

Bacteriological profile and antibiotic resistance pattern of urinary tract pathogens isolated at National Referral Laboratory, Ethiopian Public Health Institute

Abebe Aseffa*, Rajhia Abubeker, Negga Asemene, Surafel Fentew, Amete Mihret, Degefu Beyene, Elias Seyoum, Tesfa Addis, Yonas Mekonen, Mulushewa Gebre Egziabher, Dawit Assefa, Meseret Assefa and Aduugna Woyessa

Ethiopian Public Health Institute, P.O.Box 1242, Addis Ababa Ethiopia

*Corresponding author: abehea84@gmail.com

Abstract

Background: Urinary Tract Infection (UTI) most commonly affects humans next to respiratory and gastro-intestinal infections. It is caused by various ranges of bacterial pathogens with varying patterns of antibiotic resistances.

Objective: To determine the profile of bacterial pathogens causing Urinary Tract Infection and their antibiotic resistance patterns.

Methods: This is a retrospective study that used data from Clinical Bacteriology and Mycology National Reference laboratory of the Ethiopian Public Health Institute (EPHI), January 2014 to December 2015. Information on patients' demography and identified pathogens as well as their sensitivity pattern to various antibiotics are routinely documented in the laboratory registration book. The raw data was compiled and entered to spread sheet to compute proportion using Microsoft Excel and Multi-drug resistant strains were analyzed using SPSS Version 20.

Results: The overall prevalence of urinary tract infection was 30.5 % (269 of 882). Of these, above two-thirds of the infection was in females (68 %, 183 of 269). *Escherichia coli* accounted about half of bacterial pathogens (48.3%, n= 130) and below one fifths were due to *Klebsiella* species (13%, n=35.). Among Gram positive isolates, coagulase negative *Staphylococcus* and *Staphylococcus aureus* account 12.8% (n=33) and 10.4 % (n= 28), respectively. Gram negative bacteria pathogens showed extreme resistance to ampicillin (96.7 %) and less resistant to amikacin (8.8 %) while the resistance rate of Gram positive bacteria isolated in this study range from 16.2% (nitrofurantion) to 85.7 % (cotrimoxazole). Multidrug resistance was noted for *Klebsiella* spp (71 %), *E. coli* (55 %), *Enterobacter* spp (50%) and *Staphylococcus aureus* (25%)

Conclusion: This study documented high prevalence of Urinary Tract Infection caused by Gram negative bacterial pathogens. These pathogens are also highly resistant to a range of commonly used antimicrobials with significant proportion of them being Multi-drug resistant (MDR). Therefore, we recommend regular surveillance and monitoring to be put in place that would eventually provide necessary information for decision makers.

Key words: Urinary Tract Infection, bacterial pathogens profile, antibiotic resistance, Ethiopia

Introduction

The presence and growth of microorganisms anywhere in urinary tract causes urinary tract infection (UTI). It is the most common infection in human, next to respiratory and gastrointestinal tract infections. Approximately, over 150 million people experience UTI resulting in a cost of 6 billion USD for health care expenditure worldwide annually (Anejo-Okopi et al. 2015; Chang and Shortliffe 2006; Dulawa 2003; Gupta 2001). The clinical manifestations of UTI is determined by the part of urinary tract involved, the causative agents, severity of infection and patient's immune system status (Foxman and Brown 2003). Pyelonephritis, cystitis and urethritis are known to cause morbidity that leads to hospital visit in the general population (Omeregbe et al. 2008). UTI can affect all population regardless of age and sex. However, the infection is most common in women, particularly who are sexually active. Women are more susceptible to UTI than men, due to several factors

including: anatomic difference, hormonal effects and behavioral pattern (Kunin 1994; Karki et al. 2004; Hooton 2000). A range of etiological agents can cause UTI when exist in a significant number (10^5 colony forming units per milliliter of urine) (Sobel and Kaye 2010). The detection and identification of significant number of causative agent in urine is a gold standard for diagnosis of UTI (Schmiemann et al. 2010). Gram negative bacteria are the most prevalent pathogens causing UTI. More than 50 % of UTI is caused by *Escherichia coli* (Blomgran et al. 2004). In addition, Gram negative bacteria that cause UTI include: *Klebsiella* species, *Enterobacter* species, *Serratia* species, *Pseudomonas aeruginosa* and *Proteus* species. Gram positive bacteria, *Staphylococcus* species and *Enterococcus* species, can also cause of UTI. In Ethiopia, there are a number of studies done on UTI that show the prevalence and antimicrobial resistance patterns of pathogens causing the infection (Moges et

al. 2002; Abajew et al. 2014). However, periodic evaluation of antimicrobial activity of different antibiotics is essential as the pattern of antibiotic sensitivity may vary over periods (Jones and Thornsberry 1982). Moreover, an increasing trend in antibiotic resistance among urinary pathogens to commonly prescribed drugs has become a global truth (Manges et al. 2001). To provide appropriate antibiotic treatment, physicians need to know up to date local patterns of microbial susceptibility and cost effectiveness for proper drug selection (Kurin 1997). Therefore, the aim of this study was to determine the prevalence of common uropathogens and their antibiotic resistance pattern

Materials and Methods

This research was a retrospective study done at Clinical Bacteriology and Mycology Referral Laboratory, Ethiopian Public Health Institute, from 2014 to 2015. The laboratory provides referral diagnostic services for all bacterial culture tests requested from different health facilities in the country. Accordingly, the data used in this study was obtained from patients referred to the institution for urine culture. A hard copy of the urine sample records in the laboratory log book was used to retrieve data. The data was captured and computed using Microsoft Excel. Percentage of MDR strains were analyzed using SPSS Version 20.

The total number of patient's records were 882 and it included the gender, age, specimen date, names of the organisms isolated and their codes. It also included the antibiotic sensitivity pattern of each organism isolated. Tables were used to summarize the proportions and antibiotic resistant patterns of the different organisms causing UTIs. The frequency of infection by different

microbes in male and female patients was also analyzed. The ethical clearance was obtained from EPHI Scientific and Ethical Review Office

Results

A total of 882 urine samples were referred to the laboratory during the study period. About two thirds (64 % n= 563) of the study participants were female, while male account for (36% n=319). Nearly, one-thirds of the samples were culture positive (30.5% n=269). Among 269 culture positive patients 68 % (n=183) were female (Table-2). Among female patients, 21-30 year age group was more infected (27.3 %) than other age group. Males at the age of 0-20 year and the elderly (above 50 years) exhibit increased incidence of UTI while females were found to be more infected than male in all other age groups (Table-1).

Table: 1 Age and sex distribution of UTI positive patients referred to EPHI for urine culture, 2014-2015.

Age	Male (n=86)		Female (n=183)	
	n	%	N	%
0-10	4	4.7	7	3.8
11- 20	4	4.7	8	4.4
21-30	14	16.3	50	27.3
31-40	4	4.7	47	25.7
41-50	7	8.1	33	18.0
51-60	17	19.8	25	13.7
≥61	36	41.9	13	7.1

UTI: Urinary tract infection EPHI: Ethiopian Public Health Institute

Bacterial Pathogens Identified: *Escherichia coli* was the most predominant bacterial pathogens isolated (48.3%, n=130) followed by *Klebsiella* spp (13%, n= 35), Coagulase negative *Staphylococcus* (12.8 %, n=33) and *Staphylococcus aureus* (10.4 %, n=28). Other recovered isolates account for less than 5 % (Table- 2).

Table 2: The distribution of bacterial pathogens in terms of sex in UTI positive patients referred to EPHI for urine culture, 2014-2015.

Pathogens	Acin	Candida	Citr	CNS	E.coli	Entr	Ente	Kleb	Prot	Prov	Pseu	S.aureus
Sex F	3	1	4	25	95	2	8	20	2	5	1	17
M	0	0	2	8	35	3	5	15	1	4	2	11
Total	3	1	6	33	130	5	13	35	3	9	3	28
%	1.1	0.4	2.2	12.8	48.3	1.9	4.8	13.0	1.1	3.4	1.1	10.4

Acin: Acinetobacter, Citr: Citrobacterspp, CNS: Coagulase negative Staphylococcus, Entr: Enterococcus spp, Ente: Enterobacter spp, Kleb: Klebsiella spp, Prot: Proteus spp, Prov: Providence, Pseu: Pseudomonas aeruginosa, UTI: urinary tract infection, EPHI: Ethiopian Public Health Institute

Antibiotics Resistance pattern: Among tested antibiotics for Gram negative bacteria, the highest resistance was documented for ampicillin (96.7 %) followed by co-trimoxazole (86.3 %), augmentin (73%) and tetracycline (62.6 %). However, the rest of antibiotics such as norfloxacin, gentamicin, ceftriaxone, ciprofloxacin, nitrofurantion and

amikacin have show less than 50 % resistance (Table 3). The antimicrobial resistance pattern of Gram positive bacterial isolates is summarized in Table 4. The highest resistance was shown for co-trimoxazol (85.7 %) while the least resistance was for nitrofurantion (16.2 %) (Table 4).

Table 3: Percentage of antimicrobial resistant Gram negative bacterial pathogens isolated from UTI patients referred to EPHI, 2014-2015.

Gram Negative Bacteria Isolated	Antibiotics Resistance (%)									
	Amp	Cot	Gen	Nit	Amc	Nor	Tet	Cro	Cip	Amk
<i>Acinitobacter</i> spp	ND	ND	0	0	ND	0	50	0	0	ND
<i>Citrobacter</i> spp	100	ND	50	25	ND	40	75	66.7	50	33.3
<i>E.coli</i>	95	71.4	25.9	18.8	85.7	59.3	78	37.9	49.4	12.1
<i>Klebsiella</i> spp	100	87.5	52	32.1	83.3	43.5	77.8	58.3	66.7	7.1
<i>Enterobacter</i> spp	82	100	25	30.8	50	83.3	57.1	57.1	50	0
<i>Protesus</i> spp	100	ND	50	33.3	ND	0	100	0	0	0
<i>Providencia</i> spp	100	ND	50	14.3	ND	42.9	0	42.9	50	0
Overall resistance (%)	96.7	86.3	31.1	22.2	73	38.4	62.6	37.6	38	8.8

Amp: Ampicillin, Cot: Cotrimoxazol, Chl: Chloramphenicol, Gen: Gentamycin, Nit: Nitrofurantion, Amc: Amoxycillin+clavulanic acid, Tet: Tetracycline, Cro: Ceftriaxone, Cip: Ciprofloxacin, Amk: Amkacin, ND: Not done, UTI: urinary tract infection, EPHI: Ethiopian Public Health Institute

Table 4: Percentage of antimicrobial resistant of Gram positive bacterial pathogens isolated from UTI patients referred to EPHI, 2014-2015.

Gram positive bacteria isolated	Antibiotics Resistance pattern (% resistance)							
	Cot	Tet	Gen	pen	Nit	Nor	Cip	Oxa
CONS	100	50	21.4	68.8	26.7	81.8	61.9	66.7
<i>S. aureus</i>	71.4	46.7	50	81.8	5.6	70	47.8	33.3
Overall resistance (%)	85.7	48.4	35.7	75.3	16.2	75.9	54.9	50

Cot: Cotrimoxazole, Tet: Tetracycline, Gen: Gentamycine, Cli: Clindamycin, Ery: Erythromycin, Pen: Penicillin, Van: Vancomycin, Nit: Nitrofurantion, Nor: Norfloxacin, Cro: Cefriaxon, Cip: Ciprofloxacin, Oxa: Oxacillin, UTI: urinary tract infection, EPHI: Ethiopian Public Health Institute

Multi drug resistance: Isolated bacterial strains resistant to three or more antibiotics were considered as multi drug resistance (MDR). Accordingly, the following pathogens were found to be MDR: *Klebsiella* spp (71 %), *E. coli* (55 %), *Enterobacter* spp (50%) and *Staphylococcus aureus* (25%) (Table 5).

Table 5: Multidrug resistance among major bacteria isolated from UTI patients referred to EPHI, 2014-2015.

Name of bacteria	MDR bacteria (%)
<i>Escherichia coli</i>	55
<i>Klebsiella</i> spp	71
<i>Enterobacter</i> spp	50
<i>Staphylococcus aureus</i>	25

UTI: urinary tract infection, EPHI: Ethiopian Public Health Institute, MDR: Multi drug resistant

Discussion

The present study found 30.5 % prevalence of urinary tract infection. This finding is comparable to another study (30.2 %) reported from Bahir-Dar (Biadlegne and Abera 2009), but higher than previous reports from other parts of Ethiopia: Addis Ababa (23.3%) and Dessie, (27.35%) (Kabew et al. 2011; Abajew et al. 2014). On the other hand, the prevalence reported in the current study is lower than studies conducted in other countries. For instance, the prevalence of UTI in Nigeria and South Africa were 60 % and 51.1 %, respectively (Kolawole et al. 2009; Habte et al. 2009). This indicates that the prevalence of UTI may vary

among different clinical settings and geographical regions. In line with other studies, the prevalence of urinary tract infection was found to be higher in females than males. This can be explained by the fact that women and men have anatomical differences. In women, urethra is shorter and this makes it easier for normal flora microbes in the rectum to get access into urethra and potentially cause infection (Iram et al. 2012; Jatleni et al. 2015). The age group classification in the study was customized from similar retrospective study done in Nigeria (Chadi et al. 2009).

Among female patients in this study, age group between 21-30 years was the most infected. This may be because females in this age group are sexually active which is one of the risk factors for urinary tract infection. The result from the present study is in agreement with other studies done in Ethiopia and elsewhere (Gezmu et al. 2016; Chadi et al. 2009). However, among males included in this study, patients who are younger and the elderly exhibited increased occurrence of UTI. Similar result was also reported from Nigeria (Chadi et al. 2009).

Gram negative bacteria were more predominant (75.4 %) than Gram-positive (24.6 %) in the present study. Similar results have been reported from Addis Ababa (Kabew et al. 2011) and Gonder (Moges et al. 2002) as well as by other researchers outside Ethiopia (Sabrina et al. 2010). Of all Gram negative bacterial

uro-pathogens, *E coli* accounted for 64.4 % followed by *Klebsiella* spp (17.3 %) and *Enterobacter* (6.4 %). This finding is in line with other studies done in Ethiopia (Gezmu et al. 2016) and elsewhere (Murugan et al 2012). Out of the 66 Gram positive isolates, *Staphylococcus* spp account for 61(92.4 %). The high prevalence of *Staphylococcus* species may be because of poor sample collection procedure. This is due to the fact that these groups of bacteria are commensal of pre-anal and vaginal regions that can easily enable them to contaminate the urine sample during collection (Nuhu 2015). Antibiotic resistance pattern data for the isolated bacteria were compiled and analyzed for Gram negative and Gram positive separately. In this study, Gram negative bacteria showed very high resistance to ampicillin and such level of resistance was documented from studies in Jimma (Beyene and Tsegaye 2011), Addis Ababa (Kabew et al. 2011), Gonder (Moges et al. 2002) and India (Premanatham et al. 2015).

Resistant to cotrimoxazole and amoxicillin-clavulanic acid was also very high among Gram negative bacterial uropathogen. Other studies have reported such high prevalence from Ethiopia and other developing countries (Kibret and Abera 2014; Nuhu 2015). In a study done at Tikur Anbessa Specialized Hospital (Kabew et al. 2011), 37.32 % and 38.92 % of Gram negative bacteria were resistant to ciprofloxacin and norfloxacin, respectively. These findings are similar to our study results. However, it is less than the report from Nigeria: ciprofloxacin (51.2 %) and norfloxacin (67.2 %) (Nuhu 2015). On the other hand, our findings were higher than the study from Namibia (Jatleni et al. 2015): ciprofloxacin (21.3 %) and norfloxacin (7.1 %).

In the current study, 62.2 % of Gram negative bacteria were found to be resistant to tetracycline. The resistance level was lower than a similar study done in Addis Ababa (Kabew et al. 2011) and comparable with study done in Jimma (Beyene and Tsegaye 2011), while it is higher than a report from Namibia (Jatleni et al. 2015). The percentage of Gram negative bacteria resistant to ceftriaxone was 37.6 % which is nearly comparable with a report from Dessie (42.4 %) (Kibret and Abera 2014) and less than the finding from India (84.3 %) (Amarjit et al. 2014).

The prevalence of Gram negative bacteria resistant to gentamycin is lower than the reports from other studies (Kabew et al. 2011; Amarjit et al 2014). Lowest resistance was seen to amikacin and nitrofurantion in our study. These results were in agreement with a study done in Namibia (Jatleni et al. 2015). Among Gram positive bacteria, *S. aureus* and Coagulase negative *Staphylococcus* (CoNS) were the

most predominant uro-pathogens isolated. This finding is comparable with the study done in other part of Ethiopia (Demilie et al. 2012). The bacteria showed 16.2 to 85.7 % resistant rate for all tested antibiotics in this study. This resistant range is very high when compared with other studies done in Ethiopia (Kibret and Abera 2014) and elsewhere (Verma et al. 2016).

Multidrug resistance observed in the present study ranges between 25% and 71% with *Klebsiella* spp being the highest and *S. aureus* the lowest. The existence of high level of MDR *Enterobacteriaceae* sends alarming message as these group of organisms have significant clinical implication.

Conclusions and Recommendations

In this study, the overall prevalence of uro-pathogenic Gram-negative bacteria is very high. These pathogens are also highly resistant to a range of tested antimicrobials with significant proportion of them being MDR. Therefore, we recommend clinicians to be alarmed of the emergence of highly antimicrobial resistant uro-pathogens. In addition, regular surveillance and monitoring is necessary to provide information to decision making.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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