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Virulence factors and antimicrobial susceptibility pattern of uropathogenic *Escherichia coli* isolated from urinary tract infected patients at Jimma University Medical Center, Jimma, Ethiopia

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Abstract

Background: *Uropathogenic Escherichia coli (E. coli) have evolved multitude of virulence factors and strategies that facilitate bacterial growth and persistence within the adverse settings of the urinary tract of the host. Thus, it is essential to determine the virulence factors and antimicrobial susceptibility profile of uropathogenic E. coli. The purpose of this study was to determine the virulence factors and antimicrobial susceptibility profile of E. coli isolated from symptomatic urinary tract infected (UTI) patients in Jimma University Medical Center.*

Methods*: Midstream urine from suspected symptomatic UTI patients were cultured following standard bacteriological procedures. Antimicrobial susceptibility testing was done by the Kirby-Bauer disk diffusion method according to the CLSI 2021 recommendations. The virulence factor determinants of uropathogenic E. coli were tested using standard protocols. Data was analyzed using SPSS version 20 statistical software. Chi-square and binary logistic regression test results were used.*

Results*: A total of 387 urine samples were cultured, of which 154 (39.8%) showed significant bacteriuria. Uropathogenic E. coli (UPEC) was isolated from 105 (68.1%) study participants among which 77 (73.3%) were women. E. coli strains showed various virulence markers such as hemolysin production (42%), cell surface hydrophobicity (32.3%), ESBL production (32.3%) and biofilm formation (52.3%). Antimicrobial susceptibility testing revealed a higher rate of resistance against ampicillin (90.4 %%) and augmentin (60%).*

Conclusions*: E. coli was the most common cause of UTIs; many isolates showed virulence factors and multidrug resistance. Therefore, the acquaintance of virulence factors of E. coli and their antibiotic susceptibility pattern will help in better understanding of the organism and in proper management of UTI patients, thus will contribute to reduce the emergence and spread of antibiotic resistance.*

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Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections occurring among human. It can be symptomatic or asymptomatic (1,2). The infections are more common among women than men (3). Among the common etiologies, *Escherichia coli* accounts for 50% of all hospital acquired UTIs. Those serotypes of *E. coli* consistently associated with the infections are known as uropathogenic *E. coli* (UPEC) (4). Uropathogenic *E. coli* possesses a variety of virulence factors which enhance their ability to overcome host defenses, and subsequently able to colonize the urinary tract system and cause UTI (5). These bacterial factors include α-hemolysin (HlyA), capsule, adhesive appendages and endotoxin (6,7). The presentation of adhesions are the most important factors of pathogenicity which enable the bacteria bind to host cells within the urinary tract in order to invade and replicate within uroepithelial cells. *E.coli* further possesses diverse ways to use the hydrophobic effect in order to adhere to different surfaces including human cells (8). Furthermore, biofilm promote the persistence of *E. coli* in urinary mucous which protects the bacteria from antibiotics, antibodies and white blood cells (9).

The emergence of antibacterial resistant pathogens causes serious obstacle in the management of various infectious diseases including UTI (10). Although various studies conducted in Ethiopia indicated the isolation of antimicrobial resistant *E. coli* from ambulatory and admitted patients (2,3,11), they were not comprehensive and recent. Moreover, virulence factors vary from strain to strain within species of the bacterium and resistance pattern of pathogens differ with location and time (12). Therefore, thorough studies and up-to-date information on antimicrobial susceptibility and possession of different virulence determinants among UPEC strains in Ethiopia, particularly in Jimma area, is imperative. Thus, this study aimed to substantiate these gaps.

Method

Study design, period, area and study participants

An institution based cross-sectional study was conducted from February to June 2019 at Jimma University Medical Center (JUMC) to determine the virulence factors and antimicrobial susceptibility pattern of UPEC. The University Medical Center is a teaching hospital with over 760 beds capacity and approximately 20,000 in-patients and 210,000 outpatient attendants per year. All patients from the age of 15-65 years who were suspected of UTI (screened by physician) and who visited outpatient department of JUMC were included in this study. Patients on antibiotics within a week prior to data collection and those who have been seriously ill to get consent to participate in the study were excluded.

Data collection

Socio-demographic information, clinical data and associated factors for the occurrence of UTIs were collected by attending nurses using semi-structured questionnaire. For data analysis, virulence factor and antimicrobial resistance were considered as dependent variables while socio-demographic and clinical data as independent variables.

Microbiological handling and investigations

Mid-stream urine was collected from symptomatic UTI suspected patients. Study participants were given sterile, dry, wide-necked, and leak proof containers. Instructions on how to collect midstream urine samples were also given. The collected samples were processed within a maximum of an hour of delay at JUMC microbiology laboratory. Properly mixed urine specimens were quantitatively transferred (using a sterile calibrated wire loop, that holds 0.002 ml), and inoculated onto each of MacConkey (Oxoford, UK) and Blood agar plates and incubated at 37^0C for 24 hours. Urine specimen with a count of $\geq 10^5$ colony forming unit (CFU) per milliliter was considered positive for single organism (12). *E. coli* isolates which was presumptively identified as lactose fermenter on MacConkey agar plates were further identified and confirmed by conventional biochemical tests (13).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried out using disk diffusion method on Mueller Hinton Agar (Oxoid; Hampshire UK) according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) (14). Furthermore, antibacterial agents were selected based on local prescription pattern and availability. The following drugs were used including: ampicillin (10µg), amikacin (30µg), ampicillin-sulbactum (10/10µg), gentamicin (10µg), ceftriaxone (30µg), ciprofloxacin (5µg), trimethoprim-sulphamethoxazole (Cotrimoxazole) (1.25/23.75µg), norfloxacin (10µg), ceftazidime (30µg), cefepime (30µg), amoxicillin-clavulanic acid (10µg), mero-

penem (10µg), and nitrofurantoin (300µg). *E. coli* ATCC-25922, *S. aureus* ATCC-25923 and *P. aeruginosa* ATCC 27853 were employed as reference strains for quality control.

Extended spectrum beta-lactamase detection

Initial screening of *E. coli* for the production of extended spectrum beta-lactamase (ESBL) was carried out by measuring the diameters of zone of inhibition produced by ceftazidime, ceftriaxone, and cefotaxime. Besides the screening, a combined disk method was used as a confirmatory phenotypic method based on the CLSI criteria (14). Ceftazidime and Cefotaxime disks (30µg) alone as well as their combinations with clavulanic acid (30µg/10µg) were used for confirmations of ESBLs. A difference of at least (≥) 5 mm increase in inhibition zone diameters for either of the cephalosporin disks, and their respective combination disks was interpreted as ESBL positive.

Detection of virulence factors production

Pure bacterial culture was inoculated onto 5% sheep blood agar plate, and hemolysin production was detected based on the presence of a zone of complete lyses of the erythrocytes around the colony and clearing of the medium (15). UPEC strains were also tested for their hydrophobic property by using salt aggregation test (7). Bacterial cell suspension was made in phosphate-buffered saline (pH 7.2) equivalent to 10^5 CFU/ml. Then, 20µl of it was mixed with 20µl of 1M, 1.5M and 2M ammonium sulphate on separate glass slide. To define positive reaction, the aggregated clumping should be observed at ≤1.5M with naked eye (7). Quantitative determination of biofilm formation was also assessed using 96-wells microtiter plate, and optical density was read by using ELISA reader (Elisys Uno Human, Wiesbaden, Germany). An optical density cutoff value was chosen to distinguish and categorize biofilm producers from non-biofilm producers (16).

Statistical analysis

Statistical analysis was done using SPSS statistical software version 21.0. The frequencies were calculated using descriptive statistics. Logistic regression analysis or χ^2 test was used to see the relationship between dependent and independent variables. P values of < 0.05 were considered significant.

Ethical consideration

Ethical clearance was obtained from Jimma University, Health Institute Ethics Review Committee (Ref No - IHRPGD594/2019). Informed written consent was obtained from each participant after explaining the objectives of the study. Information obtained from each study participant was kept confidential. Culture positive results and their antimicrobial resistance test results were delivered to the attending physicians for management of the patients.

Result

Socio-demographic characteristics

Among the total of 387 participants, 306 (79.1%) were women, 332 (85.8%) of them married and 134 (34.6%) were illiterate. The age of the study participants ranged from 15 to 65 years with the mean age of 33.2 (SD ± 14.1) years. Two third of the study participants, 256 (66.1%), were in the age ranges of 15 and 34 years (**Table 1).**

Association between *E. coli* infection and socio-demographic characteristics

Significant bacteriuria was detected among 154 (39.8%; 95% CI [38.4-41.2]) study participants. The rate of infection was higher in females, 110 (71.4%) than in males, 44 (28.6%). *E. coli* was identified from 105 of 154 (68.1%) urinary tract infected participants, among which 77 (73.3%) were females. There was significant association between educational level (χ^2=22.637, p-value=0.000) or job status (χ^2=10.343, p-value=0.035) with the isolation of UPEC **(Table 1).**

Clinical characteristics related to urinary tract infection

From the total of 387 study participants, 317 (82%) had urine urgency, 280 (72.3%) dysuria, and 230 (59.4%) supra-pubic pain. There was significant association between dysuria, urine urgency, incontinence, flank and supra-pubic pain with the occurrence of UTI due to UPEC ($P < 0.05$) **(Table 2).**

Prevalence of bacterial uropathogens

A total of 154 bacterial species were isolated out of 387 symptomatic urinary tract infected patients. There was no multiple bacterial isolates from the cases. The dominant isolate was *E. coli* followed by *Klebsiella* species (13.6%) and the least number of isolates were *S. saprophyticus* with isolation rate of 0.6%. Gram positive bacteria accounted for only 3.9% out of the total 154 bacterial isolates **(Table 3).**

Table 1: Socio-demographic characteristics of the study participants along with *E. coli* isolation from urine and association characteristics, February to June 2019, Jimma, Ethiopia.

Socio-demographic characteristics		No. of Study participants (%)	*E. coli,* n (%)		p-value
			Absent	Present	
Gender	Male	81 (20.9)	53(65.4)	28 (34.6)	0.091
	Female	306 (79.1)	229 (74.8)	77 (25.2)	
Age(yrs.)	15-24	129 (33.3)	99 (76.7)	30 (23.3)	
	25-34	127 (32.8)	98 (77.2)	29 (22.8)	
	35-44	44 (11.4)	27 (61.4)	17 (38.6)	0.124
	45-54	37(9.6)	26 (70.3)	11 (29.7)	
	55-65	50 (12.9)	32 (64)	18 (36)	
Marital status	Single	46 (11.9)	38 (82.6)	8 (17.4)	
	Married	332 (85.8)	240 (72.3)	92 (27.7)	0.051
	Divorced	9 (2.3)	4 (44.4)	5 (55.6)	
Educational status	Illiterate	134 (34.6)	81(60.4)	53 (39.6)	
	Read & write	10 (2.6)	8 (80)	2 (20)	
	Primary	110 (28.4)	89(80.9)	21(19.1)	0.00
	Secondary	76 (19.6)	53(69.7)	23 (30.3)	
	College & Above	57 (14.7)	51(89.5)	6 (10.5)	
Job status	Farmer	226 (58.4)	174(77)	52 (23)	
	Merchant	55 (14.2)	42(76.4)	13 (23.6)	0.035
	Gov't employ	68 (17.6)	56(82.4)	12 (17.6)	
	Others	38 (9.8)	10(26.3)	28 (73.7)	
Monthly income	<2000	281 (72.6)	206(73.3)	75 (26.7)	
	2001-4000	57 (14.7)	42(73.7)	15 (26.3)	0.489
	4001-6000	36 (9.3)	23(63.9)	13 (36.1)	
	>6001	13 (3.4)	11(84.6)	2 (15.4)	

Table 2: Statistical association between *E. coli* isolation rate and clinical data from February to June 2019, Jimma, Ethiopia.

Clinical data		*E. coli*		AOR (95% C.I.)	P-value
		Positive	Negative		
Dysuria	Present	86	194	0.487 (0.279,0.850)	0.011
	Absent	19	88		
Urine urgency	Present	93	224	0.498 (0.256,0.971)	0.041
	Absent	12	58		
Urgency incontinence	Present	78	169	0.518 (0.315,0.852)	0.010
	Absent	27	113		
Supra-pubic pain	Present	72	158	0.584 (0.363,0.939)	0.026
	Absent	33	124		
Flank pain	Present	71	155	0.584 (0.365,0.939)	0.026
	Absent	34	127		
Fever (≥38ºC)	Present	48	110	0.759 (0.483,1.167)	0.233
	Absent	57	172		
Chills	Present	34	72	0.716 (0.439,1.167)	0.180
	Absent	71	210		

Table 3: Prevalence of uropathogenic bacteria among culture positive patients from February 2019 to June 2019, Jimma

S. No.	Isolated pathogen	Frequency	%
1	*E. coli*	105	68.2
3	*Citrobacter species*	8	5.2
4	*Enterobacter species*	4	2.6
5	*Klebsiella species*	21	13.6
6	*Proteus vulgaris*	2	1.3
7	*Providencia species*	6	3.9
8	*Pseudomonas aeruginosa*	2	1.3
9	*S. aureus*	5	3.2
11	*S. saprophyticus*	1	0.6
	TOTAL	154	100

Antimicrobial susceptibility pattern

All 105 UPEC isolates were tested against 13 different anti-bacterial drugs. Ampicillin resistance was accounted for the highest rate (90.4%), followed by ampicillin-sulbactum (62%), Augmentin (60%), trimethoprim-sulfamethoxazole (56.1%) and norfloxacin (55.2%). Contrary to this, the isolates showed high rate of susceptibility to meropenem (95.2%), followed by nitrofurantoin (91.4%) and gentamicin (71.4%) **(Figure 1).**

Virulence factors of uropathogenic *E. coli* isolates

Hemolysin production was observed in 44 (42%); cell surface hydrophobicity feature in 34 (32.3%); biofilm production in 52% and ESBL elaboration in 34 (32.3%) of the 105 UPEC isolates **(Figure 2).**

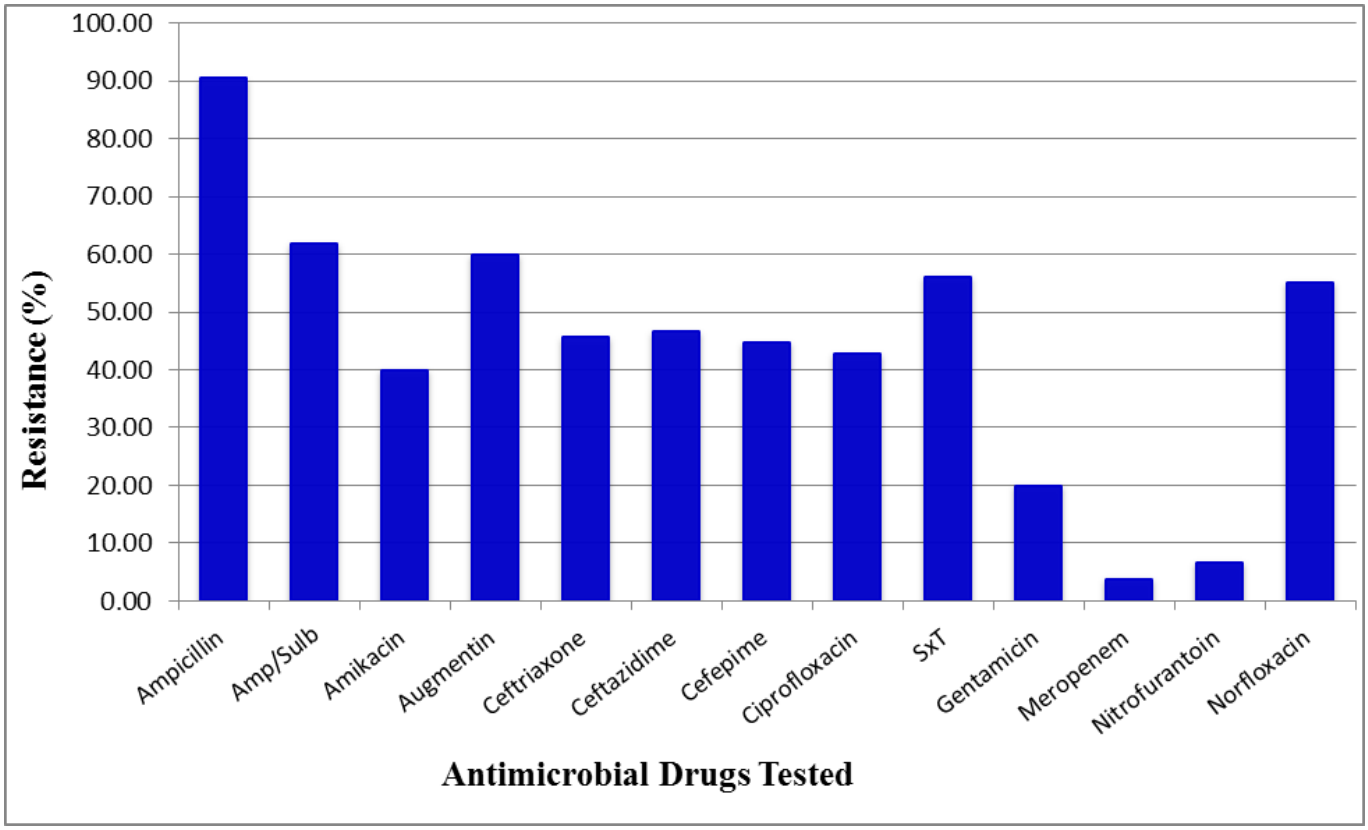

Figure 1: *Antimicrobial sensitivity pattern of UPEC culture isolates from February 2019 to June 2019, Jimma Ethiopia. Key: SxT: trimethoprim/sulfamethoxazole; Amp/Sulb: Ampicillin/sulbactam.*

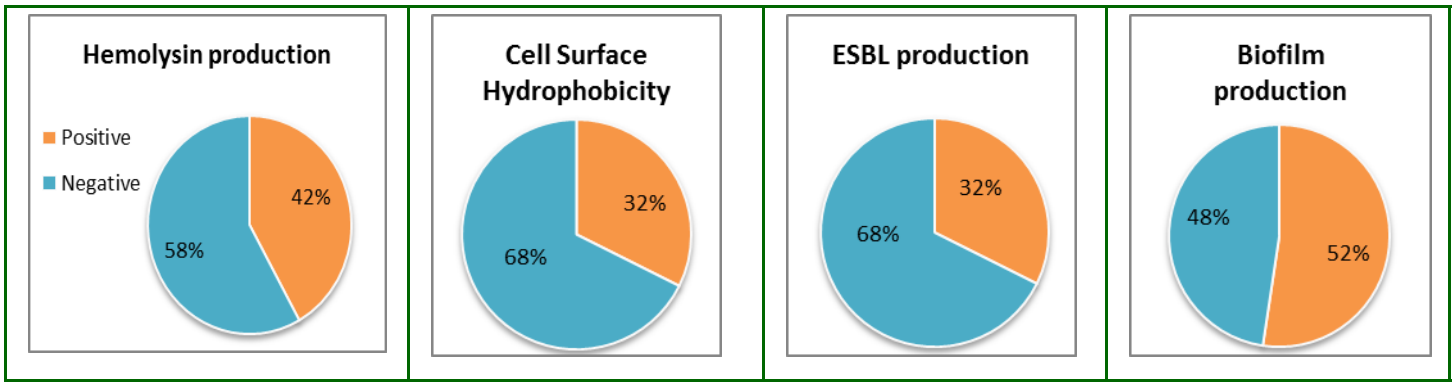

Figure 2: *Virulence factors of UPEC isolated from patients with symptomatic UTI from February 2019 to June 2019, Jimma, Ethiopia*

Discussion

Updated information about the prevalence and antimicrobial resistance (AMR) pattern is essential in the therapeutic management of UTIs due to UPEC strains for its AMR varies with geographic location and time. Moreover, understanding of the epidemiology of UPEC on the basis of the type of virulence factors expressed is little recognized in Jimma area (17). The prevalence of significant bacteriuria in this study was 39.8% (95% CI, 38.4-41.2) which is comparatively lower than reported from Shashemene, Ethiopia, 90.1% (13), and India, 60% (18), but higher than the previous study reported from Jimma, 9.2% (2). This difference in the rate of UTI may be explained due to variation in climate, geography and the methodology used. Too cold weather for instance may contribute to lack of personal and environmental hygiene of participants. However, this finding is in line with studies done in Arbaminch Ethiopia (37.2%) (19) and another study in India (36.2%) (16).

In our study, UTI was more prevalent among females constituting 71.4%. Similar findings were reported by different investigators (7,9,10). This disparity for females compared to males might be owing to lack of prostatic secretion, use of birth controls, pregnancy status, shorter urethra and proximity of the urethra to the anus. Contraceptive use is a significant risk factor for acquiring UTI, with the barrier methods being more predisposed to it.

In the current study, about two-third (68%) of all isolates were *E. coli* which is the major pathogen responsible for the occurrence of UTI. Our finding may be higher than study done in Arbaminch, 41.6% (19) which could be due to smaller sample size (129 adult cases) as compared to our study. The second dominant pathogen was *Klebsiella* species which was similar with previously reported study (19). There was significant association between the common clinical symptoms such as dysuria, urine urgency, incontinence, flank and suprapubic pain with UTI due to UPEC ($P < 0.05$) which was in agreement with studies conducted in Ethiopia (11, 20), Nigeria,(21), and Iran(22). These urologic symptoms are indicative of UTIs mainly due to UPEC.

Maximal proportion of UPEC isolates (90.4%) were non-susceptible to ampicillin which is similar with studies conducted in Jimma, Ethiopia, 100% (2); Libya, 100% (7); Jimma, Ethiopia, 86.6% (23) and India, 85.7%(24). Uropathogenic *E. coli* isolates showed 60% resistance to amoxicillin-clavulanic acid. This result is higher than the finding in Morocco (38.8%) (25), and can be attributed to frequent use of the drug. In the current study, resistance to trimethoprim-sulfamethoxazole was seen in 56.1% of UPEC isolates which is in agreement with previous study reported from Jimma, Ethiopia 56.6% (23), but higher than the finding of other similar studies conducted in Bahir Dar, Ethiopia, 45.5% (26); and South Korea, 35.9% (27). On the contrary, the study finding reported lower than that of Addis Ababa, 68.5 (20). Despite such high resistance profile, trimethoprim-sulfamethoxazole is recommended for the treatment of UTIs in the Ethiopian national standard guideline (28). Probably the existing standard guideline was prepared based on old study data, and there may be a need to review based on a large scale study. In the study area, noncompliance or self-medication is commonly observed (29). This could be one of the reasons for increased resistance to the antimicrobial drugs.

In the present study, *E. coli* showed lower resistance rate to nitrofurantoin (6.6%) which is lower than reported from India (19%) (16). However, the finding was higher than previous study from the same study area (Jimma) that reported all isolates were sensitive (2). The gradual increment of resistance may be due to over usage of this drug over the past long period. Most (80%) UPEC isolates were found to be sensitive to gentamicin, and it is almost similar with result of previous studies in Jimma (75%), Libya (87%) and Morocco (90.2%) (2,7,25). Increased sensitivity rate was also observed to meropenem (95.2%), and similar finding was reported in Iran (100%) (30). The finding may be expected as this drug is not easily available in the market and assumed to be the last resort drug in rare terminal infections.

Uropathogenic *E. coli* strains encode a number of virulence factors which enable the bacteria to selectively colonize the mucosal uroepithelium, induce inflammatory reactions and eventually proceed from lower region to upper renal urinary tracts, invade tissue and persist in the face of highly effective host defense mechanisms (31). In this study, hemolysin production was observed in 42% of the isolates. This was similar to a previous report from India with 41.3% (16) but higher than that of Libya, 60% (7). It is recognized that high concentrations of alpha hemolysin (HlyA) that possess UPEC able to lyse erythrocytes and nucleated host cells. This leads to a process that may enable extra-intestinal travel to cross mucosal barriers, damage effector immune cells, and gain enhanced access to host nutrients and iron stores (32). On the other hand, bacterial cell surface hydrophobicity property promotes the adherence of the bacteria to various surfaces like the mucosal epithelial cells (33). In the present study, 32.3% of the strains were having hydrophobic cell surfaces. Studies conducted in India and elsewhere revealed hydrophobic activity between 23% and 38.15% of isolates (34,35), and our study findings are in this range.

Biofilm producing uropathogenic bacteria is another virulence determinant responsible for many recurrent UTI's and provides resistance to antibiotic treatment (36). In the present study, the incidence of biofilm production in UPEC was 52% which is in agreement with the finding by different authors in Nepal, 51.9% (37), and Mekelle (Ethiopia), 61.7% (38). The difference in isolation of UPEC with different virulence factors in different proportion may indicate the presence of different strains of the bacterium causing symptomatic urinary tract infections.

In the current study, nearly one third (32.3%) of UPEC isolates were ESBL producing strains. The rate of ESBL elaboration in this study was lower than previously conducted study in Ethiopia where 51% to 100% of *E. coli* isolates were ESBL-positive (39,40). Similar to this work from Jimma, 28.2% of *E. coli* was positive for ESBL enzyme although these isolates were not exclusively recovered from urine samples (41).

Conclusion

This study illustrated the pathogenicity of UPEC isolates by demonstrating different types of virulent factors and antimicrobial resistance profile to commonly used drugs like ampicillin, Augmentin and Co-trimoxazole. Despite such high resistance profile (56.1%) of UPEC to trimethoprim-sulfamethoxazole, it is still recommended for the treatment of UTIs in the Ethiopian national standard guideline. Hence, this piece of study finding recommends conducting large scale antimicrobial susceptibility study and revising the national guide line accordingly. Furthermore, the acquaintance of virulence factors of *E. coli* and their antibiotic susceptibility pattern will help in better understanding of the organism and in the proper management of UTI patients; thus, serve as an input in decreasing the improper use of antibiotics.

Declarations

Ethics approval and consent to participate

This research project was approved by Institutional Review Board of Health Institute, Jimma University. Permission was also asked from the JUMC administrator. Study subjects were enrolled after being informed about the objective of the study. Each study participants gave written consent form, and for those participants under 18 years old, parent's written consent and assent was obtained. For all confirmed urinary tract infected patients, the responsible clinician was informed for proper management and follow up. All information contained within this study is kept confidential

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Consent for publication

On this research, patient's personal information was not mentioned. Thus obtaining consent for publication is not applicable for this study. All authors have consented for publication of this research manuscript.

Author's contributions

GB was the primary researcher, and involved in conceiving and designing the study. He was also, participated in the data analysis. AB and TK involved in data collection, interpretation of the results, reviewing the draft manuscript and finalizing it for publication. HA, ZM & KG participated in the design of the study, coordinated the overall work and critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interest

We, authors declared that no competing interests exist.

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