Full Length Research Paper

In vitro susceptibility of some uropathogens and a comparative assessment of antibacterial activities of local and imported multidiscs

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The in vitro antimicrobial susceptibility of recently isolated uropathogens was investigated using two locally produced brands of antibiotics and one imported brand, in a comparative study to determine their degree of effectiveness and the susceptibility profiles of these uropathogens. Seventy eight (78) bacterial strains containing 12 different species of both Gram negative and Gram positive bacteria were isolated and investigated. Escherichia coli was identified as the leading cause of urinary tract infections being the most isolated uropathogen. The activity of FD was comparable with that of AB (imported) which had the most effective antibacterial activities while those contained in JD were the least effective. Of the 18 different antibiotics employed, fluoroquinolones were the most effective antibiotics against all the bacterial isolates, followed by gentamicin > augmentin > nalidixic acid > nitrofurantoin > chloramphenicol while other antibiotics exhibited varying degree of activities on the bacterial isolates. It was therefore concluded that some locally manufactured antibiotics are as effective as imported brands while fluoroquinolones, augmentin, nitrofurantoin, gentamicin and nalidixic acid could be considered for first-line therapy in UTIs.

Key words: Uropathogens, UTIs, susceptibility, antibiotics, multidiscs.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting (Tice, 1999). It accounts for 7 million consultations and >1 million hospital admissions a year in the USA (Stamm and Hooton, 1993). It is one of the most common bacterial infections encountered by clinicians in developing countries (Tessema et al., 2007) and its isolates are much more frequent in females than in males (Hunjak et al., 2007). This may be due to anatomical predisposition or urothelial mucosa adherence to the mucopolysaccharide lining or other host factors (Schaeffer et al., 2001). Of all pathogens from patients with simple UTI, Escherichia coli is the most common cause of both complicated and uncomplicated urinary tract infections (UTIs) (Yüksel et al., 2006; Yamamoto, 2007; Tessema et al., 2007) with Enterococcus spp., Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumanii, Citrobacter spp., Serratia spp., coagulase-negative staphylococci and Klebsiella spp being the next most frequently encountered species (Rafal'skiĭ et al., 2006).

Uncomplicated urinary tract infection (UTI) caused by uropathogenic E. coli (UPEC) represents a prevalent and potentially severe infectious disease (Hagan and Mobley, 2007). While the treatment and management of uncomplicated urinary tract infections is important (Jackson, 2007), management has become more complicated in the last decade due to the trend toward increasing antimicrobial resistance to ampicillin and trimethoprim/sulfamethoxazole (TMP/SMX) (Gobernado et al., 2007), ciprofloxacin, gentamicin and ceftriaxone (Laupland et al., 2007). In order to determine the extent of drug-resistance among Enterobacteriaceae isolated from urinary tract infection, in vitro experiments becomes essentially important as the emergence of enterococci with alarming rates of resistance concomitantly to penicillins and
aminoglycosides highlights the need for a more rational and restricted use of antimicrobials. In order to minimize the selection and spread of such strains, an early detection of these problem pathogens is also important for preventing any treatment failures (Miskeen and Deodhar, 2002).

Also, the knowledge of the sensitivity of uropathogens to antimicrobials can help to initiate empirical therapy of urinary tract infections (Sanchez et al., 2004). In view of the fact that in vitro susceptibility studies have indicated that a significantly high proportion of the urinary E. coli isolates has already developed resistance to the currently prescribed empirical antibiotics, namely the fluoroquinolones, a transition in empirical therapy appears imminent. Although antimicrobial resistance is a global concern (Blondeau and Vaughan, 2000), antimicrobial resistance among Enterobacteriaceae isolated from intra-abdominal infections is a problem (Baquero et al., 2006). Worldwide data showed that there is increasing resistance among urinary tract pathogens to conventional drugs (Hryniewicz et al., 2001). Multiple drug resistance (MDR) to β-lactams, aminoglycosides and quinolones mediated through R plasmids among Gram-negative bacteria has become a major nosocomial problem worldwide (Babinchak et al., 2005). Antimicrobial treatment of nosocomial infections caused by these bacteria is compromised (Barrett, 2005). The ability of both nosocomial and community-acquired pathogens to develop resistance to powerful broad-spectrum agents, presents a great challenge for prescribing patterns and development of new drugs relatively resistant to inactivation.

Hence, with the emergence of antimicrobial resistance in bacteria as a global problem, national and international surveillance programmes have been developed to monitor resistance (Felmingham, 2002). While surveillance of Enterobacteriaceae monitors changes in antimicrobial susceptibility and prevalence of isolates resistant to multiple classes of antimicrobial agents (Karlowsky et al., 2003), selection of oral antibiotics for the management of patients with infections should be based on knowledge of the susceptibility patterns of these isolates (Murray, 1991). An early detection of these problematic uropathogens will help in preventing any treatment failure (Ishikawa et al., 2004).

To this end, this study was designed to investigate the antibiotic susceptibility patterns of recently isolated community-acquired urinary tract pathogens from some teaching hospitals in South-Western Nigeria, using two locally manufactured antibiotics and one imported antibiotics sold in Nigerian markets. Analysis of these antimicrobial data will provide information for comparison with national trends, allow the rational selection of antibiotics for empiric treatment of UTIs in this country and compare the efficacy of locally manufactured antibiotics with the imported multodisk sold in Nigerian markets.

MATERIALS AND METHODS

Specimen collection

Freshly voided midstream urine specimens were collected aseptically from some patients who attended three teaching hospitals in South Western Nigeria, either as inpatient or out patient, with symptoms suggestive of UTIs (Savas et al., 2006; Santo et al., 2007). All patients had clinical evidence of urinary tract infections, as determined by the treating physician. Only a single positive culture per patient was included in the analysis. These patients did not include those who were on antibiotics a week before the samples were collected. The urine samples were collected into labeled 20 ml calibrated sterile bottles containing boric acid (0.2 mg) added to prevent the growth of bacteria in the urine. All patients were instructed on how to collect the urine samples aseptically. They were advised to take the samples to the laboratory immediately for culturing.

Bacteriological analysis

In the hospital laboratory, each well mixed urine sample (5 µl) was inoculated on McConkey agar (Oxoid), blood agar (Oxoid), and cystine lactose electrolyte deficient agar (CLED, International Diagnostic Group). The inoculum on each plate was streaked out for discrete colonies with a wire loop following standard procedures (Cheesborough, 2006). The culture plates were incubated at 37°C for 24 h and observed for growth colonies. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesborough (2006).

Antibiotic susceptibility testing

The antibiotic susceptibility tests were performed by disc diffusion technique using three different commercially available discs on Mueller Hinton agar plates. Susceptibility testing was performed by using a standard agar dilution technique (Washington and Sutter, 1980) with Mueller Hinton agar (Lab. M; International Diagnostic Group Plc., Lancashire, UK) which is a susceptibility test medium recommended by the National Committee for Clinical Laboratory Standards, (NCCLS) (Crider and Colby, 1985) because of its low content of inhibitory substances. 100 µl (approximately 106 cfu/ml) of overnight broth culture of each test organism was dispersed into 20 ml volumes of molten Mueller Hinton Agar prepared according to manufacturer’s instruction, swirled gently to ensure an even distribution of inoculums, poured into sterile Petri dishes and allowed to set.

Each set of antibiotic discs was aseptically dispensed onto the surface of the inoculated agar plate, pressed down to ensure complete contact with the agar surface while the plates were inverted and incubated at 35°C for 24 h within 15 min after the discs were applied. The assessment of antibacterial activities was based on measurement of the diameter of the zones of inhibitions to the nearest millimeter with calibrated transparent meter rule held on the back of the inverted Petri plates. The different multodiscs respectively contained 12, 13 and 15 different antibiotics as shown in Table 1.

RESULTS

From the study, E. coli was the most isolated (34.6%), followed by Proteus morganella (10.3%), Enterobacter
Table 1. Different multidoses from different manufacturers and their various concentrations.

<table>
<thead>
<tr>
<th>Drug</th>
<th>AB</th>
<th>FD</th>
<th>JD</th>
<th>Concentration (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>AMX</td>
<td>AMX</td>
<td>na</td>
<td>25</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>OFL</td>
<td>OFL</td>
<td>OFL</td>
<td>5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CPX</td>
<td>CPX</td>
<td>CPX</td>
<td>10</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GEN</td>
<td>GEN</td>
<td>GEN</td>
<td>10</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>CHL</td>
<td>CHL</td>
<td>CHL</td>
<td>30</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>COT</td>
<td>COT</td>
<td>COT</td>
<td>25</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>ERY</td>
<td>ERY</td>
<td>ERY</td>
<td>5</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>NIT</td>
<td>NIT</td>
<td>NIT</td>
<td>300</td>
</tr>
<tr>
<td>Augmentin</td>
<td>AUG</td>
<td>AUG</td>
<td>AUG</td>
<td>30</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TET</td>
<td>TET</td>
<td>TET</td>
<td>30</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>NAL</td>
<td>na</td>
<td>na</td>
<td>25</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>CXC</td>
<td>na</td>
<td>na</td>
<td>5</td>
</tr>
<tr>
<td>Pefloxacin</td>
<td>PFX</td>
<td>PFX</td>
<td>na</td>
<td>5</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>STR</td>
<td>STR</td>
<td>STR</td>
<td>10</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>na</td>
<td>CEF</td>
<td>CEF</td>
<td>30</td>
</tr>
<tr>
<td>Ampiclox</td>
<td>na</td>
<td>na</td>
<td>APX</td>
<td>30</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>na</td>
<td>na</td>
<td>LIN</td>
<td>30</td>
</tr>
<tr>
<td>Cephalxin</td>
<td>na</td>
<td>na</td>
<td>COX</td>
<td>15</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>na</td>
<td>na</td>
<td>PN</td>
<td>15</td>
</tr>
</tbody>
</table>


Figure 1. Percentage and type of each bacterial species isolated.

intermedium and Klebsiella Edwardsii (8.9%), Citrobacter freundii and Enterobacter aerogenes (7.7%), Staphylococcus aureus (6.4%), Klebsiella pneumoniae (5.1%) as well as Proteus vulgaris, P. mirabilis, Shigella paradysenteriae and Shigella sonnei (2.6%) which were the least isolated as shown in Figure 1. The clinical
isolates were susceptible to the routinely prescribed antibiotics. Percentages of different bacterial spp. susceptible to different antibiotic discs contained in the different brands of multidisc are as shown in Figures 2 to 4.

From the bacterial susceptibility profiles presented in Figures 2 to 4, the antibiotics contained in the AB were most effective against all the bacterial isolates, followed by the antibiotics contained in FD, while those contained in JD were the least effective. Fluoroquinolones were the most effective antibiotics against all the bacterial isolates, followed by Gentamicin > Augmentin > Nalidixic acid > Nitrofurantoin > Chloramphenicol while other antibiotics exhibited varying degrees of antibacterial activities on the
isolates. With the exception of *Proteus* spp, not 100% susceptible to any of the antibiotics, many isolates were 100% susceptible to 3 to 4 different antibiotics, such as fluoroquinolones, augmentin, nitrofurantoin, gentamicin and nalidixic acid, from both AB and FD multidoscs while only few of the bacteria were 100% susceptible to gentamicin only in JD. In addition to being susceptible to fluoroquinolones, *E. intermedium* strains were 100% susceptible to chloramphenicol, gentamicin, augmentin and nalidixic acid, nitrofurantoin (83.3%), tetracycline (33.3%), amoxicillin and erythromycin (16.7%) while they were highly resistant to both cotrimoxazole and cephalaxin. *E. coli* strains were 100% susceptible to gentamicin followed by augmentin (96.4%), nitrofurantoin (92.9%), nalidixic acid (82.1%), chloramphenicol (78.6%), tetracycline and erythromycin (67.9%), amoxicillin (60.7%) and cotrimoxazole (3.6%). *Klebsiella* spp. were 100% susceptible to augmentin, nitrofurantoin and nalidixic acid, chloramphenicol (90.9%), gentamicin (81.8%), erythromycin, tetracycline and amoxicillin (54.5%) and cotrimoxazole (9.1%).

*Enterobacter aerogenes* were 100% susceptible to augmentin and nalidixic acid, chloramphenicol, gentamicin, erythromycin and nitrofurantoin (83.3%), tetracycline (50%), cotrimoxazole and amoxicillin (33.3%). *C. freundii* were 100% susceptible to augmentin and nitrofurantoin, nalidixic acid and gentamicin (83.3%), chloramphenicol (66.7%), tetracycline and amoxicillin (33.3%), cotrimoxazole, erythromycin (16.7%). While augmentin was the most effective against *Proteus* spp. Having 96.4% susceptible, *Proteus* spp. were (91.7%) susceptible to ofloxacin and nitrofurantoin, nalidixic acid and ciprofloxacin (83.3%), chloramphenicol (78.6%), gentamicin (75%), erythromycin (50%), amoxicillin (41.7%), tetracycline and cotrimoxazole (25%), and cephalaxin (8.3%). *Shigella* spp. was 100% susceptible to gentamicin and nitrofurantoin, erythromycin, chloramphenicol, augmentin, tetracycline and nalidixic acid (75%), cotrimoxazole and amoxicillin (50%) as well as cephalaxin (25%). Apart from being 100% susceptible to fluoroquinolones, gentamicin, augmentin, nitrofurantoin and nalidixic acid, most of these bacteria exhibited multidrug resistance to all other antibiotics used in this study, while cephalaxin was totally ineffective against all the tested bacterial species.

**DISCUSSION**

As a result of the fact that most UTIs are treated empirically, the selection of an antimicrobial agent is determined by the most likely pathogen and its expected susceptibility pattern. Monitoring antibiotic susceptibility patterns of uropathogens at a local level will yield important information regarding emerging problems of antibiotic resistance and provide assistance in managing empirical therapy.

In this study, the most common organisms were *E. coli* (34.6%), *Enterobacter* spp (16.7%), *Proteus* spp (15.4%) and *Klebsiella* spp (14.1%) indicating that *E. coli* is the most common cause of UTIs. This result is in agreement....
with reports from earlier investigators (Henry et al., 1998; NCCLS, 1998; Xu et al., 1999; Tice, 1999; Blondeau et al., 1999; Zhanel et al., 2005) and contrary to the report of Gruneberg (1994) who reported that E. coli, as the leading cause of uropathogen, was being replaced by other members of the Enterobacteriaceae and Enterococci. Treatment of UTIs is a major community indication of antibiotic usage (Fihn, 2003; Hooton, 2003). Fluoroquinolones (Fihn, 2003; Hooton, 2003), trimethoprim-sulphamethoxazole (cotrimoxazole) or trimethoprim alone (Warren et al., 1999; Hooton and Stamm, 1997; Nicolle, 2002), ampicillin or amoxicillin (Gupta et al., 2001) and nitrofurantoin (Nicolle, 2002), have been implicated as being frequently used for the treatment of UTIs. While resistance to nitrofurantoin among E. coli from UTIs remain low despite more than 50 years widespread use of the drug (Kahlmeter, 2000; Mazzulli et al., 2001), resistance to nalidixic acid (Kahlmeter, 2000), cotrimoxazole (Mclssac et al., 2004) have been reported. Cotrimoxazole prescriptions for UTI have declined while fluoroquinolones prescriptions have increased (Gupta et al., 2001).

This study indicated that fluoroquinolones, augmentin, nitrofurantoin, gentamicin and nalidixic acid were active against all the isolates and could be used as first-line therapeutic agents in UTIs. The use of cotrimoxazole, ceftriazone, clindamycin, ampicillin, cephalaxin and cloxacillin should be discouraged as they were ineffective against the isolates. The observed high rate of resistance to these ineffective antibiotics may be a reflection of the previous exposure of the isolates to them and acquisition of resistant genes. The level of susceptibility of E. coli and the varied degree of susceptibility of other enterobacteria to the effective antibiotics was found to be comparable with results from other investigators (Cunney et al., 1992; Jones et al., 1999; Vromen et al., 1999; Fluit et al., 2000). The susceptibility of the uropathogens to fluoroquinolones may, however, indicate that virulent strains might be less resistant to antimicrobials than strains causing only colonization or lower tract UTI as previously observed by Komp et al. (2005) and Roos and Klemm (2006).

Although the activities of two locally manufactured multidiscs were compared against an imported multidiscs AB, the obtained result indicated that FD produced a reasonably comparable result with the AB while JD was the least effective. The observed disparity could have resulted from differences in production techniques, location, quality of raw materials used during production and the shelf lives of these products. It will, thus, be worthwhile that local and international manufacturers produce multidiscs that will contain the same number, quality and types of antibiotics. They should also establish and maintain good quality standards in their productions.

In conclusion, this study indicates that some antibiotics commonly used in UTI treatments are still effective, particularly in both community and hospital infections.

These may be of immense value for use to determine drugs of choice in the treatment of UTIs prior to outcome of laboratory investigations while fluoroquinolones, augmentin, nitrofurantoin, gentamicin and nalidixic acid could be considered for first-line therapy for UTIs, in agreement with previous reports (Stamm, 2002; Cunha, 2006; Nicolle et al., 2006). Although there are some others "old antibiotics" with a role that may be underestimated for UTIs (Honderlick et al., 2006), prudent and rationale use of antibiotics must encourage prescribing fluoroquinolones and other indicated antibiotics parsimoniously for uncomplicated UTIs.

REFERENCES


