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Urinary Tract Infection: Bacterial etiologies, drug resistance profile and associated risk factors in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia

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ABSTRACT

A prospective cross sectional study was conducted to determine the causative agents of UTI in asymptomatic and symptomatic diabetic patients, associated risk factors and drug resistance pattern of the isolates. Between May and June 2010, a total of 422 diabetic patients with asymptomatic UTI (n=387) and symptomatic UTI (n=35) were investigated for urinary tract infection at Gondar University Hospital. Clean catch mid-stream urine specimens were collected from each study subjects. Urine culture, identification and sensitivity tests were done using standard microbiologic procedure. The age range of study participants was 20 to 84 years (mean age 42.3 years). Significant bacteriuria was detected in 14.7% and 51.4% of asymptomatic and symptomatic diabetic patients, respectively. The overall prevalence of significant bacteriuria in both groups was 17.8%. A total of 82 different bacterial uropathogens were isolated. Out of the 82 bacterial isolates, E. coli (31.7%), coagulase negative staphylococci (CONs) (22%), Klebsiella spp. (14.6%), Enterococcus spp. (11%) and S. aureus (8.5%) were the commonest bacterial uropathogens in both groups. The gram positive and negative bacteria accounted for 42.7% and 57.3% of the bacteria isolates, respectively. Significant bacteriuria was significantly associated with history of previous UTI, antibiotic treatment, type of diabetes and blood glucose level. Both gram positive and negative bacteria showed significant level of resistance to most antimicrobial agents tested. Multidrug resistance to two or more drugs was observed in 59.8% of bacterial isolates.

Key words: Diabetes mellitus, urinary tract infection, bacteriuria, antibiotic resistance

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INTRODUCTION

Urinary tract infection (UTI) is a major problem in diabetics. The risk of developing infection in diabetic patients is higher and urinary tract is the most common site for infection [1]. Changes in host defense mechanisms, the presence of diabetic cystopathy and micro-vascular disease in the kidneys may play a role in the higher incidence of UTI in diabetic patients [2]. Serious complications of urinary tract infection, such as emphysematous cystitis,

pyelonephritis, renal or perinephric abscess, bacteremia and renal papillary necrosis occur more commonly in diabetic patients [3]. Acute renal failure is twice as likely to develop in bacteraemic patients [4].

The successful management of patients suffering from urinary tract infections in diabetic patients depends up on the identification of the types of organisms that cause the disease and the selection of an effective antibiotic against the organism in question. The emergences of resistant bacterial strains in hospitals pose a continued challenge to treat and control the spread of infections. Moreover, the indiscriminate use of antibiotics often results in the increased resistance of urine pathogens to most commonly used antimicrobial drugs [5]. Although UTI seldom leads to complications, it can cause significant morbidity and mortality. Different studies in Ethiopia showed that the prevalence rates of UTIs are increasing. In most studies the prevalence rate is in between 10.5-39.5% [6-9]. In addition, resistance to the commonly used antibiotics was found to be very high among the isolates leaving clinicians with very few choices of drugs for the treatment of UTIs [7-9].

There is a paucity of research addressing the etiologies, risk factors and management of UTI in diabetic patients in most developing countries [4]. There is little information about the etiologies of UTI in Ethiopian diabetic patients [10]. Therefore, this study was done to identify the associated risk factors and type of organism(s) isolated in diabetic patients with UTIs attending Gondar university hospital diabetic center, northwest Ethiopia. Thus the data presented in this study will provide information to clinicians on the selection of antimicrobial agents for the treatment of diabetic patients suffering from UTIs.

MATERIALS AND METHODS

Study subjects

A cross-sectional study was conducted at diabetic clinic of Gondar university hospital during the period from May to July 2010. Informed and consented adult diabetic patients (n=422) with symptoms (n=35) and without symptoms of UTI (n=387) coming for their diabetic check-up at diabetic clinic of Gondar university hospital were investigated for UTIs. Diabetic patients on antibiotics for the last two weeks were excluded.

A symptom of UTI is defined the presence of at least two of the following complaints: dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or cost vertebral angle tenderness, fever (temp.38^{o}C) and chills.

All study participants during the study period were interviewed using pre-tested questionnaire that includes socio-demographic and clinical data by attending physicians and transferred to a questionnaire prepared for this study.

Collection, handling and transport of specimens

Each diabetic patient was instructed how to collect a 'clean-catch' mid-stream urine specimen. Accordingly, about 10 to 20 ml urine specimen was collected in a sterile screw-capped, wide-mouth container from each diabetic patient. The bottle was labeled with unique sample number, date and time of collection; then immediately delivered to bacteriology laboratory of Gondar university hospital for culture and drug susceptibility test.

Culture and identification

Urine specimens were directly inoculated onto blood agar and MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) using a standard calibrated wire loop (0.002ml). Streaked culture plates were incubated at 36^{o}C overnight. On the next day, the bacterial growth on the respective media was observed, and total colony count was done on blood agar and checked for significant bacteriuria.

Significant bacteriuria is defined as urine cultures grew $\geq 10^5$ colony-forming unit /ml midstream urine. 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 [11].

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)[12]. In brief, pure culture was transferred into a tube containing 5 ml sterile normal saline (0.85 % NaCl) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then 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 (Oxoid). 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 3-5 minutes

The antimicrobials for disc diffusion testing were obtained from Oxoid in the following concentrations: ampicillin (AMP) (10µg), amoxicillin-clavulanic acid (AMC) (30µg), ceftriaxone (CRO) (30 µg), chloramphenicol (C) (30µg), ciprofloxacin (CIP) (5 µg), erythromycin (E) (15µg), gentamicine (CN) (10µg), penicillin (P) (10 IU), trimethoprim-sulphamethoxazole (SXT) (25µg) and Tetracycline (TTC) (30 µg).

Using a sterile forceps the antibiotic discs were placed on the inoculated plates and incubated at 36^{o}C for 18-24 hours. Diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a metal caliper and the isolate were classified as sensitive, intermediate and resistant according to NCCLS (2002). As the number of intermediate susceptibility reading was very small all were consider as sensitive.

Reference strains
Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for culture and sensitivity testing.

Data analysis
The data obtained from this study were analyzed using statistical package for social science (SPSS, version 16). Percentage for proportion, and odds ratio for categorical variable were used wherever appropriate. A p-value < 0.05 were considered as statistically significant.

Ethical considerations
The research project was approved Institutional Review Board (IRB), Faculty of Medicine; Addis Ababa University. Official permission from the study site was obtained.

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

RESULTS

Study subjects
The socio-demographic characteristics of study subjects are presented in Table 1. Of the 422 diabetic patients investigated, 387 (91.7%) had no symptoms of UTIs (asymptomatic) and the remaining 35 (8.3%) presented with symptoms of UTIs (symptomatic). Type I and II diabetes was observed in 249 (59.0%) and 173 (41.0%) of the patients, respectively.

The mean age of the study participants was 42.3 years (age range 20-84 years). Majority of them (38.2%) were in the age range of 20-35 years. Out of 422 diabetic patients, 200 (47.4%) were males and 222 (52.6%) were females, resulting in male to female ratio of 0.9:1. Most of them were from urban part of Gondar (60.2%).

Significant bacteriuria
Significant bacteriuria was detected in 57/387 (14.7%) and 18/35 (51.4%) of asymptomatic and symptomatic diabetic patients, respectively (p=0.000). The overall prevalence of significant bacteriuria in both groups was 75/422 (17.8%).

Etiologic agents
A total of 82 bacterial uropathogens were isolated from 422 diabetic patients investigated for UTIs. Of these, 64/82 (78%) were from asymptomatic diabetic patients and the remaining 18/82 (22%) were from symptomatic diabetic patients (p=0.000) (Table 2). Out of the 82 bacterial isolates, *E. coli* (31.7%), coagulase negative staphylococci (CONs) (22%), *Klebsiella* spp. (14.6%), *Enterococcus* spp. (11%) and *S .aureus* (8.5%) were the commonest bacterial uropathogens in both groups.

Table 1. Socio-demographic characteristics of diabetic patients investigated for UTIs in Gondar University Hospital (May to July 2010)

Characteristics	Total (n=422) No. (%)	Asymptomatic diabetic patient (n=387) No. (%)	Symptomatic diabetic patient (n=35) No. (%)
Age			
20-35	161(38.2)	157 (40.6)	4(11.4)
36-45	81(19.2)	75(19.4)	6(17.1)
46-55	93(22.0)	83(21.4)	10(28.6)
>56	87(20.6)	72(18.6)	15(42.9)
Sex			
Male	200 (47.4)	193 (49.9)	7 (20.0)
Female	222 (52.6)	194 (50.1)	28 (80.0)
Address			
Urban	254 (60.2)	224 (57.9)	30 (85.7)
Rural	167 (39.8)	163 (42.1)	5 (14.3)
Type of diabetes			
Type I	249(59.0)	241(62.3)	8(22.9)
Type II	173(41.0)	146(37.7)	27(77.1)
History of previous UTI			
Yes	63 (14.9)	37 (9.6)	26 (74.3)
No	359 (85.1)	350 (90.4)	9 (25.7)
History of previous antibiotic Rx			
Yes	31 (7.3)	16 (4.1)	15 (42.9)
No	391 (92.7)	371 (95.9)	20 (57.1)
Duration of diabetes			
< 5 years	256(60.7)	245(63.3)	11(34.4)
> 5 years	166(39.3)	142(36.7)	24(68.6)
Blood glucose level (mg/dl)			
< 126	87(20.6)	86(22.2)	1(2.9)
>126	335(79.4)	301(77.7)	34(97.1)

Table 2. Frequency and types of bacterial species isolated from asymptomatic and symptomatic diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010).

Types of bacterial spp.	Asymptomatic diabetic patient	Symptomatic diabetic patient	Total
	Nº(%)	**Nº (%)**	**Nº(%)**
Escherichia coli	21(32.8)	5 (27.8)	26(31.7)
CONS	16 (25.0)	2 (11.1)	18 (22.0)
Klebsiella spp.	7 (11.0)	5 (27.8)	12 (14.6)
Enterococcus spp	7 (11.0)	2 (11.1)	9 (11.0)
Staphylococcus aureus	3 (4.7)	4 (22.2)	7 (8.5)
Enterobacter spp.	3 (4.7)	-	3(3.7)
Providencia spp.	2 (3.1)	-	2 (2.4)
Pseudomonas aeruginosa	2 (3.1)	-	2 (2.4)
Proteus spp.	1(1.6)	-	1(1.2)
Non-group A,β.HS	1 (1.6)	-	1 (1.2)
Citrobacter spp.	1(1.6)	-	1(1.2)
Total	64 (78.0)	18 (22.0)	82(100)

CONS= Coagulase negative Staphylococci β.HS = Beta Hemolytic streptococci

Others found in small numbers included *Proteus* spp., *P. aeruginosa*, non-group A-β-haemolytic streptococcus, *Providencia*, *Enterobacter* and *Citrobacter* species as shown in Table 2. The gram positive and negative bacteria accounted for 35/82 (42.7%) and 47/82 (57.3%) of the bacteria isolates, respectively (p=0.000).

More than one type of bacteria (mixed type) was isolated in seven urine specimens cultured. Of these, two bacterial spp. were isolated in 5 study subjects and 3 bacterial spp. were isolated in 2 study subjects.

In general no statistically significant differences were observed in the isolation frequency of each pathogen in the two groups (p>0.05).

Risk factors

Significant bacteriuria was strongly associated with history of previous UTI, antibiotic treatment, type of diabetes, and blood glucose level ($p<0.05$) as shown in Table 3.

Table 3. Variables associated with symptomatic and asymptomatic bacteriuria in diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010)

Characteristics	UTI			Crude-OR (95.0% CI)	Adjusted-OR (95.0% CI)	
	SSB	ASB	Total	OR(Lower-Upper)	OR(Lower-Upper)	P-value
Sex						
Male	3(10.7)	25(89.3)	28(37.3)	1	1	
Female	15(31.9)	32(68.1)	47(62.7)	0.526 (.296-.937)	0.606 (.363-.1.012)	0.056
History of previous UTI						
Yes	13(68.4)	6(31.6)	19(25.3)	1	1	
No	5(8.9)	51(91.1)	56(74.7)	1.117 (.456-2.738)	2.336 (1.271-4.295)	0.006
History of previous antibiotic						
Yes	9 (75.0)	3(25.0)	12(16.0)	1	1	0.002
No	9 (14.3)	54(85.7)	63(84.0)	2.898 (.954-8.805)	3.288 (1.520 – 7.111)	
Types of diabetes						
Type I	4 (15.4)	22(84.6)	26(34.5)	1	1	
Type II	14(28.6)	35(71.4)	49(65.3)	0.230 (.114-.462)	0.295 (.175-.498)	0.000
Duration of diabetes						
< 5 years	5(12.8)	34(87.2)	39(52.0)	1	1	
> 5years	13(36.1)	23(63.9)	36(48.0)	1.009 (.559 - 1.823)	0.649(.393- 1.073	0.092
Blood glucose level (mg/dl)						
<126	-	5(100)	5(6.7)	1	1	0.002
>126	18(25.7)	52(74.3)	70(93.3)	0.344 (.125 - .943)	0.231 (.090-.591)	
Age						
20-35	4(17.0)	19(82.6)	23(30.7)	1	1	
36-45	4(40.0)	6 (60.0)	10(13.3)	1.766 (.713 – 4.375)	1.183 (.534-2.622)	0.678
46-55	2(9.1)	20(90.9)	22(29.3)	1.239 (.519- 2.958)	0.538 (.281-1.031)	0.062
>56	8(40.0)	12(60.0)	20(26.7)	1.238 (.498 – 3.076)	0.558 (287-1.087)	0.087

SSB: Symptomatic Significant Bacteriuria
ASB: Asymptomatic Significant Bacteriuria
OR: Odds ratio
CI: Confidence interval

Table 4. Antimicrobial susceptibility pattern of Gram-negative bacteria isolated from urine culture of diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010)

Bacteria isolated	Total Nº.	S/R	Antimicrobial agents tested							
			AMP	AMC	CRO	CIP	C	CN	SXT	TTC
			Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº.%
E. coli	26	S	10(38.5)	17(65.4)	20(76.9)	24(92.3)	11(42.3)	23(88.5)	20(76.9)	5(19.2)
		R	16(61.5)	9(34.6)	6(23.1)	2(7.7)	15(57.7)	3(11.5)	6(23.1)	21(80.8)
Klebsiella spp,	12	S	-	4(33.3)	5(41.7)	9(75.0)	2(16.7)	2(16.7)	2(16.7)	2(16.7)
		R	12(100)	8(66.7)	7(58.3)	3(25.0)	10(83.3)	10(83.3)	10(83.3)	10(83.3)
P. aeruogenosa	2	S	-	1(50.0)	1(50.0)	2(100)	1(50.0)	-	1(50.0)	-
		R	2(100)	1(50.0)	1(50.0)	-	1(50.0)	2(100)	1(50.0)	2(100)
Providencia spp.	2	S	1(50.0)	1(50.0)	2(100)	2(100)	1(50.0)	-1(50.0)	1(50.0)	-
		R	1(50.0)	1(50.0)	-	-	1(50.0)	1(50.0)	1(50.0)	2(100)
Proteus spp	1	S	-	-	1(100)	1(100)	-	1(100)	1(100)	-
		R	1(100)	1(100)	-	-	1(100)	-	-	1(100)
Enterobacter spp	3	S	2(75.0)	3(100)	3(100)	3(100)	1(25.0)	2(66.7)	2(66.7)	-
		R	1(25.0)	-	-	-	2(75.0)	1(33.3)	1(33.3)	3(100)
Citrobacter spp	1	S	-	1(100)	1(100)	1(100)	1(100)	1(100)	-	-
		R	1(100)	-	-	-	-	-	1(100)	1(100)
Total	**47**	**S**	**15(31.9)**	**28(59.6)**	**29(61.7)**	**40(85.1)**	**16(34.0)**	**30(63.8)**	**27(57.4)**	**8(17.0)**
		R	**32(68.1)**	**19(40.4)**	**18(38.3)**	**7(14.9)**	**31(66.0)**	**17(36.2)**	**20(42.6)**	**39(83.0)**

AMP= Ampicillin; AMC=Amoxicillin-clavulanic acid; CRO=Ceftriaxone; CIP=Ciprofloxacin C=Chloramphenicol; CN= Gentamicin; SXT=Trimethoprim-sulphamethoxazole; TTC= Tetracycline

Table 5. Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from urine culture of diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010)

Bacteria isolated	Total Nº.	S/R	Antimicrobial agents tested									
			AMP	AMC	CRO	CIP	C	E	CN	P	SXT	TTC
			Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)
S.aureus	7	S	2(28.6)	4(57.1)	4(57.1)	-	2(28.6)	3(42.9)	4(57.1)	-	-	-
		R	5(71.4)	3(42.9)	3(42.9)	7(100)	5(71.4)	4(57.1)	3(42.9)	7(100)	7(100)	7(100)
CONS	18	S	18(100)	18(100)	18(100)	15(83.3)	18(100)	15(83.3)	17(94.4)	13(72.2)	10(55.6)	5(27.8)
		R	-	-	-	3(16.7)	-	3(16.7)	1(5.6)	5(27.8)	8(44.4)	13(72.2)
***Enterococcus* spp**	9	S	8(88.9)	9(100)	5(55.6)	7(77.8)	7(77.8)	7(77.8)	5(55.6)	3(33.3)	4(44.4)	4(44.4)
		R	1(11.1)	-	4(44.4)	2(22.2)	2(22.2)	2(22.2)	4(44.4)	6(66.7)	5(55.6)	5(55.6)
Non-group A,β.HS	1	S	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
		R	-	-	-	-	-	-	-	-	-	-
Total	**35**	**S**	**29(82.9**	**32(91.4)**	**28(80.0)**	**23(65.7)**	**27(77.1)**	**26(74.3)**	**27(77.1)**	**17(48.6)**	**15(42.9)**	**10(28.6)**
		R	**6(17.1)**	**3(8.6)**	**7(20.0)**	**12(34.3)**	**8(22.9)**	**9(25.7)**	**8(22.9)**	**18(51.4)**	**20(57.1)**	**25(71.4)**

CONS= Coagulase negative Staphylococci β.HS = β .Hemolytic streptococci
AMP=Ampcillin; AMC=Amoxicillin-clavulanic acid; CRO=Ceftriaxone; CIP=Ciprofloxacin; C=Chloramphenicol
CN= Gentamicin; E=Erythromycin; P= Penicillin
SXT= Trimethoprim-sulphamethoxazole
TTC= Tetracycline

Table 6. Multi-drug resistance pattern of Gram-positive bacteria isolated from urine culture of diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010)

Combination of antibacterial agent	Total	*S.aureus*	*CONS*	*Enterococcus* spp	*B.hemolytic streptococci*
	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)
CIP , SXT	1(6.3)	-	1(20.0)	-	-
P, SXT ,TTC	2(12.5)	-	2(40.0)	-	-
E, SXT , TTC	1(6.3)	-	1(20.0)	-	-
P, CIP , SXT , TTC	3(18.9)	2(28.6)	1(20.0)	-	-
P, CRO , SXT , TTC	2(12.5)	-	-	2(50.0)	-
P, AMC , C, SXT ,TTC	1(6.3)	-	-	1(25.0)	-
P, AMC , CIP , C, E, SXT , TTC	1(6.3)	1(14.3)	-	-	-
AMP ,AMC, CIP , C, E, SXT , TTC	1(6.3)	1(14.3)	-	-	-
AMP,CRO, CIP , C, E, CN , SXT , TTC	1(6.3)	-	-	1(25.0)	-
AMP , AMC , CRO , CIP , C, CN , P, SXT , TTC	1(6.3)	1(14.3)	-	-	-
AMP , AMC , CRO , CIP , C, E, CN , P, SXT , TTC	2(12.5)	2(28.6)	-	-	-
Total	16(100)	7(100)	5(100)	4(100)	-

AMP=Ampicillin AMC=Amoxicillin-clavulanic acid CRO=Ceftriaxone CIP=Ciprofloxacin
C=Chloramphenicol CN= Gentamicin E=Erythromycin P= Penicillin
SXT= Trimethoprim-sulphamethoxazole TTC= Tetracycline

Antimicrobial susceptibility

Gram negative bacteria

The antimicrobial susceptibility pattern of gram-negative bacteria (n=47) is presented in Table 4. All isolates showed intermediate level of resistance (60-80%) against ampicillin and chloramphenicol. Low level of resistance (<60%) was observed against amoxicillin-clavulanicacid, ciprofloxacin, ceftriaxone, gentamicin and trimethoprim-sulphamethoxazole. High level of resistance (>80%) was observed against tetracycline

Gram positive bacteria

The antimicrobial susceptibility pattern of gram positive bacteria (n=35) is presented in Table 5. Gram-positive bacteria showed low level of resistance (<60%) to all antimicrobials tested except for tetracycline.

Multi drug resistance

Multidrug resistance (MDR) to two or more drugs was observed in 16/35 (45.7%) and 33/47 (70.2%) of gram-positive and gram negative bacteria, respectively (Tables 6 and 7). The overall prevalence of MDR in both groups was 49/82 (59.8%).

Table 7. Multi-drug resistance pattern of Gram-negative bacteria isolated from urine culture of diabetic patients attending at GUH diabetic center, Gondar, Ethiopia (May-July 2010)

Antibiotics	Total	*E. coli*	*Klebsiella* spp	*P.aerugenosa*	*Providencia* spp	*Proteus* spp	*Entrobacter* spp	*Citrobacter* spp
	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)	Nº. (%)
C , TTC	2(6.1)	2(12.5)	-	-	-	-	-	-
AMP , AMC, C	2(6.1)	2(12.5)	-	-	-	-	-	-
AMP , C , TTC	2(6.1)	2(12.5)	-	-	-	-	-	-
AMP , AMC , TTC	2(6.1)	1(6.3)	-	-	-	1(100)	-	-
CRO , C , TTC	2(6.1)	-	-	-	1(100)	-	-	1(100)
C , CN , SXT , TTC	1(3.0)	-	-	-	-	-	1(33.3)	-
AMP , C , SXT , TTC	1(3.0)	1(6.3)	-	-	-	-	-	-
AMP , AMC , C , TTC	1(3.0)	1(6.3)	-	-	-	-	-	-
AMC , C , SXT , TTC	1(3.0)	1(6.3)	-	-	-	-	-	-
AMP , AMC , CRO , C , TTC	2(6.1)	2(12.5)	-	-	-	-	-	-
AMP , CRO , C , CN , TTC	2(6.1)	2(12.5)	-	-	-	-	-	-
AMP , AMC , CRO , SXT , TTC	1(3.0)	1(6.3)	-	-	-	-	-	-
AMP , AMC , CRO , CN , TTC	1(3.0)	-	-	1(50.0)	-	-	-	-
AMP , C , CN , SXT , TTC	3(9.1)	-	2(22.2)	1(50.0)	-	-	-	-
CRO , CIP , C , SXT , TTC	1(3.0)	-	-	-	-	-	1(33.3)	-
CRO , CIP , C , CN , SXT , TTC	1(3.0)	-	-	-	-	-	1(33.3)	-
AMP , AMC , C, CN , SXT , TTC	1(3.0)	-	1(11.1)	-	-	-	-	-
AMP , AMC , CRO , CIP , SXT , TTC	1(3.0)	1(6.3)	-	-	-	-	-	-
AMP , AMC , CRO , C , CN , SXT , TTC	5(15.2)	-	5(55.6)	-	-	-	-	-
AMP, AMC , CRO , C , CN , CIP, SXT , TTC	1(3.0)	-	1(11.1)	-	-	-	-	-
Total	33(100)	16(100)	9(100)	2(100)	1(100)	1(100)	3(100)	1(100)

AMP=Ampicillin; AMC=Amoxicillin-clavulanic acid; CRO=Ceftriaxone; CIP=Ciprofloxacin C=Chloramphenicol; CN= Gentamicin; SXT=Trimethoprim-sulphamethoxazole; TTC= Tetracycline

DISCUSSION

In this study, the overall prevalence of significant bacteriuria (SB) in both symptomatic and asymptomatic diabetic patients was 17.8%. Similar findings have been reported in previous study conducted in Ethiopia (14%) [10] and other countries e.g. in Kenya (17%) [13], Pakistan (21%) [14] and Germany (22.5%) [15]. However lower prevalence of SB has been reported in another study conducted in pregnant women from Addis Ababa (11.6%) [9]. Diabetes mellitus has been considered a predisposing factor for UTI, especially in women, in whom the prevalence of asymptomatic bacteriuria is four fold higher when compared to women without diabetes [16]. This is not true for men with diabetes, in whom the prevalence of SB is similar to that in the general population. In this study, SB was more common in females (21.2%) than males (14%) (p=0.05) (Table3). This is in agreement with previous studies done in Ethiopia [7] and the Netherlands [17]. The high prevalence of UTI among female population may be due to decrease of normal vaginal flora (*Lactobacilli*), less acidic pH of vaginal surface, poor hygienic conditions, short and wide urethra and proximity to anus.

Different studies in the general population showed that the etiologic agents of UTIs belonged mainly to gram negative enteric bacteria [16]. In the present investigation, *E. coli* was the most frequent isolate (31.7%) (Table 2), substantiating earlier reports from the same study area [7, 8] and Manaal et.al., reported 30% prevalence of *E. coli* [36]. The second most common isolate was coagulase negative staphylococci (CONs) (22.0%). This is in contrast the findings from previous studies conducted in Ethiopia where *S. aureus* was the second commonest isolate [7, 8]. The high isolation rate of CONs in the present study could be explained as contamination during specimen collection or processing and/or could be change in pattern of infection in diabetic patients.

The other common isolates were *Klebsiella* (14.6%) and *Enterococcus spp.* (11%). This is also in agreement with previous studies conducted in Ethiopia and India [7, 8 and 37]. More than one type of bacteria (mixed type) was isolated in seven urine specimens cultured in this study. Some microbiologists regard polymicrobial growth as contamination [18]. However, polymicrobial growth from mid-stream urine has been found among patients with

confirmed bladder infection and is more likely to occur in patients with underlying disorders that interfere with free urine flow and is frequent also in patients with indwelling catheters [19]. In general, the present study confirmed that almost no difference in the type and frequency of bacteria isolated in diabetic and non-diabetic patients investigated for UTIs when compared with the findings from the general population observed in Ethiopia [7, 9-10]. In the present study, significant bacteriuria (SB) was detected in 14.7% of asymptomatic diabetic patients. Similar findings have been reported from diabetic patients in Kenya (11.1%) (Kayima *et al.*, 1996) and Iran (11.1%) [20]. But in contrast, Alebiosu *et al*. [21] and Adeyba *et al*. [22] from Nigeria reported a higher prevalence of 26.6% and 21%, respectively.

In the present investigation significant bacteriuria (SB) was detected in 51.4% of symptomatic diabetic patients, which is higher than reports from Gondar in the general population (39.5%) [8] and (28.1%) [7]; and from Addis Ababa in pregnant women (20%) [9]. The finding of this study shows that diabetic patients are more prone to UTIs than others.

Urinary tract infection appears to be multifactorial in subjects with diabetes and various diabetes-related risk factors have been proposed. In this study, history of previous UTI, previous antibiotic treatment, type II diabetes and blood sugar level) have strong association with significant bacteriuria in both symptomatic and asymptomatic diabetic patients (Table 3). Similar findings have been reported in studies conducted elsewhere [16, 23-24].

Previous UTI as a risk factor for ASB indicates that bacteriuria can be present with or without symptoms of UTI. In some reports the presence of UTI during past year, has also been postulated as important risk factor for ASB in diabetics [23, 25-26]. It can be concluded that, the colonization of uropathogens in urinary tract of diabetics after episodes of UTI, decrease local secretion of cytokines and increased adherence of bacteria to uroepithelial cells can accelerate the prolonged release of bacteria from urinary tract resulting in bacteriuria.

In the present study, both gram-negative and positive bacteria (except for non-group A beta hemolytic streptococci) showed intermediate to low level resistance to one or more antimicrobial agents except for tetracycline (Tables 4 and 5). Similar findings have been reported in previous studies conducted in Ethiopia [27,8, 7, 28] and elsewhere [29-30]. The high level of resistance to tetracycline may be due to easy availability and low cost of the antibiotic. These factors are common in the study area where some patients buy drugs without prescription.

In this study, gram negative bacteria were relatively susceptible to ciprofloxacin (85.1%) and gentamicine (63.8%) as shown in Table 4. But compared to previous studies conducted in Ethiopia where ciprofloxacin susceptibility among the isolates was 98.3 % [8]. This implies resistance to this drug has alarmingly increased in the present study. In contrast to the present study, 97.3% gram negative bacteria isolated from pregnant women in Addis Ababa, Ethiopia were susceptible to gentamicine [9].

In the present study most of the gram negatives isolates (59.6%) were susceptible to amoxicillin-clavulanic acid. This is in contrast to 70% susceptibility observed to the same drug in a previous study from Ethiopia [9]. Decreased susceptibility to this drug in the present study may be due to self-medication and indiscriminate use like any other antibiotics in the study area.

Amoxicillin-clavulanic acid is recommended for treatment of urinary tract infection as it acts on all urinary isolates of *E. coli* instead of using other broad-spectrum antibiotics [31]. The susceptibility of *E. coli* to amoxicillin-clavulanic acid (65.4%) in this study was not as high as expected. Studies have shown that the increased resistance of *E .coli* to amoxicillin -clavulanic acid is the result of hyper production of TEM-β lactamase, production of pencillinase resistant to inhibitors and production of cephalosporinase [32].

Klebsiella species showed high level of resistance to most antimicrobial agents tested except for ciprofloxacin in the present study (Table 4). It is a well-known fact that *Klebsiella* spp. is inherently resistant to ampicillin, cephalosporins and aminoglycosides due to increasing acquisition of R- plasmids [33]. *Klebsiella* spp. also produces SHV, a chromosomally mediated pencillinase which can hydrolyze ampicillin and first generation cephalosporin's [34].

In this study, *S. aureus* was resistant to penicillin (100%) as shown in Table 5. It is an established fact most *S. aureus* strains produce pencillinase and alternative penicillin binding proteins (PBP-2A) helps the organisms to become resistant to most beta lactam antibiotics [35].

Multidrug resistance (MDR) to two or more drugs was observed in 59.8% of the isolates in this study (Tables 6 and 7). This is in contrast with the previous findings reported in Ethiopia, where MDR ranges from 74 to 85% [7, 9].

In conclusion, significant bacteriuria was detected in 14.7% and 51.4% of asymptomatic and symptomatic diabetic patients, respectively. Base on this, UTI in asymptomatic diabetic patients should not be neglected and follow up studies are required to supplement the present findings for appropriate management of asymptomatic UTI's in diabetic patients. As a complication of diabetes, UTI may be preventable with better glucose control and unnecessary use of antimicrobials. Gram-negative organisms were the commonest organisms isolated; among which *E. coli* was the principal urinary pathogen. Most of the isolates showed intermediate to low level of resistance to one or more antimicrobials tested. This indicates that regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of diabetic patients with UTI.

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