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Bacteriuria and their Antibiotic Susceptibility Patterns among People Living with HIV Attending Tikur Anbessa Specialized and Zewditu Memorial Hospital ART Clinics, Addis Ababa, Ethiopia

Genet Molla Fenta[1], Melese Hailu Legese[2*] and Gebru Mulugeta Weldearegay[2]

[1]Department of Medical Laboratory Sciences, College of Medicine & Health Sciences; Wollo University, Ethiopia

[2]Department of Clinical Laboratory Science, School of Allied Health Sciences, College of Health Science, Addis Ababa University, Ethiopia

[*]**Corresponding author:** Melese Hailu Legese, Department of Clinical Laboratory Science, School of Allied Health Sciences, College of Health Science, Addis Ababa University, Ethiopia, E-mail: melerose85@gmail.com

Abstract

Background: Urinary tract infections are major causes of morbidity in people living with HIV. Hence the study aimed to determine the prevalence of bacteriuria and their antibiotic susceptibility patterns among people living with HIV.

Method: A prospective cross-sectional study conducted from April to June 2015. A total of 297 and153 participants were from Zewditu Memorial Hospital and Tikur Anbessa Specialized Hospital, respectively. First morning urine samples were collected and cultured on Blood and MacConkey agar. Culture positives were characterized by Gram stain and standard biochemical tests and Kirby-Bauer method was used for antimicrobial susceptibility patterns of the isolates. Chi-square test was used to see the relation between dependent variables and independent variables. P-value <0.05 were taken as statistically significance. Data was entered and analyzed using SPSS version 20.

Result: Overall prevalence of bacteriuria was 11.3% (n=51/450). Isolated bacteria from HAART naïve and on HAART participants were 7% (n=9/131) and 13% (n=42/319) respectively. *E. coli* 25(49%), *S. aureus* 10(19.6%) and Enterococcus species 7 (13.7%) were the predominant isolated bacteria. The highest proportion of bacteria were isolated from patients having a CD4 count of less than 500 cells/mm^3 (22.5%; n=38/169). Most bacterial isolates were sensitive to amikacin (100%), ceftriaxone (96%); resistant to ampicillin (81%), sulfamethoxazole-trimethoprim (71%) and amoxicillin-clavulanic acid (61%). Multiple drug resistance was 78.4% (n=40/51). Gram positives and gram negatives accounts 65% (n=13/20) and 87% (n=27/31) of multiple drug resistance level respectively.

Conclusion: HAART users with low CD4 counts were more frequently infected with urinary pathogens compared with HAART naïve who had higher CD4 counts. More than three quarters of all isolated bacteria were resistant to two or more commonly prescribed antimicrobial drugs. Thus, regular monitoring of bacteriuria and their antimicrobial susceptibility patterns among this group of individuals is recommended to provide effective therapy and thereby prevent renal complications.

Keywords: UTI; HIV; CD4; HAART; MDR; Ethiopia

Introduction

Urinary tract infection (UTI) refers to the presence of microbial pathogens within the urinary tract and is usually classified by the site of infection: bladder [cystitis], kidney [pyelonephritis], UTI always requires the presence of bacteria in the urine (bacteriuria), but can be both asymptomatic or symptomatic, and is characterized by a wide spectrum of symptoms ranging from mild irritative voiding to bacteremia, sepsis, or even death [1]. HIV infection is associated with a variety of renal syndromes; patients with low CD4 counts are at risk of the neurological complications of: hyperreflexia and hyporeflexia which can lead to urinary stasis and ultimately infection [2].

Urinary tract infections accounts for a significant proportion of patients daily hospital visits in HIV patients. Untreated UTIs account for 7-60% of opportunistic infections and could be a source for

ascending urinary tract infection and septicemia in immuno-compromised hosts [3]. UTIs start when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. More than 90% of UTIs are due to enteric Gram positive and Gram negative bacteria including *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [4].

Bacterial UTIs are more common in HIV positive individuals than HIV-negative people, although the difference is driven in large part by those HIV-positive patients with CD4 counts less than 500 cells/mm^3 [5,6]. Symptoms and signs of UTI vary depending on sex, age, immune status, and the area of the urinary tract that is infected; some unique symptoms develop depending on the infecting agent. Females have a higher risk for UTIs than most males, probably because of their anatomy; other risk factors for UTIs include any condition that may impede urine flow (e.g., enlarged prostate, congenital urinary tract abnormalities, and inflammation) [7].

The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health problem in the globe, particularly in the developing world [8]. Antimicrobial treatment of UTIs in HIV patients is necessary; both to alleviate symptoms and to reduce the risk of renal complications like HIV associated nephropathy, pyelonephritis and acute and chronic kidney diseases [9]. Since bacterial pathogens of UTIs are variable regionally, infection control and treatment depends on knowledge of common causative organisms and their antibiotic resistance level in local scenario [10]. In Ethiopia very limited data are available regarding UTIs in the HIV positive population. Therefore we conducted a study providing information on the prevalence of bacteriuria and their antibiotic susceptibility patterns among people living with HIV.

Materials and Methods

Study area, design and population

A prospective cross-sectional study was conducted at Tikur Anbessa specialized Hospital and Zewditu Memorial hospital from April to June 2015 in Addis Ababa, Ethiopia.

Eligibility criteria's

All HIV patients who were HAART and non-HAART users attending ART clinics; gave blood for CD4 counts and urine for culture and the absence and presence of UTIs symptoms recruited based on physician decision were included. Whereas, patients who were on antibiotic therapy for two weeks prior to data collection, those under 18 years old and those who didn't gave consent were excluded.

Sample size and sampling technique

We estimated a total of 450 study participants for a 10% non-response rate, 297 from Zewditu Memorial Hospital and 153 from Tikur Anbessa Specialized Hospital, were recruited using convenient sampling technique. The sample size was calculated based on single population proportion sample size estimation at 95% confidence interval with +/-5% tolerable errors using a previous study conducted in Ethiopia [11].

Sample collection and processing

From eligible participants data on socio-demographic characteristics and other associated variables were collected using a structured, pretested questionnaire. Five milliliter mid-stream urine specimens were collected from every study participants using a sterile wide mouth container. The study participants were given appropriate sample collection instructions before providing urine samples. All urine specimens were brought to microbiology laboratory within one hour of collection for culture and drug susceptibility testing. Also CD4 counts of these patients were done using BD-FACS Count.

Culture and antimicrobial susceptibility pattern

Using a sterile calibrated wire loop (0.001 ml), all urine samples were inoculated on blood and MacConkey agar plates (Oxoid, England) and were incubated at 37°C for 24 h. The number of colonies and number of different colony morphologies were counted; allowing estimation of colony-forming units (CFU) per ml of urine, $\geq 10^5$ CFU/ml of urine was considered as significant bacteriuria. Positive cultures were characterized by colony characteristics, Gram stain and

standard biochemical tests following standard operating procedures [12]. For the identification of *S. aureus* Mannitol salt agar (Oxoid, England) and DNAse agar (Oxoid, England) were used.

Drug susceptibility testing

The disk diffusion was performed and after 16-18 h of incubation at 37°C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI). Using a sterile wire loop, 3-5 pure colonies were picked from blood agar for Gram positives and MacConkey agar for Gram negatives and emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland using McFarland Densitometer was swabbed onto Muller-Hinton agar (dispensed on 100 mm plate). Accordingly the CLSI guideline for each category of bacteria, drug susceptibility testing was performed against penicillin G (10 unit Oxoid), ampicillin (10 µg Oxoid), amoxicillin-clavulanic acid (20/10 µg Oxoid), ceftazidime (30 µg, Oxoid), ceftriaxone (30 µg, Oxoid), gentamicin (10 µg, Oxoid), nitrofurantoin (300 µg, Oxoid), cefotaxime (30 µg, Oxoid), sulfamethoxazole-trimethoprim (1.25/23.75 µg, Oxoid), ciprofloxacin (5 µg, Oxoid), tobramycin (10 µg, Oxoid), Vancomycin (30 µg, Oxoid), novobiocin (5 µg, Oxoid), cefoxitin (30 µg, Oxoid). Sulfamethoxazole (Oxoid, England) and Tellurite disk (Oxoid, England) were used for identification of Enterococcus species [13]. Availability and frequency of prescriptions were given attention to select those antibiotics used for the management of bacterial infections in Ethiopia keeping the CLSI guidelines. In this study multidrug resistance was defined as simultaneous resistance to two or more classes of antimicrobial agents.

Quality control (QC)

Standard Operating Procedures (SOP) were strictly followed verifying that media meet expiration date and quality control parameters per CLSI. Visual inspections of cracks in media or plastic petridishes, unequal fill, hemolysis, evidence of freezing, bubbles, and contamination was done. Quality control was performed to check the quality of medium. Each new lot was quality controlled before use by testing the *Escherichia coli* ATCC 25922 and/or *Staphylococcus aureus* ATCC25923 standard control strains.

Data analysis and interpretation

The data was analyzed using SPSS version 20. The descriptive statistics (mean, percentages or frequency) was calculated. The bi-variant logistic regression analysis was used to see the relation between dependent variable and independent variables. Variables that showed a significant association were selected for further analysis using multiple logistic regression models with a p-value<0.05 considered statistically significant.

Data quality assurance

Socio-demographic characteristics of patients were collected using structured data collection sheets after getting informed consent. Urine specimens were collected in accordance with SOPs and brought to bacteriology laboratory immediately for bacteriological analysis. Culture results were recorded carefully before data entry and the data was double checked by a different person before analysis.

Ethical consideration

The study was approved by "Department Research and Ethical Review Committee (DRERC)" of the Department of Medical Laboratory Science (MLS/483/16), School of Allied Health Sciences, College of Health Sciences, Addis Ababa University. Written permission letter was also obtained from the study site. The purpose and procedures of the study was explained to the study participants within the study period. Those participants who gave informed consent were selected and enrolled as the participants of the study. A patient result was communicated to the attending physicians.

Results

A total of four hundred fifty urine samples were collected from HIV patients attending Tikur Anbessa Specialized Hospital (n=153) and Zewditu Memorial Hospital (n=297) ART Clinics. Of these, 71% (n=319/450) and 29% (n=131/450) were HAART and HAART naïve

participants respectively. Two thirds; 68.7% (n=309/450) of the participants were females. Among the total participants 12% (n=52/450) had symptoms of UTI.

The overall prevalence of UTI was 11.3% (n=51/450) distributed equally on males and females though the prevalence of UTI among males 7% (n=10/141) was lower than females 13% (n= 41/309). In relation to symptoms bacteriuria was detected from 7% (n= 29/398) of asymptomatic participants. On the other hand bacteriuria was confirmed from 42% (n=22/52) of participants with symptom for UTI.

The prevalence of UTI among participants on HAART and HAART naïve was 13% (n=42/319) and 7% (n=9/131) respectively. Based on CD4 count of the patients, the highest proportion of bacteria were isolated from patients having a CD4 count of less than 300 mm 350% (n=9/18) followed by 300-500 mm 319% (n=29/151) and greater than 500 mm 35% (n=13/281). There was an association between level of CD4 count and bacterial UTI (P= 0.001) (Table 1).

Variables		Bacteriuria Positive No. (%)	Bacteriuria Negative No. (%)	Total No. (%)	P-value
Sex					
Female		41 (13)	268 (87)	309 (69)	
Male		10 (7)	131 (93)	141 (31)	0.036
Age					
18-26		1 (5)	20 (95)	21 (5)	
27-36		21 (14)	133 (86)	154 (34)	
37-46		20 (11)	166 (89)	186 (42)	0.526
47-56		9 (12)	69 (88)	78 (17)	
57-66		0 (0)	11 (100)	11 (2)	
CD4 Count/mm^3					
<300		9(50)	9 (50)	18 (4)	
300-500		29(19)	122 (81)	151 (34)	
>500		13(5)	268 (95)	281 (62)	0.001
HAART					
Yes		42 (13)	277 (87)	319 (71)	
No		9 (7)	122 (93)	131 (29)	0.071
UTI symptoms					
Yes		22 (42)	30 (58)	52 (12)	
No		29 (7)	369 (93)	398 (88)	0.029

Table 1: Prevalence of bacterial UTI among people living with HIV

Majority of isolated bacteria were Gram negative 60.8% (n=31/51). *Escherichia coli* 49% (n=25/51), *Staphylococcus aureus* 19.6% (n=10/51) and *Enterococcus* species 13.7% (n=7/51) were the most frequent isolates whereas *Staphylococcus saprophyticus* 3 (5.9%), *Citrobacter* 2 (3.9%), *Serratia* 1 (1.9%) and *Acinetobacter* 1 (1.9%) species were the least frequent isolates from cultures of all study participants (Figure 1).

Gram negative isolates were 100% sensitive to amikacin and nitrofurantoin while they showed high rate of resistance to ampicillin (81%) and sulfamethoxazole-trimethoprim (71%) (Table 2). Similarly most Gram positive isolates were 100% sensitive to nitrofurantoin however they were 100% cresistant to penicillin and ampicillin (Table 3).

Most frequent isolates; *E. coli* were 84% sensitive to cefotaxime, ceftriaxone and ceftazidime but it showed 76% of resistance to sulfamethoxazole-trimethoprim. All isolated *S. aureus* were 90% sensitive to amoxicillin-clavulanic acid, gentamicin and cefoxitin however they were 100% resistance to ampicillin and penicillin. All isolated *Enterococcus* species were 100% sensitive to Vancomycin and ciprofloxacin but 100% resistance to ampicillin. Among the total isolated bacteria 78.4% (n=40/51) had multidrug resistance. Gram positives and Gram negatives showed an MDR rate of 65% (n=13/20) and 87% (n=27/31), respectively (Table 4).
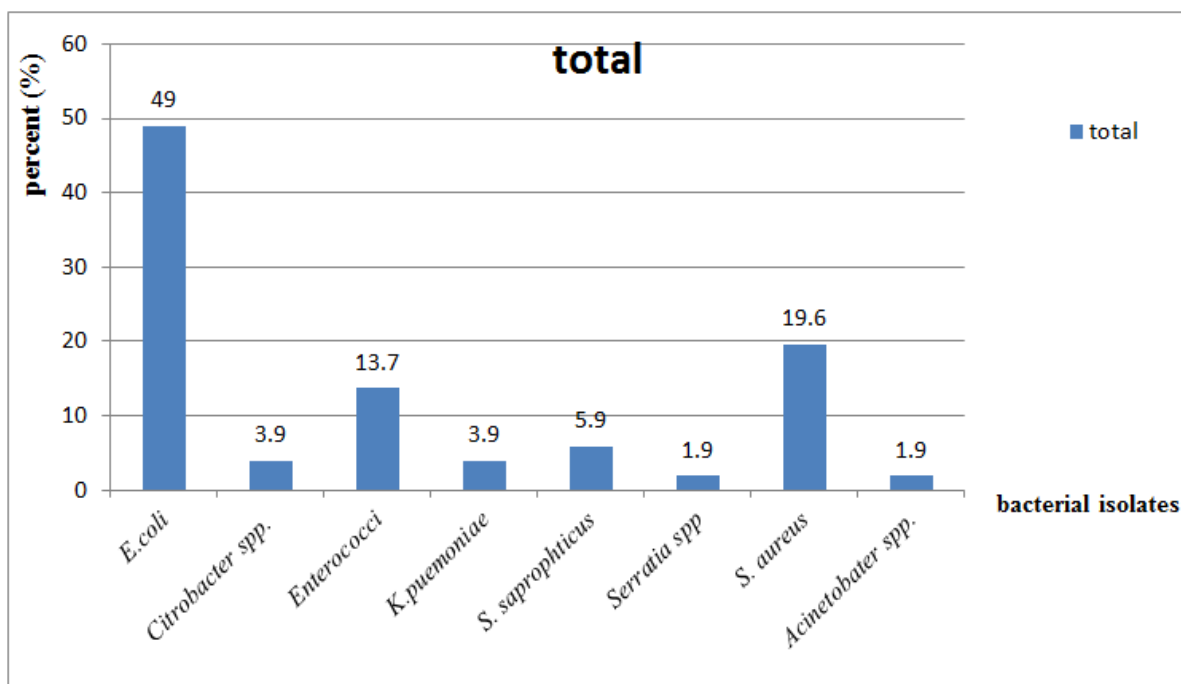

Figure 1: Frequency of bacterial species in cases with significant bacteriuria.

Isolated bacteria	Pattern	AMP	AMC	SXT	GN	CPR	CTX	CPZ	CRO	FN	TO	AMK
HAART Naïve												
E. coli (3)	S	0 (0)	0 (0)	0 (0)	2 (66.7)	2 (66.7)	3 (100)	3 (100)	3 (100)	3 (100)	3(100)	3 (100)
	R	3 (100)	3 (100)	3 (100)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)	0 (0)
K. pneumoniae (1)	S	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
	R	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
On HAART												
E. coli (22)	S	3 (13.6)	9 (40.9)	6 (27.3)	22 (100)	17 (77.3)	18 (81.8)	18 (81.8)	18 (81.8)	21 (95.5)	20 (90.9)	22 (100)
	R	19 (86.4)	13 (59.1)	16 (72.7)	0 (0)	5 (22.7)	4 (18.2)	4 (18.2)	4 (18.2)	1 (4.5)	2 (9)	0 (0)
K. pneumoniae(1)	S	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)
	R	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
Citrobacter spp. (2)	S	1 (50)	1 (50)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
	R	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Serratia (1)	S	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
	R	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)

Acinetobacter spp. (1)	S	NA	NA	1 (100)	1 (100)	1 (100)	NA	NA	NA	NA	1 (100)	1 (100)
	R	NA	NA	0 (0)	0 (0)	0 (0)	NA	NA	NA	NA	0 (0)	0(0)

NA: Not applicable; AMK: Amikacin; AMP: Ampicillin; AMC: Amoxicillin-Clavulanic acid; SXT: Sulphamethoxazole-trimethoprim; CRO: Ceftriaxone CTX: Cefotaxime; GN: Gentamycin; CPZ: - Ceftazidime; FN: Nitrofurantoin; TO: Tobramycin; CPR: Ciprofloxacillin

Table 2: Antimicrobial susceptibility pattern of Gram-negative bacteria isolated from urine from HIV positive patients.

Isolated bacteria	Pattern	P-G	AMP	CXT	AMC	SXT	GN	CPR	CTX	CRO	NF	VA
HAART Naïve												
S. aureus (2)	S	0 (0)	0 (0)	2 (100)	2 (100)	0 (0)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	NA
	R	2 (100)	2 (100)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Enterococci (3)	S	NA	0 (0)	NA	NA	NA	NA	3 (100)	NA	NA	3 (100)	3 (100)
	R	NA	3 (100)	NA	NA	NA	NA	0 (0)	NA	NA	0 (0)	0 (0)
On HAART												
S. aureus (8)	S	0 (0)	0 (0)	7 (87.5)	7 (87.5)	1 (12.5)	7 (87.5)	5 (62.5)	7 (87.5)	7 (87.5)	8 (100)	NA
	R	8 (100)	8 (100)	1 (12.5)	1 (12.5)	7 (87.5)	1 (12.5)	3 (37.5)	1 (12.5)	1 (12.5)	0 (0)	NA
S. saprophyticus (3)	S	0 (0)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.6)	2 (66.6)	3 (100)	3 (100)	3 (100)	NA
	R	3 (100)	3 (100)	2 (66.6)	2 (66.6)	2 (66.6)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	0 (0)	NA
Enterococci (4)	S	NA	0 (0)	NA	NA	NA	NA	4 (100)	NA	NA	4 (100)	4 (100)
	R	NA	4 (100)	NA	NA	NA	NA	0 (0)	NA	NA	0 (0)	0 (0)

NA: Not applicable; AMP: Ampicillin; AMC--Amoxicillin-Clavulanic acid; SXT—Sulphamethoxazole-trimethoprim; CRO-Ceftriaxone; CTX--Cefotaxime; CPR—Ciprofloxacillin; GN--Gentamycin; FN—Nitrofurantoin; VA--Vancomycin; CXT: Cefoxitin; P-G: Penicillin G

Table 3: Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from urine from HIV positive patients.

Isolated bacteria	Total (%)	R0	R1	R2	R3	R4	≥ R5	MDR(≥ 2 drug classes)no (%)
Gram negative	31 (60.8)	2 (6.5)	2 (6.5)	8 (25.8)	10 (32.1)	2 (6.5)	7 (22.6)	27 (87)
E. coli	25 (80.5)	1 (4)	2 (8)	7 (28)	8 (32)	1 (4)	6 (24)	22 (88)
K. pneumoniae	2 (6.5)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)	2 (100)
Citrobacter spp	2 (6.5)	1 (50)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	1 (50)
Serratia spp	1 (3.2)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Acinetobacter spp	1 (3.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)
Gram positive	20 (39.2)	0 (0)	7 (35)	0 (0)	4 (20)	6 (30)	3 (15)	13 (65)
S. aureus	10 (50)	0 (0)	0 (0)	0 (0)	2 (20)	5 (50)	3 (30)	10 (100)
S. saprophyticus	3 (15)	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)	3 (100)
Enterococcus spp	7 (35)	0 (0)	7 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	51 (100)	2 (3.9)	9 (17.6)	8 (15.7)	14 (27.5)	8 (15.7)	10 (19.6)	40 (78)

R0: no antibiotic resistance, R1=resistance to one antibiotic class, R2= resistance to two antibiotic classes, R3= resistance to three antibiotic classes, R4= resistance to four antibiotic classes, ≥ R5= resistance to five or more antibiotic classes

Table 4: Multidrug resistance patterns of bacterial isolates from urine from HIV positive individuals.

Discussion

In our study the overall 11.3% (n=51/450) prevalence of bacteriuria was consistent with previous reports from Ethiopia [11,14]. On the other hand our finding was lower than a study done in Nigeria [15] though it was a higher finding than a study done in Benin City [16]. These differences might be due to methodological differences, study design and included study populations.

We found asymptomatic bacteriuria rate of 7% (n=29/398) which was comparable to findings of other studies in Ethiopia [14]. However a different finding was recorded in studies done from India and Nigeria [5,17]. Rates of bacteriuria were higher in females 13% (n=41/51) than males 7% (n= 10/141) similarly with other studies from Ethiopia and other countries [11,14,18,19]. This might be due to the anatomy of females (short proximity of the urethra to the anus) that exposed them for a higher risk of UTI compared to males [20,21].

Bacteriuria in our study among HAART 13% (n=42/319) and HAART naive 7% (n=9/131) participants was similar with other studies done from Ethiopia [11,14]. However, an Indian study reported a higher prevalence of bacteriuria among both HAART and HAART naive participants [22-24]. This difference could be due to lowered CD4 counts among included patients in their study and fits our finding of a higher bacteriuria rate in participants with CD4 counts less than 500 cells/mm^3.

In this study, Gram negative isolates 60.8% (n=31/51) were more prevalent than gram positive isolates. This finding was comparable with other studies done in Ethiopia; in Gondar [13] and Jimma [10]. In addition it showed similarity with a study from South Africa [8]. This might be due to the presence of unique structure in Gram negative bacteria used for attachments to uroepithelial cells and prevent them from urinary lavage, allowing for multiplication and tissue invasion resulting in invasive infection and pyelonephritis [25,26]. In contrary to studies from Calabar, India and Nigeria [15,25,26] where *S. aureus* were the most frequent isolates, *Escherichia coli* (49%) were the most frequent isolates in our study which was correlated with other findings in Ethiopia [11,14].

Our findings of Gram negative AST patterns were comparable with other findings from Ethiopia [11,14]. However, it contradicted to a study done in Nigeria [26] where all isolated organisms were highly sensitive to gentamicin, ciprofloxacin, ampicillin and amoxicillin-clavulanic acid. This disagreement could be due to variations among antibiotic usage patterns in Nigeria. The findings of our study showed that most Gram positive isolates were sensitive to nitrofurantoin (100%), gentamicin (90%) and ceftriaxone (90%) that was similar to other studies done in different parts of Ethiopia [11,14].

The overall 78% MDR level (n=40/51) was a relatively lower finding as compared to other study done in Ethiopia [27]. Gram positive and Gram negative isolates showed 65% (n=13/20) and 87% (n=27/31) MDR level respectively. These findings were lower findings compared to other studies from Gondar and Bahir Dar, Ethiopia [14,27]. However, our findings were higher than study findings from Dessie, Ethiopia [28].

Conclusion

Bacteriuria was common among HAART users compared with HAART naïve. Participants with low CD4 counts were more frequently infected with bacterial urinary pathogens compared with who had higher CD4 counts. More than three quarters of all isolated bacteria were resistant to two or more commonly prescribed antimicrobial drugs. Thus, regular monitoring of bacteriuria and their antimicrobial susceptibility patterns among this group of individuals is recommended.

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