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Full Length Research Paper

Susceptibility of common urinary isolates to the commonly used antibiotics in a tertiary hospital in southern Nigeria

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Antimicrobial resistance in the treatment of urinary tract infections is a major health problem. This study evaluates the pattern of susceptibility of pathogens commonly responsible for urinary tract infections (UTIs) to commonly used antimicrobial agents in Benin City. Midstream urine samples of 700 patients (300 males and 400 females), who were attending clinics in a 550-bed University of Benin Teaching Hospital, Benin City, between April 2003 to March 2004 were examined. Susceptibility of the urine bacteria isolates to twelve commonly used antibiotics was investigated. Eight bacteria isolates were recovered from 49.5% of the patients (18.1% of males and 31.4% of females). These were *Escherichia coli* (19.7%), *Klebsiella aerogenes* (15.1%), *Proteus mirabilis* (6.7%), *Acinetobacter calcoaceticus* (2.3%), *Pseudomonas aeruginosa* (2.3%), *Streptococcus faecalis* (1.3%), *Providence stuartii* (1%), and *Alkaligenes faecalis* (1%). All the isolates exhibited a significantly high resistance to tetracycline, co-trimoxazole, amoxycillin and cefuroxime but were either moderately or highly sensitive to the quinolones and nitrofurantoin. We conclude that majority of the antimicrobial agents that are commonly used to treat UTIs in the hospitals are no longer effective. Therefore, the development and strict management of antimicrobial policy, and surveillance for resistant organisms should be given priority in Nigeria.

Key words: Urinary tract infections, antimicrobial agents, bacterial isolates.

INTRODUCTION

Urinary tract infections (UTIs) are common type of bacterial infection accounting for reasonably high health care expenditures in people of all ages, with more than 35 million medically treated infections each year (Mindbranch Inc, 2004). This translates into sales in excess of $1.1billion across key markets in 2003, with the majority of value derived from community management (Mindbranch Inc, 2004). Young women, the elderly and those undergoing genitourinary instrumentation or catheterization, among others, are also at risk (Kunin, 1994). UTIs are the leading cause of gram-negative bacteremia. In 1991, these infections accounted for approximately 7 million office visits and more than 1 million hospitalizations, for an overall annual cost in excess of $1 billion, in the United States (Patton et al., 1991).

UTIs are usually treated with antibiotics including nalidixic acid, nitrofurantoin, ofloxacin, perfloxacin, ciprofloxacin, gentamycin, etc. In the last couple of years, there has been a lot of focus in scientific literature on inappropriate use of antimicrobial agents and the spread of bacterial resistance (Gold and Moellering, 1996; Tenover and Hughes, 1996; Tenover and McGowan, 1996). The widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents (Mincey and Parkulo, 2001). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial

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resistance to that agent. For example, the consumption of macrolides correlates with the rates of macrolide-resistance pneumococci (Granizo et al., 2000). There are numerous reports in the literature on the incidence of growing resistance of urinary isolates to available antibiotics (Amyes, 1987; Chesborough, 1985; Gupto et al., 1999; Jacoby and Archer, 1991). The emergence of antimicrobial resistance in the management of urinary tract infections is an important public health issue. While many antibiotics including penicillin, macrolides and tetracyclines were very useful in the treatment of urinary tract infections in the past, the rates of bacterial resistance to antimicrobial agents has significantly increased and are increasing in many countries in recent times (Ho et al., 2004). Despite the well-publicized concerns about the problems of inappropriate use of antimicrobial agents, or use of broad spectrum antibiotics (when narrow spectrum drugs would be effective), increasing resistance of bacteria causing urinary tract infections to antimicrobial agents remains a serious problem (McCaig and Hughes, 1995). With the high prevalence of fake and substandard drugs available in Nigeria (Raufu, 2002), the rate of bacterial resistance to antimicrobial agents in the management of UTIs is likely to be much higher than many other countries.

This study therefore seeks not only to highlight the types of urinary tract pathogens treated in a tertiary health care facility (University of Benin Teaching Hospital) in Benin City, Nigeria, but the degree of susceptibility of the pathogens to the commonly used antibiotics in the area. The result of this study is expected to provide useful information which would assist physicians in their good prescribing habits towards a better management of urinary tract infections.

MATERIALS AND METHODS

Collection of specimen

Midstream urine samples were collected from 700 patients (300 males and 400 females) attending the clinics (family practice clinic, consultant out-patient clinic, obstetrics and gynaecology clinic, and accident and emergency clinic) in a 550-bed University of Benin Teaching Hospital, Benin City with an out patient attendance of over 100,000 patients per annum. These patients did not include those who were on any antibiotic a week before the samples were collected. The urine samples were collected into labelled 20 ml calibrated sterile universal containers distribution to the patients suspected by the attending physicians to have UTIs. In each container, boric acid (0.2 mg) was added to arrest multiplication of bacteria in urine. All patients were instructed on how to collect the sample aseptically and taken to the laboratory immediately for culture.

Bacteriology

Each well-mixed urine sample (4.0 µl) was inoculated (in duplicates) into the appropriate culture media used with a standard wire loop (30 morse guage) having a diameter of 3.26 mm. The general culture media used was McConkey (Oxoid No Cm 7) and cysteine lactose electrolyte deficient agar (CLED, International Diagnostic Group). Blood agar (Oxoid No cm 271) was used in the case of some fastidious organisms that cannot grow on McConkey or CLED. The inoculum on the plate was streaked out for discrete colonies with a wire loop using standard method (Onanuga et al., 2005; Banjo et al., 2005). The culture plates were incubated at a temperature of 37°C for a period of 24 h. All the organisms isolated were identified by standard technique (Onanuga et al., 2005; Cowan and Steel, 1974).

Antibiotic susceptibility testing

The antibiotic susceptibility pattern of all the bacterial isolates to colistin sulphate 25 µg, nalidixic acid 30 µg, gentamicin 10 µg, co-trimoxazole 15 µg, nitrofurantoin 200 µg, tetracycline 25 µg, amoxycillin 25 µg, cefuroxine 30 µg, Ciproxin 5 µg, Tarivid 5 µg and Peflacin 5 µg were determined by the modified Kirby-Bauer diffusion technique as earlier described (Onanuga et al., 2005). A standard sensitive strain, *Escherichia coli* Cw3310, was used as the control organism. The inoculated plates carrying the antibiotics were incubated at 37°C for 24 h after which the diameter of the zone of inhibition around each antibiotic disc was measured, and isolates were classified as “resistant”, “intermediate sensitive” or “sensitive” based on the standard intermediate chart updated according to the current NCCLS standard and Fluka zone interpretative chart in accordance with WHO requirements (Onanuga et al., 2005).

RESULTS

Of the 700 urine samples collected from the patients, 347 samples (49.5%), made up of 220 (31.4%) samples from females and 127 (18.1%) from males, had infections and yielded eight different bacterial isolates (Table 1). The highest proportions of bacterial isolates from the samples investigated were *E. coli* (19.7%) and *Klebsiella aerogenes* (15.1%) accounting for 70.3% of the total number of isolates recovered from the urine samples. The standard strain was sensitive to all the antibiotics used. However, all the isolates were “resistant” to tetracycline, co-trimoxazole, amoxycillin and cefuroxime but were either “intermediate sensitive” or “sensitive” to the quinolones, and nitrofurantoin.

While nitrofurantoin effectively inhibited the growth of *Providence stuartii* and *E. coli*, the drug intermediately inhibited the growth of *Proteus mirabilis*, *Klebsiella aerogenes*, *Alkaligenes faecalis* and *Acinetobacter calcoaceticus* (Table 2). Apart from *A. calcoaceticus* that was “sensitive” to ofloxacin and perflacin, the quinolones mainly “intermediately” inhibited the growth of the bacterial isolates. Nalidixic acid “intermediately” inhibited the growth of *K. aerogenes* while gentamicin moderately inhibited the growth of *E. coli*, *aerogenes* and *P. stuartii*. Other antimicrobial agents (amoxicillin, tetracycline, colistin, and co-trimoxazole) did not effectively inhibit the growth of the bacterial isolates (Table 2).

DISCUSSION

Over the years, antimicrobial agents that are commonly used in the treatment of UTIs include trimethoprim-sulpha

Table 1. Distribution of bacteria isolates from urine samples collected from 700 patients in a tertiary hospital.

Isolates	No of patients	% of isolates
Proteus mirabilis	47	6.7
Escherichia coli	138	19.7
Klebsiella aerogenes	106	15.1
Providence stuartii	8	1.0
Alkaligens faecalis	7	1.0
Acinotobacter calcoaceticus	16	2.3
Psuedomonas aeruginosa	16	2.3
Streptococcus faecalis	9	1.3

Table 2. Susceptibility of urine bacteria isolated from 700 patients attending a tertiary hospital to commonly used antimicrobial agents.

Organisms	Number of bacterial isolates	% susceptibility to specified antimicrobial agent										
		Nitrofurantoin (F)	Oflorxacin (OFX)	Ciprofloxcin (CIP)	Peflaxcin (Pef)	Nalidixate (Na)	Gentamicin (CN)	Cefuroxine (CXM)	Amoxicillin (Amx)	Treyracucline (Te)	Colistin sulphate (CT)	Co-trimoxazole (SXT)
Proteus mirabilis	47	76.6	70.2	70.2	55.3	38.3	42.6	2.1	4.3	12.8	27.7	2.1
Escherichia coli	138	93.5	64.5	60.9	68.8	56.5	76.1	23.2	1.4	2.2	11.6	0
Klebsiella aerogenes	106	79.2	74.5	70.8	71.7	61.3	66.0	33.0	8.5	5.7	18.9	1.9
Providence stuartii	8	100	87.5	62.5	50.0	50.0	62.5	37.5	0	0	25.0	0
Alkaligenes faecalis	7	71.4	85.7	71.4	57.1	42.9	42.9	28.6	14.3	0	0	0
Acinetobacter *calcoaceticus*	16	62.5	100	62.5	100	0	0	6.3	0	0	0	0
Pseudomonas aeruginosa	16	0	56.3	56.3	31.3	0	18.8	0	0	0	56.3	0
Streptococcus faecalis	9	55.6	77.8	88.9	55.6	11.1	11.1	11.1	0	0	0	0

methoxazole (co-trimoxazole), ciprofloxacin, ofloxacin, nitrofurantoin, gentamicin, ampicillin (Orenstein and Wong, 1999), tetracycline, amoxicillin, and nalidixic acid (Romac, 1992). Our study has shown a steady and generalized increase in bacteria resistance to almost all these commonly used antimicrobial agents. For example, the sensitivity of *E. coli* urinary isolates to gentamicin, ciprofloxacin, nalidixic acid, tetracycline, and co-trimoxazole previously reported to be 97.8, 93, 84.6, 63.2, and 41.6%, respectively (Mansouri and Shareifi, 2002), has declined considerably. Although amoxicillin was frequently earlier suggested as the agent of choice for *E. coli* infections, the bacteria is now commonly resistant to the drug as shown in our results. Trimethoprim-sulfamethoxazole (co-trimoxazole) has also earlier been considered the treatment of choice in *E. coli* infections of the urinary tract but complete resistance of *E. coli* to this drug in recent times, as in our study, has previously been reported (Orenstein and Wong, 1999; Obaseiki-Ebor, 1988).

E. coli has the highest occurrence in the bacterial isolates from the urine samples we studied and was followed by *K. aerogenes*. This result differs from that reported by Akerele et al. (2000), in a five-year (1992-1996) retrospective study, which indicated that *Staph. aureus* (35.6%) was the most frequently encountered single bacteria species in urinary tract infections (accounting for 35.65% of the bacteria isolates) and was followed by *E. coli* (accounting for 24.98% of the isolates). The high frequency of occurrence of *E. coli* in urinary tract infection in our result may be related to the fact that it is most predominant in the gut. An important finding in our study is that *E. coli* and *K. aerogenes* accounted for over 70% of the bacteria isolates presenting a challenge to physicians in the management of UTIs.

In the mid 80s, ciprofloxacin proved itself as highly effective drug against urinary tract pathogens (Barba et al., 1985). Like most other drugs reported in this study, the quinolones' (ciprofloxacin, ofloxacin, and perflacin) effectiveness against urinary tract pathogens has considerably reduced. Apart from ciprofloxacin exhibiting high potency against *Streptococcus faecalis*, ofloxacin exhibiting high potency against Providence species, *Alkaligenes faecalis* and *Acinetobacter calcoaceticus*, and perflacin exhibiting high potency against *Acinetobacter calcoaceticus*, the other isolates are now only moderately sensitive to the quinolones we investigated. While nalidixic acid has only shown to be moderately effective against *E. coli* and *K. aerogenes* in our investigation, an earlier report has shown only as low as 23% of UTI bacteria showing resistant to the drug (Barba et al., 1985). Although gentamicin has been a very effective drug for the treatment of Gram-negative UTI bacteria, including *Ps. aeruginosa* in the past (Romac, 1992), we have observed resistance of *Proteus mirabilis*, *Alkaligenes faecalis*, *Acinetobacter calcoaceticus*, *Ps. aeruginosa* and *Strep. faecalis* against the drug.

When our results are compared with earlier report ((Romac, 1992), nitrofurantoin has shown continuous effectiveness against most urinary tract pathogens. This may be explained by the low rate of prescription of the drugs in the hospital where this work was carried. Our results indicate that the sensitivity of the isolated bacteria to cefuroxime, amoxicillin, tetracycline, colistin sulphate and co-trimoxazole has declined to a point that these antimicrobial agents that were previously relatively highly effective against the bacteria are no longer effective as single drug in the treatment of any of the bacteria causing UTI in the hospital. Unfortunately, amoxicillin, tetracycline, and co-trimoxazole are the commonly used antimicrobial agents for this purpose by many individuals, particularly those using them for self medication. While the high resistance to cefuroxime, amoxicillin, tetracycline, and co-trimoxazole can be explained on the basis of indiscriminate use of the antimicrobial agents in the community where this study was carried out, colistin sulphate may be ineffective because of cross-resistance.

Previous reports have indicated that the high resistance of urinary tract pathogens to antimicrobial agents in developing countries (Lester et al., 1990) is often due to self-medication, the suboptimal quality of antimicrobial drugs, and poor community and patient hygiene (Walson et al., 2001). In spite of the continued introduction of a variety of antimicrobial agents in multiple unrelated drug classes, resistance continues to emerge and poses a great challenge for the management of UTIs. In this study, many reasons can be adduced for the increasing resistance of UTI bacteria to the antimicrobial agents. First, there is high prevalence of fake and substandard drugs available in Nigeria (Romac, 1992) which is exposing unsuspecting patients to sub therapeutic doses of drugs. Second, inappropriate use of antimicrobial agents in Nigeria is widespread as many people can easily buy antibiotics from some pharmacy stores and patent medicine stores, with or without prescriptions. This widespread and inappropriate use of antibiotics is recognized as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents (Mincey and Parkulo, 2001). Third, there is evidence that for most bacteria, increased usage of a particular antimicrobial agent correlates with increased levels of bacterial resistance to that agent (Granizo et al., 2000). Bacterial resistance can results from contaminated food, antimicrobial drug exposure, and cross-contamination from humans or animals (Lester et al., 1990).

In conclusion, our results further confirm the numerous reports (Amyes, 1987; Chesborough, 1985; Gupto et al., 1999; Jacoby and Archer, 1991; Ho et al., 2004) in the literature on the incidence of growing resistance of urinary isolates to available antimicrobial agents. Although pharmaceutical companies continue to introduce new drugs for the treatment of UTIs, the rapidity of emergence of antimicrobial-resistant organisms is not being reflected by the same rate of development of new antimicrobial agents. It is, therefore, conceivable that patients with serious infections will soon no longer be treatable with currently available antimicrobials. Therefore, the development of antimicrobial policy (which is currently non-existence in Nigeria) is a priority. Strict management of such antibiotic policies and surveillance programmes for resistant organisms, together with infection control procedures, need to be implemented and continuously audited as suggested by Elliott and Lambert (1999). The pharmaceutical industry must respond to these clinical challenges by bringing forward a stream of new agents with antimicrobial activity against resistant UTI bacteria.

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