E3 Journal of Microbiology Research Vol. 1(1). pp. 009-015, June, 2013
Available online http://www.e3journals.org

Full length research paper

Escherichia coli isolated from patients suspected for urinary tract infections in Hawassa Referral Hospital, Southern Ethiopia: An institution based cross sectional study

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Accepted May 3, 2013

To investigate antimicrobial susceptibility and virulence patterns of uropathogenic *E. coli* isolated from patients visiting Hawassa Hospital, South Ethiopia. Cross-sectional study was conducted from December2011-March2012. Midstream urines were collected and streaked on MacConkey agar (Oxoid). Colony counts of 10^5 or more/ml of urine were regarded as significant bacteriuria. Identification was done using cultural characteristics and biochemical tests. Agar disc diffusion was used to test drug susceptibility pattern. Virulence tests were done using conventional methods. Data generated were analyzed using SPSS version 13. Ninety urine specimens were analyzed, 35.5% showed significant growth of *E. coli.* Ampicillin showed 100% drug resistance, Sulphamethoxazole/Trimethoprim (81.25%) and Ciprofloxacin and Chloramphenicol, both 43.8%. Multi-drug resistance showed in 81.25% isolates. Of those who had history of urinary tract infection, 77.77% exhibited resistance to three or more drugs. However, 96.87% susceptibility was seen to Nitrofurantion, Ceftriaxion (84.4%), Cefotaxime (81.25%) and Gentamicin (75.0%). Twenty eight percent isolates displayed hemolytic characters whereas 37.5% showed hydrophobicity. Many uropathogenic *Escherichia coli* showed haemolysin and cell surface hydrophobicity. Previous exposure to UTI was found to be the risk factor ($p<0.05$). Most uroisolates illustrated high level of drug resistance. So, clinicians shall base their decision on culture and drug susceptibility tests.

Key words: Urinary tract infection;Antimicrobial susceptibility test;Virulence factor; Ethiopia

INTRODUCTION

Escherichia coli (E. coli) is capable of causing widespread infectious diseases like intestinal and extra intestinal types. Extraintestinal infections are urinary tract infection (UTI), neonatal meningitis, septicemia, pneumonia and wound infections (Aberra et al., 1994; Dawit and Worku, 1997; Santo et al., 2006). However, UTI is the most commonly caused bacterial infection by *E. coli* (Fantahun and Bayeh, 2009).*Escherichia coli* present in the gastrointestinal tract as commensals provide the pool for initiation of UTI (Raksha et al., 2003). The organism is implicated in more than 90% of all uncomplicated cases of UTI (Silveira et al., 2001).

The strains of *E. coli* causing UTI have been termed as Uropathogenic *E. coli* (UPEC) (Smith et al., 2008). In UPEC strains, virulence factors include the ability to adhere to uroepithelial cells, some O and K antigens, and resistance to phagocytosis and to the bactericidal action of human serum (Santo et al., 2006; Lule, 2005;Naveen

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and Mathai, 2005). Other factors known to contribute to the virulence of *E. coli* include the production of hemolysin, colicins, siderophores, cytotoxic necrotizing factor 1 (CNF 1) and cell surface hydrophobicity (Santo et al., 2006; Najar et al., 2007; Piatti et al., 2008). Adherence to uroepithelial cells is mediated by fimbrial and non-fimbrial adhesions (Raksha et al., 2003; Siegfried et al., 1994; Addisu et al., 2008; Sharma et al., 2007). These factors are generally lacking in the commensal or non-pathogenic *E. coli. Escherichia coli* caused UTI is a common infection encountered daily in medical practice and demanded correct diagnosis for proper management (Fantahun and Bayeh, 2009; Alemayehu and Messele, 1981) and appropriate antibiotic use has unquestionable benefit. Different studies have shown that *E. coli* accounted for approximately 70 to 95% of community-acquired cases and about 30 to 50% of all nosocomial infections (Piatti et al., 2008; Abilo et al., 2007; Foxman, 2005; Kahlmeter, 2003; Roos et al., 2008; Lau et al., 2008). They also reported that these organisms were responsible for significant social and economic costs for both communities and public health resources. For instance, it has been estimated that 150 million cases of UTI occur on a global basis per year and cost about 6 billion dollars for national health resources as (Roos et al., 2008; Lau et al., 2008) reviewed in their study. Besides these, a study in Ethiopia showed that 78.9% of the total *E. coli* isolated from different specimen of outpatients was the uropathogens (Messele and Alebachew, 1983).

In another study, the incidence of this isolate from urine of both nosocomial and community acquired was recorded as approximately 54% (Yeshi et al., 1994). A recent report from Bahir Dar, Ethiopia also showed that of the total isolation rate of UTIs cases, 72.5% were caused by gram negative bacteria with the most predominant of *E. coli* (Fantahun and Bayeh, 2009). However, in the present context, there is dearth of authentic data in Southern Region of Ethiopia particularly to Hawassa Referral Hospital which is supposed to serve millions of people coming from different regions. This probably may reflect poor addresal to this field of work and the need for data on UTI caused by *E. coli* to this particular site. This study is therefore sought to determine the prevalence of UPEC, its antibiotic susceptibility patterns and virulence factors in Hawassa Referral Hospital, Southern Ethiopia.

MATERIALS AND METHODS

Study design, area and period

A prospective institution based cross-sectional study was conducted in Hawassa Referral Hospital; Southern Ethiopia from December 2011 to March 2012. Hawassa is located in South Ethiopia regional state, 275 km away from the capital, Addis Ababa.

Study population

Study participants were patients directed to laboratory for microbiological analysis of urine at the hospital during the study period. Patients not willing to participate in the study were excluded from this study.

Data collection techniques

Socio-demographic data were collected using questionnaire after the written informed consent was obtained. Then, 10ml of early morning midstream urine was collected with wide mouthed sterile container having lids after cleansing their genitals. Samples were analyzed within an hour in the microbiology laboratory of the hospital using standard methods (Graham and Galloway 2001; Cheesbrough, 2000). No transport media as well as refrigerator for urine preservation were used as patients provided the specimens without delay.

Bacteriological investigation

Culture

For bacteriological examination, one loopful (10µl) of urine sample was streaked on MacConkey`s (Oxoid) agar. It was then incubated in aerobic atmosphere at 37^0C for 24 hours. A positive culture was defined as colony count greater than 10^5 CFU/ml of midstream urine. Positive cultures were further identified by their colony characteristics appearance on the respective medium, Gram staining technique, and confirmed by the pattern of a series of standard biochemical tests using the standard procedures (Cheesbrough, 2000).

Antibiotic susceptibility test

Antimicrobial susceptibility testing was done on all isolates according to the criteria of Clinical and Laboratory Standards Institute (CLSI) (2011). The antibiotic discs obtained from Oxoid (Hampshire, England) were tested include: ampicillin(AMP 10 µg,), amoxicillin/clavulanic acid(AMC 30 µg), cefotaxime (CTX 30 µg), cefoxitin (FOX 30 µg), ceftriaxon (CRO 30 µg), ciprofloxacin (CIP 5 µg), chloramphenicol(C30 µg), gentamicin (CN 10 µg), nitrofurantoin (F 300 µg), norfloxacin (10 µg) and sulphamethoxazole/trimethoprim (SXT 25 µg).

Hemolysin production

Hemolysin production was assessed using plate hemolysis test for the determination of presence of

hemolysin produced by the UPEC (Raksha et al., 2003; Siegfried et al., 1994; Sharma et al., 2007). Hemolysin production was evaluated visually by the presence of clear zone around each colony on 5% sheep blood agar plates following overnight incubation at 37°C(Najar et al., 2007; Piatti et al., 2008; Sharma et al., 2007).

Cell surface hydrophobicity

Cell Surface Hydrophobicity of the isolates was determined by using Salt Aggregation Test (SAT) (Raksha et al., 2003; Siegfried et al., 1994; Sharma et al., 2007). Ten µl of the isolates` suspension made in phosphate buffer was mixed with equal amounts of ammonium sulphate solution of different molar concentrations (0.2, 0.4, 1, 1.4, 2m) on a glass slide, and the visible clumping or aggregation of the organism was observed for one minute while rotating. The highest dilution of ammonium sulphate solution giving visible clumping of isolates was considered as SAT value (Siegfried et al., 1994). UPEC strains that had SAT value less than or equal to 1.4 m were considered hydrophobic. But strains showing aggregation in 0.002 m phosphate buffer alone (P^H 6.8) were considered as auto agglutination (Najar et al., 2007; Sharma et al., 2007)

Quality control

Culture media were tested for sterility and performance. Standard strain of *E.coli* ATCC 25922 was used during culture and antimicrobial susceptibility testing.

Data management and analysis

Data were checked for completeness, cleaned manually, entered and analyzed using SPSS version 13.0 statistical package. Data were summarized using frequency tables. Pearson chi-square test was used to analyze the association between socio-demographic factors and UPEC prevalence. Statistical significance was inferred at P-value <0 .05.

Ethical consideration

This study was approved by Ethical review committee of Hawassa University, College of Medicine and Health Sciences. Informed and written consent was obtained from each patient prior to study participation. In order to keep confidentiality of any information provided by study participants, the data collection procedure were anonymous. Results of culture and sensitivity were given to the attending physicians for subsequent management of patients.

Results

Socio-demographic characteristics

A total of 90 patients were enrolled in this study with the age range of 6-79 years with the mean (standard deviation) of 34.83($\pm$14.838) years. Among the study participants, 59 (65.55%) were married. Forty seven (52.2%) had educational level of below secondary ($\leq 8^{th}$ grade) and 51 (56.7%) were urban dwellers. sixty two (68.9%) were females, and 27 (30.0%) were with age range of 25-34 years. Thirteen (14.4%) of study participants had history of urinary tract infection (Table 2).

Urinary tract infection

Of the total 90 urine specimens examined, 35.5% showed significant UPEC with mono-microbial growth of counts of greater than 10^5 CFU/ml of the specimen. *E. coli* was more commonly (46.8%) isolated from 25-44 age groups followed by 55-64 (33.3%) and 15-24 (27.8%) (table2). Of those who had history UTI, 69.2% were again culture positive in this study and it showed statistically significant association ($p<0.05$) (Table 2).

Antibiotic susceptibility and virulence factors test results

The result of antimicrobial susceptibility pattern of the isolate against to 11 drugs is indicated on Table 1 below. Rates of bacterial resistant against antibiotics range from 0% to 100%. *E. coli* isolated in this study showed 100.0% resistance for ampicillin and 81.25% for sulphamethoxazol/trimethoprim followed by 43.8% resistance for both ciprofloxacin and chloramphenicol, 40.6% for norfloxacine. Of isolates tested, 81.25% showed resistance for three or more commonly used drugs(ampicillin,sulphamethoxazole/trimethoprim,ciprofloxacin,chloramphenicol,norfloxacin,amoxicillin/clavulanic acid, and/or cefoxitin, whereas 15.6% were only resistance for two routinely prescribed drugs, ampicillin and sulphamethoxazol/trmethoprim, and/or amoxicillin/ clavulanic acid; however, 3.13% was resistance for ampicillin.

About 96.87% susceptibility was observed for nitrofurantion followed by ceftriaxion (84.4%), cefotaxime (81.3%) and gentamicin (75.0%). Most of those who had of previous UTI treatment exposure, 77.77% showed multi-resistance (three or more drugs) whereas 22.2% were resistant for two drugs, ampicillin and sulphamethoxazole/trimethoprim or amoxicillin/clavulanic acid. About 28.1% and 37.5% were hemolytic and hydrophobic respectively; however, 12.5% showed both hemolytic and hydrophobocity (Table 3).

Table 1. Antimicrobial susceptibility pattern of UPEC+ isolated from urine culture of study participants (n= 32) at Hawassa University Hospital, December 2011 to March 2012.

Organism		Antimicrobial agents (%)										
		AMP	AMC	CTX	CRO	FOX	CIP	C	CN	F	NOR	SXT
E.coli (n=32)	S*	0.0	43.75	81.25	84.4	34.4	50.0	46.9	75.0	96.87	53.1	15.6
	I*	0.0	28.1	3.12	9.4	40.6	6.3	9.37	3.13	3.13	6.3	3.12
	R*	100.0	28.1	15.6	6.3	25.0	43.8	43.8	21.87	0.0	40.6	81.25
	T*	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

*= Sensitive, Intermediate, Resistant, Total, + = Uropathogenic E. coli

Discussion

In this study, the prevalence of UPEC caused UTI (35.5%) was comparatively higher than a report from Jimma, Ethiopia (22.2%) (Lule, 2005), but lower than other previous studies conducted in Addis Ababa, Ethiopia (46.0%) (Solomon, 1998) and Gondar, Ethiopia (40.0%) (Moges et al., 2002). This is possibly due to geographical variations, presence of urinary tract problems, having a previous urinary catheter, medical conditions and previous UTIs exposure. Mean age of the participants was 34.83 with a range of 6 to 79 years. UPEC was more commonly isolated from middle adult age groups followed by old and young adults (Table 2) respectively; however, a study at Jimma, Ethiopia showed UTI was more common in ages between 11-20 years. Another study from the globe explained that UTI increases with age (Ahmad et al., 2008). This is probably because increases in age causes to physiological alteration and deteriorations of the immune system, and also previous UTI exposure, sexual activities, pregnancy, going to menopause, use of birth controls, and some medical conditions may lead to UTIs at such specified age ranges in this study.

Maximum number of participants observed in this study was females and the isolation rate of bacterium across sexes was also higher in females (table 2). Related findings have been reported in earlier studies done in Ethiopia (Dawit and Worku, 1997; Fantahun and Bayeh, 2009; Lule, 2005; Messele, 1983) and in other areas (Ahmad et al., 2008; Ramzan et al., 2004; Amiri et al., 2009; Haider et al., 2010). This variation might be owing to lack of prostatic secretion, use of birth controls, pregnancy, shorter (~4cm) and wider urethra, and its proximity to anus. In addition, sexual intercourse may access entry of bacteria in to bladder and may cause such an infection. More participants were from urban and they showed more culture positive (Table 2). Findings revealed that half of the isolates were presented in participants who had a better educational level which describes as education is not found to be the risk factors. However, it is believed to be knowledge is vital to create awareness to the mechanisms of UTIs management and controls of predisposing factors. Similarly, studies (Ahmad et al., 2008; Sheikh et al., 2000) explained that people who have low socioeconomic, low education and poor medical facilities might be susceptible to UTIs. Of all, more UPECs were isolated from married followed by unmarried and divorced participants (Table 2) respectively. This might be because married people may have frequent coitus and pregnancy. Results on those who had previous UTI exposure specify that UPEC is high among those who have past drug exposure for UTI (Table 2). It is comparable with reports from Gondar, Ethiopia (Moges et al., 2002) (54%), and studies in other areas (Ahmad *et al*., 2008; Amiri *et al*., 2009; Haider *et al*., 2010) who found history of UTI exposure was the strongest risk factor. This is might be because of recurrent (relapsing) infection and failures of drugs in removing the etiology.

Drug susceptibility pattern was studied for all isolates. They illustrated high resistance for routinely used antibiotics (Table 1) which is similar to studies conducted at Jimma (Lule, 2005), and Mekele (Zeamanuel, 2007), Ethiopia. This may perhaps these drugs were most commonly used in this hospital and around, hence resistant UPECs may successfully develop. Results on those who had previous UTI chemotherapy exposure (Table 1) specify that there is high resistance for three or more drugs which is comparable to the study done at Gondar, Ethiopia (Moges et al., 2002) and elsewhere in the world (Amiri et al., 2009). Multiple-drug resistance to

Table 2. Demographic characteristics of study participants (N=90) and UPEC^{+} isolates (n=32) at Hawassa University Hospital, December 2011 to March 2012

Characteristics	Negative for *E. coli* (%)	Positive for *E. coli* (%)	X^2	p-value
Age (years)				
5-14	4(100.0)	0(0.0)	7.031	0.318
15-24	13(72.2)	5(27.8)		
25-34	15(55.0)	12(44.4)		
35-44	10(50.0)	10(50.0)		
45-54	8(80.0)	2(20.0)		
55-64	4(66.7)	2(33.3)		
>65	4(80.0)	1(20.0)		
Total	**58(64.4)**	**32(35.5)**		
Sex				
Male	22(78.6)	6(21.4)	3.54	0.06
Female	36(58.1)	26(41.9)		
Residence				
Urban	31(60.8)	20(39.2)	0.688	0.407
Rural	27(69.2)	12(30.8)		
Total	**58(64.4)**	**32(35.5)**		
Educational status				
Illiterate	17(60.8)	6(26.1)	3.986	0,408
Grade 1-4	10(76.9)	3(23.1)		
Grade 5-8	7(63.6)	4(36.4)		
Grade 9-12	12(63.2)	7(36.8)		
Grade12+	12(50.0)	12(50.0)		
Marital status				
Married	37(62.7)		.699	0.259
Unmarried	13(59.1)	9(40.9)		
Divorced	8(88.9)	1(11.1)		
Total	**58(64.4)**	**32(35.5)**		
Monthly income				
<100	11(78.6)	3(21.4)	10.546	0.061
100-300	20(76.9)	6(23.1)		
301-600	8(50.0)	8(50.0)		
601-1000	7(50.0)	7(50.0)		
>1000	5(100.0)	0(0.0)		
Others	7(46.7)	8(53.3)		
Total	**58(64.4)**	**32(35.5)**		
Previous UTI				
Yes	4(30.8)	9(69.2)	7.520	0.006
No	54(70.1)	23(29.8)		
Total	**58(64.4)**	**32(35.5)**		

commonly used drugs was observed (Table 1) and it concurs with other studies (Lule, 2005; Moges et al., 2002) (68%). But 3.13% and 15.6% UPECs in the present study were resistance for one and two drugs respectively which is in agreement with a study done in Gondar Ethiopia (Moges et al., 2002)(15.7%). Possible reason for this is that these drugs might be easily available and may be used indiscriminately in the area. This is illustrated by about 14.4% of the participants who used drugs previously for UTI treatment and of these,

Table 3. Virulence factors pattern of UPEC^{+} isolated from urine culture of study participants (n= 32) at Hawassa University Hospital, December 2011 to March 2012.

Virulence factors	Conditions	No.	%
Hemolysin production	Hemolytic	9	28.1
	Nonhemolytic	23	71.9
	Total	32	100.0
Hydrophobiicity(CSH)	Hydrophobic	12	37.5
	Nonhydrophobic	20	62.5
	Total	32	100.0
Hemolysin + CSH		4	12.5

69.2% were again positive and most of them showed multi-resistance and it is similar to reports of others (Sharma et al., 2007; Moges et al., 2002; Ahmad et al., 2008).

The present finding shows that (Table 3) the isolate produced hemolysin which is in agreement with studies conducted in the globe (21.0%-28.0%) (Najar et al., 2007; Kausar et al., 2009). Another study (Raksha et al.,2003) also depicted that 41.36% of UPEC were hemolytic which is equivalent to findings of this study. In addition, comparable result with this study was reported from (Sharma et al., 2007) (39.5%). But the report of (Siegfried et al., 1994) showed 80% were hemolytic which is quite different from magnitude of hemolysin producers seen in this study. On the other hand, 37.5% were hydrophobic and it supports findings of previous study (Sharma et al., 2007) (33.4%). Some isolates expressed both of these markers simultaneously and it is in line with other reports (Sharma et al., 2007) (11.2%). More isolates (Table 3) had at least one or two virulence factors which is agreed with the works of others (Kausar et al., 2009) (80%) and (Raksha et al., 2003) (68.6%). Presence of these factors may aid the organisms to circumvent and suppress immune systems of the host. This may happen through either lysis the hosts` erythrocytes or cells and cause lose of nutrients like iron in case of hemolytic UPEC or mediating adherence to the host cells in case of hydrophobic isolates.

Conclusion

Results of this study have shown the burden of UPEC caused UTIs with a prevalence of 35.5%. Previous exposure to UTI chemotherapy was found to be the risk factor ($p<0.05$). Most of the uroisolates displayed high level of resistance to commonly used drugs. Multidrug resistance for the routinely used antibiotics was also high. It is therefore, understanding the effect of different factors on UTIs and their drug resistance will aid the proper management of this case. High rates of multi-drug resistance and resistance for many individual antibiotics studied should cause alarm and proper selection of drugs for treatment has to be depends on the results of antibiotic sensitivity test. It is especially important in low-income countries where options for safe, effective, and affordable chemotherapy are already limited. Hemolysin production and CSH were detected and some isolates expressed both of these markers simultaneously. On the basis of these findings, (1) When chemotherapy for UTIs is thought necessary, clinicians shall base their decision on positive urine culture and drug susceptibility patterns of uropathogens, (2) There is a need to develop locally useful guidelines for UTI management to disseminate current information about susceptibility patterns of uropathogens in the local health settings and to supervise drug usage in order to promote rational drug use, (3) Further studies including other uropathogens with more isolates on better understanding of interaction of different virulence factors at molecular level are necessary as most UPECs expressed at least one or the two virulence markers simultaneously, are recommended.

Abbreviations

AMP, Ampicillin; AMC, Amoxicillin/Clavulanic acid; CTX, Cefotaxime, CRO, Ceftriaxone; FOX, Cefoxitin; CIP, Ciprofloxacin; C, Chloramphenicol; CN, Gentamicin; F, Nitrofurantion; NOR, Norfloxacin; SXT, Sulphamethoxazole/trimethoprim; CSH, cell surface hydrophobicity; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; km, kilo meter; µl, micro liter; cm, centimeter; Others, house wife/student/dependent;

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

MG: initiation of the study, design, implementation, analysis and writing of the manuscript. MK: design, implementation, analysis and co-writing. YM: design, implementation of the study and co-writing. YM: initiation of the study, analysis and writing of the manuscript. MT: helping in writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We acknowledged the financial and technical support provided by University of Gondar and Hawassa

University. All participants and health personnel who participated in this study were also highly acknowledged.

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