriminated bacteria (*E. coli*). Therefore this antibiotic can be use for empiric treatment against UTI where laboratory diagnosis is not available or in case of urgent management.

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