



Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections

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ABSTRACT

Objective: *Escherichia coli* is the greatest cause of primary urinary tract infections (UTI). Antimicrobial susceptibility testing provides information that allows physicians to select the most appropriate antimicrobial agents for treating a specific infection. This study aimed to assess the current status of multidrug resistance among urinary *Escherichia coli* isolates in Kenya.

Methodology and results: A total of 3,341 urine samples were collected from in and out-patients attending Thika district hospital in Kenya between January and December 2008. The samples were cultured on Cystein lysine electrolytes deficiency (CLED) media and the bacterial isolates recovered were tested against Trimethoprim-sulfamethoxazole, Cefuroxime, Augmentin®, Nitrofurantoin, Nalidixic acid, Gentamycin, Cephaloxin, Norfloxacin, Ciproxin®, Ceftazidime, Amikacin, Ofloxacin, Centriaxone, Perfloxacin, Ticarcillin, Pipril and Roceph using Kirby Bauer disc diffusion technique. Among the 3,341 samples examined, 24% had *Escherichia coli* isolates with 64% of them being from female patients compared to 36% that were from men aged above 21 years. In children aged >10yrs, boys had the highest prevalence (55%) compared to girls (45%). Those in age categories <21yrs had the more isolates (73%) followed by 5-10yrs (46%), 1-4yrs (16%), and the least 11-20yrs (5%). Upto 75% of the isolates were resistant to Trimethoprim-sulfamethoxazole; all (100%) were susceptible to Ticarcillin, Peril/Tazo, Amikacin, Ofloxacin and Roceph; and 80% of the isolates were susceptible to Cephalexin, Ceftriaxime, Nalidixic acid, Gentamycin, Norfloxacin, Ciproxin®, Ceftazidime/fortum and Centriaxone.

Conclusion and application of findings: Considering the relatively high rates of UTI and drug resistance observed in this study, continued local, regional, and national surveillance is warranted. Antibiotics should only be issued when prescribed by physicians.

Key words: Urinary tract infection, drug resistance, *Escherichia coli*

INTRODUCTION

Urinary tract infections (UTI) are among the most common infections with an increasing resistance to antimicrobial agents (Samra *et al.*, 2005). These ailments affect patients in all age groups and both

sexes (Gupta *et al.*, 1999). *Escherichia coli* has been documented as the most important pathogen associated with urinary tract infections in many countries (Samra *et al.*, 2001). It is estimated that



half of all women will have recurrent episodes of acute cystitis during adult life (Nicolle, 2001). UTI in males, though not rare, occurs in much less frequency (Mazzuli, 2001). Almost 15% of all antibiotic prescriptions are for the management of UTI (Mazzuli, 2001). Increasing concurrent resistance to antimicrobials of different classes has arisen in different bacterial species, which may complicate therapeutic management of infections, including those of the urinary tract (Daniel *et al.*, 2001).

The use of narrow spectrum, inexpensive antimicrobial agents is becoming less feasible, affecting both cost and access to health care by patients. However, inappropriate and excessive use of antibiotics contributes significantly to antimicrobial resistance (Jones *et al.*, 1999). Therefore, it is desirable to prescribe antibiotics only when necessary and also the most appropriate one for the known or likely organism(s) causing the infection (Hooton & Stamm, 1997).

Knowledge regarding common uropathogens and their susceptibility patterns to drugs will help in improving prescribing decisions in general practice, since one of the most

important factors to consider when selecting appropriate antimicrobial therapy is the prevalence of resistance in the community (Nicolle, 2002). The vast majority of UTIs arise in female outpatients, many of whom are treated empirically (before urine culture verification) by physicians if their symptoms suggest acute uncomplicated bacterial cystitis (Daniel *et al.*, 2001).

The pathogens causing UTIs are almost always predictable (Daniel *et al.*, 2001), with *Escherichia coli* being the primary etiologic agent among both outpatients and inpatients accounting for 75 to 90% of urinary tract infection isolates (Nicolle, 2001). Therefore, constant monitoring or drug resistance is required because only limited data describing multidrug resistance among UTI isolates is available (Daniel *et al.*, 2001).

The present study was carried out to isolate *Escherichia coli* from urine samples from in and outpatients at Thika District Hospital Kenya, presenting with UTI. The susceptibility patterns of the bacterial isolates to antimicrobial agents was determined to generate information to guide treatment of UTI.

agar (Oxoid N.Y., USA) and incubated for 24 h at 37°C aerobically. Organisms were selected for inclusion in further study when they occurred as pure culture and in concentrations greater than 10⁵ CFU/ml. Isolations and identifications were performed by use of biochemical tests using API20 method Beckton Dickson USA (Murray *et al.*, 2003).

Antimicrobial susceptibility testing: Kirby Bauer disc diffusion technique (Bauer *et al.*, 1996) was used and 0.5 MacFarland's 10⁸/ml employed in inoculum suspensions preparation according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (Murray *et al.*, 2003). These discs include; Trimethoprim-sulfamethoxazole, Cefuroxime, Augmentin®, Nitrofurantoin, Nalidixic acid, Gentamycin, Cephalexin, Norfloxacin, Ciproxin®, Ceftazidime, Amikacin, Ofloxacin, Centriaxone, Perfloxacin, Ticarcillin, Pipril and Roceph (Table 1) and were tested against the isolates. The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates (Iroha *et al.*, 2009).

MATERIALS AND METHODS

Study design: The study was carried out at Thika District hospital, Kenya. A prospective analysis was done on 3,341 patients who presented symptoms of urinary tract infections. All patients were within ages 1 to 60 years, comprising of both male and female, either out and inpatients. All samples received consisted 2138 of female and 1203 from men. The diagnosis of urinary tract infection was based on microscopic findings of more than 5 White Blood Cells per high power field (1000x for high power) Leitz microscope and a colony count of 10⁵ CFU/ml of a single pathogen. The urine of adult patients was sampled by clean catch of midstream urine, while in neonates the urine was collected through suprapubic approach (Mbata, 2007). For the neonates ages and children aged >3yrs the urine was sampled using sterile urine bags. Urine samples were delivered to the laboratory within 1 h of collection and processed within 2-4 hours.

Isolation and identification of *Escherichia coli*: A loopful 0.01ml of urine sample was cultured on Cystine Lactose Electrolyte-Deficiency (CLED) and MacConkey



Table 1: Antibiotics used for the disc diffusion technique against *Escherichia coli* from urine samples in Kenya.

Antibiotic	Concentration level	Antibiotic	Concentration level
Trimethoprim-sulfamethoxazole	200µg	Ciproxin®	10 µg
Cefuroxime	30 µg	Ceftazidime	30 µg
Augmentin®	30 µg	Amikacin	30 µg
Nitrofurantoin,	200 µg	Ofloxacin	10µg
Nalidixic acid	30 µg	Perfloxacin	5µg
Gentamycin	10 µg	Ticarcillin	75 µg
Cephalexin	30µg	Roceph	30 µg
Norfloxacin	10 µg	Pipril	100 µg

Commercial antibiotic discs of Hi media laboratories PVT Ltd and Difco Laboratories (Detroit, Mich) were used.

RESULTS

For the twelve months of the study, 3,341 urine samples were received and cultured. Among the cultures screened, bacteriuria of 10^5 per millilitre of urine was found in 831 (24%) of the samples having *Escherichia coli*, and of this (831) 30% occurred with other uropathogens. A total of 2610 (76%) of the samples were culture negative.

Urinary tract infection distribution among patients demonstrated that females presented the highest prevalence of the cases (64%) compared to males at 36% in all age groups. In the category aged below 10 years old, boys had the highest prevalence of 55% and girls 45%. The age distribution of the study subjects were 1-4years (16%), 5-10yrs (46%), 11-20yrs (5%) and above 21yrs (73%).

The levels of resistance to antimicrobial agents among the 831 *E. coli* isolates analyzed are

DISCUSSION

In the community, bacterial infection of the urinary tract is one of the common causes for seeking medical attention. Demonstration of bacteria by appropriate culture methods is one of the methods in diagnosis of UTI. Traditionally, $> 10^5$ bacteria/ml of urine showing a single isolate is taken to indicate bacteriuria and distinguishes infection from contamination in asymptomatic patients (Orrett & Shurland, 1998; Graham, 2001). The pathogens causing UTIs are almost always predictable, with *Escherichia coli* being the primary etiological agent among both outpatient and inpatients (Hooton Stamm, 1997; Gupta *et al.*, 1999; Jones *et al.*, 1999; Gales *et al.*, 2000). Despite the widespread availability of antibiotics, UTIs remain the most common bacterial infections in human populations (Phillippon *et al.*, 1989). In the United States for instance, each year about 11% of women experience at least one urinary tract infection (Manges *et al.*, 2001).

provided in (Table 2). Trimethoprim-sulfamethoxazole had the highest number of resistant isolates (64%) and Norfloxacin, Ciproxin®, Perfloxacin, Centriaxone shared the least number (5%). All *Escherichia coli* isolates (100%) were found to be susceptible to Ticarcilin, Peril/Tazo, Amikacin, ofloxacin and Roceph. In addition, 96% of the isolates were susceptible to Ceftazidime/Fortum followed by 95% susceptible to Norfloxacin, Ciproxin® and Centriaxone. In addition, 94.6% of the isolates were sensitive to Perfloxacin, Cefuroxime (94%), Gentamycin (93%), Nalidixic acid (91%), and Cephalexin (87%). However, with Nitrofurantoin (77%) isolates were susceptible followed by Augmentin (75%); with Trimethoprim-sulfamethoxazole, 36% showed the lowest percentage of susceptibility (Table 2).

In this study in Kenya, 24% prevalence of UTI was detected with more females being infected. The predominance of infection (64%) in females above 21 years old agreed with the report of Orrett and Shurland (1998). The high prevalence of infection in females is usually related to anatomical and pathogenic factors, e.g. the short length of the urethra hence lesser distance of bacteria ascending up the tract, lack of antimicrobial properties of prostatic fluid as in males, hormonal changes affecting the adherence of bacteria to the mucosa and urethra trauma during sexual intercourse. The latter factor accounts for the well recognised "honeymoon cystitis" that is associated with UTI infections. Recent infection has also been found to be a predisposing factor to UTI acquisition.

In early infancy, 58% of females as compared to 42% boys were detected to have urinary tract infection while in neonates 55% UTI prevalence



occurred in boys and 45% in girls. This confirms the results from previous studies (Hooton, 1990; Kathy *et al.*, 1998; Linda & Jack, 2002). High rates of UTI in boys has been associated with the high incidence of congenital malformations and also the uncircumcised genitalia that often gets contamination from the prepuce or introtial area that is not always clean.

It is worth noting the considerable reduction in the activity of Trimethoprim-sulfamethoxazole followed by Augmentin and also nitrofurantoin among the commonly used drugs in treatment of UTI. This was also noted by Sana *et al.* (2000) in a study done in Kuwait and also in the U.S., southern Europe, Israel, and Bangladesh with up to 50% of *E. coli* strains being resistant to antibiotics used (Manges *et al.*, 2001). In our study, 25% of the isolates were resistant to

Nitrofurantoin, which is above 10% what is common in most areas (Sana *et al.*, 2005). This suggests a likelihood of significant increase in resistance to this drug (Warren *et al.*, 1999).

Based on the findings of this study, it is concluded that acute uncomplicated UTI affects a large proportion of the population in the study area, and is more prevalent in females. As reported previously, our study confirmed *Escherichia coli* to be a major uropathogen. The study further detected increasing resistance of *Escherichia coli* strains to trimethoprim-sulfamethoxazole. The results of this study indicate a need for continued surveillance of antimicrobial resistance among uropathogens causing UTI, so as to increase positive outcomes of clinical interventions.

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Table 2: Resistance patterns to seventeen antimicrobial agents among 831 *E. coli* isolates from the urinary tract.

Antimicrobial agent	Total (no) of isolates	Resistant isolates		Susceptible isolates	
		Total (no.)	%	Total (no.)	%
Trimethoprim-sulfamethoxazole	795	506	64	289	36
Ticarcillin	4	0	0	4	100
Peril/Tazo	16	0	0	16	100
Amikacin	22	0	0	22	100
Ofloxacin	29	0	0	29	100
Rocep	14	0	0	14	100
Cefuroxime	759	47	6	710	94
Nalidixic acid	804	72	9	732	91
Gentamycin	799	58	7	741	93
Norfloxacin	775	37	5	738	95
Ciproxin®	145	7	5	138	95
Ceftazidime/Fortum	227	9	4	218	96
Perfloxacin	55	3	5	52	95
Centriaxone	86	4	5	82	95
Augmentin	801	234	25	567	75
Nitrofurantoin	820	192	23	628	77
Cephalexin	749	98	13	651	87

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