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Antibiotic Resistance in Urinary Tract Bacteria in Ouagadougou

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Abstract: The present study aimed to ascertain for the current situation of antimicrobial resistance of major urinary tract bacteria in Saint Camille Medical Centre. During two consecutive years, 794 urine specimens were analyzed for microorganism isolation and identification. The microorganisms were identified by conventional methods used in the centre and antimicrobial assays were performed by the NCCLS agar disk diffusion. Pathogenic microorganism's isolation was attempted for 89.04% samples. *Escherichia coli* (32.76%) was the most frequently isolated microorganism followed by *Staphylococcus aureus* (22.74%) and *Klebsiella pneumoniae* (10.45%). The antimicrobial screenings revealed very high antimicrobial resistance, to β-lactams. The resistance rates recorded with *E. coli* were 76.64, 74.01, 25 and 74.34% for ampicillin, amoxicillin amoxicillin/clavulanic acid and trimethoprime-sulfamethoxazole, respectively. Microorganisms were still susceptible to quinolones however, attention should be paid, because, the resistance rate already reached 10% for nalidixic acid and ciprofloxacin. Periodic performance of prevalence studies is a useful tool to know the current situation of microorganisms and their resistance patterns in an institution and it helps to access the emergence and the spread of antibiotic resistance.

Key words: Antibiotics, resistance, microorganisms, urinary tract infection

INTRODUCTION

Urinary Tract Infection (UTI) is a problem that is frequently encountered by health care providers. Over recent decades, the importance of UTI has been increasingly recognized, in particular, the role of UTI as an occult cause of febrile illness in infants. UTI is defined by the presence of organisms in the urinary tract, which is usually sterile (Gérôme *et al*., 2009). However, since asymptomatic colonization of the urinary tract can occur, other features such as the presence of inflammatory markers or follow-up cultures may be needed to definitively diagnose a UTI (Rubin *et al*., 1992). Clinically important infections usually occur due to bacteria, although viruses, fungi and parasites can also cause infections. Common nonbacterial causes of UTI include hemorrhagic cystitis from *Adenovirus* and *Candida* infections in immunocompromised individuals. Common bacterial pathogens include gram-negative species such as *E. coli*, *Klebsiella*, *Proteus*, *Enterobacter*, *Pseudomonas* and *Serratia* and gram-positive organisms, including group B *Streptococci*, *Enterococcus* and *S. aureus* (Zorc *et al*., 2005a).

The epidemiology of UTI during childhood varies by age, gender and other factors. The incidence of UTI is highest in the first year of life for all children but decreases substantially among boys after infancy (Jakobsson *et al*., 1999). Estimates of UTI incidence among infant boys have varied in different populations, likely due to factors such as circumcision, which has been associated with a reduction in risk of UTI. The clinical significance of UTI has been controversial (Foxman, 2007). In the preantibiotic era, UTI had a mortality rate as high as 20% although, acute complications in healthy children are now uncommon except in young infants, who may progress to systemic infection. Long-term complications of UTI have been associated with renal scarring and include hypertension, chronic renal failure and toxaemia in pregnancy. Long-term follow-up data are limited, however, it has been

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reported that children diagnosed with renal scarring due to pyelonephritis during the 1950s and 1960s developed high rates of hypertension and end-stage renal disease. More recent studies questioned the association between pyelonephritis and end-stage renal disease (Grünberg, 1998).

Although, the individual risks associated with UTI remain unclear, the high prevalence of UTI and potential morbidity associated with complications require careful attention to diagnosis and management. The appropriate treatment of UTI has been relied on antibiotic use. The choice of antibiotic may be affected by local resistance patterns and other considerations. Amoxicillin was traditionally the first-line therapy for outpatient treatment of UTI (Wagenlehner and Naber, 2006). However, increased rates of *E. coli* resistance have made amoxicillin a less acceptable choice and studies have found higher cure rates for trimethoprim-sulfamethoxazole (Fritsche *et al*., 2009; Borsari *et al*., 2008; Zorc *et al*., 2005b). The present study was conducted to evaluate the prevalence of the microorganisms frequently involved in UTI and to access for the antimicrobial resistance patterns of the most commonly implicated bacteria.

MATERIALS AND METHODS

Microorganism isolation and identification: From January 2006 to December 2007, a total of 794 urine specimens were collected from patients attending Saint Camille Medical Centre in Ouagadougou with urinary tract infection suspicion. Samples were cultured in appropriated media according to the conventional methods used in the centre. Yeast were isolated from sabouraud agar. Chapman medium was used for selective isolation of staphylococci. The other gram-positive cocci were isolated from blood agar and gram-negative bacilli specially *Escherichia coli* from Eosin Methylene Blue (EMB) agar. Microorganisms numeration was performed on with Cystine Lactose Electrolyte Deficient (CLED) medium. They were identified according to their colonies and their morphological characteristics. Identities were confirmed by API 20 system (Bio Merieux, France).

Antimicrobial assay: The antimicrobial screenings were performed using Muller Hinton (MH) agar disk diffusion assay as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). Briefly, microorganisms from growth on nutrient agar incubated at 37°C for 18 h were suspended in saline solution 0.85% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (10^8 cfu mL^{-1}). The suspension was used to inoculate 90 mm diameter petri plates with a sterile non toxic cotton swab on a wooden applicator. The antibiotic discs were directly placed onto the bacterial culture. After 24 h incubation in air at 37°C, antimicrobial activities were assayed by measuring the inhibition zone diameter around the disk using a calliper. Antibiotic break points were determined according to NCCLS guidelines. *Escherichia coli* CIP 105182, *S. aureus* ATCC 25923 and *S. aureus* CIP 53154 were used as control. All antibiotics and media were purchased from Bio Rad (France). The following antibiotics were tested: ampicillin, amoxicillin, amoxicillin/clavulanic acid, oxacillin, cefazolin, cefotaxim, oleandomycin, pristinamycin, erythromycin, lincomycin, nalidixic acid, norfloxacin, ciprofloxacin, gentamicin, trimethoprime-sulfamethoxazole and chloramphenicol.

RESULTS

The present study covered 2 consecutive years. During this period time, a total of 794 urine specimens were collected from patients with UTI suspicion. Pathogenic microorganism's isolation was attempted for 89.04% (707/794) samples. Cultivation of the other samples did not yield pathogens. Positive sample cultivation allowed us to isolate 928 microorganisms, indeed some samples showed dual or trial infections. Among the isolated microorganisms, 9.81% (91/928) were *Candida* species and 90.19% (883/928) were bacteria. Table 1 displays microorganism distribution. Overall microorganisms, *E. coli* (32.76%) was the most frequently isolated followed by *S. aureus* (22.74%) and *K. pneumoniae* (10.45%).

The most common microorganisms were tested for their susceptibilities to several antimicrobial used in the centre. Table 2 shows the resistance rates of gram positive cocci. According to the Table 2, *S. aureus* has developed high resistance (resistance rate >10%) to the main β-lactams (ampicillin, amoxicillin and amoxicillin/clavulanic acid) including cephalosporin (cefazolin and cefotaxim). The highest rates were recorded with ampicillin and amoxicillin (45.02 and 22.27%,

Table 1: Isolated microorganisms distribution

Microorganisms	Percentage
Escherichia coli	32.76
Klebsiella pneumoniae	10.45
Klebsiella oxytoca	1.50
Proteus sp.	1.29
Enterobacter cloacae	4.42
Acinetonacter sp.	9.81
Staphylococcus aureus	22.74
Staphylococcus sp.	1.51
Group D *Streptococcus*	5.17
Candida albicans	5.17
Non *Candida albicans*	4.64
Others	0.54
Total No. of microorganisms	928.00

Table 2: Resistance rates of the isolated gram-positive cocci

Gram-positive cocci	*S. aureus* (n = 211)	GDS (n = 48)
Ampicillin	45.02	2.08
Amoxicillin	22.27	6.25
AMC	11.37	6.25
Oxacillin	18.96	20.83
Cefazolin	11.85	20.83
Cefotaxim	15.17	14.58
Oleandomycin	24.64	25.00
Pristinamycin	14.69	10.42
Erythromycin	10.90	8.33
Lincomycin	22.75	47.92
Gentamicin	6.64	16.67

AMC: Amoxicillin/clavulanic acid, GDS: Group D *Streptococcus*

Table 3: Antimicrobial resistance rates of the gram-negative bacilli

Gram-negative bacilli	*E. coli* (n = 304)	*K. pneumoniae* (n = 97)	*Acinetobacter* sp. (n = 91)	*E. cloacae* (n = 41)
Ampicillin	76.64	85.57	39.56	63.41
Amoxicillin	74.01	76.29	16.48	48.78
AMC	25.00	19.59	18.68	29.27
Cefazolin	0.66	4.12	2.20	0.00
Nalidixic acid	11.51	2.06	1.10	2.44
Ciprofloxacin	10.86	0.00	2.20	2.44
Norfloxacin	8.89	0.00	0.00	0.00
Pristinamycin	5.26	2.06	1.10	7.31
Gentamicin	5.59	2.06	3.30	0.00
SXT	74.34	36.08	36.26	29.27
Chloramphenicol	19.08	18.56	34.07	34.15

AMC: Amoxicillin/clavulanic acid, SXT: Trimethoprim-sulfamethoxazole

respectively). Group D *Streptococcus* (GDS) remained susceptible to ampicillin, amoxicillin and amoxicillin/clavulanic acid (resistance rate <10%), but it has developed high resistance to oxacillin and cephalosporin (20.83, 20.83 and 14.58% for oxacillin, cefazolin and cefotaxim, respectively). The two microorganisms have developed high resistance to macrolides (oleandomycin, pristinamicin, erythromycin and lincomycin). Resistance rates of 24.64 and 22.75% were recorded for oleandomycin and lincomycin in *S. aureus*; 25 and 47.92% were recorded for these same antibiotics in GDS. Finally, *S. aureus* was still susceptible to gentamicin while there was an increasing resistance to this antibiotic in GDS (6.64% vs. 16.67%).

Four gram-negative bacteria, *E. coli*, *K. pneumoniae*, *E. cloacae* and *Acinetobacter* sp. were tested for there susceptibility to the antibiotics. According to Table 3, resistance to β-lactams (ampicillin, amoxicillin and amoxicillin/clavulanic acid) is common within these gram negative bacilli. The highest resistance rates were recorded with *E. coli* (76.64 and 71.01%) and *K. pneumoniae* (85.57 and 76.29%) for ampicillin and amoxicillin, respectively. Resistance to trimethoprim-sulfamethoxazole was also common among these microorganisms, the rates were greater than 25%, the highest rate (74.34%) being recorded with *E. coli*. For chloramphenicol, the resistance rates were greater than 15% and the highest rates were recorded with *E. cloacae* and *Acinetobacter* sp. (34.15 and 34.07%, respectively). Excepted *E. coli* which displayed resistance rates of 10.86 and 11.51% for ciprofloxacin and nalidixic acid respectively, all these gram-negative bacteria were globally susceptible to quinolones (norfloxacin, nalidixic acid and ciprofloxacin) to cefazolin, pristinamycin and gentamicin.

DISCUSSION

The present study was undertaken to evaluate the prevalence of microorganisms implicated in UTI and to ascertain for their antimicrobial resistance patterns. During two consecutive years study, 794 urine samples were analyzed and 928 microorganisms were identified and isolated.

According to these results, *E. coli* (32.76%) was the leading agent responsible for the UTI, followed by *S. aureus* (22.74%) and *K. pneumoniae* (10.45%). These observations are supported by several studies conducted previously. In a prospective study in Benin, a border country from 534 samples, Anagounou *et al.* (1995) isolated *E. coli* in 36% cases. In another study conducted on UTI in a geatric hospital, Haber *et al.* (2007) found *E. coli* and *Proteus mirabilis* to be the main bacterial species involved in 57 and 14%, respectively. A study conducted in France, revealed that *E. coli* was the most prevalent pathogen followed by *P. mirabilis*, *Klebsiella* sp., *S. aureus*, *Staphylococcus saprophyticus* and *Streptococcus agalactiae* (De Mouy *et al.*, 2007). Similar observations were made in Tunis by Larabi *et al.* (2003), who found that Enterobacteriaceae were the most frequently identified strains including *E. coli*, while gram-positive strains were often *S. saprophyticus*. However, it should be noted that there was a high prevalence of *S. aureus* in the samples in comparison with the prevalence recorded in these earlier studies.

In order to elucidate the current situation of antimicrobial resistance in the centre the antimicrobial tests were performed for the most common isolated. The results revealed that the problem of antimicrobial resistance is effective in the centre. Antimicrobial resistance is a direct consequence of antimicrobial use. Both continue to escalate despite many calls for moderation of antibiotic use in hospitals and in community. Many surveillance studies have demonstrated an increase in antimicrobial resistance in *S. aureus*, although they are based on limited number of referral institutions per country (Fluit *et al.*, 2001). Similarly, uniformly high levels of methicillin resistance and resistance to other antibiotics have been observed among *S. aureus* isolates. This situation underlines the need for surveillance studies in different geographical

areas including all types of institutions. According to present results, the resistance rates in *S. aureus* are less than what was observed in Spain by Cuevas *et al.* (2004). In their study the resistance rates were 31.2, 31.7 and 16.9% for oxacillin, erythromycin and gentamicin, respectively. However, we must consider that our centre is in high methicillin resistance area (oxacillin resistance rate >10%). The frequency of Methicillin Resistant *Staphylococcus aureus* (MRSA) varies among countries and hospital to hospital within each country. For example, in Europe MRSA are rare in Nordic countries (<1%), but it is more frequent in Southern Europe even exceeding 40% in France (Ferrara, 2007).

Three Enterobacteria (*E. coli*, *K. pneumoniae* and *E. cloacae*) and *Acinetobacter* sp. were tested in the present study. The results revealed that both *Acinetobacter* and the Enterobacteria have developed resistance to many antibiotics principally β-lactams. *Acinetobacter* species are opportunistic pathogens of low virulence. However, their contribution to nosocomial infection has been increasing over the past 30 years. Though widely prevalent in nature and generally regarded as commensals of human skin and respiratory and genitourinary tracts, they have been implicated as the cause of serious infectious diseases such as meningitis, pneumonia, tracheobronchitis, endocarditis, wound infections, septicaemia and UTI, mostly involving patients with impaired host defenses. We should note that in the present study *Acinetobacter* is the fourth microorganism implicated in UTI with a prevalence of 9.81%. Treatment of serious infections due to *Acinetobacter* sp. is complicated by the widespread multidrug resistance of the organism. Over the last 15 years, interest in *Acinetobacter* species has grown rapidly, due to the emergence and outbreak of multidrug-resistant *Acinetobacter* isolates. They are intrinsically resistant to many antimicrobial agents and resistance to β-lactams is most commonly associated with the production of high levels of naturally produced cephalosporinase (Yong *et al.*, 2003).

In the present study the resistance rate of enterobacteria to ampicillin and amoxicillin were globally greater than 50%. The same findings were reported by several studies. Working on microorganisms isolated from UTI, Haber *et al.* (2007) found that *E. coli* strains were 59% resistant to amoxicillin, 55% resistant to amoxicillin-clavulanic acid and 39% resistant to fluoroquinolones. In a similar study of Larabi *et al.* (2003) found resistance rates of 57.9% to β-lactams and 46.9% to trimethoprim-sulfamethoxazole in *E. coli*, 13.8% *K. pneumoniae* were found to produce extended spectrum bêtalactamase.

By the end of 1990s the increasing resistance to antibiotics such as β-lactams in the country and in particular in our centre became very crucial. This prompted studies that enable the identification of *K. pneumoniae* and *E. coli* with atypical bla_{SHV-11} that was responsible for the high resistance of these microorganisms to β-lactams. In addition isolates of *Chryseobacterium indologenes* producing metallo-β-lactamase were also identified (Zeba *et al.*, 2005). In 2002, in the centre, a large number of *E. coli* strains were resistant to aminopenicillins (>90%) and cotrimoxazole (80%), only norfloxacin appeared to be very efficient, however, the antibiotic was not widely used in the country (Bonfiglio *et al.*, 2002). According to the present results, there is a decrease of resistance to β-lactams. This may be due to the fact that by the end of 1990s a nationwide restriction in the prescription of the main β-lactams was adopted with the introduction of ciprofloxacin and norfloxacin that were first found to be too expensive for the patients. The decrease of resistance to β-lactams and the susceptibility to the quinolones may be the results of the selective pressure. Reduction of antimicrobial resistance by the modulation of antibiotic policies was found in France, where studies have demonstrated reduced prevalence of gentamicin-resistant MRSA on reducing gentamicin prescribing (Aubry-Damon *et al.*, 1997). In Finland also, a trebling of community prescribing of erythromycin in the late 1980s and early 1990s, to treat group A streptococcal throat and skin infections, led to an increase in erythromycin resistance from 5% in 1988 to 19% in 1993. A subsequent 50% reduction in erythromycin consumption then led to resistance rates falling to 8.6% in 1996, indeed resistance rates may decline without total removal of the antibiotic, probably by reduced selection allowing suppressed, sensitive strains to become dominant (Gould, 1999).

Antimicrobial resistance is a major challenge to take up in the near future. The phenomenon results in the increase of mortality and morbidity, as well as an increase in the cost for health care system. In Burkina Faso as in many other developing countries, where, there is a lack of bacteriology laboratories and where antibiograms are not affordable for the majority of the patients, clinicians only relied on empirical therapy to prescribe antibiotics. This is the first study conducted on the ethiology of urinary tract infection and the antimicrobial resistance pattern of the implicated bacteria in the country. Present results indicate that a periodical survey on the susceptibility of the major pathogenic microorganisms is useful for the therapeutic choice and the rationale use of antimicrobial agents.

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