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URINARY TRACT INFECTIONS
Original Article

Prevalence and associated factors of urinary tract infections among diabetic patients in Arba Minch Hospital, Arba Minch province, South Ethiopia

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ABSTRACT

Objective: Urinary tract infection (UTI) is a common and grave health problem in the world. In fact, patients with diabetes mellitus have an immense risk for developing UTI. The development of resistance among uropathogens to antibiotics is a major crisis which limits the use of drug of choice for the treatment of UTI. On this view point, the aim of the present study is to elucidate the prevalence of UTI, associated factors, causative agents and their antimicrobial susceptibility amongst diabetic patients attending Arba Minch Hospital, Arba Minch, Ethiopia.

Material and methods: A facility based cross-sectional study was carried out in diabetic patients visiting the Internal Medicine Unit of Arba Minch Hospital (AMH) during the study period (March to May 2016). Pre-tested structured questionnaire was used for collecting the data pertaining to socio-demographic characteristics and possible risk factors. In order to quantify the uropathogens, midstream urine samples were collected in sterile leak proof culture bottles and streaked onto diverse bacteriological media. All the positive urine cultures showing significant bacteriuria as per the Kass count (>10^5 organisms/mL) were further subjected to biochemical tests. The antimicrobial susceptibility test was performed to determine the resistance/susceptibility pattern of isolated uropathogens. Data entry and analysis were done using Statistical Package for Social Services, version 20.

Results: In total, 239 diabetic patients were included in the study of which 60.2% (n=144) were females. A total of 81 (33.8%) diabetic patients had positive urine cultures. Sixty-eight (83.9%) female diabetic patients had significant bacteriuria (p=0.000). Fifty-two (64.1%) participants had drinking habit and 79 (97.5%) of respondents had higher glucose levels (≥126 mg/dL) (p=0.004 and p=0.003), respectively. According to the biochemical tests, in a total of 90 isolates from patients with significant bacteriuria, eight species of uropathogens such as *Escherichia coli, Klebsiella* sp., *Proteus* sp., *Citrobacter* spp., *Staphylococcus aureus*, Coagulase negative *Staphylococcus* (CNS), *Enterococcus faecalis* and yeast isolates were identified. The antibiogram evidenced that 79.6% (n=51) of Gram-negative bacteria were invariably resistant to amoxicillin and penicillin whereas 73.4% (n=47) and 65.6 % (n=42) of them were resistant to trimethoprim, erythromycin and chloramphenicol, respectively. Regarding the Gram-positive bacteria, high degree of resistance was exhibited towards penicillin and trimethoprim (100%, n=24) followed by amoxicillin (83.3%, n=20) and gentamicin (62.5%, n=15). Invariably, all the Gram-positive cocci and Gram-negative bacilli were susceptible (100%) to amikacin, doxycycline, ceftriaxone and nitrofurantoin.

Conclusion: The prevalence of UTI is higher in diabetic patients. Results revealed that the predominant pathogens of UTI were Gram-negative bacilli *(Enterobacteriaceae),* particularly *E. coli*. Significant bacteriuria had an association with the consumption of alcohol, gender and glucose level. Based on the results of antimicrobial susceptibility tests, it might be inferred that the antibiotics such as amikacin, doxycycline, ceftriaxone and nitrofurantoin are the drugs of choice for the management of both Gram-negative and Gram-positive uropathogenic bacteria in the study area.

Keywords: Arba Minch; diabetes mellitus; urinary tract infections; South Ethiopia.

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Introduction

Urinary tract infection (UTI) is one of the most prevalent diseases in human beings with diverse etiological agents annually affecting 250 million people worldwide.[1] Albeit great diversity of etiological agents is attributed to UTIs, bacteria are the major causative organisms which are responsible for more than 95% of UTIs.[2] The most common bacterial species that are implicated in UTIs are *E. coli, Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa* and *Proteus mirabilis*.[3] The incidence of UTIs depends upon diverse risk factors such as diabetes mellitus (DM), advanced age, urinary tract obstructions, immunosuppression, and neurological disorders.[4] It has been documented that DM is one of the widely known risk factor for developing UTI.[5] Numerous studies corroborated that patients with DM are quite vulnerable to the adverse effects of UTIs as compared to non-diabetics.[6-8] In diabetic patients, urinary tract is the primary site of infection which carries the risk of variable complications such as emphysematous cystitis, pyelonephritis, renal or perinephric abscess, bacteremia, and renal papillary necrosis.[9] The higher prevalence of UTI in diabetic patients was ascribed to the differences in host immunity between diabetic and non-diabetic patients, or to a dissimilarities among infecting etiological agents.[5]

Diabetes mellitus imposes a significant burden in developing countries including Ethiopia. For instance, epidemiological studies in Ethiopia evinced that the prevalence rate of UTI is increasing in diabetic patients.[10,11] This rise in the prevalence has been surmised due to emerging antibiotic resistance among urogenital pathogenic bacteria. Albeit, the prevalence, etiological profile and antibiotic susceptibility pattern of bacterial uropathogens among general people of Arba Minch has been elucidated[1], a similar study pertained to diabetic patients is seldom being investigated. In this background, the present study was intended to delineate the prevalence and associated factors of UTI among diabetic patients attending Arba Minch Hospital (AMH). Besides, etiological profile and antibiotic susceptibility pattern of bacterial uropathogens were also included in this investigation.

Material and methods

Study area and participants

This study was conducted at AMH, Arba Minch located 505 km southwest of Addis Ababa, Ethiopia. A cross-sectional study was developed to elucidate the prevalence and associated factors of UTI among diabetic patients attended the Internal Medicine Unit of AMH between March and May, 2016. The patients who were treated with antibiotics for UTIs within three months, and those who were too ill to respond to the questions were excluded from the study. In order to identify the risk factors, interviews of all the suspected cases of UTI was performed using a structured questionnaire. Prior to the interview, informed verbal consent from each study participants was obtained after lucidly briefing about the purpose of the study. Details on study participants' sociodemographic (age, education, occupation, marital status, drinking and smoking habit) and clinical characteristics (glucose level, previous history of UTI and other chronic diseases) were solicited. The study protocol was ethically approved by the Arba Minch University Institutional Ethical Review Committee.

Sample size determination and sampling technique

The sample size was computed using a sample size determination formula for the estimation of single population proportion. The p-value of 0.21 was opted from the previous study.[12] After considering 95% of confidence interval (z=1.96) and 5% of marginal error (d=0.05); the initial sample size was estimated as 217 participants, and finally by computing a 10% (≈ 22 subjects) of non response rate, the final sample size was consolidated as 239. Systematic random sampling technique was opted. With the reference of last year's diabetic patient's number (~500), K^{th} value was calculated and subjects were preferably selected by lottery method.

Sample collection and processing

For the quantitative evaluation of uropathogens, ten ml of first voided midstream urine samples were collected into 50 mL of sterile Falcon™ tubes. Collected samples were labeled and immediately transported at ambient temperature to the laboratory following appropriate safety precautions and standard operating procedures (SOPs) as described in our earlier study.[1] The processing and culture of urine was performed within 1 hr of sampling to avoid contamination. The urine culture was performed in our Microbiology and Parasitology Laboratory, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Arba Minch University.

Isolation of uropathogenic bacteria

Aliquot of urine samples were seeded using a calibrated inoculation needle onto diverse isolation media such as 5% blood agar, MacConkey, and Mannitol Salt Agar (MSA) agar (Oxoid Ltd, Bashingstore, Hampire, UK). The inoculated plates were incubated at 37°C for 24 to 48 hours. Following incubation, plates were inspected for the bacterial growth. The samples displaying prominent bacterial growth according to Kass count ($>10^5$ organisms/mL) were considered as culture-positive for UTI.[13] Only the strains retrieved from the patients with significant bacteriuria ($\geq 10^5$ CFU/mL) (Colony Forming Unit) were chosen for the bacteriological analysis. The exclusion criteria were contamination (polybacterial growth) and negative results ($<10^5$ CFU/mL Kass count). The isolated bacteria were maintained on nutrient agar media at 4°C.

Identification of uropathogenic bacterial isolates

The pure cultures of respective uropathogens were subsequently subjected to identification and confirmation processes for species. Biochemical, morphological and physiological characteristics of isolated uropathogens were ascertained by adopting standard laboratory methods as described elsewhere.[14] Corresponding American Type Culture Collection (ATCC) strains were used as reference standard to validate the biochemical

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Table 1. Prevalence of UTI associated with sociodemographic characteristics of diabetic patients

Characteristics		Frequency	Percent	Significant bacteriuria		p
				Positive	Negative	
Age of respondents (years)	20-35	47	19.66	16	31	0.884
	36-45	106	44.35	34	72	
	46-55	55	23.0	22	33	
	≥56	31	12.9	9	22	
Respondents' residences	Urban	197	82.4	68	129	0.551
	Rural	42	17.6	13	29	
Gender	Male	95	39.7	13	82	0.000
	Female	144	60.25	68	76	
Marital status	Unmarried	14	5.85	4	10	0.923
	Married	211	88.28	72	139	
	Other relation	14	5.85	5	9	
Educational status	Illiterate	26	10.87	14	33	0.403
	Primary	64	26.77	39	63	
	Secondary	102	42.67	11	15	
	Tertiary	47	19.66	17	47	
Occupational status	Government employee	65	27.19	20	45	
	Merchant	66	27.61	23	43	
	Farmer	28	11.71	6	22	
	Student	24	10.04	9	15	
	Housewife	50	20.92	21	29	
	Laborer	6	2.51	2	4	

UTI: urinary tract infection

Table 2. Prevalence of UTI associated with clinical characteristics of diabetic patients

Characteristics		Frequency	Percent	Significant bacteriuria		p
				Positive	Negative	
History of UTI	Yes	191	79.91	64	127	0.897
	No	48	20.08	17	31	
Other chronic' diseases	Yes	161	67.36	51	110	0.127
	No	78	32.63	30	48	
Hospitalization periods	None	187	78.2	60	127	0.788
	1-15 days	35	14.66	18	17	
	>15 days	17	7.1	3	14	
Presence of UTI symptoms	Symptomatic	155	64.53	52	103	0.879
	Asymptomatic	84	35.14	29	55	
Smoking status	Smoker	217	90.79	74	143	0.449
	Nonsmoker	22	9.2	7	15	
Drinking habits	Yes	122	51.0	52	70	0.004
	No	117	48.95	29	88	
Fasting blood glucose levels	<126 mg/dL	39	16.31	2	37	0.003
	≥126 mg/dL	200	83.68	79	121	

UTI: urinary tract infection

identification of isolated uropathogens. Antibiotic sensitivity profile of each isolate was determined by adopting Kirby-Bauer disc diffusion method.[15] Eleven commercially available antibiotic discs (Himedia®, Mumbai) were used for the determination of antibiotic susceptibilities.

Statistical analysis

The data were analyzed using IBM Statistical Package for Social Services (IBM SPSS Corp.; Armonk, NY, USA) for Windows, version 20. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for each pre-

Table 3. Multivariate logistic regression analyses for factors associated with UTI among diabetic patients

Variables		Frequency	%	Crude OR	Adjusted OR
Sex	Male Female	95 144	39.74 60.25	5.644 (2.887,11.033)	6.549 (3.183,13.476)
Drinking status	No Yes	117 122	48.95 51.0	2.254 (1.298,3.915)	2.825 (1.502,5.314)
Fasting blood glucose	<126 mg/dL ≥126 mg/dL	39 200	16.31 83.68	12.079 (2.831,51.531)	8.789 (1.978,39.046)

UTI: urinary tract infection

Table 4. Drug resistance patterns of Gram-negative bacilli sourced from the urine samples of diabetic patients with UTI

Uropathogenic Gram-negative bacilli	Total no of isolates	Drug resistant uropathogens										
		AX	P	E	CP	CL	AK	G	TP	D	C	N
E. coli	43	36	36	30	8	31	0	10	31	0	0	0
Klebsiella spp.	11	5	5	6	2	1	0	1	6	0	0	0
Proteus spp.	6	6	6	6	4	6	0	6	6	0	0	0
Citrobacter spp.	4	4	4	0	0	4	0	0	4	0	0	0
	64	51 (79.6%)	51 (79.6%)	42 (65.6%)	14 (21.8%)	42 (65.6%)	0 (0%)	8 (12.5%)	7 (73.4%)	0 0 (0%)	0 0 (0%)	0 0 (0%)

UTI: urinary tract infection; AX: amoxicillin; P: penicillin; E: erythromycin; CP: ciprofloxacin; CL: chloramphenicol; AK: amikacin; G: gentamicin; TP: trimethoprim; D: doxycycline; C: ceftriaxone; N: nitrofurantoin

Table 5. Drug resistance patterns of Gram-positive cocci sourced from the urine samples of diabetic patients with UTI

Uropathogenic Gram-positive cocci	No of isolates	Drug resistant uropathogens										
		AX	P	E	CP	CL	AK	G	TP	D	C	N
S. aureus	17	14	17	5	5	8	0	10	17	0	0	0
CNS	5	4	5	1	1	1	0	3	5	0	0	0
E. faecalis	2	2	2	2	1	1	0	2	2	0	0	0
	24	20 (83.3%)	24 (100%)	8 8 (33.3%)	7 (29.1%)	10 (41.6%)	0 (0%)	15 (62.5%)	24 (100%)	0 (0%)	0 (0%)	0 (0%)

UTI: urinary tract infection; AX: amoxicillin; P: penicillin; E: erythromycin; CP: ciprofloxacin; CL: chloramphenicol; AK: amikacin; G: gentamicin; TP: trimethoprim; D: doxycycline; C: ceftriaxone; N: nitrofurantoin

dictor. The p-value of ≤0.05 was considered statistically significant.

Results

Sociodemographic characteristics, clinical data, overall prevalence and associated factors

During the study period of three months, diabetic patients with or without clinical symptoms of UTI attending at AMH were randomly selected for the participation in accordance to the inclusion criteria. In total, 239 diabetic patients were included in the study. The detailed sociodemographic and clinical characteristics of the patients were listed in the Tables 1 and 2. Amid the eligible study participants, a total of 81 (33.8%) were tested positive for the urine culture according to Kass count. Amongst the positive cultures, significant bacteriuria was detected in 35.8% (n=29) of asymptomatic and 64.1% (n=52) of symptomatic diabetic patients. Gender of participants, drinking habit and high level of glucose were significantly associated with UTI (Table 3).

Of the 81 diabetic patients who had significant bacteriuria, 83.9% (n=68) were females (p=0.000). Fifty-two (64.1%) par-

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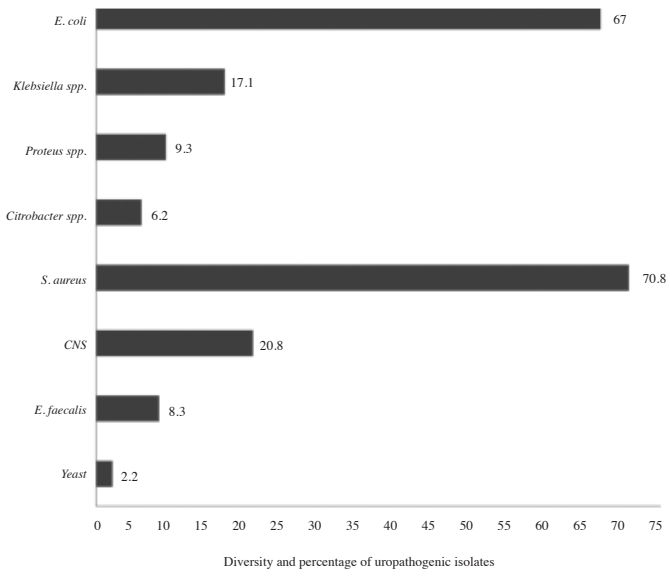

Figure 1. Percentage of uropathogens isolated from urine samples (n=90) of diabetic patients with UTI

ticipants had drinking habit and 79 (97.5%) respondents had higher glucose levels (≥126 mg/dL) (p=0.004 and p=0.003, respectively (Table 2). No statistically significant correlation was found between significant bacteriuria and respondent's age, marital status, occupation, educational status, residence and hospital stay (Table 2). The culture-positive samples displaying discrete colonies were further preferably selected for biochemical characterization and antimicrobial susceptibility test.

Diversity of uropathogens isolated from diabetic patients

According to the biochemical tests, a total of 90 isolates from patients with significant bacteriuria were tentatively identified. The diversity and percentage of uropathogens retrieved from the midstream urine samples are depicted in Figure 1. Among the diverse bacterial isolates, 72.7% (n=64) consisted of Gram-negative bacilli and 27.2% (n=24) of them were Gram-positive cocci. In consideration of the colony morphology, culture and biochemical characteristics, and comparisons with previous reports, isolates were identified and sorted into eight species of uropathogens including Gram-negative bacilli such as *E. coli*, *Klebsiella* sp., *Proteus* sp., *Citrobacter* sp., and Gram-positive cocci such as *S. aureus*, coagulase-negative *Staphylococci* (CNS), *E. faecalis* and yeast isolates. Of the culture-positive samples, species of *Enterobacteriaceae* were generally observed to be the predominant group.

Antibiogram

The antibiotic sensitivity pattern of uropathogenic Gram-negative bacilli and Gram-positive cocci are appended in Tables 4 and 5. The isolated uropathogens showed broad variation in their resistance/susceptibility to the tested antibiotics. The antibiogram evinced that in 79.6% of the cases Gram-negative bacilli were invariably resistant to amoxicillin and penicillin whereas in 73.4% and 65.6% of the cases they were resistant to trimethoprim, erythromycin and chloramphenicol, respectively. Resistance of *E. coli* (83.7%, n=36), *Klebsiella* spp. (45.4%, n=5), *Proteus* spp., (n=6) and *Citrobacter* spp. (100%, n=4) to amoxicillin and penicillin was noted in indicated percentages of cases. Resistance of *E. coli* (72.0%, n=31), *Klebsiella* spp. (54.5%, n=6), and *Proteus* spp., and *Citrobacter* spp. (100%, n=4) to trimethoprim was noted in indicated percentages of cases. Pertaining to the susceptibility pattern, 76.7% (n=33) of *E. coli,* 90.9% (n=10) of *Klebsiella* spp. and 100% (n=6) of *Proteus* spp. showed sensitivity to gentamicin. Whilst 81.4.7% (n=35) of *E. coli* and 90.9% of *Klebsiella* spp. showed susceptibility to ciprofloxacin. Notable result is that all the Gram-negative bacilli (100%) were susceptible to four antibiotics tested including amikacin, nitrofurantoin, doxycycline and ceftriaxone.

Regarding the three species of uropathogenic Gram-positive cocci, high degree of resistance was exhibited towards penicillin and trimethoprim (100%) followed by amoxicillin (83.3%, n=20) and gentamicin (62.5%, n=15). Resistance to penicillin and trimethoprim was displayed for *S. aureus*, CNS and *E. faecalis* in 100% of cases. The resistance to amoxicillin was also seen for *S. aureus*, CNS, and *E. faecalis* in 82.3, 80, and 100% of the cases respectively. Resistance to gentamicin was also observed for *S. aureus*, CNS, and *E. faecalis* in 58.8, 60, and 100% of the cases, respectively. In contrast, susceptibility results demonstrated that indicated percentages of cases were *S. aureus* (70.5%, n=12), CNS (80%, n=4), and *E. faecalis* (50%, n=1) were sensitive to ciprofloxacin. Whilst, 70.5% of *S. aureus*, 80% of CNS and 100% of *E. faecalis* strains showed susceptibility to erythromycin. Invariably all the three Gram-positive cocci were susceptible (100%) to amikacin, nitrofurantoin, doxycycline and ceftriaxone.

Discussion

Diabetes mellitus has long been implicated as a predisposing factor for the UTI. Moreover, it is a well-established fact that urinary tract is the primary site of infection in diabetic patients with increased risk of complications of UTI. Findings of the present study provided baseline information on the prevalence of UTI in diabetic patients, socioeconomic status, clinical characteristics, etiological profile and antibiotic susceptibility test results. Survey of literature indicated that no systematic studies have been undertaken so far to delineate the prevalence of UTI in diabetic patients in Arba Minch province of Ethiopia.

The overall prevalence of significant bacteriuria in both symptomatic and asymptomatic diabetic patients was 33.9%. Previous studies done in Ethiopia showed that the prevalence of DM associated complications such as UTIs increased from 7.1% in 2005

to 34.1% in 2009.[11] A similar pattern of prevalence was reported in a study conducted in Kuwait (35.0%).[16] Howbeit, in contrast to our findings, the prevalence rate of UTI was higher in a study reported from Nepal (54.76%)[17], and lower in another studies cited from Nigeria (17.3%)[18] and southern part of Ethiopia (17.8%).[12] Results revealed that the age of the respondents was one of the factors that were not significantly associated with UTI. Majority of the respondents were within the age range of 20-35 years which agreed with the previous study conducted at Gondar University Hospital, Gondar, Ethiopia.[12] It was found that majority of the study participants had higher glucose levels which might lead to the development of UTIs. Our results were confirmed with an earlier study reported from Kuwait.[16] In the present study it was evidenced that females were 6.5 times more susceptible to bacteriuria than males [6.549 (3.183,13.476)] which well-nigh resembled to a study conducted in Nigeria. [18] Moreover it's universally averred that incidence of UTI is particularly higher amongst women ascribed to their reproductive physiology.[19] In addition to these factors, the respondents with a habit of drinking were 2.8-fold [2.825(1.502,5.314)] more vulnerable to UTIs. In the present study, the most commonly isolated uropathogens were *E. coli* (53%), *S. aureus* (19.7%,), *Klebsiella* spp. (12.2%) and *Proteus* spp. (7.4%). Similar types of isolates with different frequency of isolation were previously reported from the various regions of Ethiopia.[12,20,21] Based on the present observation, it could be inferred that *E. coli* is one of the most prevalent Gram-negative bacterial pathogen causing UTIs in the study area. In accordance with our results, numerous studies from various locales corroborated that *E. coli* is the most predominant uropathogenic isolate.[20,21-24] The second most prevalent isolate amongst the Gram-positive bacilli was *K. pneumoniae*. Similar pattern of prevalence was noted from other regions of Ethiopia.[21] The prevalence of Gram-negative cocci was moderate in our study. *S. aureus* was the most frequently isolated uropathogen amongst the Gram-positive cocci, which was also in concordance with a previous study done in Ethiopia.[25] In fact, the most common form of fungal infection of urinary tract is caused by the *Candida* species. In the present study, species of yeast were also observed but in a lower frequency. In fact, for the effective management of urinary tract infections, delineating the drug resistance pattern of uropathogens is essential. The development of resistances among uropathogens to multiple drugs is a major crisis which limits the drug of choice for the treatment of UTI.[1] The antibiotic susceptibilities of the uropathogenic isolates against the commonly used antibiotics aid in selection and prescription of antibiotics. It has been surmised that the results of the antimicrobial susceptibility tests obtained in the present study probably reflect the actual antibiotic resistance pattern in the study area. Findings evinced that Gram-negative uropathogenic bacilli showed high degree of resistance to amoxicillin and penicillin which ultimately indicated low susceptibility of these pathogens to these drugs. Our results rather tally with those of earlier studies reported from other parts of Ethiopia.[10,12,26] Especially, Gram-negative bacilli, *E. coli* exhibited broadest spectrum of resistance against amoxicillin, penicillin and trimethoprim curtailing their empirical usages. Apart from *E. coli,* other Gram-negative bacilli such as *K. pneumoniae, Proteus* spp., and *Citrobacter* spp. also exhibited resistance against the similar antibiotics with varying degrees. Since the prevalence of resistance exhibited by the Gram-negative uropathogens against routinely used antibiotics such as amoxicillin penicillin and trimethoprim is at high levels, it is a major setback for the effective management of UTI. In case of Gram-positive bacteria, highest level of resistance was exhibited against erythromycin, penicillin and trimethoprim whereas these pathogens showed sensitivity to other antibiotics tested. The similar trend of resistance was reported from the various prefectures of Ethiopia.[10,12,26] It was averred that trimethoprim is the first-line drug for treating UTI in general practice. Invariably, all the isolates showed resistance against trimethoprim. It could be inferred from the results that the high degree of resistance exhibited by the uropathogens was a consequence of long term usage of these antibiotics for the treatment of UTI and other bacterial diseases in the study area. Conversely, notable result of the present study was, invariably all the bacterial isolates showed 100% sensitivity to nitrofurantoin, amikacin, doxycycline and ceftriaxone, indicating the possibilities of administrating these antibiotics for the management of UTI in the study area.

In conclusion, as no earlier reports were available pertaining to the prevalence of UTI in diabetic patients in Arba Minch province, this study represents the first report on the prevalence of UTI in the same study area of interest. The overall prevalence of UTI among diabetic patients was 33.9%. This study evidenced that the predominant pathogen of UTI was *E. coli* which accounted for 67% of the isolated uropathogens. Other uropathogens such as *S. aureus* and *Kliebsiella* spp. was the second and the third dominant bacteria isolated respectively. Bacteriuria was significantly associated with gender, glucose level and the habit of drinking. Based on the results of antimicrobial susceptibility tests it might be inferred that the antibiotics such as nitrofurantoin, amikacin, doxycycline and ceftriaxone were the drugs of choice for the management of both Gram-negative and Gram-positive uropathogenic bacteria in the study area. The overall results of the present study have envisaged that selection, prescription and usage of antibiotics for the management of UTIs in diabetic patients should be vigilantly monitored not only by periodic inspection of local resistance patterns, but also with reference to gender and blood glucose level.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Arba Minch University, College of Medicine and Health Sciences (GOV/AMU/TH5/CMHS/MLS/05/07).

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