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ORIGINAL ARTICLE

MULTI-DRUG RESISTANT BACTERIA AND ASSOCIATED FACTORS AMONG REPRODUCTIVE AGE WOMEN WITH SIGNIFICANT BACTERIURIA

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ABSTRACT

Background: *Urinary tract infection (UTI), an infection that disproportionately affects women, is commonly caused by bacteria. Emergence of multi-drug resistant urinary tract infections is a serious health issue with significant maternal morbidity and mortality.*

Objective: *The aim of this study was to assess the prevalence of multi-drug resistant bacteria and associated factors among reproductive age women with significant bacteriuria.*

Methods: *Cross-sectional study was conducted from April to August 2016 on 424 study subjects in Jimma University Specialized Hospital. Data were collected using pre-tested questionnaire. Morning midstream urine samples were collected and processed following standard operating procedures. Antimicrobial susceptibility testing was done following Clinical Laboratory Standards Institute 2014 guidelines. Samples were tested for cell surface hydrophobicity, biofilm production, extended spectrum beta-lactamases and carbapenemases production.*

Results*: The prevalence of UTI among suspected reproductive age women was 22.9%. E. coli was the most frequent isolate with a rate of 57% among isolated bacteria followed by Klebsiella species (24.7%). Over 90% of the isolates were multi-drug resistant. Resistance pattern for ampicillin was 100% followed by ticarcillin (92.4%) and colistin (86%) while less resistance rate was found for imipenem* (13%). *Multivariate analysis revealed that risk factors such as previous history of hospitalization, extended spectrum beta-lactamase production and strong biofilm production were significantly associated with multidrug resistance (p <0.05).*

Conclusion: *The prevalence of multi-drug resistance (MDR) among isolates of UTI in the study was high and this correlates with the prevalence of virulence phenotypes. Gram-negative organisms were the most common causes of UTIs.*

Key words*: Multi-drug resistance, Urinary tract infection, Reproductive age women*

INTRODUCTION

Microorganisms that resist antibiotics are getting highly prevalent and emergence of multi-drug resistant (MDR) urinary tract infecting bacteria has become a significant public health threat (1-3). Urinary tract infection (UTI) is more common in females compared to males (4, 5). The prevalence of UTI pathogens and their resistance to different antibiotics may have changed over the years (6). There are particular factors that make each population in the world heterogeneous. Considering this fact, various studies in different parts of the world and in our country tried to test for the association of different socio-demographic and economic factors with the prevalence of UTI and some for drug resistance, including age, residence area, marital status, occupation, level of education, monthly income, parity and gravidity (7-11).

Mechanisms for emergence of resistance such as extended spectrum beta lactamase (ESBL) and carbapenemases production challenge use of empiric antibiotics due to associated resistance (10, 12-14).

Many studies examined antibiotic resistant UTI-causing bacteria which were not considered MDR and their related virulence phenotypes associated with drug resistance. The problem of UTI in reproductive age women who account for the highest proportion of UTI episodes was not addressed in the study areas (13, 15, 16). This study was therefore conducted to assess the prevalence of MDR uropathogenic bacteria and to define associated virulence factors among reproductive age women with significant bacteriuria in Jimma University Specialized Hospital (JUSH), Southwest Ethiopia, from April to August 2016.

PATIENTS AND METHODS

A prospective hospital based cross-sectional study was conducted in Jimma University Specialized Hospital on reproductive age women who had been consecutively attending JUSH during the study period with sign and symptom of UTI and without antibiotics therapy within the past two weeks at the study time (7, 8).

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Women who were unable to give information due to illness during the data collection were excluded (8). The sample size was determined using the formula for single population proportion. The study considered bacteria isolated from urine and multi drug resistance as dependent variables considering age, residence, educational status, occupation, marital status, monthly income, parity, gravidity, history of hospitalization, history of previous UTI, history of catheterization, history of chronic disease, extended spectrum beta-lactamase (ESBL) production, biofilm production and cell surface hydrophobicity (CSH) as independent variables.

Multi-drug resistance was defined as bacteria that were non-susceptibility to at least one agent in three or more antimicrobial categories (17). Patient information was obtained after informed consent and study participants were selected based on their clinical history of UTI signs and symptoms exhibiting supra-pubic/pelvic/flank pain, discomfort or a burning sensation during urination, pain during sexual intercourse, an intense urge to urinate, usual passage of frequent and small amounts of urine, a sign of blood in the urine, urine that looks cloudy or smells foul or unusually strong etc. (18,19).

From 10 ml of morning midstream urine collected, 1µL was cultivated (10, 20-22) on to MacConkey agar (HiMmedia, India) and 5% sheep blood agar (Oxoid, Hampshire United Kingdom) and incubated in aerobic atmosphere at 37 ^{0}C for 18 - 24 hours. A plate with colony counts of $\geq 10^{5}$colony forming unit (CFU)/ml of midstream urine was taken as significant bacteriuria (8). Organisms were identified on the basis of their growth characters, Gram staining and series of biochemical tests as per the standard recommended procedures to identify the isolates at species level (9, 23). Antimicrobial sensitivity of the isolates was done on pure colony inoculums prepared in physiological saline by adjusting the turbidity of bacterial suspension to give 0.5 McFarland's standard by using Kirby Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) 2014 protocol (24) on Mueller–Hinton agar (Oxoid, Hampshire United Kingdom) plate surface (5, 25). The antibiotic discs used for susceptibility testing in this study were selected following CLSI 2014 (24), European Society of Clinical Microbiology and Infectious Diseases standard selection criteria (17) and CLSI 2014 (24) recommendation; however, only those currently available in local research institutes or market were used. Cell surface hydrophobicity test was done by ammonium sulfate salt aggregation test (26-28).

Biofilm production test was done using the Modified Christensen method in micro-titer plates (26, 29-31).

ESBL detection was performed using double antibiotic disc synergy test (26, 31-33) and carbapenemase production was detected by modified Hodge test (24, 34-36). Quality control was done along with the test sample according to the manufacturer's instruction and CLSI 2014.

RESULTS

From 424 consecutive UTI suspected reproductive age women who attended JUSH from April to August 2016, 97 (22.9%) had significant unmixed bacteriuria. The mean age of the 97 women with significant bacteriuria was 29.6 yrs (SD + 8.033) and over 76% of them were between 15 to 35 years; 62% were urban dwellers, the majority (74%) were married and 74% had studied up to primary level education or below **(Table 1).**

Out of the total 97 isolates, 92 (95%) were Gram-negative bacteria of which *E. coli* (56.7%) was the most prevalent isolate followed by *Klebsiella species* (25%) with other organisms making 18% (Table 2).

Among the total of 97 isolates, all the 5 (100%) Gram-positive strains were MDR. Except for some *E. coli* (5.5%) and *Klebsiella species* (25%), all the other isolates were also MDR. Rates of resistance for antibiotics were 100% for ampicillin, 92.4% for ticarcillin, 86% to colistin and 13% for imipenem. Out of the 18 commonly used antibiotics tested against both Gram-negative and Gram-positive isolates in this study, 15 (83.3%) showed resistance ranging from 56.5% for cefepime to 100% for ampicillin.

With regard to the antibiotic resistance pattern of *E. coli* and *Klebsiella species* which were the most dominant isolates of the study, all 55 (100%) *E.coli* isolates were resistant to ampicillin, followed by 94.5% resistance to ticarcillin and 91% resistance to tobramycin. *Klebsiella species* were highly resistant to ticarcillin (83.3%) followed by 66.7% to cephalothin and 75% to colistin. Both species showed low resistance to imipenem; 11% in *E.coli* and 8.3% for *Klebsiella species* (**Table 3).**

Table 1: Socio-demographic and economic characteristics of reproductive age women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Variables	Category	Frequency (%)	Variables	Category	Frequency (%)
Age	15-25	31 (32.0)	**Marital status**	Married	72 (74.2)
	26-35	43 (44.3)		Single	20 (20.6)
	36-45	20 (20.6)		Divorced	4 (4.1)
	46-50	3 (3.1)		Widowed	1 (1)
Residence	Rural	37 (38.1)	**Family monthly in-come**	≤500	9 (9.3)
	Urban	60 (61.9)		501-1000	15 (15.5)
Educational status	Illiterate	9 (9.3)		1001-1500	21 (21.6)
	Can read and write	13 (13.4)		1501-2000	15 (15.5)
	Grade 1-4	20 (20.6)		2001-3000	20 (20.6)
	Grade 5-8	9 (9.3)		>3000	17 (17.5)
	Grade 9-12	14 (14.4)	**Pregnancy history**	Yes	63 (64.)
	College and above	32 (33.0)		No	34 (35.1)
Occupation	Farmer	8 (8.2)	**Parity**	Nullipara	34 (35.1)
	House wife	17 (17.5)		One	6 (6.2)
	Student	16 (16.5)		Multipara	57 (58.8)
	Government or NGO employee	36 (37.1)		No	34 (35.1)
	Merchant	20 (20.6)	**Gravidity**	1-3	42 (43.3)
				≥4	21 (21.6)

NGO = non-governmental organization

Table 2: Bacterial profile among reproductive aged women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Species isolated	No (percent)
E. coli	55 (56.7)
K. oxytoca	11 (11.3)
K. pneumoniae	10 (10.3)
C. freundii	5 (5.2)
P. vulgaris	5 (5.2)
K. ozaenae	3 (3.1)
S. aureus	3 (3.1)
P. aeruginosa	2 (2.1)
E. aerogenes	1 (1)
Enterecoccus species	1 (1)
S. saprophyticus	1 (1)
Total	97 (100)

Table 3: Antibiotic resistance pattern of isolates from reproductive age women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Anti-biotics Used	Number resistant for each species											Total R (% R)
	E. coli (n=55)	K. pneu-monia e (n=10)	K. oxy-toca (n = 11)	K. ozae nae (n= 3)	C. freu ndii (n =5)	E. aero-genes (n =1)	P. aeru gino sa (n =2)	P. vul-garis (n = 5)	S. aure us (n = 3)	S. sap-rophy ticus (n=1)	En-terec occus spp. (n=1)	
GM	39	8	6	0	4	1	0	3	3	1	-	65 (67.7)
TOB	50	8	7	1	5	1	0	5	-	-	-	77 (83.7)
TIC	52	9	9	2	5	1	2	5	-	-	-	85 (92.4)
MEM	23	5	5	1	2	1	0	4	-	-	-	41 (44.6)
IPM	6	1	1	0	1	1	0	2	-	-	-	12 (13)
CLT	41	9	7	0	4	1	-	3	-	-	-	65 (72.2)
FOX	31	6	7	0	4	1	-	4	3	1	-	57 (60.6)
CRO	43	7	7	1	5	1	-	4	-	-	-	68 (75.6)
CAZ	44	8	6	1	5	1	1	5	-	-	-	71 (77.2)
CFP	30	5	5	1	4	1	1	5	-	-	-	52 (56.5)
CIP	31	6	6	0	3	1	0	4	3	1	1	56 (57.7)
SXT	44	7	7	0	5	1	-	5	3	1	-	73 (77.7)
AMP	55	-	-	-	-	-	-	-	-	-	1	56 (100)
AUG	44	4	8	0	4	1	0	2	-	-	-	63 (68.5)
C	24	6	4	0	4	1	-	4	3	1	-	47 (50)
CL	50	9	8	1	5	1	0	5	-	-	-	79 (85.87)
TE	35	5	8	0	4	1	-	5	3	1	1	63 (66.3)
DO	40	6	9	0	4	1	-	5	3	1	1	70 (73.68)
MDR No (%)	52 (94.5)	9 (90)	8 (72.7)	1	5 (100)	1	2	5 (100)	3	1	1	88 (90.7)

NB:GM: Gentamicin; TOB: Tobramycin; TIC: Ticarcillin; MEM: Meropenem; IPM: Imipenem; CLT: Cephalothin; FOX: Cefoxitin; CRO: Ceftriaxone; CFP: Cefepime; CIP: Ciprofloxacin; CAZ: Ceftazidime; SXT: Trimethoprim-sulphamethoxazole; AUG:Amoxycillin/clavulanic acid; AMP: Ampicillin; CL: Colistin; C: Chloramphenicol; TE: Tetracycline and DO: Doxycycline; R = resistant; MDR = multidrug resistant; No = number

Empty cells in the Table show isolates to which an antibiotic in the respective column is not recommended for susceptibility testing.

Virulent phenotypic characteristics of bacterial isolates including ESBL and carbapenemase production tests were performed for all Gram-negative isolates (n=92) whereas cell surface hydrophobicity and biofilm production tests were performed for all 97 bacterial strains.

The study findings showed that 53 (57.6%) of the Gram-negative isolates were ESBL producing bacteria and 41 (44.6%) were carbapenemase producing strains. From the total of 97 isolates, 64 (66%) were positive for cell surface hydrophobicity, while 63 (65%) were strong biofilm producers **(Table 4).**

Table 4: Phenotypic characters of uropathogens from reproductive age women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Species	ESBL producers	Carbapenamase producers	CSH producers	Strong biofilm producers
	No (%)	No (%)	No (%)	No (%)
E. coli (n=55)	31 (56.4)	23 (42)	40 (73)	39 (71)
Klebsiella species (n=24)	12 (50)	11 (46)	14 (58.3)	14 (58.3)
C. freundii (n=5)	4*	2*	4*	3*
P. vulgaris (n=5)	4*	4*	4*	4*
P. aeruginosa (n= 2)	1*	0	0	0
E. aerogenes (n=1)	1*	1*	1*	0
S. aureus (n=3)	-	-	0	3*
Enterococcus species (n=1)	-	-	0	0
S. saprophyticus (n=1)	-	-	1*	0
Total (n=97)	53 (57.6)	41 (44.6)	64 (66)	63 (65)

ESBL = extended spectrum beta lactamase CSH = cell surface hydrophobicity. Empty cells (-) in the Table show isolates for which phenotypes were not determined and the column totals did not include them.
*Denotes only number without percentage because of small value

Bacterial virulence phenotypic characteristics were compared with antibiotic resistance patterns demonstrating wide ranging variations for multiple classes of antibiotics in the study ranging from 100% resistance for all phenotypes expressed for Ampicillin to 13% for Imipenem for strong biofilm producers (Table 5).
Different socio-demographic and clinical risk factors were compared with phenotypic characteristics of bacteria in patients with and without MDR UTI for possible association with prevalence of MDR UTI pathogens.

But none of the factors considered were significantly associated with the prevalence of MDR (Table 6). This finding is different from other studies conducted elsewhere, may be due to variation in socio-demographic, spatial, clinical or other underlining features between study populations and strain variation among isolates (7-11).

Table 5: Antibiotic resistance pattern and virulence phenotypic characteristics of uropathogens from reproductive age women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Antibiotics used	Resistance % for virulence phenotypes			
	ESBL	CAP	CSH	Biofilm
GM	73.6	70.7	72	76
TOB	94	92.7	92	91.7
TIC	96	92.7	97	96.7
MEM	73.6	100	46	43
IPM	19	22	14	13
CLT	82.7	83	77.8	76.7
FOX	63.5	66	68.8	68
CRO	88.5	83	84	85
CAZ	88.7	80.5	85.8	83
CFP	62	58.5	68	63
CIP	68	70.7	64	62
SXT	86.5	83	84	87
AMP	100	100	100	100
AUG	70	73	74.6	75
C	54	49	58	58.7
CL	88.7	92.7	89	91.7
TE	71	68	72	77.8
DO	80.8	78	78	84
MDR %	100	95	97	97

ESBL: extended spectrum beta lactamase CAP: Carbapenemase, CSH: Cell surface hydrophobicity; Biofilm: Strong biofilm producers; GM: Gentamicin; TOB: Tobramycin; TIC: Ticarcillin; MEM: Meropenem; IPM: Imipenem; CLT: Cephalothin; FOX: Cefoxitin; CRO: Ceftriaxone; CFP: Cefepime; CIP: Ciprofloxacin; CAZ: Ceftazidime; SXT: Trimethoprim-sulphamethoxazole; AUG: Amoxycillin/clavulanic acid; AMP: Ampicillin; CL: Colistin; C: Chloramphenicol; TE: Tetracycline and DO: Doxycycline

Table 6: Association of sociodemographic variables with urinary tract infection (UTI) by multidrug resistant uro-pathogens in reproductive age women with UTI in Jimma University Specialized Hospital, 2016.

Variables	Total/MDR No (%)	X2 statistics		Variables	Total/MDR No (%)	X2 statistics	
		Value	p-value			Value	p-value
Age (years)		1.138	0.268	Marital status		1.401	0.845
15-25	31/28 (32)			Married	72/64 (72.7)		
26-35	43/41 (47)			Single	20/19 (21.6)		
36-44	20/17 (19)			Divorced	4/4 (4.5)		
45-50	3/2 (2)			Widowed	1/1 (1.1)		
Residence		0.452	0.501	Educational status		0.821	0.365
Rural	37/35 (40)			Cannot read and write	9/8 (9.1)		
Urban	60/53 (60)			Can read and write	13/12 (13.6)		
Occupation		4.565	0.299	Grade 1-4	20 /19(21.6)		
Farmer	8/8 (9.1)			Grade 5-8	9/9 (10)		
House wife	17/14 (15.9)			Grade 9-12	14/13 (15)		
Student	16/15 (17)			College and above	32/27 (30.7)		
				Pregnancy history		0.231	0.631
Gov't/NGO employee	36/31(35.2)			Yes	63/56 (63.6)		
Merchant	20 /20(22.7)			No	34/32 (36.4)		
Month. income (birr)		0.861	0.216	Gravidity		1.129	0.288
≤ 500	9/9 (10)			None	34/32 (36.4)		
501-1000	15/15 (17)			1-3	6/6 (6.8)		
1001-1500	21/17 (19)			≥4	57/50 (56.8)		
1501-2000	15/14 (16)			Parity		2.269	0.132
2001-3000	20/18 (21)			Nulliparous	34/32 (36.4)		
>3000	17/15 (17)			Monoparous	42/39 (44.3)		
Catheterization history		0.00	1.00	Multiparous	21/17 (29.3)		
Yes	1/1 (1.0)			UTI history		1.067	.302
No	96/87 (99.0)			Yes	31/30 (34)		
Chronic disease history		0.091	0.763	No	66/58 (66)		
Yes	13/11 (12.5)						
No	84/77 (87.5)						

Note: % was calculated with 88 MDR isolates as denominator
Total/MDR No (%) = Total number of patients with the specified study variable/number of MDR (% of MDR)

After multivariate logistic regression analysis, significant risk factors for MDR UTI in the study were previous history of hospitalization (AOR 12.038; 95% CI 1.683 - 86.131; P = 0.013), ability of isolates for ESBL production (AOR 5.446; 95% CI 1.223 - 24.24; P = 0.026) and strong biofilm production ability of isolates (AOR 16.537; 95% CI 1.932 – 141.550; P = 0.010) (Table 7).

Table 7: Association of clinical and bacterial variables with multidrug resistance of uropathogens in reproductive age women with urinary tract infection in Jimma University Specialized Hospital, 2016.

Variables	Total/ MDR No (%)	X2 statistics Value	p-value	Bivariate analysis p-value	COR (95% CI)	Multivariate analysis p-value	AOR (95% CI)
Hospitalization history		5.023	0.025	0.017	6.0 (1.383-26.030)	0.013*	12.038 (1.683 - 86.131)
Yes	69/65(74)						
No	28/23 (26)						
ESBL production		14.238	<.001	0.016	4.07(1.305-12.692)	0.026*	5.446 (1.223 - 24.240)
Yes	53/53 (64)						
No	39/30 (36)						
Cell surface hydrophobicity		6.45	0.011	0.011	8.346(1.624-42.890)	0.098	5.114 (.741 - 35.284)
Yes	67/62 (70.5)						
No	30/26 (29.5)						
Biofilm production		6.021	0.014	0.013	7.907(1.541-40.580)	0.010*	16.537 (1.932–141.550)
Strong	63/61 (69)						
Weak	34/27 (31)						

* Statistically significant values at $p < 0.05$
% was calculated considering 88 MDR as denominator
Total/MDR No (%) = Total number of patients with the specified study variable/Number of MDR (% of MDR)

DISCUSSION

From a total of 424 UTI suspected reproductive age women, 97 (22.9%) had significant bacteriuria and over 76% of women with significant bacteriuria were in the age range of 15 to 35 years. The overall prevalence of confirmed UTI in this study is consistent with previous studies conducted in Addis Ababa and Dessie whose findings showed a rate of 23.4% (37) and 27.1% (15), respectively. Comparable findings were also reported from other countries (5, 38, 39).

In contrast, the prevalence of UTI among reproductive age groups was 10.4% in Gondar, Ethiopia (8), 9% in Bangladesh (40) and 8.9% in India (41). On the other hand, it is lower compared with studies which reported 38.6% from Pakistan (13), 43.3% and 30% from Nepal (42, 43), 69% from Kenya (44) and 91.6% and 49% from India (45, 46). This difference could be related to variation in socio-demographic, geographic, clinical or other underlying features between the study populations. The higher prevalence of UTI in the younger age group in this study might be due to active sexual or other behavioral factors (8, 13, 16, 47).

A 95% prevalence of Gram-negative isolates identified in this study is consistent with another study reported from Addis Ababa, 85.3% (37). Similarly, comparable results were reported from Kenya 90% (44), 95.3% and 91.3% from Pakistan (13, 48). Gram -negative bacteria possess multiple unique structures or virulence factors that help them for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion (16).

E.coli was found to be predominant in this study which is in agreement with reports from Dessie (15) and Gondar in Ethiopia (8). Comparable results which were reported from elsewhere (37), (43), (5) did not show agreement with 32.6% coagulase negative staphylococci (CoNS) dominance reported from Hawassa Ethiopia (49) and 48.7% *K. pneumoniae* from Romania (50). The latter difference may be due to variation in clinical or underlying condition of patients since CNS and *Klebsiella species* assume greater prevalence in recurrent infections associated with urologic manipulations (47, 51).

Antibiotic resistance of bacterial isolates in this study was very high ranging from 100% resistance for Ampicillin to 56.5% resistance for Cefepime. The 100% resistance to Ampicillin is consistent with the result reported from Gondar and Mekelle in Ethiopia, in which all isolates were 100% resistant (8, 52). Similarly comparable results were reported in Romania (50) and India (45). Lower proportions of Ampicillin resistance such as 90.3% (5) and 56.4% (53) were reported from Iran and Nepal, respectively. The complete resistance to Ampicillin observed in this study could be attributed to the presence of ESBLs in the isolates.

In this study, uropathogenic bacteria showed 13% resistance to Imipenem. This is high compared to 0% in India (54) and 0.6% in Libya (37). It is however lower in comparison with 25% from Pakistan (13), 28% in India (45) and 41% in Iran (5). The lower level of resistance observed to Imipenem (13%) and Meropenem (44.6%) might be due to less frequent use of these antibiotics in our study area.

The 90.7% MDR proportion among UTI isolates reported in this study is very high in magnitude. However, it is consistent with a previous study reported from Mekelle, which was 90% (52). Similarly, comparable findings were reported from elsewhere in Ethiopia: 90% and 87% in Gondar, (8, 10) and 88.5% in Addis Ababa (37).

This is much higher than rates reported from several other places such as 75.8% from India (55), 72% from Nepal (43, 53), 44.8% from Romania (50) and 47.5% from Pakistan (48). The difference might be due to methods employed to define MDR, number and types of antibiotics used, variation in pattern of bacterial strains tested and difference in the socio-demographic characteristics and life style of study population. The high prevalence of MDR UTI bacterial isolates identified in our study population might be attributed to the increased rate of improper drug utilization that might have allowed organisms to be selected with plasmid encoded resistance genes.

In this study, 57.6% of isolates were ESBL producers, 44.6% were carbapenemase producers, 66% were CSH positive isolates and 65% were strong biofilm producers. The prevalence of ESBL in our finding was very high, and it is comparable with 56% reported from Iran (56). Comparable results were reported from India, 66% (57) and Iraq (58) and much higher proportions, 92% and 81.8% from Iran (29) and India (59). But lower resistance rates have also been recorded from Jimma, in which 38.4% of isolates were ESBL producers (33). Other reports included 36.2% from India (55), 7.3% from Nepal (53) and 2.4% from Pakistan (48). Generally, the prevalence of ESBL expression among UTI isolates was high in our study and this might be due to lack of control over antibiotic use and prescription, and extensive use of antibiotics in the study population, and especially the use of β-lactams that enhances acquisition of ESBL producing strain (57,60).

Carbapenems are considered to be the best alternative for treating antibiotic resistant pathogens, but over 44% of the isolates in our study were carbapenemase producer Gram-negative strains including 42% of *E. coli* and 46% of *Klebsiella species*. This is a bad sign for future options of treatment in Ethiopia since carbapenems are not protected as alternatives for managing resistant infections in the treatment guidelines of the country and this needs serious consideration. The overall prevalence of carbapenem resistance in this study is very high compared with 2.7% from Gondar, Ethiopia (10) and 6.5% from India (55) but low compared to 69% in Pakistan (34). The differences observed for ESBLs and carbapenemases could be due to the different methods used for detection of the enzymes, differences among isolates tested, target population and drug use habits in study populations. International travel, migration and importation of food products may also contribute to the emergence and spread of resistant organisms far beyond their countries of origin.

From the total isolates reported in this study, 66% were CSH isolates and 65% were strong biofilm producers. Cell surface hydrophobicity in our study is comparable with 61.5% in a study reported from India (61) but higher than 43% in another study from India (28). The finding on the biofilm producers in this study is consistent with that of India in which 66.6% of the isolates were strong biofilm producers (30) but lower than 16.6% reported from another study in India for strong biofilm producers (62). The differences observed for CSH and biofilm production between this study and others may be due to the different methods followed and strain variation among isolates.

The antibiotic resistance pattern among isolates in relation with phenotypes observed was consistent with their number i.e. all 100% ESBL producers, 93% cell surface hydrophobic and 97% strong biofilm producer isolates showed MDR for many commonly used antibiotics in the study area corresponding with the prevalence of virulence phenotypes expressed and also for broad spectrum antibiotics used in the study. This was also seen in many study reports in different areas and can be explained by the ability of these factors to increase drug resistance by reducing drug efficacy (10, 26, 29, 31, 33, 35, 59, 61, 63).

This study also attempted to test for the association of several factors with the prevalence of MDR bacterial isolates from UTI. Accordingly, among the many variables tested in this study, MDR was found to be associated with previous history of hospitalization, ESBL production and strong biofilm production. The prevalence of MDR UTI in reproductive age women with previous history of hospitalization was significantly higher than those without ($p = 0.013$). Similar results were documented in Gondar, Ethiopia and the United States of America (10, 64) though the categories of study participants were different in these studies. This can be explained by the contribution of nosocomial pathogens that resist multiple classes of antibiotics possibly acquired during previous hospitalization (10, 65).

ESBL and biofilm producing isolates were also found to be significantly more resistant to three or more drugs compared with non ESBL elaborators and non-biofilm producers ($p < 0.05$) (Table 7). The association seen in this study between MDR and biofilm production, and ESBL and MDR has also been observed in India (30, 57). A report from Iraq has also shown that MDR rates of ESBL producers were higher than those of ESBL non-producers (58).

This is because of the ESBL genes that have the ability of carrying resistance to multiple antibiotics (31, 33, 66). The significant association of strong biofilm producing isolates and MDR in this study can be explained by the ability of strong biofilm formers to be embedded within self-produced extracellular hexo -polysaccharides matrix. The complex communities of millions of adherent bacterial cells helps them to withstand unfavorable circumstances due to cell to cell communication, decreased growth and altered metabolism. Gene expression and facilitation of plasmid mediated exchange of resistance genes between cells within the biofilm makes the isolates more resistant to multiple classes of antibiotics (29, 67).

Conclusion

The overall prevalence of MDR UTI among reproductive age women was high. Gram-negative organisms were the most common causes of UTI and *E. coli* was the most predominant organism. High prevalence of virulence phenotypes of isolates correlated with increased antibiotic resistance. The prevalence of MDR is significantly associated with previous history of hospitalization, ESBL expression and strong biofilm production ability of the isolates. Considering the high prevalence of MDR revealed in this study, it is recommended that the treatment guidelines in the study area are routinely revised based on resistance trends over time.

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