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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATES FROM URINE OF URINARY TRACT INFECTION PATIENTS IN NORTHWEST ETHIOPIA

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Abstract

Urinary tract infection continues to be an important public health problem worldwide in terms of both morbidity and mortality. Nowadays urinary pathogenic agents which were susceptible to common antibiotics became resistance and their number also increased gradually. It has been shown that with the development and widespread use of antimicrobial drugs, the types of the pathogenic organism and their antibiotic susceptibility have changed over time, making continuous and periodic surveillance necessarily in guiding appropriate antimicrobial therapy Therefore, this study was carried out to determine the bacterial profile and their antibiotic susceptibility pattern of patients with suspected cases of urinary tract infections in University of Gondar teaching hospital Northwest Ethiopia. A total of 975 urinary tract infections suspected patients were recruited for this study. Clean catch midstream urine was collected into sterile universal containers and were examined using culture. Of 975 urinary tract infections suspected patients, 250(25.6%) were positive for significant bacteriuria. Escherichia coli was the most predominant gram negative bacteria and Staphylococcus aureus from gram positive bacteria. All isolates of Gram negative bacteria and most Gram-positives (85.1%) isolates showed multiple drug resistance. Chloramphenicol (62.3%), ciprofloxacin (50.9%) and norfloxacin (50%) were effective drugs for Escherichia coli and ciprofloxacin (61.9%) and ampicilline and norfloxacin (57.1%) for Staphylococcus aureus compared to other tested drugs. The prevalence of bacteriuria was comparable to pervious similar study. However, most current isolates were resistant to multiple drugs and the effectiveness of most drugs like ciprofloxacin, amoxicillin, erythromycin were reduced.

Keywords: Antimicrobial, Susceptibility, Urinary tract infection, Ethiopia.

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Introduction

Urinary tract infection (UTI) continues to be an important public health problem worldwide in terms of both morbidity and mortality. Many different microorganisms can infect the urinary tract but by far the most common agents are the gram-negative bacilli where *Escherichia coli* are the predominant one although the distribution of pathogens that cause UTI is changing[1]. Most urinary tract infections are initiated by organisms that gain entrance from the natural environment to the bladder through the urethra and are more common in women than men[2].

Bacteriuria can be divided into two: Asymptomatic bacteriuria (It is a condition in which urine culture reveals a significant growth of pathogens that is greater than 10^5 bacteria/ml, but without the patient showing symptoms of urinary tract infection (UTI)) and symptomatic bacteriuria (UTI was diagnosed when patients had fever (>38°C), urgency, frequency, dysuria, or suprapubic tenderness and positive urine culture, i.e. ≥ 105cfu / ml)[3].

Studies in different part of the world indicated the prevalence of UTI and hospital stays following infection is high. It has been estimated that symptomatic urinary tract infections occurs in as many as 7 million visits to emergency units and 100,000 hospitalizations annually in USA[4]. Similarly in Sweden the occurrence of first time symptomatic UTIs are highest for both boys and girls. The minimum cumulative incidence in both boys and girls aged two years is slightly over 2% and the cumulative incidence of symptomatic UTI in boys during the first 10 years of life is 1.1 to 1.6 %[5].

UTIs are also a serious problem in many developing countries including Ethiopia. In Ethiopia there are reporting rates of UTIs prevalence in range of 29.1% to 39.5% and the number of multiple drug resistant strains becomes increased[6-8]. Nowadays urinary pathogenic agents which were susceptible to common antibiotics became resistance and their number also increased gradually. According to the National Nosocomial Infections Surveillance (NNIS) System report, in 2003, 20.6% of all *Klebsiella species* isolates from patients in intensive care units (ICUs) in the United States were non susceptible to third-generation cephalosporin. This represented a 47% increase compared with resistance rates for 1998 to 2002. Non susceptibility to third generation cephalosporin was also observed in 31.1% of *Enterobacter species* and 5.8% of *Escherichia coli* isolated from patients in ICUs in 2003[9].

There are many factors in the tropical environment that may contribute to the high prevalence of antimicrobial resistance, although few formal studies showing causal relationships exist. These factors include: widespread availability of non-prescription antimicrobials, sub-optimal therapeutic regimens, poly-antimicrobial therapy, lack of laboratory facilities to guide prescribing, mobile bacterial resistance genes, spread of resistant strains in the community and spread of resistant strains in hospitals[10].

In tropical countries, UTIs caused by bacteria associated with an increased antimicrobial resistant species in all age groups and emerging resistance in *Enterobacteriace* (major causative agents of UTI like *Escherichia coli*, *Klebsiella species*) and gram –positive cocci (like *Staphylococcus auerus)* are a significant problem that requires immediate attention[11]. Spread of resistant organisms that cause UTIs in a community including hospital will require the implementation of effective infection-control programmes. These should include the improvement of personal, environmental and hospital hygiene and the reduction of risks of contamination of the environment and equipment. Health staff training in basic infection-control procedures and rational use of antibiotics will help to reduce the incidence of UTIs and resistant strains[10]. To achieve these up-to-date and continual information which show the distribution of different etiological agents that cause UTIs and their antibiotic susceptibility pattern are crucial.

In Ethiopia there are few published reports concerning the antibiotic susceptibility pattern of UTI causing bacteria. It has been shown that with the development and widespread use of antimicrobial drugs, the types of the pathogenic organism and their antibiotic susceptibility have changed over time[12], making continuous and periodic surveillance necessarily in guiding appropriate antimicrobial therapy. To readdress this situation this study was conducted to identify the commonest bacterial agents and their antibiotic

susceptibility pattern from patients with urinary tract infection visiting University of Gondar teaching hospital Northwest Ethiopia. The finding also provides up-to-date information for appropriate management of urinary tract infection.

Methods and Materials

Study area

The study was conducted in University of Gondar teaching hospitals, Gondar, Northwest Ethiopia which is located 738 Km far from the capital city, Addis Ababa to the Northwest of Ethiopia. Gondar town has an average temperature of 24^{o}c and an altitude of 2133m above sea level. The hospital provides health service to over 3million inhabitants in and around Gondar.

Study Population

A total of 975 patients clinically diagnosed for UTIs were assessed for bacteriuria from September 2010 to May 2012. Informed written consent was obtained from each patient prior to sample collection. Personal interviews were used to get socio-demographic data. Ethical approval and permission to conduct the study were obtained from the Ethical review board of the University of Gondar and Medical directors of the hospitals.

Sample collection and processing

Sample collection

Early morning, 5 ml of midstream urine specimens were collected using lick proof re-usable plastic containers. All of the specimens were analyzed within an hour of collection.

Bacteriological investigation

Culture

Using calibrated wire loop (0.001 ml) samples were inoculated in to Cystine Lactose Electrolyte Deficient medium (CLED). After incubation at 37°C for 24–48 hours colonies were counted to check significant growth. Colony counts yielding bacterial growth of 10^5/ml of urine were regarded as significant for bacteriuria. Colonies from CLED were sub cultured into MacConkey agar and blood agar plates (BAP) (Oxoid, LTD) and incubated at 37°C for 24–48 hours. Identification of bacteria was done using colony characteristics, gram reaction of the organisms and biochemical test following standard procedure[13].

Antimicrobial susceptibility testing

Susceptibility testing was performed on all isolates according to the criteria of National Committee for Clinical Laboratory Standards (NCCLs)[14]. The drugs that were tested include amoxicillin (25 µg), ampicillin (10 µg), erythromycin (15 µg), ciprofloxacin (5 µg), Norfloxacin (10 µg), tetracycline (30 µg), co-trimoxazole (25 µg) and chloroamphenicol (30 µg). Diameters of the zone of inhibition around the disc were measured to the nearest millimetre using an electronic digital calliper, and the isolates were classified as sensitive, intermediate, and resistant according to the standardized table supplied by the NCCLs[14]. High, intermediate and low level of resistance is defined when the percentage of resistance is >80%, 60-80% and < 60% respectively.

Quality control

Culture media were tested for sterility and performance. Standard strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used during culture and antimicrobial susceptibility testing.

Data management and analysis

Data were entered into a database designed using MS Excel spreadsheet and analyzed using SPSS statistical software package (version 16). Study findings were explained in words and tables.

Results

A total of 975 urine samples were examined for suspected cases of UTIs during the study period. Of these, 250 were positive for significant bacteriuria with a prevalence of 25.6%. From the isolated 250 bacteria, *Escherichia coli* (45.6 %) was the most predominant organism followed by *Klebsiella pneumoniae* (8.8 %) and *Staphylococcus aureus* (8.4%) (Table 1).

Two hundred and fifty bacteria were tested against eight antimicrobial agents commonly used in study area for the treatment of UTIs. The result of antimicrobial susceptibility pattern of the isolate is shown on Tables 2 and 3 below.

Table No. 01: Type of Bacteria isolated from suspected Urinary tract infections.

Organism isolated	Organism number	(%) prevalence
Escherichia coli	114	45.6
Klebsiella pneumoniae	22	8.8
Staphylococcus aureus	21	8.4
Proteus mirabilis	10	4
Coagulase-Negative Staphylococci	10	4
Klebsiella species	10	4
Citrobacter species	10	4
Providencia species	9	3.6
Enterobacter species	9	3.6
Enterococcus faecalis	7	2.8
Streptococci species	6	2.4
Pseudomonas aeruginosa	4	1.6
Proteus vulgaris	4	1.6
Streptococcus pyogenes	3	1.2
Morganella morganii	3	1.2
Salmonella species	3	1.2
Shigella species	3	1.2
Serratia species	1	0.4
Edwardeseilla species	1	0.4
Total	250	100

Among the total isolates (n = 250) multi drug resistance (MDR = resistance in ≥ 2 drugs) were recorded in 238 (95.2 %) of all bacterial uropathogens. All isolates of Gram negative bacteria and most Gram-positives isolates 40/47(85.1%) showed multiple drug resistance (resistance to two or more drugs) (data not presented here). Rates of susceptibility of Gram-negatives range from 10.8 % - 60.6 %. All isolates showed high level of resistance (>80%) to ampicilline, erythromycine, tetracycline, intermediate level of resistance (60-80%) to amoxicillin and co-trimoxazole and low level of resistance (<60%) to the rest of tested drugs. Among the Gram negatives, the predominant isolate was E. coli, 114 (56.2 % of the Gram-negatives and, 45.6 % of all isolates) demonstrated high level of resistance to erythromycin (92.1) ampicillin (88.6%) and tetracycline (82.5 %). Better susceptibility can be achieved using chloramphenicol (62.3%), ciprofloxacin (50.9%) and norfloxacin (50%) compared to other tested drugs (Table 2).

In the same manner the rates of susceptibility of Gram-positives range from 6.4 % - 68.1%. All isolates showed high level of resistance (>80%) to co-trimoxazole, intermediate level of resistance (60-80%) to amoxicillin, chloramphenicol, norfloxacin and tetracycline and low level of resistance (<60%) to the rest of tested drugs. Among the Gram-positives, the predominant isolate was *Staphylococcus aureus*, 21 (44.7 % of the Gram-positives and, 8.4 % of all isolates) demonstrated high level of resistance to co-trimoxazole (90.5) and intermediate level of resistance to amoxicillin and erythromycin (61.9%), chloramphenico (71.4%), and tetracycline (76.2%). Better susceptibility can be achieved using ciprofloxacin (61.9%) and ampicilline and norfloxacin (57.1%) compared to other tested drugs (Table 3).

Discussion

The prevalence of bacteriuria in this study was 25.6%. This was higher than the 10.9% from the study in Aligarh, India[15] and lower than the 71.1% western Nepal[16] and comparable to Previous report from Gondar (28.1%)[8]. From all isolates, gram negative bacteria compromised 81.2%. The most common organisms were *Escherichia coli* (45.6%) and *Klebsiella pneumoniae* (8.8%). This finding is in agreement with earlier reports [6, 8, 17,18] which indicated the same predominant trend in *Escherichia coli* infection pattern. Among the gram positive isolates, *Staphylococcus aureus* (8.4%) was the most common one. This agrees with previous studies[6, 8, 17] which observed an increasing trend in the prevalence of *Staphylococcus aureus* infection among symptomatic as well as asymptomatic patients. Other organisms that were isolated include: coagulase-negative *Staphylococci*, *Klebsiella* species, *Proteus* species, *Citrobacter* species, *Providencia* species,*Pseudomonas aeruginosa* and others (Table 1). They are less common organisms causing UTI[19].

Table No. 02: Antimicrobial susceptibility pattern of Gram negative bacteria isolates from UTIs

Organism isolated	Number tested		Antimicrobial Susceptibility No(%)							
			AMOX	**AMP**	**CAF**	**CIP**	**ERY**	**CO**	**NOR**	**TTC**
Escherichia coli	114	S	35(30.7)	13(11.4)	71(62.3)	58(50.9)	9(7.9)	32(28.1)	57(50)	20(17.5)
		R	79(69.3)	101(88.6)	43(37.7)	56(49.1)	105(92.1)	82(71.9)	57(50)	94(82.5)
Klebsiella pneumoniae	22	S	4(18.2)	0(0)	18(81.8)	18(81.8)	3(16.7)	0(0)	15(66.7)	3(12.5)
		R	18(81.8)	22(100)	4(18.2)	4(18.2)	19(83.3)	22(100)	7(33.3)	19(83.3)
Proteus mirabilis	10	S	10(100)	0(0)	2(20)	8(80)	1(10)	2(20)	5(50)	0(0)
		R	0(0)	10(100)	8(80)	2(20)	9(90)	8(80)	5(50)	10(100)
Klebsiella species	10	S	0(0)	3(30)	0(0)	8(80)	2(20)	0(0)	8(80)	3(30)
		R	10(100)	7(70)	10(100)	2(20)	8(80)	10(100)	2(20)	7(70)
Citrobacter species	10	S	3(30)	3(30)	3(30)	6(60)	2(20)	3(30)	7(70)	0(0)
		R	7(70)	7(70)	7(70)	4(40)	8(80)	7(70)	3(30)	10(100)
Providencia species	9	S	0(0)	2(22.2)	2(22.2)	4(44.4)	1(11.1)	3(33.3)	5(55.6)	6(66.7)
		R	9(100)	7(77.8)	7(77.8)	5(55.6)	8(88.9)	6(66.7)	4(44.4)	3(33.3)
Enterobacter species	9	S	0(0)	0(0)	3(33.3)	8(88.9)	2(22.2)	1(11.1)	5(55.6)	0(0)
		R	9(100)	9(100)	6(66.7)	1(11.1)	7(77.8)	8(88.9)	4(44.4)	9(100)
Pseudomonas aeruginosa	4	S	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	4(25)	1(25)
		R	4(100)	4(100)	4(100)	3(75)	4(100)	4(100)	0(0)	3(75)
Proteus vulgaris	4	S	0(0)	0(0)	1(25)	2(50)	1(25)	2(50)	2(50)	0(0)
		R	4(100)	4(100)	3(75)	2(50)	3(75)	2(50)	2(50)	4(100)
Morganella morganii	3	S	0(0)	1(33.3)	3(100)	3(100)	1(33.3)	1(33.3)	0(0)	0(0)
		R	3(100)	2(66.7)	0(0)	0(0)	2(66.7)	2(66.7)	3(100)	3(100)
Salmonella species	3	S	0(0)	0(0)	1(33.3)	3(100)	0(0)	0(0)	1(33.3)	2(66.7)
		R	3(100)	3(100)	2(66.7)	0(0)	3(100)	3(100)	2(66.7)	1(33.3)
Shigella species	3	S	3(100)	1(33.3)	2(66.7)	2(66.7)	0(0)	0(0)	0(0)	0(0)
		R	0(0)	2(66.7)	1(33.3)	1(33.3)	3(100)	3(100)	3(100)	3(100)
Serratia species	1	S	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)
		R	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)
Edwardeseilla species	1	S	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)
		R	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)
Total	203	S	73(36)	23(11.3)	106(52.2)	123(60.6)	22(10.8)	44(21.7)	111(54.7)	35(17.2)
		R	1309(64)	180(89.7)	97(47.8)	80(39.4)	181(89.2)	159(78.3)	92(45.3)	168(82.8)

S=Susceptible, R=Resistance

AMOX= Amoxicillin, AMP = Ampicillin, CAF= Chloramphenicol. , CIP = ciprofloxacin,

ERY= Erythromycin, Co= Co-trimoxazole, NOR=Norfloxacillin, TTC= Tetracycline

Table No. 03: Antimicrobial susceptibility pattern of Gram positive bacteria isolates from UTIs.

Organism isolated	Number tested		Antimicrobial Susceptibility No(%)							
			AMOX	AMP	CAF	CIP	ERY	CO	NOR	TTC
Staphylococcus aureus	21	S	8(38.1)	12(57.1)	6(28.6)	13(61.9)	8(38.1)	2(9.5)	12(57.1)	5(23.8)
		R	13(61.9)	9(42.9)	15(71.4)	8(38.1)	13(61.9)	19(90.5)	9(42.9)	16(76.2)
Coagulase-Negative Staphylococci	10	S	2(20)	1(10)	3(30)	10(100)	5(50)	0(0)	0(0)	0(0)
		R	8(80)	9(90)	7(70)	0(0)	5(50)	10(100)	10(100)	10(100)
Enterococcus faecalis	7	S	0(0)	3(42.9)	4(57.1)	3(42.9)	2(28.6)	0(0)	3(42.9)	3(42.9)
		R	7(100)	4(57.1)	3(42.9)	4(57.1)	5(71.4)	7(100)	4(57.1)	4(57.1)
Streptococci species	6	S	0(0)	3(50)	2(33.3)	3(50)	3(50)	1(16.7)	0(0)	3(50)
		R	6(100)	3(50)	4(66.7)	3(50)	3(50)	5(83.3)	6(100)	3(50)
Streptococcus pyogenes	3	S	0(0)	3(100)	3(100)	3(100)	3(100)	0(0)	0(0)	0(0)
		R	3(100)	0(0)	0(0)	0(100)	0(0)	3(100)	3(100)	3(100)
Total	47	S	10(21.3)	22(46.8)	18(38.3)	32(68.1)	21(44.7)	3(6.4)	15(31.9)	11(23.4)
		R	37(78.7)	25(53.2)	29(61.7)	15(31.9)	26(55.3)	44(93.6)	32(68.1)	36(76.6)

S=Susceptible, R=Resistance

AMOX= Amoxicillin, AMP = Ampicillin, CAF= Chloramphenicol. , CIP = ciprofloxacin,
ERY= Erythromycin,Co= Co-trimoxazole,NOR=Norfloxacillin,TTC= Tetracycline

Antimicrobial resistance among uropathogens to the commonly used antibiotics become increasing that make clinicians with very few choices of drugs for the treatment of urinary tract infection[20,21]. In this study, susceptibility pattern of Gram-negative bacteria showed that high to intermediate level of resistance to most of the tested drugs like ampicilline, erythromycine, tetracycline, amoxicillin and co-trimoxazole. In the same manner all isolates of Gram-positives also showed high to intermediate level of resistance to half of the tested drugs including co-trimoxazole, amoxicillin, chloramphenicol, norfloxacin and tetracycline. This agrees with previous studies[6, 8, 15] which observed resistant to most of the tested drugs. Rates of susceptibility of the isolates range from 6.4 % - 68.1% and 95.2% of them developed resistance for at least two of the tested drugs. This indicates that multi drug resistance was found to be very high to the commonly used antibiotics. Antibiotic resistance has been recognized as the consequence of antibiotic use and abuse[22, 23]. Therefore, the reasons for this alarming phenomenon might be inappropriate and incorrect administration of antimicrobial agents in empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community.

Among the Gram negatives, the predominant isolate was *Escherichia coli*, which respond better to chloramphenicol (62.3%), ciprofloxacin (50.9%) and norfloxacin (50%) compared to other tested drugs. Likewise among Gram-positives, the predominant isolate was *Staphylococcus aureus*, which respond better to ciprofloxacin (61.9%) and ampicilline and norfloxacin (57.1%) compared to other tested drugs. The effectiveness of these drugs against the tested organisms in our study could be the reflection of infrequency prescription of these drugs by the physicians in Ethiopia and expense of the drugs in addition to the difficulty of administration (personal communication). Even though the above drugs still better compared to other drugs for treatment of UTI, the result showed in reduction of their effectiveness. Previous studies in our set up showed, 98.3% of the organisms were controlled using ciprofloxacin[8]. However, ciprofloxacin which is routinely prescribed for treatment of UTIs in our study area was effective only for 62% of the present isolates. Further more drugs like amoxicillin, erythromycin , chloramphenico, and tetracycline which previously showed good result for almost all bacteria, their effects currently highly reduced[6,8]. The upsurge in antibiotic resistant pattern seen in this study could be due to antibiotic abuse and self medication being practiced in many developing countries including Ethiopia. In addition, exhaustively usage of first line drugs, low cost and availability of these drugs could be another contributing factor for antibiotic resistance in our locality.

Conclusion

This study revealed 25.6% prevalence of symptomatic bacteriuria among UTI suspected patients. This is worrisome because UTI may have serious consequences like renal failure that cost the life of an individual. Multi drug resistances bacteria were common and even the effectiveness of some

drugs like ciprofloxacin, amoxicillin, erythromycin are reduce overtime. The findings have no doubt highlighted the need for constant monitoring of susceptibility of specific pathogens in different populations to commonly used antimicrobial agents. These data may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies, to compare local with national data and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse or overuse of antibiotics. Finally, since the hospital environment is a sort of collection agency for many pathogenic microorganisms by virtue of the many seriously ill patients who passes through it, it is extremely important for the hospital managements to do everything possible to minimize the spread of these organisms to other patients.

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