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Research Article

Urinary Tract Infection: Bacterial Etiologies, Drug Resistance Profile and Associated Risk Factors among Diabetic Patients Attending NRH

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Abstract:
In diabetic patients, there is increased risk of urinary tract infections (UTIs). Hence, current information on antimicrobial resistance and the prevalence of the pathogenic bacteria need to be available at national and local levels to guide the rational use of the existing antimicrobials. This study was conducted to determine the prevalence of UTIs, risk factors and antimicrobial resistance pattern of the bacterial isolates from diabetic patients in Nekemte Referral Hospital (NRH). An institution based cross-sectional study was conducted on 200 diabetic patients, who were attending the study setting for a clinical check-up during the study period. The overall prevalence of UTIs was 16.5% among the study population with the prevalence being higher among aged patients and females. Multivariate analysis revealed that level of education, history of UTIs and glucosuria was significantly associated with UTIs. Thirty-three bacterial uropathogens, total, were isolated with *S. aureus* (24.2%) and *CN Staphylococcus spp* (24.2%), *E. coli* (12.1%) and *K. pneumonia* (12.1%) being the most common isolates. The microbes showed highest level of resistance to penicillin G (97.0%), and ampicillin (93.9%); and lowest level of resistance to ceftriaxone (50.0%) and vancomycin (52.9%). In conclusion, majority of isolated bacteria were resistant to antibiotics used in the study setting. This calls for attention of health professionals to consider the resistance pattern in their clinical practice. Most importantly, this finding may be used to control trends of antibiotic-resistance, to develop local antibiotic policies and to assist clinicians in the rational choice of antibiotics therapy; thus, to prevent indiscriminate use of antibiotics.

Keywords: Antimicrobial resistance; Ethiopia; prevalence; risk factors; urinary tract infections; uropathogens
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Introduction

The prevalence of diabetes mellitus has increased over the past decades, and it is now approaching epidemic proportions [1]. Worldwide, 382 million people had diabetes in 2013; this number is expected to rise to 592 million by 2035. Most people with diabetes live in low- and middle-income countries [2]. Changes in lifestyle, aging of the population and the increasing prevalence of obesity are responsible for this dramatic situation [1,2]

In diabetic patients, it is generally accepted that infections are frequent; Urinary tract infections (UTIs) being the most common bacterial infections [3]. Risk factors for UTI among patients with and without diabetes mellitus (DM) have been identified: female sex, lower education level, low immunity, glycosuria, employment status, poor diabetic control, obesity, incomplete bladder emptying due to autonomic neuropathy, bladder dysfunction and prostate syndrome in men [4–8]. UTIs are much more common in elderly and females than younger individuals and men for a variety of reasons such as short and straight anatomy of the urethra in females, and termination of female urethra beneath the labia resulting in colonization by uropathogens [5].

Although fungi are occasional etiological agents, UTIs are predominantly caused by bacteria [3]. The most common bacteria implicated as causative agents of UTI generally originate in the intestine and include but not limited to *E. coli, Klebsiella spp., Staphylococcus spp, Streptococcus spp, Citrobacter spp. Proteus spp., Pseudomonas aeruginosa,* and *Candida spp* [3,9,10]. *E. coli* and *S. aureus* are the most frequently recovered gram-negative and gram-positive uropathogens, respectively, in diabetic patients [9–11].

The antimicrobial resistance patterns of bacteria isolated from UTIs differed for different bacteria and antibiotics with sensitivity to ciprofloxacin, gentamicin and ceftriaxone being usual while resistance is often observed to ampicillin, amoxicillin, penicillin G, and tetracycline [9,12] in different parts of the country. So far, *E. coli* has been reported as being resistant to majority of routinely used antibiotics including cotrimoxazole, amoxicillin, ceftriaxone, chloramphenicol [10,13] leading to recurrent infections [14]; but found to be susceptible for nitrofurantoin, gentamicin and ciprofloxacin (10,13,15). Irrational drug use such as long term use and low-dose antibiotic use due to lack of protocol for antibiotic use and empiric therapy due to lack of laboratory facility to determine sensitivity are the possible reasons for resistance and thus, recurrent infections and complicated UTIs [14].

Hence, reporting common etiologic agents and respective antibiotic resistance pattern is crucial for stakeholders to search for preventive and control measures against antibiotic resistance. There are few research reports regarding etiologic agents and respective antibiotic resistance pattern in our country [9,10,12,13,15]. However, to our knowledge, there was no published data on etiologic agents and antibiotic resistance pattern of UTIs among diabetic patients in East wollega. Thus, the aim of this study was to determine the prevalence of common etiologic agents in UTI and the respective antimicrobial resistance pattern in diabetic patients who were attending NRH during study period.

Material and methods

Materials and chemicals

Materials: McFarland 0.5 standard, sterile screw-capped wide-mouth container, Forceps, wire loop (1 µl), Bunsen burner, ruler, Sterile cotton swabs, inoculating wire, glass slides, **Culture Media and**

chemicals: Sodium chloride, Mueller-Hinton agar, 5% defibrinated sterile sheep blood, MacConkey agar, Cystein-Lactose Electrolyte Deficient (CLED) medium. **Antibiotic discs:** amoxicillin (10µg), ampicillin (10µg), penicillin G (10IU), erythromycin (15µg), vancomycin (30µg), gentamicin (10µg), ceftriaxone (30µg), tetracycline (30µg), ciprofloxacin (5µg), norfloxacin (10µg), chloramphenicol (30µg), cotrimoxazole (25µg), nitrofurantoin (50µg); Reference strains: Escherichia coli (ATCC 25922). Almost all the culture media (Titan Biotec Ltd) and majority of the antibiotic discs were purchased from pharmaceutical fund and supply agency (PFSA) of Ethiopia, Nekemte branch; and the standard bacterial strain was obtained from Ethiopian public health institute (EPHI). The remaining antibiotic discs and some culture media were obtained from Nekemte Regional laboratory (NRL).

Study setting and period

This study was conducted in NRH which is located in Nekemte town, 315 Kms away from Addis Ababa, western Ethiopia. It is one of the oldest public hospitals in the country; and currently, it is the teaching and referral hospital in East Wollega. The hospital delivers health services in many specialty areas, namely: gynecology and obstetrics, surgery, pediatrics and child health, ophthalmology, psychiatry and internal medicine. The study was conducted from June to September 2016.

Study Design

An institution based cross-sectional study was conducted to assess the antimicrobial drugs resistance pattern of urinary tract bacterial pathogens from urine samples of diabetic patients.

Source population

The source population of the study was all diabetic patients visiting the study setting.

Study subjects

All diabetic patients who were visiting NRH during the study period and were fulfilled the inclusion criteria.

Inclusion and exclusion criteria

Inclusion criteria

- All diabetic patients who were attending the diabetic clinic of the hospital, during the study period, were included in the study.

Exclusion criteria

- Diabetic patients on antibiotics for the last two weeks
- Diabetic patients who were not voluntary to participate in the study

Study Variables

Independent Variables

- Socio-demographic characteristics (age, sex, address, occupation, level of education) of the participants
- Clinical characteristics (Type of DM, duration of DM, Blood glucose level, History of UTI, Urine glucose level, symptom of UTIs) of the participants

Dependent Variables

- Prevalence of significant bacteriuria
- Risk factors for UTIs in diabetic patients
- Bacterial uropathogens
- Antimicrobial-resistance pattern

Sample size determination and sampling techniques

The total number of patients with DM who attended the study setting in past seven years was 941. The sample size for this study was determined by using a single proportion population equation (eqn.1) with correction formula for finite population (eqn.2) by considering N=941, the prevalence of UTI, 17.8% from a study conducted in Gonder, Ethiopia [10] and with margin of error (d) 0.05 and 95% confidence-interval critical value (Zά/2=1.96) .

$$n_0 = \frac{Z^2_{\frac{\alpha}{2}} * P(1-P)}{d^2} \quad \text{---------- eqn.1}$$

n_o= $(1.96)^2$ *0.178 *(1-0.178) / $(0.05)^2$

$\boldsymbol{n_0}$**=225**

$$n = N * \frac{n_0}{(N-1)+ n_0} \quad \text{---------- eqn.2}$$

n=941*225/((941-1) + 225)

n= 182

By considering 10% non-response rate (177*10% ≈ 18), the total number of sample for this study was **n= 18+182=200**

Data collection and processing

Socio-demographic and clinical history Information

A structured questionnaire, developed by reviewing different journal articles in the topic of study, was employed to collect information regarding the socio-demographic status and clinical history of the study participants.

Collection, handling and transportation of urine specimen

Each diabetic patient was instructed how to collect a 'clean-catch' mid-stream urine specimen. Accordingly, about 10 to 15 ml of urine specimen was collected in a sterile screw-capped, wide-mouth container from each diabetic patient. The sample container was labeled with unique sample number, date and time of collection and then the sample was delivered to NRL for culturing, isolation, biochemical test and drug-resistance test.

Culturing and identification of the Uropathogens

Urine specimens were directly inoculated onto cysteine-Lactose electrolyte deficient (CLED) medium plates, and MacConkey agar (Titan Biotec. Ltd) using a standard calibrated wire loop (1µL). The streaked culture plates were then incubated at 37^{o}C for 18-24 hours. On the next day, the bacterial growth on the respective media was observed, and total colony count was done and checked for significant bacteriuria.

Significant bacteriuria is defined as urine cultures grew >10^5 CFU/ml midstream urine. For Staphylococcus *aureus*, its pure culture was considered significant regardless of the number of CFUs. All positive urine cultures showing significant bacteriuria were further identified by their characteristics appearance on their respective media and confirmed by the pattern of biochemical reactions using the standard procedures [19]. The pathogens were isolated and identified using phenotypic methods including biochemical testing like catalase, oxidase, coagulase, triple sugar iron agar (TSI), hydrogen sulfide (H_2s), indole, motility, citrate, urease and lysine iron agar.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for bacterial isolates using agar disc- diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS) [20]. In brief, the pure culture was transferred to a tube containing 5ml sterile normal saline (0.85% NaCl) and mixed gently until it formed a homogeneous suspension. The turbidity of the suspension was adjusted to the optical density of McFarland 0.5 tubes in order to standardize the inoculums size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria suspension evenly over the entire surface of Mueller-Hinton agar. For antimicrobial testing of streptococci, 5% defibrinated sterile sheep blood was aseptically added to Mueller-Hinton medium. The inoculated plates were left at room temperature to dry for 3 to 5 minutes.

The antimicrobial discs representative of penicillin group, cephalosporins, aminoglycosides, tetracycline, quinolones, antimetabolites and nitrofurantoin was tested in the following concentrations: amoxicillin (10µg), ampicillin (10µg), penicillin G (10IU), erythromycin (15µg), Vancomycin (30µg), gentamicin (10µg), ceftriaxone (30µg), tetracycline (30µg), ciprofloxacin (5µg), norfloxacin (10µg), chloramphenicol (30µg), cotrimoxazole (25µg), nitrofurantoin (50µg). These groups of antibiotics have been in use for management of UTI in the study setting.

By using a sterile forceps, the antibiotic discs was placed on the inoculated plates and incubated at 37^{o}C for 18-24 hours. The diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a ruler and the isolate was classified as sensitive, intermediate and resistant according to NCCLS [20].

Reference strains

E.coli (ATCC 25922) was used as reference strains for culture and sensitivity testing quality control.

Data analysis

The data was double entered into Epidata (version 3.21) software by different data clerics and was checked to minimize error committed during the data entry. The entered data was then transferred to SPSS (Version 20, for window) for analysis. Bivariate and multivariate analysis was applied to show an association between urine culture status of diabetic patients and socio-demographic characteristics and clinical history of the patients. The percentage for proportion, chi-square test and odds ratio for the categorical variable was used wherever appropriate. P-values of <0.05 was considered as statistically significant for all results.

Ethical considerations

The research project was approved by Institutional Review Board (IRB), college of medical and health sciences, Wollega University. Official permission from NRL and NRH was obtained by providing a letter of cooperation written from the research and technology transfer vice-presdent, Wollega University.

All diabetic patients consulting for their diabetic check-up during the study period was informed about the purpose of the study and their oral consent was obtained for the study. Any information related to the patient and clinical history was kept confidential.

Results

Socio-demographic Characteristics and Clinical Profile of the Study Participants Screened for Bacterial UTIs in NRH

A total of 200 diabetic patients with or without symptoms of UTI were screened for UTIs during the study period. The majority 128(64.0%) of the study participants were female with mean age of 39.63(±15.78) years, and mean BMI of 26.18(±5.05). As comparted to male, female participants had above normal (unhealthy) bodyweight to height ration (40.3% Vs 54.7%). About 110(60.0%) of the study population was affected by type-one diabetes mellitus with uncontrolled fasting blood glucose level of 136(72.0%) and being lived with diabetes mellitus on average for 5.09(±4.71) years. The proportion of the participants with glucosuria were 10(14.3%) and 21(16.7%) among male and female respectively (Table 1).

Table 1 Socio-demographic and clinical characteristics of diabetic patients tested for UTIs in NRH, East Wollega, Ethiopia (June to September 2016)

S.No	Variables		Male n (%)	Female n (%)	Total n (%)
1	Age in years (n=200)	≤ 35	32 (44.4)	48 (37.5)	80 (40.0)
		> 35	40 (55.6)	80 (62.5)	120 (60.0)
		Mn ± SD	41.06±16.23	39.63±15.78	40.15±15.92
2	Level of education (n=197)***	Illiterate	8 (11.3)	49 (38.9)	57 (28.9)
		Primary (1-8) school	26(36.6)	41 (32.5)	67 (34.0)
		High (9-12) school	23 (32.4)	21 (16.7)	44 (22.3)
		College/University	14 (19.7)	15 (11.9)	29 (14.7)
3	Body mass index (BMI) (n=200)*	<18.5 (underweight)	5 (6.9)	6 (4.7)	11 (5.5)
		[18.5-25) (healthy)	38 (52.8)	52 (40.6)	90 (45.0)
		[25-30) (overweight)	25 (34.7)	42 (32.8)	67 (33.5)
		≥ 30 (obese)	4 (5.6)	28 (21.9)	32 (16.0)
		Mn ± SD	23.90±4.15	26.18±5.05	25.36±4.86
4	Type of Diabetes Mellitus (n=185)	Type-I	38 (56.7)	72 (61.0)	110 (59.5)
		Type-II	29 (43.3)	46 (39.0)	75 (40.5)
5	Urine glucose (n=196)	Positive (glucosuria)	10 (14.3)	21 (16.7)	31 (15.8)
		Negative (none)	60 (85.7)	105 (83.3)	165 (84.2)
6	Fasting Blood glucose level (mg/dl)** (n=189)	≤ 126 (controlled)	26 (40.0)	27 (21.8)	53 (28.0)
		>126 (uncontrolled)	39 (60.0)	97 (78.2)	136 (72.0)
		Mn ± SD	182.23±106.44	187.81±85.94	185.89±93.25
7	Duration of DM (in years) (n=198)	≤5	52 (74.3)	82 (64.1)	134 (67.7)
		>5	18 (25.7)	46 (35.9)	64 (32.3)
		Mn ± SD	4.63±4.94	5.34±4.58	5.09±4.71
8	History of UTI in previous time (n=193)	Yes	12 (16.9)	17 (13.9)	29 (15.0)
		No	59 (83.1)	105 (86.1)	164 (85.0)

*p<0.05,**p<0.01,***p<0.001, BMI-body mass index, Mn±SD-Mean±Standard deviation

Prevalence of UTIs among diabetic patients in NRH

As depicted in

Figure 1, the over all prevalence of UTIs was 16.5% among the study population. The prevalence of UTIs were 3.0% and 27.3% for age group ≤ 35 years; and 21.2% and 48.5% for age group >35 years among female and male participants, respectively. The proportion of participants with UTIs were higher among older patients for both gender. On top of that, in all age groups, females participants were more affected by UTIs than male participants.

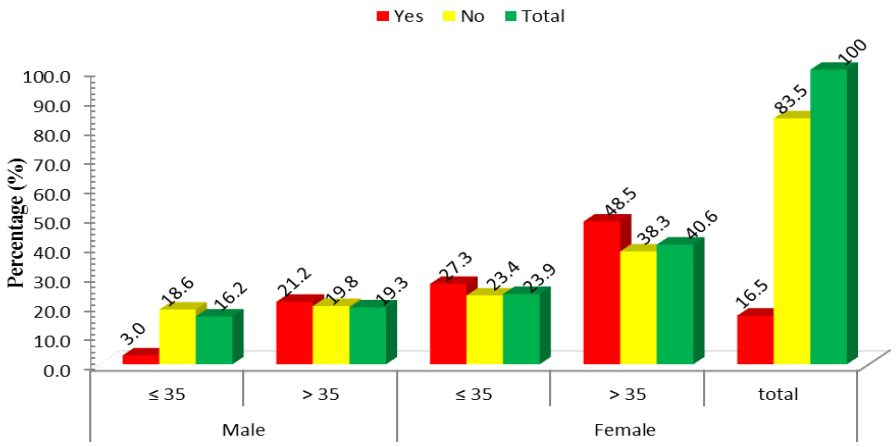

Figure 1 Prevalence of UTIs among diabetic patients (n=200) by gender and age group in NRH, East Wollega, Ethiopia (June to September 2016)

Risk factors for UTIs among diabetic patients in NRH

Multivariable analysis revealed that among socio-demographic characteristics and clinical history of the study participants: only level of education, history of UTIs and glucosuria was significantly associated with UTIs). Regarding level of education, illiterate diabetic patients had an odds of 9.3 (AOR = 9.3, 95 % CI (1.1–79.2) for being UTIs positive than those who had university/college level of education. Moreover, the study participants with history of UTIs and glucosuria had odds of 3.2 (AOR = 3.2, 95 % CI (1.2–8.7) and 3.2 (AOR = 3.2, 95 % CI (1.2–8.1) for being UTIs positive, respectively, than those who did not have history of UTIs and glucosuria.

Table 2 Association of socio-demographic and clinical profile of the study participants on UTIs in NRH, June to September 2016

S. No.	Variables		UTI			COR (95%CI)	AOR (95%CI)
			Total n (%)	**Yes** n (%)	**No** n (%)	OR (Lower, Upper)	OR (Lower, Upper)
1	Level of education (n=197)	Illiterate	57 (28.9)	16 (50.0)	41 (24.8)	10.9 (1.4, 87.2)*	9.3 (1.1, 79.2)*
		Primary school	67 (34.0)	9 (28.1)	58 (35.2)	4.3 (0.5, 36.0)	4.8 (0.5, 42.3)
		High school	44 (22.3)	6 (18.8)	38 (23.0)	4.4 (0.5, 38.8)	4.3 (0.5, 40.7)
		College and above	29 (14.7)	1 (3.1)	28 (17.0)	1.0	1.0
2	History of UTIs in previous time (n=193)	Yes	29 (15.0)	11 (33.3)	18 (11.2)	3.9 (1.7, 9.5)**	3.2 (1.2, 8.7)*
		No	164 (85.0)	22 (66.7)	142 (88.8)	1.0	1.0
3	Urine glucose (n=196)	Positive (glicosuria)	31(15.8)	12 (36.4)	19 (11.7)	4.3 (1.8, 10.2)**	3.2 (1.2, 8.1)*
		Negative (none)	165 (84.2)	21 (63.6)	144 (88.3)	1.0	1.0

Data was analyzed by binary and multivariate logistic regression; COR-Crude odds ratio; AOR- Adjusted odds ratio; *p<0.05, **p<0.01

Bacterial isolates from urine specimen of diabetic patients in NRH

A total of 33 bacterial uropathogens, a single bacterial isolate from each urine-culture specimen, were isolated from 200 diabetic patients tested for UTIs. Of these, 17(51.5%) were gram-positive bacteria and 16(48.5%) were gram-negative bacteria. Out of the gram-positive bacterial isolates, the most common isolates were *S. Aureus* 8(24.2%) and CN *Staphylococcus spp* 8(24.2%). Besides that 16(48.5%) of the isolates were gram-negative bacteria with the most common isolates being *E. coli* 4(12.1%) and *K. pneumonia* 4(12.1%). Majority of both gram-positive 12(70.6%) and gram-negative 13(81.3%) bacterial isolates were recovered from female participants. *Streptococcus spp* from gram-positive bacteria; and *P. merablis, K. ozanae, K. rhinoscleromatis* and *Citrobacter spp* from gram-negative bacteria were exclusively isolated from female participants.

Table 3 Bacterial isolates (n=33) from midstream urine samples of DM patients Screened for Bacterial UTIs in NRH, East Wollega, Ethiopia (June to September 2016)

S.No	Bacteria Isolated	Male n (%)	Female n(%)	Total n (%)
	I. Gram-positive			
1	*S. aureus*	3(37.5)	5(62.5)	8(24.2)
2	*CN Staphylococcus spp*	2(25.0)	6(75.0)	8(24.2)
3	*Streptococcus spp*	0	1(100)	1(3.1)
	Total	5(29.4)	12 (70.6)	17(51.5)
	II. Gram-negative			
5	*E. coli*	1(25.0)	3(75.0)	4(12.1)
6	*K. pneumonia*	1 (25.0)	3(75.0)	4(12.1)
7	*P. Merablis*	0	2(100)	2(6.1)
8	*K. ozanae*	0	2(100)	2(6.1)
9	*Citrobacter spp*	0	2(100)	2(6.1)
10	*P. rettgeri*	1(100)	0	1(3.0)
11	*K. rhinoscleromatis*	0	1 (100)	1(3.0)
	Total	3(18.7)	13 (81.3)	16(48.5)

CN: *Coagulase negative, K: Klebsiella, P: Providencia/Proteus*

Resistance Pattern of Bacterial Isolates to Antimicrobial Agents

The bacterial uropathogens, isolated from the urine specimen of diabetic patients, showed different degree of resistance to commonly used antimicrobial agents in the study setting. Among antimicrobial-discs tested on bacterial uropathogens, the isolates showed highest level of resistance (97.0%, 93.9%, and 90.9%) to penicillin G, ampicillin, and erythromycin, respectively; and lowest level of resistance (50.0%) to ceftriaxone followed by vancomycin (52.9%), norfloxacin (54.5%), and gentamicin (54.5%). The proportion of sensitive bacterial uropathogens was obtained by combining susceptible and intermediate readings of the susceptibility test result. Accordingly, majority of the tested bacterial isolates displayed highest level of sensitivity to ceftriaxone (50.0%) followed by vancomycin (47.1%) (Figure2).

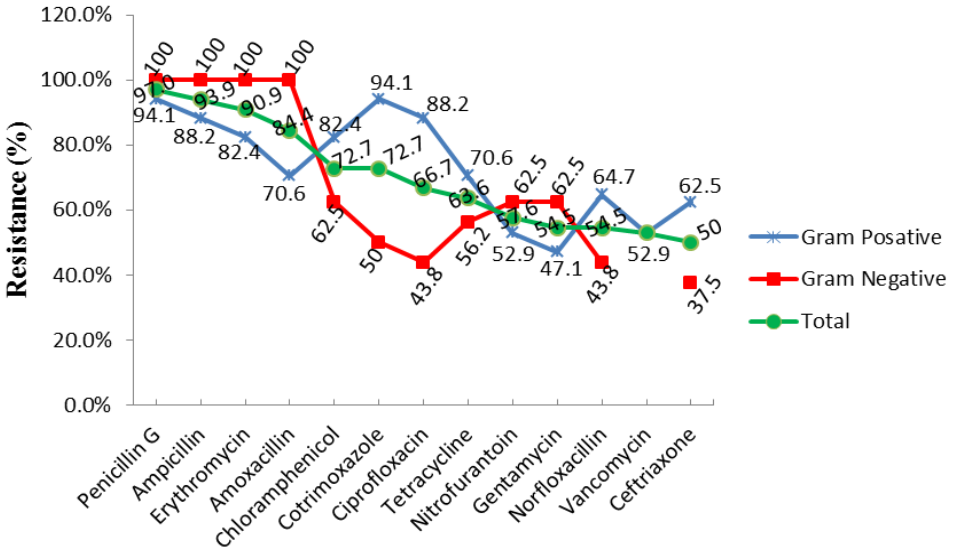

Figure 2 The antimicrobial resistance pattern of bacterial uropathogens to commonly used antimicrobials in NRH, June to September 2016

Bacterial uropathogens isolated from diabetic patients revealed the presence of high levels of single and multiple antimicrobial resistances against commonly prescribed drugs. S. *Aureus*, which is the predominant cause of UTI, showed highest proportion of resistance (100%) to ciprofloxacin, chloramphenicol, cotrimoxazole, and pencillin G; and lowest level of resistance (25%) to Vancomycin. Besides that CN Staphylococcus spp, another most frequent gram-positive uropathogens, showed 100% resistant to ampicillin, cotrimoxazole and pencillin G; and low level of resistance to nitrofurantoin (37.5%), gentamicin (50.0%), and ceftriaxone (57.1%). Moreover, *E.coli* and K. pneumonia, the most prevalent uropathogens, among gram-negative bacteria, displayed highest level of resistance (100%) to amoxicillin, ampicillin, Erythromycin, and penicillin G; and lowest level of resistance to ceftriaxone (25% vs 0%) and nitrofurantoin (25% vs 25%).

Table 4 Antimicrobial resistance profile of individual bacterial isolates to specific antimicrobials in NRH, East Wollega, Ethiopia (June to September 2016)

S. No.	Individual bacterial isolates		Number of strains (%) resistant to												
			Amx	Amp	Cfx	Cip	CAF	Cot	Ert	Gent	Nrf	Nitr	PnG	TTC	Van
1	*E.coli* (n=4)	#T	4	4	4	4	4	4	4	4	4	4	4	4	NA
		%R	100	100	25.0	75.0	75.0	50.0	100	50.0	75.0	25.0	100	75.0	NA
2	K. *pneumonia* (n=4)	#T	3	4	4	4	4	4	4	4	4	4	4	4	NA
		%R	100	100	0	75.0	50.0	50.0	100	100	25.0	100	100	50.0	NA
3	P. *Merabilis* (n=2)	#T	2	2	2	2	2	2	2	2	2	2	2	2	NA
		%R	100	100	100	0	100	100	100	100	0	100	100	0	NA
4	K. *Ozanae* (n=2)	#T	2	2	2	2	2	2	2	2	2	2	2	2	NA
		%R	100	100	50.0	0	50.0	0	100	50.0	0	50.0	100	50.0	NA
5	Citrobacter spp (n=2)	#T	2	2	2	2	2	2	2	2	2	2	2	2	NA
		%R	100	100	100	100	100	50.0	100	0	100	0	100	100	NA
6	K. *Rhino* (n=1)	#T	1	1	1	1	1	1	1	1	1	1	1	1	NA
		%R	100	100	0	0	0	0	100	0	0	100	100	0	NA
7	P. *Rettegeri* (n=1)	#T	1	1	1	1	1	1	1	1	1	1	1	1	NA
		%R	100	100	100	100	0	100	100	100	100	100	100	100	NA
8	S. *Aureus* (n=8)	#T	8	8	8	8	8	8	8	8	8	8	8	8	8
		%R	87.5	87.5	75.0	100	100	100	87.5	37.5	62.5	75.0	100	75.0	25.0
9	CN Staph. Spp (n=8)	#T	8	8	8	8	8	8	8	8	8	8	8	8	8
		%R	62.5	100	57.1	75.0	62.5	100	87.5	50.0	62.5	37.5	100	75.0	75.0
10	Strep. Spp (n=1)	#T	1	1	1	1	1	1	1	1	1	1	1	1	1
		%R	0	100	0	100	100	0	0	100	100	0	0	0	100

#T-Total number of bacterial isolated, %R-% of resistant bacteria, Amx-Amoxacillin, Amp-Ampicillin, Cfx-Ceftriaxone, Cip-Ciprofloxacin, CAF-Chloramphenicol, Cot-Cotrimoxazole, Ert-Erythromycin, Gent-Gentamicin, Nrf-Norfloxacine, Nitr-Nitrofurantoin, PnG-Pencillin G., TTC-Tetracycline, Vanc-Vancomycin, NA-Not Applicable.

Discussion

In communities with limited resources, it is common to see preventable diseases like urinary tract infections (UTIs) devastating the lives of ordinary citizens. The situation becomes even complicated if the infected individual harbors underlying chronic debilitating disease like diabetes and the tendency of the involved microorganism to develop resistance to routine treatment regimen [21].

In our study, the overall prevalence of UTIs among diabetic patients was 16.5%, which is higher than reports from other study in Ethiopia: Jimma (9.2%), and many other countries, such as Roumania (10.7%), Nepal (10.37%), and Uganda (13.3%), [9,22–24]; but lower than the studies from Kuwait (35%), India (32%) and Uganda (31.1%) [21,25,26]. The disagreement of our finding with other studies might be either due to variation in sample size, the environment, social habits of the community, the standard of personal hygiene, or level of education. However, the findings is similar with the report from previous study in Gondar (17.8%) [8].

The proportion of participants with UTIs was higher, though it was not statistically significant, among females and older (age >35years) diabetic patients than men and younger (age <=35) ones (
Figure **1**). This finding is in concordance with study done in Saudi Arabia, Taiwan and Nigeria [4,6,27]. The gender related difference can be explained by a variety of anatomical and physiological factors, such as the shorter length of the urethra, the smaller distance between the urogenital meatus and the anus and the easy contamination of the urinary tract with fecal flora in females; and the antibacterial properties of the prostatic fluid in men [28]. In aged diabetic patients, the increased risk of UTIs could be associated with dysfunctional bladder and increased post-void residual volumes [6]. Along with this, age-associated changes in immune function, exposure to nosocomial pathogens and an increasing number of comorbidities put the elderly at an increased risk for developing the infection [29].

Furthermore, a number of other factors have been proposed as risk factors for acquisition of UTIs among diabetic patients. In this study, the contributing factors that showed a significant association were lower level of education (illiteracy), history of UTIs and glucosuria. In accordance with prior study [10,11], the history of UTIs is significantly associated with UTIs among diabetic patients. This could be due to relapse of the infection as a result of ineffective treatment of prior UTIs. In addition, similar finding have been reported elsewhere regarding lower level of education and glucosuria as risk factors for UTIs [5,27,30]. This might be due to lower level of awareness of illiterate diabetic patients on how to keep their general health, particularly their personal hygiene [27]; and growth promoting effect of glucosuria to bacterial pathogens typically associated with UTIs. Personal hygiene may be important in reducing the incidence of UTIs since most bacteria causing UTI are commensals of perianal and vaginal regions.

In this study, gram-positive bacteria were recovered more frequently than gram-negative bacteria as etiological agents of UTIs in diabetic patients (Table 3). This finding is accordant with results reported from Uganda [21]; but discordant with studies done in Ethiopia (9,10) and other parts of the world [11,24]. The discrepancy of the reports with our finding might be because of geographical variation and may even variation over time within a population [18]. Samuel *et al.* (2014) [11] reported S. aureus as being the predominant gram-positive uropathogen among diabetic patients. Our result also confirmed this finding. On the other hand, a number of other studies reported *E. coli* followed by K. *pneumoniae* as a predominant etiological agent of UTIs in both diabetic patients and general population [9,11,24]. The possible reason for dominance of S. aureus in UTIs might be because of the ability of the pathogen as a prime competitor of *E. coli* and K. *pneumoniae* during the course of infection [31]. Although diabetic persons may be more susceptible to infection by

uncommon organisms, we found most of their infections to be due to typical uropathogens, which suggests that diabetes facilitates the same route of infection as that for UTI in non-diabetic persons (i.e., ascending infection from the urethra).

In addition, majority of both gram-positive and gram-negative bacterial isolates were recovered from female participants. This might be related with the demography of our study population since gram-positive bacteria are found more often as etiologic agents of community-acquired UTIs in elderly and females [32,33].

Resistance to antimicrobial agents has been noticed since the first use of these agents and is an increasing world-wide problem [9]. In current study, both gram-positive and gram-negative bacteria showed high (>80%) to intermediate (80% to 60%) level of resistance to most of antimicrobial agents being tested (Figure 2 and Table 4). Similar findings have been reported in previous studies conducted in Ethiopia [13] and Nigeria [27].

Antimicrobial resistance of S. *aureus*, the predominant uropathogen, is a great concern in management UTIs among diabetic patients in the study setting. This uropathogen showed high rate of resistance to routinely used antimicrobials such as amoxicillin, ampicillin, erythromycin, penicillin G, trimethoprim-sulphimethoxazole, chloramphenicol and ciprofloxacin. This finding is in agreement with a number of prior studies in the country and other parts of the world [9,10,13]. The resistance to most beta-lactam antibiotics of the bacteria might be due to its ability to produce penicillinase and alternative penicillin binding proteins (PBP-2A) [11]. However, the bacteria depicted low rate of resistance to gentamicin and vancomycin (Table 4) as it is supported by other studies [11,24]. On top of that E. *coli*, the most common gram-negative uropathogen, showed high rate of resistance to amoxicillin, ampicillin, erythromycin, penicillin G, chloramphenicol and ciprofloxacin, norfloxacin and tetracycline; but low level of resistance to nitrofurantoin and ceftriaxone. Similar data were reported in other parts of the country and elsewhere in the world [9,13,21,24].

The high rate of resistance might be due to the easy availability and erroneous and indiscriminate use of the antimicrobials in empirical treatment of UTIs. Since, antibiotic resistance has been recognized as the consequence of antibiotic use and abuse [34]. Alternatively, the increase in resistance may be attributed to inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies, which may result in increased prevalence of resistant organism in the community [24].

In this study, most isolates of both gram-positive and gram-negative bacteria were resistant to two or more drugs, multi-drug resistance (Table 4). Similar findings were reported in other parts of the country [9,10,12,15]. This indicates that multi-drug resistance is increasingly becoming a major problem in the management of uropathogens in Ethiopia. This warns the country to implement a nationwide antimicrobial surveillance and in-vitro susceptibility testing with strict adherence to antibiotic policy to prevent the spread of drug-resistant microbes in the country. Moreover, considering antibiotics with a resistance level of 10% or more are less suitable for empiric therapy [13]. Since the resistance level of antibiotics reported in this study were more than 10%, for most common causative agents of UTIs, these antibiotics are no longer appropriate for empiric management of UTIs. Hence, calls for nationwide study to know the exact level of antibiotics resistance among pathogenic bacteria is vital to make the right recommendation of alternative antibiotics to treat UTIs among diabetic patients.

As a limitation, this study may not represent general population who live in East Wollega area since only those who visited the diabetic clinic during the study period were included in the study. Only those antibiotics that were available in the market, during the study period, were included in the study. Thus, it may not include all antibiotics used in clinical practice.

Conclusion

In conclusion, the prevalence of UTIs among diabetic patients was 16.5% with S. *aureus*, CN Staph. Spp, E. *coli* and K. *Pneumonia* being the four commonly isolated microorganisms; and illiteracy, history of UTIs and glucosuria determined as the risk factors for UTIs. Besides, majority of isolated bacterial microbes were resistant to antimicrobials commonly used in the study setting. This calls for attention of health professionals and policy makers to consider the resistance pattern in their clinical practice, and policy making process, respectively. Most importantly, these data may be used to control trends of antibiotic resistance, to develop local antibiotic policies and to assist clinicians in the rational choice of antibiotic therapy; thus, to prevent indiscriminate use of antibiotics.

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