JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Drug resistance and plasmid profile of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Abeba

Belayneh Regasa Dadi[1], Tamrat Abebe[2], Lixin Zhang[3], Adane Mihret[2], Workeabeba Abebe[2], Wondwossen Amogne[2]

[1] *Arba Minch University, Arba Minch, Ethiopia*
[2] *Addis Ababa University, Addis Abeba, Ethiopia*
[3] *Michigan State University, East Lansing, MI, United States*

Abstract

Introduction: Urinary tract infection is a major cause of morbidity and mortality worldwide. Uropathogenic *Escherichia coli* bacteria are the most common cause of urinary tract infections. Drug resistant *Escherichia coli* is results in high levels of treatment failure and can be a significant threat to survival of patients.

Methodology: *Escherichia coli* bacteria were isolated using culture and conventional biochemical tests. Antimicrobial susceptibility testing and plasmid profile were performed using the Kirby Bauer disc diffusion method and plasmid analysis. Data was processed with SPSS version 16.0 and Epi-info version 3.4.1 software.

Results: The highest proportion of *Escherichia coli* isolates was resistant to (86.5%) to ampicillin, followed by ceftazidime (84%), ceftriaxone (80.5%), tetracycline (80%), trimethoprim-sulfamethoxazole (68.5%) and cefotaxime (66%). *Escherichia coli* isolates were most susceptible to meropenem (100%), imipenem (100%), amikacin (97.5%), nitrofurantoin (95%), ciprofloxacin (85.5%), norfloxacin (85%), chloramphenicol (83.5%), gentamycin (80%) and nalidixic acid (79%). Multidrug resistance (MDR) was observed in most (96.5%) *E. coli* isolates. Plasmid analysis revealed the presence of plasmid(s) in 165 (82.5%) of the E. coli isolates many of which had a plasmid size of 23 kb.

Conclusions: The overall incidence of antibiotic resistance (including MDR) among *E. coli* in this study was high to commonly used antibiotics, but no drug resistance to meropenem and imipenem was observed. Periodic monitoring of the drug resistance pattern is essential for better management of urinary tract infections, which has direct impact on the outcome of the patient.

Key words: uropathogenic *Escherichia coli*; urinary tract infections; drug resistance; plasmid profile.

J Infect Dev Ctries 2018; 12(8):608-615. doi:10.3855/jidc.9916

(Received 07 November 2017 – Accepted 17 March 2018)

Introduction

Urinary tract infections (UTIs) are one of the most common human infections and a major cause of morbidity and mortality worldwide [1-2]. UTIs are also a major cause of sepsis, which has a mortality rate of 25% and results in more than 36,000 deaths per year in the USA [3]. It has been estimated that globally, symptomatic UTIs result in as many as seven million visits to outpatient clinics, one million visits to emergency departments, and 100,000 hospitalizations annually [4]. In Africa, urinary tract infections are the most common causes of morbidity and mortality [5-7]. Drug resistance in urinary tract infection, is a major concern in Africa including in Ethiopia [5-7]. *Escherichia coli* is the main agent causing urinary tract infections, accounting for up to 80% of cases. Serotypes of *Escherichia coli* consistently associated with UTI are designated as uropathogenic *E. coli* (UPEC) [8]. Uropathogenic *E. coli* are implicated in 70-90% of community acquired UTIs and 50% of nosocomial UTIs [9].

Antibiotic resistance, including multidrug resistance, is an increasingly serious problem in UPEC [10]. The high antimicrobial resistance of UPEC significantly reduces the therapeutic options and increases the treatment costs and mortality rates [11]. Drug resistance ofUPEC to commonly used antibiotics such as ampicillin, amoxicillin-clavulanic acid, norfloxacin, cefuroxime, ceftriaxone and co-trimoxazole[6,7,11,12] has been reported. So, determination UPEC drug resistance patternsis important for appropriate treatment of urinary tract infections. The aim of this study was to assessdrug resistance, including plasmid profiles, of uropathogenic *Escherichia coli* among urinary tract infection patients in selected health facilities of Addis Ababa, Ethiopia.

Methodology

Subjects and uropathogenic E. coli isolates

A total of 780 subjects in three hospitals (Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu Hospital) in Addis Abeba, Ethiopia participated in this study. All subjects had been diagnosed with urinary tract infections. Socio-demographic and clinical data were collected by a questionnaire completed by interview. Mid-stream urine samples (10-20 mL) were collected using sterile containers. A sample was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ CFU (colony forming unit) per milliliter of urine. *Escherichia coli* isolates were presumptively identified by colonial morphology on MacConkey agar (Oxoid, Hampshire, UK), and further identified and confirmed by conventional biochemical tests. *E. coli* bacteria were recognized by the following: negative for urease, citrate utilization and hydrogen sulfide generation; and positive for motility, lysine decarboxylase, lactose fermentation, glucose fermentation and indole test [13].

Antimicrobial Susceptibility Testing

In vitro susceptibility testing of the bacterial isolates was performed by the Kirby-Bauer disc diffusion method [14]. The following antimicrobial agents were used at the concentrations shown: trimethoprim-sulfamethoxazole (TMP-STX) (1.25/23.75 μg), ampicillin (AMP) (10 μg), nalidixic acid (NA) (30 μg), amoxicillin-clavulanate (AMC) (20/10 μg), ceftazidime (CAZ) (30 μg), cefotaxime (CTX) (30 μg), ceftriaxone (CRO) (30 μg), gentamycin (CN) (10 μg), ciprofloxacin (CIP) (5 μg), tetracycline (TE) (30 μg), amikacin (AK) (30 μg), norfloxacin (NOR) (10 μg), nitrofurantoin (F) (300 μg), meropenem (MEM, 10 μg), imipenem (IM, 10 μg) and chloramphenicol (C) (30 μg) (Oxoid, Hampshire, UK). The antimicrobials were selected based on the antimicrobial agents commonly used locally to treat urinary tract infections, as well as the recommended antimicrobial agents for *E. coli* by Clinical and Laboratory Standard Institutes [32]. Isolates resistant to two or more antibiotics were classified as multidrug-resistant (MDR).

Plasmid DNA extraction and analysis

Plasmid DNA was extracted from *E. coli* isolates by the alkaline lysis method using Wizard Plus SV Minipreps DNA Purification Systems kit (Promega Corporation, Madison, USA). The manufacturer's instructions (protocol from Promega, USA for Wizard Plus SV Minipreps DNA Purification Systems) were strictly followed. The alkaline lysis method makes plasmid DNA linearized before it is placed in gel electrophoresis.

Twenty microliters (20μl) of the extracted plasmid DNA was mixed with 5μl of 6x loading dye on parafilm and loaded on slots of 0.7% agarose gel electrophoresis stained with 10μL 10,000x GelRed. After electrophoresis for 4 hours at 100 Volt on TAE buffer system, the gel was imaged under UV light (E-gel Imager; life technologies, Waltham, USA). Plasmid number/s wereobtained by counting the number of bands observed on the agarose gel. Standard DNA

Table 1. Study participants' socio-demographic status and site of data collection, and association with *E.coli* isolation rate.

Variables		Frequency (%)	χ^2 (P-value)
Gender	Male	265 (34)	0.734 (0.392)
	Female	515 (66)	
Age	< 18	71 (9)	3.227 (0.665)
	18-25	132 (17)	
	26-45	430 (55)	
	46-65	127 (16)	
	> 65	19 (3)	
Educational status	Illiterate	23 (3)	4.121 (0.532)
	Read and write	103 (13)	
	Grade 1 to 6	135 (18)	
	Grade 7 to 8	173 (23)	
	Grade 9 to 12	210 (27)	
	Above grade 12	125 (16)	
Marital status	Single	218 (31)	1.526 (0.466)
	Married	438 (63)	
	Divorced	41 (6)	
Site of data collection	Tikur Anbessa Specialized Hospital	580 (74)	1.429 (0.489)
	Yekatit 12 Hospital	30 (4)	
	Zewditu Memorial Hospital	170 (22)	

molecular weight markers (1 kb DNA ladder (Invitrogen, Waltham, USA and Lambda (λ) DNA/HindIII marker (Promega Corporation, Madison, USA) were used to estimate the plasmid size [23].

Data analysis

SPSS version 16.0 and Epi-info version 3.4.1 were used for data analysis. A p value <0.05 was considered as significant.

Ethical approval and informed consent

The proposal of this study was approved by the Institutional Review Board of Addis Ababa University, College of Health Sciences. Permission was also obtained from the medical directors of Tikur Anbessa Specialized Hospital, Yekatit 12 Hospital and Zewditu Hospital. Written informed consent was obtained from each patient who participated in the study.

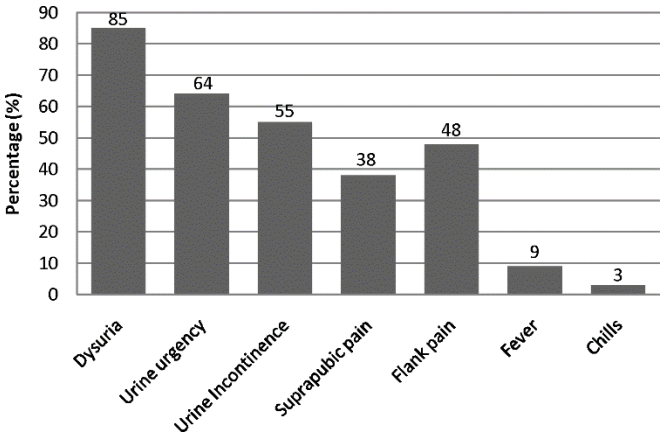
Results

The mean age of study participants was 33.95 years ± 14 SD; two thirds were female (Table 1). Most of the patients came from the Outpatient Department (OPD) but others were inpatients on the wards (13.5%); there was no difference in the *E. coli* isolation rate between these two groups (χ^2 0.067 p = 0.796). No significant association was found between gender, age, educational status or marital status, and *Escherichia coli* isolation rate). There was no significant association between site of data collection and *E. coli* isolation rates

Clinical data of study participants

The study participants had at least two of the following urologic symptoms; the most common complaint was dysuria, followed by urine urgency, urine incontinence, and flank pain at 50-65%, then suprapubic pain and a smaller proportion with fever and chills (Figure 1).

Figure 1. Clinical data of study participants.



Proportion of clinical symptoms of urinary tract infection patients. The most common clinical symptom was dysuria followed by urine urgency, urine incontinence, flank pain, suprapubic pain, fever and chills.

There was significant association between *Escherichia coli* isolation rate and the symptoms: urine urgency, fever, and chills (p = 0.002, 0.026, 0.033 respectively) (Table 2).

Bacterial isolation

Urine samples were cultured from all patients, and the 200 (25.6%) *Escherichia coli* isolates were identified by biochemical tests.

Antimicrobial susceptibility patterns

The antibiotic resistance and susceptibility patterns of the 200 *Escherichia coli* isolates revealed highest resistance to ampicillin followed by ceftazidime, ceftriaxone and tetracycline at similar levels, and to a lesser extent, to trimethoprim-sulfamethoxazole and

Table 2. Association between *Escherichia coli* isolation rate and clinical data, 2017.

Clinical data		*E. coli*		OR (95% C.I.)	P-value
		Positive	Negative		
Dysuria	Present	175	489	1.303 (0.810, 2.095)	0.274
	Absent	25	91		
Urine urgency	Present	146	352	1.751 (1.229, 2.495)	0.002
	Absent	54	228		
Urine incontinence	Present	122	309	1.372 (0.989, 1.904)	0.058
	Absent	78	271		
Suprapubic pain	Present	85	215	1.255 (0.905, 1.740)	0.173
	Absent	115	365		
Flank pain	Present	105	274	1.234 (0.895, 1.703)	0.199
	Absent	95	306		
Fever	Present	26	45	1.777 (1.064, 2.965)	0.026
	Absent	174	535		
Chills	Present	11	14	2.353 (1.050, 5.272)	0.033
	Absent	189	566		

cefotaxime. The *E. coli* isolates were most susceptible to meropenem, imipenem, amikacin, nitrofurantoin, and somewhat less to ciprofloxacin, norfloxacin, chloramphenicol, gentamycin and nalidixic acid (Table 3). Antibiotic resistance profiles showed that almost all of the local uropathogenic *E. coli* were resistant to two or more antibiotics, i.e., were multidrug resistant.

Multidrug resistance (MDR) in uropathogenic E. coli

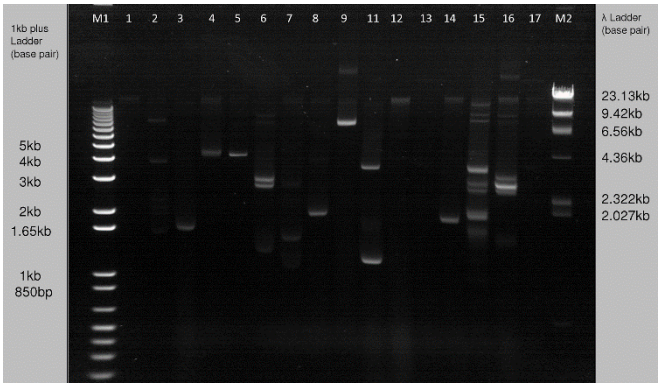
The most common multidrug resistance combinations found were to TMP, CRO, AMP, TE, CAZ and CTX, followed by CRO, AMP, TE and CAZ, and CIP, NOR, TMP, CRO, AMP, TE, CAZ and CTX combinations (Table 4).

Plasmid profile

Plasmid analysis showed presence of plasmid/s in more than 80% of the *E. coli* isolates but they were absent in nearly one-fifth.

One kilo base plus (1 kb plus) DNA ladder and Lambda/HindIII markers were used to determine the size of plasmids (Figure 2). The majority of the isolates (44.5%) carried 1 to 2 plasmids, while the maximum number of plasmids found was 10 (Figure 3).

Figure 2. Representative 0.7% agarose gel electrophoresis of uropathogenic *Escherichia coli* plasmids isolated from urinary tract infection patients. Lane M1, 1-kb plus DNA ladder; lane 1-16, plasmids of Escherichia coli isolates from sample 1-17; Lane M2, Lambda/HindIII marker.



One kilo base plus (1 kb plus) DNA ladder and Lambda/HindIII markers were used to determine the plasmid size of *Escherichia coli* isolates.

Table 3. Antimicrobial susceptibility patterns of *Escherichia coli* isolates, 2017.

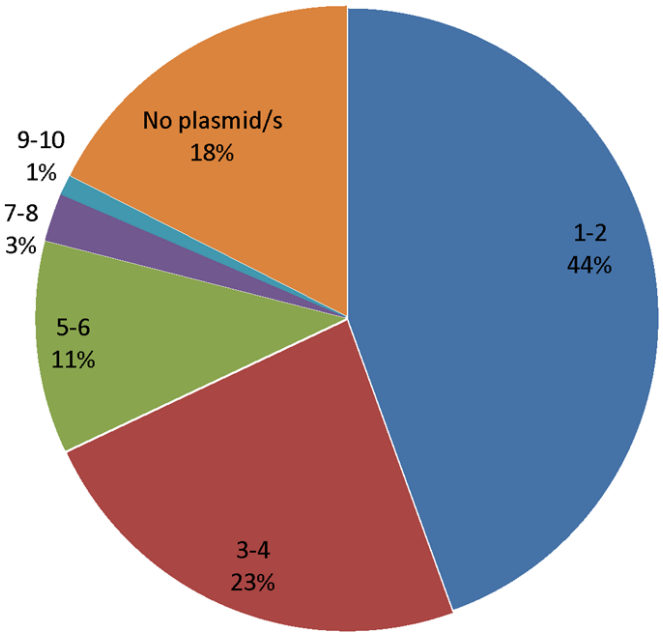
Antimicrobial agents	Number of resistant (%)	Number of intermediate (%)	Number of susceptible (%)
Ciprofloxacin	29 (14.5)	0	171 (85.5)
Norfloxacin	30 (15)	0	170 (85)
Nitrofurantoin	10 (5)	0	190 (95)
Trimethoprim-sulfamethoxazole	137 (68.5)	0	63 (31.5)
Tetracycline	160 (80)	0	40 (20)
Ceftriaxone	161 (80.5)	9 (4.5)	30 (15)
Ampicillin	173 (86.5)	6 (3)	21 (10.5)
Nalidixic acid	42 (21)	0	158 (79)
Amoxicillin-clavulanate	58 (29)	36 (18)	106 (53)
Ceftazidime	168 (84)	19 (9.5)	13 (6.5)
Cefotaxime	132 (66)	2 (1)	66 (33)
Amikacin	5 (2.5)	0	195 (97.5)
Meropenem	0	0	200 (100)
Imipenem	0	0	200 (100)
Chloramphenicol	33 (16.5)	0	167 (83.5)
Gentamycin	40 (20)	0	160 (80)

Table 4. Multidrug resistance (MDR) uropathogenic *E. coli* combinations from UTI patients, 2017.

MDR combinations	Frequency
TMP-STX, CRO, AMP, TE, CAZ, CTX	50
CRO, AMP, TE, CAZ	32
CIP, NOR, TMP, CRO, AMP, TE, CAZ, CTX	20
TMP-STX, AMP, TE, CAZ, CTX	17
CRO, CAZ	11
TMP-STX, CRO, AMP, AMC, TE, CAZ, CTX	10
TMP-STX, CRO, TE, CTX	8
AMP, TE, CAZ	4

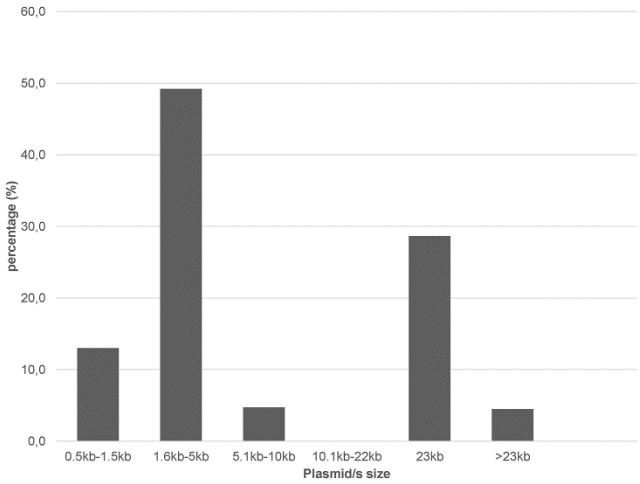
TMP-STX: trimethoprim-sulfamethoxazole, CRO: ceftriaxone, AMP: ampicillin, TE: tetracycline, CAZ: ceftazidime, CIP: ciprofloxacin, NOR: norfloxacin,

Figure 3. Number of Plasmid/s carried by uropathogenic *Escherichia coli* isolates.



Majority of *Escherichia coli* isolates carried 1 to 2 plasmids and the maximum number of plasmids carried by *Escherichia coli* isolate was 10.

Figure 4. Plasmid/s size carried by *Escherichia coli* isolates.



The plasmid size carried by *E. coli* isolates varies from 0.5 kb to >23 kb.

Table 5. Association between presence of plasmids and drug resistance of uropathogenic *E. coli*, 2017.

Drug resistance		Plasmids		OR (95% C.I.)	P-value
		Positive	Negative		
Ciprofloxacin	Present	25	4	1.384 (0.449, 4.262)	0.570
	Absent	140	31		
Norfloxacin	Present	26	4	1.450 (0.472, 4.453)	0.515
	Absent	139	31		
Nitrofurantoin	Present	8	2	0.841 (0.171, 4.141)	0.688
	Absent	157	33		
Trimethoprim-sulfamethoxazole	Present	114	23	1.166 (0.539, 2.524)	0.696
	Absent	51	12		
Ceftriaxone	Present	133	28	1.039 (0.417, 2.591)	0.934
	Absent	32	7		
Ampicillin	Present	143	30	1.083 (0.380, 3.089)	0.792
	Absent	22	5		
Nalidixic acid	Present	36	6	1.349 (0.520, 3.500)	0.537
	Absent	129	29		
Tetracycline	Present	132	27	1.185 (0.493, 2.847)	0.704
	Absent	33	8		
Amoxicillin-clavulanate	Present	49	9	1.220 (0.533, 2.794)	0.637
	Absent	116	26		
Ceftazidime	Present	137	31	0.631 (0.206, 1.931)	0.417
	Absent	28	4		
Cefotaxime	Present	107	25	0.738 (0.332, 1.642)	0.455
	Absent	58	10		
Amikacin	Present	5	0	0.821 (0.768, 0.876)	0.589
	Absent	160	35		
Chloramphenicol	Present	26	7	0.748 (0.296, 1.893)	0.539
	Absent	139	28		
Gentamycin	Present	32	8	0.812 (0.337, 1.954)	0.642
	Absent	133	27		

The plasmid size carried by *E. coli* isolates varied from 0.5 kb to > 23 kb. The majority, about half, carried plasmid sizes of 1.6 kb to 5 kb, while a third had a plasmid size of 23 kb. A few isolates had plasmids of a large size (> 23 kb) (Figure 4).

We found no significant association between drug resistance and presence of plasmids (Table 5).

Discussion

E. coli has been reported to be the most common cause of urinary tract infections [15]. We found a higher proportion of UTI in females than in males, as previously reported [16,17,25], and, possibly because of the shorter urethra in females, or injury during sexual intercourse and proximity to the anus[16,25]. The highest incidence of UTI was observed in the age groups 26-45, also reported from other studies [18,25].

The participants in our study had at least two of the following urologic symptoms; the most frequent complaint was dysuria followed by urine urgency, urine incontinence, flank pain, suprapubic pain, and a few with fever and chills. These findings are in agreement with results from studies conducted in South Korea [21] and Nigeria [24].

The overall incidence of antibiotic resistance in the *E. coli* in this study was high and almost all of the strains were resistant to two or more antibiotics. This result is comparable to the high rates of resistance found in India (92.5%) [19], (100%) [20], China (78.1%) [16], (82.6%) [25], but quite a lot higher than observed in South Korea (21.9%) [21], Mexico (30.2%) [22] and another study in India (50%) [23]. The high prevalence of multidrug resistance *E. coli* strains in our study may be explained by the fact that he majority of the isolates has been exposed to several.

The most frequent resistance found was against ampicillin, at a similar high rate of over 80% as was found in other countries: Mexico [22], Nigeria [26], India [18], and Ethiopia [7]. Resistance to trimethoprim- sulfamethoxazole, also frequently used in the treatment of UTI, was also common, found in more than two-thirds of strains; comparable frequencies were reported from Nigeria [24] and India [18]. Resistance to Ceftriaxone was high, over 80%, as observed in China [16] and Nigeria [26], all of which were much higher than results from other studies in Mexico at 10.2% [22] and Nigeria at 23.3% [24]. The high frequency of resistance to Ceftazidime at over 80% was similar to India [27] and China [16], but much higher than found in Mexico (8.5%) [22] and Nigeria (15.8%) [24]. These differences in antibiotic resistance patterns could be due to variations in antibiotic prescribing habits among different countries; for example, in some areas people can purchaseand use antibiotics without a prescription and may use it incorrectly, leading to resistance (see below).

No drug resistance was found to Meropenem and Imipenem, and this result has also been reported from South Korea [21], China [16]and Iran [28]. Low resistance was observed for Ciprofloxacin (14.5%), Nitrofurantoin (5%), Norfloxacin (15%) and Amikacin (2.5%) which is comparable to other studies in Ethiopia [7,29] and Iran [11,28].

Indiscriminate use of antimicrobials by healthcare providers or because of self-prescribing and over-the-counter availability are major risk factors for the development of high levels of antimicrobial resistance, which is common in both developed and developing countries [5,6,10]. Other factors contributing to resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion [5,6,24,30]. Therefore, proper use of antibiotics could be helpful to tackle antibiotic resistance. That means both implementing good prescribing and dispensing practices, and correct use by patients, who should take the antibiotics for specified period and within a specified time interval as prescribed by the physician.

Clinical isolates of *E. coli* are known to harbor plasmids of different molecular size ranging from 2-3 kb to 6.5 kb, with a maximum 26 kb [23]. The majority of our *E. coli* isolates carried plasmids with the size between1.6 kb and 5 kb, as was also found in India [23].; Similarly, studies in Nigeria revealed that clinical isolates of *E. coli* which showed multiple drug resistance harbored plasmids with molecular sizes ranging from 2 kb to 6.5 -23 kb to a maximum of 26 kb [24,30]. In Iran, reported plasmid sizes ranged from 1 kb to 33 kb [28], similar to our results and to findings in Nepal where the 23 kb plasmid size was common [31]. We found no significant association between the presence of plasmids and drug resistance, which is in agreement with a study conducted in Nepal [31].

Conclusion

In this study *E. coli* isolates from urinary tract infections werehighly resistant to one or more of: Trimethoprim- sulfamethoxazole, Ampicillin, Ceftriaxone, Ceftazidime and Cefotaxime. They still had susceptibility to Amikacin, Nitrofurantoin, Ciprofloxacin, Norfloxacin, Chloramphenicol and Gentamycin. There was no resistance to Meropenem and Imipenem. It is important to periodically monitor

the antibiotic resistance patterns to support the choice of treatments for better management of urinary tract infections.The judicious use of antibiotics and the correct implementation of an antibiotic policy in hospitals will help in limiting the emergence and spread of drug resistance.

Acknowledgements

The authors would like to thank those who were involved in this research. The authors also thank Arba Minch University, Addis Ababa University and Michigan State University for their support throughout the research process.

Authors' contributions

All authors contributed equally for this work. All authors read and approved the final manuscript.

References

1. Rajni E, Rawat U, Malhotra VL Mehta G (2008) Occurrence and detection of Amp C β-lactamases among clinical isolates of *Escherichia coli*and *K.* pneumoniae causing UTI. J Commn Dis 40: 21-25.
2. Foxman B (2002) Epidemiology of urinary tract infections: incidence, morbidity and economic costs. Am J Med 113: Suppl 1A:5-13.
3. Stamm WE, Norrby SR (2001) Urinary tract infections: disease panorama and challenges. J Infect Dis 183Suppl 1: 1-4.
4. Razak SK, Gurushantappa V (2012) Bacteriology of urinary tract infection and antibiotic susceptibility pattern in a tertiary care hospital in South India. Int J Med Sci PublicHealth 1:109-112.
5. Romanus II, Eze AT (2011) Antibiotics susceptibility patterns and clonal relatedness of uropathogenic *Escherichia coli*in abakaliki, ebonyi state. Canad J Pure ApplSci 5:1475-1479.
6. Mbanga J, Mudzana R (2014) Virulence factors and antibiotic resistance patterns of uropathogenic *Escherichia coli*. Afr J Microbiol Res 8: 3678-3686.
7. Biadglegne F, Abera B (2009) Antimicrobial resistance of bacteria isolates from UTIs at FelegeHiwot referral hospital. Ethiop J Health Dev 2:236-238.
8. Raksha R, Srinivasa H, Macaden RS (2003) Occurrence and characterization of uropathogenic *Escherichia coli*in urinary tract infections. Ind J Med Microbiol 21: 102-110.
9. Johnson JR, Russo TA (2005) Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol 295: 383-404.
10. Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M (2012) Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from Northwest England. J Antimicrob Chemother67:346–356.
11. Momtaz H, Karimian A, Madani M, Dehkordi FS, Ranjbar R, SarsharM,Souod N(2013) Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann ClinMicrobiolAntimicrob12:8.
12. Derakhshandeh A, Firouzi R, Motamedifar M, Boroojeni AM, Bahadori M, Arabshahi S, Novinrooz A, Heidari S(2015) Virulence Characteristics and antibiotic resistance patterns among various phylogenetic groups of uropathogenic *Escherichia coli* isolates.Jpn J Infect Dis 68:428–431.
13. Farmer JJ (1999) Enterobacteriaceae: Introduction and identification. In Murray PR, Baron EJ, Pfaller MA, Tenover FC, YolkenRH, Editors. Manual of Clinical Microbiology. Washington DC: ASM Press. 442-540.
14. Cheesbrough M (2006) Microbiological tests. In Davies S, Mortlock S, Hardy S, Bridson E, Tofiq M, Editors. District Laboratory Practice in Tropical Countries. Cambridge: Cambridge University Press. 106-143.
15. Raksha R, Srinivasa H, Macaden RS (2003) Occurrence and characterization of uropathogenic *Escherichia coli*in urinary tract infections. Ind J Med Microbiol 21: 102-110.
16. Wang Y, Zhao Sh, Han L, Guo X, Chen M, Ni Y, Zhang Y, Cui Z, He P(2014) Drug resistance and virulence of uropathogenic *Escherichia coli* from Shanghai, China. J Antibiot67: 799–805.
17. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, Gandham N, Wieler LH, Ewers CH, Ahmed N(2011) Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* from a semi urban locality in India. PLoS ONE 6: e18063.
18. Kumar Y, Sood S, Sharma A, Mani KR (2013)Antibiogram and characterization of resistance markers among *Escherichia coli*isolates from urinary tract infections. J Infect Dev Ctries7:513-519.doi: 10.3855/jidc.2706.
19. Mukherjee M, BasuSH, Mukherjee S,Majumder M (2013) Multi drug resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli*which were isolated from hospitalized patients in Kolkata, India. J Clinic DiagResear 7: 449-453.
20. Dash SK, Chakraborty SP, Mandal D, Roy S (2012) Isolation and characterization of multi drug resistant uropathogenic *Escherichia coli*from urine sample of urinary tract infected patients. Int J Life Sci Pharm Res 2: l25-l39.
21. Yun KW, Kim DS, Kim W, MD, Lim IS (2015) Molecular typing of uropathogenic *Escherichia coli*isolated from Korean children with urinary tract infection. Korean J Pediatr58:20-27
22. Molina-López J, Aparicio-Ozores G, Ribas-Aparicio RM, Gavilanes-Parra S, Chávez-Berrocal ME, Hernández-Castro R, Manjarrez-Hernández HÁ(2011)Drug resistance, serotypes, and phylogenetic groups among uropathogenic *Escherichia coli* including O25-ST131 in Mexico City.J Infect Dev Ctries 5:840-849.doi: https://doi.org/10.3855/jidc.1703
23. Jan N, Meshram S, Kulkarni A (2009) Plasmid profile analysis of multidrug resistant *E. coli*isolated from UTI patients of Nagpur City, India. Rom Biotechnol Lett14: 4635-4640.
24. Akingbade O, Balogun S, Ojo D, Akinduti P, Okerentugba PO, Nwanze JC, Okonko IO(2014)Resistant plasmid profile analysis of multidrug resistant *Escherichia coli* isolated from urinary tract infections in Abeokuta, Nigeria.Afr Health Sci14: 821-828.
25. Ranjini CY, Kasukurthi LR, Madhumati B, Rajendran R (2015) Prevalence of multidrug resistance and extended spectrum beta-lactamases among uropathogenic *Escherichia coli* isolates in a tertiary care hospital in South India: An alarming trend. Community Acquir Infect 2:19-24.
26. Abiodun AO, Olufunke OA, Dunah FC, Oladiran F (2014) Phenotypic identification and phylogenetic characterization of uropathogenic *Escherichia coli*in symptomatic pregnant women with urinary tract infections in South-Western Nigeria. Int J Biol 6: 145-155.

27. Annapurna YV, Reddy BS, Lakshmi VV (2014) Multidrug resistance and virulence phenotypes among uropathogenic *Escherichia coli*. Int J CurrMicrobiol App Sci3: 222-229
28. Farshad S, Ranjbar R, Japoni A, Hosseini M, Anvarinejad M, Mohammadzadegan R (2012) Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic *Escherichia coli* strains isolated from children in Jahrom, Iran. Arch Iran Med15:312–316.
29. Beyene G, Tsegaye W (2011) Bacterial uropathogens in UTI and antibiotic susceptibility pattern in Jimma University Specialized Hospital. Ethiop J Health Sci21: 141-146.
30. Umolu PI, Omigie O, Tatfeng Y, Omorogbe FI, Aisabokhale F, Ugbodagah OP (2006) Antimicrobial susceptibility and plasmid profile of *Escherichia coli* isolates obtained from different human clinical specimens in Lagos, Nigeria. J Am Sci2: 70-76.
31. Khadgi S, Timilsina U, Shrestha B (2013) Plasmid profiling of multidrug resistant *Escherichia coli* strains isolated from urinary tract infection patients. Int J ApplSciBiotechnol1: 1-4.
32. Clinical and Laboratory standards institute (CLSI) (2017) Performance Standards for Antimicrobial Susceptibility Testing, 27th informational supplement. CLSI document M100-S26 (ISBN 1-56238-804-5).

Corresponding author

Belayneh Regasa Dadi
Arba Minch University, Department of Medical Microbiology
Arba Minch, 1000, Ethiopia
Phone: +251-911-721624
Email: belayjanimen@gmail.com

Conflict of interests: No conflict of interests is declared.