ORIGINAL ARTICLE

Antibiotic Resistance Patterns of Common Gram-negative Uropathogens in St. Paul's Hospital Millennium Medical College

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ABSTRACT

BACKGROUND: The resistance of bacteria causing urinary tract infection (UTI) to commonly prescribed antibiotics is increasing both in developing and developed countries. Resistance has emerged even to more potent antimicrobial agents. This study was undertaken to determine the current antibiotic resistance pattern among common bacterial uropathogens in St.paul's Hospital Millennium Medical College.
METHODS: Using cross sectional study design, a total of 217 female and 207 male participants were consecutively recruited. Mid-urine samples were collected from all patients using wide mouthed urine cup. Inoculation was performed onto blood agar and MacConkey agar symoultaniously, and isolated organisms were identified by conventional methods. Antibiotic susceptibility was done by Kirby Bauer disk diffusion method. Thirteen different antibiotics representing different families of antibiotics were tested on all isolated organisms.
RESULT: Of the total 424 samples, 95(22.4%) showed significant growth. Gram negative organisms totaled 85(20.05%), and 10(2.4%) isolates were gram positive. The most frequently isolated gram negative bacterium was E. coli followed by Protues and Klebsiella spp. 53(12.5%), 8(8.4%), and 7(7.4%) respectively. Resistance to Tetracyclin, Ampicilin, Amoxycilin and Nalidixic Acid was more than 70% of all isolates of E.coli strains. There was relatively low resistance rate to Nitrofurantoin, Gentamycin and Trimethoprim-Sulfamethoxazole. However, there was emerging resistance to Ciprofloxacilin and Ceftriaxone especially for common bacteruria.
CONCLUSIONS: In this study setting, resistant rates to Tetracyclin, Ampicilin, Amoxycilin and Nalidixic Acid were high. Since most isolates were sensitive for Nitrofurantoin, Gentamycin and Trimethoprim-Sulfamethoxazole, they are considered as appropriate antimicrobials for empirical treatment for urinary tract infections with the absence of culture and sensitivity setting. Increasing antibiotic resistance trends indicate that it is imperative to rationalize the use of antimicrobials in the community and use these conservatively.
KEYWORDS: Antibiotic Resistance, Uropathogen, Addis Ababa, Ethiopia

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INTRODUCTION

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria in the urine to sever form of the kidney with sepsis (1). UTIs are one of the most common bacterial infections in humans both in the community and hospital settings (2). Worldwide, approximately 150 million people are diagnosed with UTIs resulting in 6 billion USD health care expenditures (1). UTIs are the most common bacterial infections encountered by clinicians in developing countries (3).

Most UTIs are caused by Gram-negative bacteria like *Escherichia coli (E. coli), Klebsiella spp.*, *Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter spp.*, and *Serratia spp.* and Gram-

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positive bacteria such as *Enterococcus spp.* and *Staphylococcus spp.*(4). The commonest bacterial agent involved in causation of UTIs is *Escherichia coli*, the principal pathogen both in the community as well as in the hospital (5).

The treatment of UTIs varies according to age, sex, underlying disease, infecting agent and involvement of lower or upper urinary tract. According to the Infectious Diseases Society of America (IDSA) guidelines, the recommended drug is Trimethoprim/sulphamethoxazole for the treatment of UTIs in settings where the prevalence of resistance is < 10-20 percent and ciprofloxacin is recommended where this resistance is > 20 percent (6). The other agents used in the treatment of UTI include fluoroquinolones, cephalosporins and other β-lactams with or without β-lactamase inhibitors and nitrofurantoin (7).

Drug resistance among bacteria causing UTI has increased since introduction to UTI chemotherapy (8). The etiological agents and their susceptibility patterns of UTI vary in regions and geographical locations. Besides, the etiology and drug resistance change through time (9). Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that They can give updated recommendation for optimal empirical therapy of UTI (10). In Ethiopia, a number of studies have been done on the prevalence and antimicrobial resistance patterns of UTIs (4, 11-13). However, as the pattern of bacterial resistance is constantly changing, monitoring antimicrobial susceptibilities is important. It provides information on the pathogenic organisms isolated from patients, and assists in choosing the most appropriate empirical antimicrobial therapy. In addition, continuous surveys of antimicrobial resistance are crucial for monitoring changes in this resistance. The aim of this study was therefore to determine the resistance patterns of gram negative isolates from suspected UTI for the most commonly used antimicrobials.

MATERIALS AND METHODS

A cross sectional study was conducted in St.Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia. It is an urban setting and tertiary hospital for Ethiopian and teaching hospital for national and international students. The hospital provides out- and in-patient services with 370 beds. Accordingly, patients being seen at SPHMMC come from all over the Northern, Western, Southern and Eastern parts of Ethiopia. However, most patients seeking medication at SPHMMC are predominantly from Northern Oromia Regional State and Addis Ababa city administrations.

Sample Size Calculation and Sampling Technique: The sample size for the study was calculated using the formula (n = (zα/2)2 p (1-p)/ d2) for estimating a single population proportion at 95% confidence interval (CI) (Zα/2 = 1.96), 5% margin of error, and 10% non-respondence rate by taking 50% prevalence since there is no current study in the setting. Therefore, the total sample size for this survey was 424. However, a total of 200 pregnant women were consecutively selected to increase the finding or detection rates of cytomegalovirus infection rate in the study settings.

Data Collection and Sampling Procedure: Age and sex have been taken from patients' request forms. A total of 424 participants suspected for UTI were recruited using consecutive sampling techniques. Clean catch mid-stream urine samples were collected using sterile wide mouth container from enrolled patients. The minimum acceptable volume of urine sample was 10ml. All the samples were analyzed immediately after arrival to the laboratory to ensure that the pathogenic organisms present in the urine are isolated and to avoid over population of the pathogenic organism.

Culture and Identification{ Urine specimens were directly inoculated onto blood agar (Oxoid, England) and MacConkey agar (BD, USA) using a sterile standard calibrated wire loop (0.001), and streaked culture plates were incubated at 37ºC aerobically for 24hrs. Number and type of colony count was done on blood agar plate, and then significant bacteriuia was determined. Cultures with colony counts greater than 10^5cfu/ml, for a single isolated bacteria was consider significant. Identification of bacterial isolates was done using colony characteristics on blood agar, MacConkey agar and gram reaction of the bacteria and biochemical tests in accordance with standard procedures.

Organisms isolated from urine specimens of hospitalized and outpatients over a six month period (August 2013 to January 2014) were identified and tested for antimicrobial susceptibilities. In this study, only samples with significant growth were studied (significant growth was defined as the presence of $\geq 10^5$ colony-forming units per milliliter (cfu/mL) of urine (14). After obtaining the pure strains, the strains were subjected to conventional biochemical identification methods to identify different gram-negative uropathognes.

Antimicrobial Susceptibility Tests: According to the standard operational procedures, antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, England) using Kirby-Bauer disk diffusion method (15). Briefly, using a sterile wire loop, 3-5 pure colonies were picked from blood agar plate or MacConkey agar and emulsified in nutrient broth (Oxoid, England) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of McFarland 0.5 tubes in order to standardize the inoculums size. A sterile cotton swab was then dipped into the suspension and distributed the bacteria suspension evenly over the entire surface of Mueller-Hinton agar (Oxoid, England). The antimicrobial agents tested were: Ampicillin (Amp), Amoxycillin (10 µg), Chloramphenicol (30µg), Nalidixic Acid (NA), Nitrofurantoin (300 µg), Gentamicin (10µg), Ciprofloxacillin (5µg), Cephalothin (30µg), Ceftriaxone (30 µg), Norfloxacin (NOR), Doxycycline (30µg), Trimethoprim-Sulfamethoxazole (25µg), and Tetracycline (30 µg). Resistance data were interpreted according to Clinical Laboratory Standards Institute. Reference strains of E. coli ATCC 25922 and Staphylococcus aureus ATCC 25923 (S. aureus) were used for quality control for antimicrobial susceptibility (16).

Data Analysis: The data was entered and analyzed using SPSS statistical software package (version 20). The descriptive statistics and proportion of the findings in relation with age and sex were computed by percentages.

Ethical Clearance: Ethical approval was obtained from St. Paul's Hospital Millennium Medical College Institutional Review Board (IRB). Letter of permission was also obtained from St.paul's Hospital Millennium Medical College. Written and informed verbal consent was taken from study participants after clear explanations about the purpose and aims of the project were given. The study participants were given verbal assurance for the confidentiality of their responses. Based on the findings the results were communicated to clinicians.

RESULTS

A total of 424 [217(51.2%) female and 207 (48.8%) male] participants were enrolled. The age range of the participants was 1-78 years, with mean age of 32.8 ± 17.9 years. Thirty five of them were under twenty, 62.5% between 20-35 and 29.2% were above 36 years (Table 1).

In August 2013 to January 2014, a total of 424 urine specimens were examined for isolations and identifications of bacteria and susceptibility testing. Of these, 95(22.4%) urine samples showed significant bacterial growth, with 32/262 (12.2%), and 63/162 (38.9%) were pointed out for asymptomatic and symptomatic UTI patients respectively. The proportions of isolates were gram negative 85(20.05%), and 10(2.4%) gram positive.

Table 1: Age and sex distribution of patients with suspected UTI in St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2013/2014.

Variables	Category	Total (N/%)	Positive (N/%)	Negative (N/%)
Sex	Female	217(51.2)	56(25.8)	161(74.2)
	Male	207(48.8)	41(19.8)	166(80.2)
Age group	≤ 20	35(8.3)	12(34.3)	23(65.7)
	20-35	265(62.5)	49(18.5)	216(81.5)
	+ 36	124(29.2)	44(35.5)	80(64.5)

Species of the bacteria were: *E. coli* 53(55.8%), *Proteus* spp. 8(8.4%), *Klebsiella* spp. 7(7.4%), *Enterobacter* spp. 6(6.3%), *Citrobacter* spp. 5(5.3%), *Providenicia* spp. 3(3.2%) and *Pseudomonas* spp. 3(3.2%). Among the gram-negative, *E. coli* was the most frequently isolated organism (62.4%) followed by *Proteus* spp. (9.4%) and *Klebsiella* spp. (8.2%) (Table 2). Resistance to DO, TE, Amp, Aml and NA was observed in 71.7, 83.0, 79.2, 75.5, and 73.6% of the *E .coli* isolates respectively. Low resistance SXT, F and CN rate was observed in 22.6, 20.8, and 22.6% respectively (Table 3).

Fifty-four per cent of all *E.coli* isolates were found to be resistant to ciprofloxacin. Ciprofloxacin resistance was comparatively less among the other Gram-negative uropathogens like *Proteus* spp and *Entrobacter spp*, but higher in *Klebsiella spp* as mentioned (Table 1). Resistance to the gentamicin was also considerable especially among isolates of *Citrobacter* spp and *Entrobacter spp* with as many as 60.0, and 50.0 per cent of all isolates showing resistance to gentamicin respectively (Table 3).

The rates of resistance among Gram-negative uropathogens to third generation cephalosporins like ceftriaxone were high. Ceftriaxone resistances were seen in 45.3 and 71.4 percent of all isolates of *E. coli* and *Klebsilla spp* respectively (Table 3).

In this study, the overall resistance rates to two and more antimicrobials was 77.6%, and only 4(4.7%) were sensitive to all antimicrobials tested. The resistances to two and more antimicrobial agents were 100.0%, 81.2%, 85.8% and 100.0% to *Entrobacter spp*., *E. coli*, *Klebsiella*, and *Pseudomonas* respectively (Table 4).

Table 2: Frequency of isolated species among a study (n = 424) participants in St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2013/2014

Bacterial Isolates n(%)		**Frequency**	**Percent**
	S.aurues	5	5.3
Gram – positive 10(2.4)	S.saprofiticus	3	3.2
	S.epidermides	2	2.1
	E.coli spp.	53	55.8
	Proteus spp.	8	8.4
	Klebsiella spp.	7	7.4
Gram – negative 85 (20.05)	*Enterobacter* spp.	6	6.3
	Citrobacter spp.	5	5.3
	Providenicia spp.	3	3.1
	Non-Fermenter	3	3.1

Note: S.aureus = staph auereus, E.coli = Ectieritia coli

Table 3: Antimicrobial susceptibility of bacterial isolates from patients with suspected UTI at St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia 2013/2014.

Bacteria	No.isolates	Amp	C	Aml	SXT	CIP	CRO	F	CN	NA	KF	NOR	DO	TE
E.coli Spp.	53	79.2	30.2	75.5	22.6	54.7	45.3	20.8	22.6	73.6	32.1	67.9	71.7	83.0
Proteus spp.	8	62.5	37.5	62.5	3.8	12.5	0	37.5	25.0	50.0	62.5	12.5	50.0	62.5
Klebsiella spp.	7	85.7	57.1	71.4	57.1	57.1	71.4	42.9	28.6	42.9	71.4	71.4	71.4	28.6
Enterobacter spp.	6	83.3	50.0	66.7	50.0	16.7	0	16.7	50.0	66.7	66.7	16.7	16.7	33.3
Citrobacter spp.	5	60.0	20.0	80.0	40.0	40.0	20.0	40.0	60.0	60.0	40.0	20.0	60.0	80.0
Providenicia spp.	3	66.7	0	33.3	33.3	33.3	33.3	0	33.3	66.7	33.3	33.3	66.7	100.0
Pseudomonas spp. (n=3)	3	100.0	33.3	100.0	66.7	33.3	33.3	33.3	66.7	0	66.7	33.3	33.3	66.7

Note: Amp=Ampicillin, Aml=Amoxycillin, C=Chloramphenicol, NA=Nalidixic Acid, F=Nitrofurantoin, CN=Gentamicin, CIP=Ciprofloxacillin, KF=Cephalothin, CRO=Ceftriaxone, NOR=Norfloxacin, DO=Doxycycline, SXT=Trimethoprim-Sulfamethoxazole, TE=Tetracycline

Table 4: Multiple antimicrobial resistance patterns of bacterial isolates from patients with suspected UTI at St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2013/2014.

Bacteria	R0	R1	R2	R3	R4	R5	$\geq$R6
E.coli Spp.(n=53)	1(1.9)	9(17.0)	18(34.0)	13(24.5)	7(13.2)	2(3.8)	3(5.7)
Proteus spp. (n=8)	0	3(37.5)	3(37.5)	1(12.5)	0	1(12.5)	0
Klebsiella spp. (n=7)	1(14.3)	0	3(42.9)	0	0	2(28.6)	1(14.3)
Enterobacter spp. (n=6)	0	0	0	2(33.3)	1(16.7)	1(16.7)	2(33.3)
Citrobacter spp. (n=5)	1(20.0)	2(40.0)	0	1(20.0)	0	0	1(20.0)
Providenicia spp. (n=3)	0	1(33.3)	1(33.3)	0	1(33.3)	0	0
Pseudomonas spp. (n=3)	0	0	1(33.3)	0	0	0	2(66.7)
Total isolates (n=85)	4(4.7)	15(17.6)	26(30.6)	17(20.0)	9(10.6)	6(7.1)	9(10.6)

DISCUSSION

E. coli is the commonest uro-pathogen causing complicated and uncomplicated UTI as described previously (5). There are several organisms known to cause UTIs, including *P. aeruginosa, S. saprophyticus, S.epidermidis, Enterococcus* spp, *P. mirabilis, Klebsiella* spp., *Citrobacter* spp, *etc.* as reported by earlier workers (17).

The prevalence of bacteriuria in our study 22.4% was almost similar with what had been previously reported in Gondar (17.8%) (11), Sudan (19.5 %) (18), Nigeria (17.3%) (19) and Nepal (21%) (20). However, this finding is not in agreement with the results from studies done in Addis Ababa Ethiopia (10.9%) (12), and other parts of the world: India (32%) (21), Iraq (49.1%) (22). and Pakistan (51.03%) (23). The difference in rate of uropathogens in different studies may be explained by differences in methodology used, the environment, social habits of the community, the standard of personal hygiene and education.

In this study, *E. coli* was by far the most frequently isolated bacterium that occured in 85(20.1%) of the Gram negative organisms, (55.8% of all isolates). This is comparable with a finding in Sudan, where *E. coli* was 42.4% of the Gram negative isolate (19/9). This also goes with results that obtained in Tanzania where *E. coli* was 38% of the Gram-negative isolates and 25% of all isolate (24). Likewise, many authors have the same findings e.g. in Pakistan and India (25, 26).

Given that the majority of therapy for UTIs is empiric and that urinary tract pathogens are demonstrating increasing antimicrobial resistance, continuously updated data on antimicrobial susceptibility patterns is beneficial to guide empiric treatment. The purpose of this study was to describe the susceptibility profiles of isolates of *E. coli* from patients in St.Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. The percentage of isolates of *E. coli* resistant to ampicillin was found to be as much as 79.2 percent in our set up. Such a high level of resistance to ampicillin has been quoted by many other studies from different parts of the world. For instance, studies in India showed that 80 and 76 percent resistance strains of *E. coli* for ampicillin was observed (27, 28). In Africa (e.g. Sudan, Tanzania, Kenya and Senegal), it has been reported that, *E. coli* in urinary isolates have a high antimicrobial resistance pattern (18, 29, 30, 31). Likewise, other studies have been reported high resistance of *E. coli* towards different antimicrobials was observed in Latin American and Costa Rica (32, 33).

The other gram-negative isolates, *Proteus spp.*, in our study showed 62.5% resistance towards Ampicillin and Amoxicillin, and 96.2% susceptibility to Trimethoprim-sulphamethoxazole respectively. This is different from the results from Gondar in which 100% resistance to Ampicillin and Amoxicillin-clavulanicacid and 100% susceptible to Trimethoprim-sulphamethoxazole (11) was reported.

K.pneumoniae showed 85.2% resistance to Ampicillin. This is more or less similar with studies conducted in Gondar (11), Nigeria (19) and Libya (34).

Multi drug resistance (MDR= resistance in ≥2 drugs) was observed in 77.6% of the isolated bacterial uropathogens in this study. This is more or less similar with what had been found in previous findings reported in Ethiopia, Addis Ababa (71.7%) (12), but higher than a study carried out in Gondar (59.8%) (11). This indicates that multi drug resistance was found to be very high to the commonly used antibiotics. Antibiotic resistance has been recognized as the consequence of antibiotic use and abuse (35). Therefore, the reasons for this alarming event might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies.

In general, there was a high prevalence of gram-negative bacterial pathogens with high resistance patterns of commonly used antibiotics. However, most isolates were less resistant for Nitrofurantoin, Gentamycin and Trimethoprim-Sulfamethoxazole. They are considered as appropriate antimicrobials for empirical treatment for urinary tract infections with the absence of culture and sensitivity setting. Increasing antibiotic resistance trends indicate that it is imperative to rationalize the use of antimicrobials in the community and also use these conservatively.

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