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Antibiotic Sensitivity Patterns of Bacterial Pathogens Associated with Urinary Tract Infections in Three General Hospitals in Lagos, Nigeria

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ABSTRACT This study was carried out to identify the common bacterial pathogens associated with urinary tract infections and their susceptibility patterns to common antimicrobial agents. One hundred (100) clean catch midstream urine samples from patients attending three different hospitals in Lagos, Nigeria were analyzed. A standard microbiologic procedure was used to culture the urine and the identification was done using appropriate biochemical tests. Antibiotic sensitivity of the isolates ones was determined using the disk diffusion technique on the Mueller-Hinton agar. Out of the one hundred (100) samples analyzed, 74 yielded significant bacteriuria and out of these, 83.79 percent were Gram negative bacteria. The most prevalent isolate identified were *Klebsiella pneumonia* (40.54%), followed by *Escherichia coli* (35.14%), *Staphylococcus aureus* (16.22%), *Enterobacter spp* (5.40%) and *Proteus* (2.70%). The bacterial isolates were more sensitive to Nitrofurantoin with percentage sensitivity of 76.67 percent, 73.1 percent, 58.3 percent and 50.0 percent for *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus,* and *Enterobacter spp* respectively, but resistance to Amoxicillin, Cloxacillin and Augmentin was quite high. One hundred (100%) percent resistance was recorded for Lincomycin, Oxacillin, Cotrimoxazole, Tetracycline, and Nalidixic acid. In the present study, Nitrofurantoin was the most effective therapeutic agent against bacterial pathogens causing UTI.

INTRODUCTION

Microbial invasion of the kidneys, ureters, bladder and urethra is known as Urinary Tract Infection (UTI) (Beer et al. 2006). This may be characterized by an inflammation of the urothelium (Andersson 2004) and the infecting pathogens affecting the entire urinary tract, or be restricted to either the upper region or lower region (Stamm and Norrby 2001). The bacterial that cause UTI are primarily of the Gram negative group with prevalence of eighty to eighty-five percent. The most frequently reported members of these bacteria are *E.coli and Klebsiella* (Eghafona et al. 1988; Ebie et al. 2001; Omonigho et al. 2001). However, in complicated UTI, the prevalence of other antibiotic resistance organisms such as *Proteus, Serratia, Enterobacter* and *Pseudomonas* increases. The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment (Omonigho et al. 2001). This is because they easily gain access to the urethral opening due to proximity to the anus and they constitute a serious problem in UTI in many parts of the world (Naeem 2001). In Nigeria, *E.coli*, *Proteus* sp and *Klebsiella* sp have been isolated in ninety percent of UTI reported cases (Obaseiki-Ebor 1988). Other bacterial pathogens frequently isolated include *S. aureus, Staphylococcus epidermidis* and *Streptococcus faecalis* (Omonigho et al. 2001).

The prevalence and incidence of urinary tract infections are higher in women than in men. This is due to several clinical factors in women such as hormonal effects, behavior patterns or the presence of a short urethra and vaginal vestibule, which can be easily contaminated (Ebie et al. 2001; Kolawole et al. 2009). Men who are uncircumcised are more at risk to become infected because the bacteria can build up much more easily in the folds of the extra skin of the penis as are men who engage in anal intercourse (Bhat et al. 2011).

The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health problem particularly in the

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developing world where apart from high level of poverty, ignorance and poor hygiene practices, there is also a high prevalence of counterfeit drugs of questionable quality in circulation (Abubakar 2009). Hence, the changing pattern of microorganisms involved in urinary tract infections and resistance across institutions and locations have necessitated the conduct of antibiotics susceptibility testing study of UTI pathogens in various regions from time to time.

There is a considerable disparity among various reports as to which of *E. coli* and/or *Klebsiella* spp is/are predominant in UTI cases. While some workers reported *E. coli* as the most occurring (Obiogbolu et al. 2009; Oladeinde et al. 2011; Oluremi et al. 2011), other workers maintained that *Klebsiella* spp was the most implicated (Omonigho et al. 2001; Nwadioha et al. 2010; Mazokopakis and Patolidis 2012). The etiological agents causing UTI differ in localities over a certain period of time. Hence, for effective treatment of a patient, it becomes important to determine the etiological agents and their antimicrobial sensitivity pattern.

Objective

The objective of this study was to investigate the bacteria pathogen associated with urinary tract infection and their antimicrobial susceptibilities patterns among patients attending three (3) metropolitan hospitals in Lagos state.

MATERIAL AND METHODS

Study Population

One hundred (100) patients presented with clinical symptoms of UTI from three different hospitals (Randle General Hospital, Surulere, General Hospital Mushin and Onikan Health Center, Ireland) in Lagos state, Nigeria were involved in this study. They comprise 76 females and 24 males.

Sample Collection

Permission to carry out this study and collect appropriate samples at the various study centers were obtained from the hospitals. Informed consents of the patients were also obtained for sample collection. Sterile, leak-proof, universal, plastic containers were used for collection of freshly voided midstream urine from the patients and transported to the laboratory for cultural analysis.

Isolation of Bacterial Pathogens

A sterile loop was dipped vertically just below the surface of a well-mixed urine sample and a loop full of the urine sample was taken avoiding air bubbles. The sample was then inoculated onto duplicate sterile agar plates of Chocolate and MacConkey media by streaking technique for isolation of the bacterial etiologic agents. Thereafter, inoculated plates were incubated at a temperature of 37^{o}C for 18-24 hours.

Identification of Bacterial Isolates

All cultured plates with bacterial growths were selected and examined macroscopically and microscopically. Cultural characteristics of discrete bacterial colonies on the plates with significant bacterial growths were noted and subcultured on Nutrient agar for purification. Phenotypic identification of the isolates was confirmed based on selected biochemical reactions using the standard procedures of Barrow and Feltham (1995) and Cheesbrough (2000).

Antibiotic Susceptibility Pattern

The isolated bacterial pathogens were screened for their *in vitro* susceptibilities to antibiotics using the disk diffusion method as described by the Clinical and Laboratory Standard Institute (CLSI; formerly NCCLS) guidelines (NCCL 1999) on Mueller-Hinton agar plates. Overnight pure cultures of the isolates were employed for this purpose. Few colonies of each isolate were suspended in 5 ml sterile normal saline (0.85% w/v NaCl) and mixed gently to obtain a uniform suspension. The inoculum concentration was standardized by adjusting the turbidity of the suspension to the optical density of 0.5 McFarland Standards.

Each bacterial isolate was then seeded into the Mueller-Hinton agar medium using a sterile cotton swab dipped into the standardized suspension, drained, and used for inoculating the medium. Inoculated plates were allowed to stand for about 10 minutes before sterile forceps were used to aseptically transfer some antibiotic sensitivity disks onto the surface of the cultured

plates. The plates were incubated aerobically at 37^{0}C for 18-24 hours. After incubation, the diameters of the zones of inhibition were observed and measured (Zinnah 2008) with a ruler and compared with a zone-interpretation chart (Bauer et al. 1966). *Es.coli* ATCC 25922 were used as control for Gram negative, while *S. aureus* (NCTC 6571) was used as control for Gram positive bacteria.

The following antibiotics discs from Oxoid were used for the susceptibility test: Ceftazidime (Caz 30µg), Cefuroxime (Crx 30µg), Oxacillin (Oxc 10µg), Lincomycin (Lin 2µg), Cloxacillin (Cxc 20µg), Ofloxacin (Ofl 5µg), Gentamicin (Gen 10µg), Nitrofurantoin (Nit 200µg), Nalidixic acid (Nal 30µg), Augmentin (Aug 30µg), Cotrimoxazole (Cot 25µg), Amoxicillin (Amox 25µg), and Tetracycline (Tet 25µg).

RESULTS

Of the one hundred (100) samples examined in this study, 74 showed significant bacteriuria ($\geq 10^5$ cfu/ml). Sixty-five (65) bacteria were obtained from female subjects while 9 were from males (Table 1).

Table 1 : Sex distribution of patients with urinary tract infection (N= 100)

Sex	*Number*	*Positive sample*	*% of occurrence*
Female	76	65	85.5
Male	24	9	37.5

The frequency of occurrence of Gram negative bacteria was higher (83.78%) than Gram positive bacteria (16.22%) and higher numbers of Gram negative were recovered from female urine samples as compared to male urine samples. As evident from Table 2, the highest number of Gram negative bacteria found in urine sample was *K. pnuemoniae* 30 (40.5 %) followed by *E. coli* 26 (35.1%), *Proteus mirabilis* 2 (2.7%), *Enterobacter* spp 4 (5.4%) and *S. aureus* 12 (16.2%).

Table 2: Percentage of occurrence and distribution of bacterial pathogens in relation to sex of patients with UTI (n= 74)

S. No.	*Isolates*	*Male*	*Female*	*Total*	*Percentage (%)*
1.	*K. pneumonia*	1	29	30	40.5
2.	*E. coli*	3	23	26	35.1
3.	*S. aureus*	4	8	12	16.2
4.	*Enterobacter* spp	Nil	4	4	5.4
5.	*P. mirabilis*	1	1	2	2.7
	Total	9	65	74	100

Furthermore, the isolated bacteria were subjected to antimicrobial susceptibility testing and the susceptibility patterns are illustrated in Table 3. All the isolated pathogens were one hundred percent resistant to Lincomycin, Oxacillin, Cotrimoxazole, Tetracycline and Nalidixic acid while all the Gram negative bacteria were one hundred percent resistant to Augumentin. None of the 13 tested antibiotics were one hundred percent sensitive to all the isolated bacteria but

Table 3: Percentage sensitivity of bacteria isolates to antibiotics

Organism	*E.coli (n=26)*	*K. Pneumoniae (n=30)*	*S.aureus (n=12)*	*Enterobacter spp(n=4)*	*P. mirabilis (n=2)*
CAZ	19 (73.1)	21 (70)	12 (100)	3 (75)	2 (100)
AUG	26 (100)	30 (100)	11 (91.7)	4 (100)	2 (100)
LIN	26 (100)	30 (100)	12 (100)	4 (100)	2 (100)
OXC	26 (100)	30 (100)	12 (100)	4 (100)	2 (100)
NIT	7 (26.9)	7 (23.3)	5 (41.67)	2 (50)	2 (100)
CXC	25 (96)	30(100)	9 (75)	4 (100)	1 (50)
COT	26 (100)	30 (100)	12 (100)	4 (100)	2 (100)
AMX	21.4 (82.2)	30 (100)	12 (100)	3 (75)	1 (50)
GEN	15 (57.6)	25 (93.3)	10 (83.3)	2 (50)	1 (100)
TET	26 (100)	30 (100)	12 (100)	4 (100)	2 (100)
CRX	20 (76.9)	26 (86.7)	9 (76.7)	3 (75)	1 (50)
OFL	11 (42.3)	18 (60)	4 (33.3)	2 (50)	1 (50)
NAL	26 (100)	30 (100)	30 (100)	4 (100)	2 (100)

Key: CAZ- Ceftazidime ; AUG -Augmentin; NIT –Nitrofurantoin; CXC – Cloxacillin; AMX -Amoxicillin; GEN – Gentamicin; CRX – Cefuroxime; OFL – Ofloxacin; LIN-Lincomycin; COT –Cotrimoxazole; TET-Tetracycline; NAL-Nalidixic acid; OXC-Oxacillin

Nitrofurantoin was found to be sensitive to most of the isolated bacteria.

K. pneumonia isolates were the most sensitive to Nitrofurantoin while *S. aureus* showed more resistance as compared to other isolates Tables 3 and 4.

Table 4: Susceptibility pattern of bacterial pathogens to Nitrofurantoin

Bacteria pathogens	*Susceptibility pattern (%)*
K. pneumoniae	76.7
E. coli	73.1
S.aureus	58.3
Enterobacter spp	50.0
Proteus mirabilis	0

DISCUSSION

The management of urinary tract infection is an issue that needs to be addressed urgently. Effective management can be instituted through proper identification of the etiological agents and administration of the appropriate antimicrobial agents (Kebira et al. 2009). The prevalence of UTI in the population was seventy-four percent. This figure is higher than the prevalence rate of 25.6 percent and twenty-two percent recorded in Jos and Ibadan, respectively (Nedolisa 1998; Ekweozor 1996). It is also higher and different from the prevalence rate of sixty percent obtained in another study in the North-Central region of Nigeria (Kolawole et al. 2009) but agrees with Mbata (2007) who recorded 77.9 percent among prison inmates in Nigeria. The high frequency could be due to genuine population susceptibility as a result of factors like sexual intercourse, peer-group influence, pregnancy, low socio-economic status which are common among Nigerian men and women (Akinyemi et al. 1997). It is important to know that the incidence and prevalence rate cannot be the same as a result of factors like geographical differences and the particular group been considered (pregnant women, sex, prison inmates, diabetes patients, catheter patients, age group and so on). The prevalence of UTI infection is higher in females than in males. Of the 74 isolates obtained, 65 were from female patients while 9 were from males (Table 1). This is in agreement with other reports, which stress that UTI is more frequent in females than in males, during youth and childhood because of differences in anatomical structure, sexual maturation and the changes that occur during pregnancy and childbirth as well as the presence of tumors (Asinobi et al. 2003; Olaitan 2006; Mbata, 2007). Majority of the bacteria isolated in this study were Gram negative and this is in accordance with the results obtained in other studies (Laupland et al. 2007; Mbata 2007). But unlike the findings of other researchers who reported *E. coli* to be the predominant pathogen associated with UTI (Obiogbolu et al. 2009; Hamdan et al. 2011; Oladeinde et al. 2011; Oluremi et al.*;* 2011) a high prevalent rate of 40.54 percent was recorded for *K. pneumoniae* in this study. This frequency is quite high compared to what has been reported for *K. pneumoniae* in other studies (Akinjogunla et al. 2010; Mahmood et al. 2012; Pondei et al. 2012). The high occurrence of *Enterobacteriaceae* and some of the isolated pathogens being coliforms show that a high percentage of urinary tract infection may be due to fecal contamination arising from poor unhygienic practices, poor safety habits and poor sanitary lifestyle. This is due to the fact that these bacteria occur in the perineum of the large intestine as commensals (Behzadi et al. 2008; Moore et al. 2002; Anyamene et al. 2002). Also, commensals of the intestine are more involved in UTIs because of the anatomy proximity to the genitourinary area (Obiogbolu 2004).

In the present study, resistance of the isolates to most of the antibiotics could be an indication of earlier exposure of the organisms to these antimicrobial agents. Several factors have been known to greatly contributed to this problem, which include, improper and irrational drug misuse by general practitioner in clinical practices, inexperienced practitioner, the public, low quality drugs, fake and expired drugs among several others. The use of antibiotics without prior knowledge of the etiological agents is another serious factor contributing to this growing global problem (Okeke et al. 1999).

The most sensitive antibiotic in this study was Nitrofurantoin because least resistance was developed by the bacterial isolates to this antimicrobial agent. This result is in conformity with the results of other researchers (Bean et al. 2008; Rajesh et al. 2010; Mahmood 2011; Pondei et al. 2012). Nitrofurantoin is not a frequently prescribed antibiotic and in some countries prescription is restricted (Schmiemann et al. 2010). It is thus not a commonly abused antibiotic. This could be responsible for the little resistance

showed by the bacterial isolates to Nitrofurantoin. This observation disagree with the findings of Kolawole et al. (2009), that reported ineffectiveness of Nitrofurantoin against UTI bacterial pathogen. In contrast, most of the isolates exhibited high resistance to Cloxacillin, Augmentin, Tetracycline, Cotrimoxazole, Nalidixic acid, Ofloxacin, Lincomycin, Oxacillin, Ceftazidime, Cefuroxime, Amoxicillin and Gentamicin. Drug misuse by the public has been surveyed in Nigeria, with Ampicillin and Tetracycline being the most abused antibiotics (Obaseiki-Ebor et al. 1987; Yah et al. 2008). Cloxacillin is a component of Ampiclox, which is one of the most popular antibiotics involved in self-medication in Nigeria (Yah et al. 2008). High resistant to Cotrimoxazole and Nalidixic acid was also reported in the work of Mahmood (2011).

The occurrence of multiple drug resistant bacterial isolates obtained in this study is a serious health issue, as this can result in increased morbidity and mortality, which implicates increased costs of treatment in the studied population. There is an urgent need to look into the increasing problem of antimicrobial resistance. New resistance mechanisms, such as the Extended Spectrum Beta Lactamases (ESBL) and the New Delhi metallo-beta lactamase 1(NDM-1), have also been reported. This calls for appropriate measures for antibiotics usage in the treatment of UTI. Reducing antibiotic prescription and dispensing has been associated with the reduced local antibiotic resistance (Schmiemann et al. 2010).

CONCLUSION

It is evident that variations exist in the causative agents and antimicrobial susceptibility patterns of the etiological agents of urinary tract infection. *K. pnuemoniae* and *E. coli* were the major causative agents of UTI observed in this study apparently due to poor safety habit, poor hygiene and poor sanitary lifestyle. Most of the isolates obtained are multidrug resistant with nitrofurantoin as the most effective therapeutic agent against the bacterial pathogens and the most appropriate for use in the treatment of UTI in the three health institutions under study in Lagos State, Nigeria. Determining the causative agents and the appropriate drug of choice should be the first step before commencement of treatment. This will greatly help in reducing the development of multiple drug resistant bacterial pathogens.

RECOMMENDATIONS

There is need to implement infection control measures in most healthcare institutions, which has been shown to be lacking. Also, there is need to establish disease control and surveillance agencies like the Centers for Disease Control and Prevention (CDC) and the Health Protection Agency (HPA) as obtained in the USA and the UK, respectively. Physicians and clinical microbiology laboratory need to work hand in hand so that information obtained on susceptibility testing in the laboratory can be utilized in prescribing the appropriate antibiotics to patients.

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