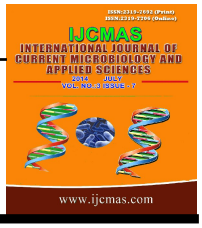
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 7 (2014) pp. 984-994**
http://www.ijcmas.com



Original Research Article

Urinary Tract Infection of Patients and Antibiotic Susceptibility Patterns of Enterobacteriaceae in IBB city Yemen

Gamal A. A-Ameri[1], Mawhoob N.O.Alkadasi[2], A.M.H.Sallam[1], Abdulrahman S.Naji[2] and Abdulbaset A. Zaid[3*]

[1]Microbiology Department of Medical collage, Taiz Univeresity
[2]Department of Chemistry, Zabid education collage, Hudaiadah University
[3]Post graduate and research centre, Maulana Azad College, Aurangabad, India
**Corresponding author*

Keywords

Urinary tract infection and antibiotic susceptibility in Ibb city, Yemen

A B S T R A C T

The primary objective of this study was to identification and determination of the prevalence urinary tract infection of patient and antibiotic auscetibility pattern of Enterobacteriaceae. total of 113 midstream urine specimens were collected from patient during the period from December 2008 to May 2009 to investigate the prevalence of bacterial rate in UTI patients.. Antimicrobial susceptibility testing was performed by using disc diffusion method. A total of 113 specimens UTI, distributed (39/113), (34.5%) males and (74/113), (65.49%) females. Total 94% (106/113) urine samples showed significant bacterial growth. The highest urinary tract infection was detected in the age groups 20-40 with percentage 51.3%, which distributed among 16/58male (27.58%) and 42/58 female (72.41%).The most common pathogens were Escherichia coli 38 (58%), followed by klebsiella 14 (21%), proteus pps 4 (6%) and others10 (15%). Ampicilln(I)was more resistant for (18/41) E.coli with a ratio 43.9% , (8/9) Klebsiella with a ratio 88.9% and (6/8) Proteus with a ratio 75% , (AM) for (15/41) E.coli with a ratio 36.6% ,(8/9) Klebsiella with a ratio 88.9% and (6/8) Proteus with a ratio 75% ,and (CP) for (11/41) E.coli with a ratio 26.8% ,(7/9) Klebsiella with a ratio 77.8% and (5/8) Proteus with a ratio 62.5% however, Ofloxacin (OF) was more sensitivity for (24/41) E.coli with a ratio 58.5% , (8/9) Klebsiella with a ratio 88.9% and (5/8) Proteus with a ratio 62.5% , Ciprofloxacin (CL) for (21/41) E.coli with a ratio 51.2% ,(5/9) Klebsiella with a ratio 55.6% and (5/8) Proteus with a ratio 62.5%, and Gentamycin (J) for (19/41) *E.coli* with a ratio 46.3% ,(8/9) Klebsiella with a ratio 88.9% and (5/8) Proteus with a ratio 62.5% .

Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases and serious ailment in humans due to the frequency, recurrence and difficulty in eradication poses stiff challenge to the medical professionals.

It is important because it may involve kidneys, ureters, bladder and urethra [1]. It has been reported in all age groups and in both sexes [2]. However, women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy

and easy contamination of the urinary tract with fecal flora [3]. UTIs are the most common infections seen in the hospital settings, and second most common infections in the general population [4]. UTIs are also commonly acquired, often due to contaminated urinary catheters, such infections tend to be more serious because the bacteria that cause them are often resistant to drug treatment and patients are often in poor general health [5].

In most cases UTIs are not life threatening and causes reversible damage, however, when a main urinary organ kidneys are involved the risk of irreparable tissue damage and bacteremia increased [6]. Gram negative bacteria play an important role in UTI. *Escherichia coli* remained the most common causative agent of uncomplicated UTI for many years with 75-90% causes of UTI infection [7, 8, 9]. The other gram negative pathogens causing UTI are *Klebsiella spp., Proteus mirabilis and Pseudomonas aeruginosa,* however, *Enterococci* and coagulase negative *Staphylococci* are the most frequently encountered gram positive bacteria in UTI [10].

Proteus mirabilis causes urinary tract infections (UTI) primarily in the complicated urinary tract, most frequently in patients with indwelling catheters or structural abnormalities of the urinary tract. However, in patients with structural or functional abnormalities in their urinary tracts or patients with long-term catheterization, up to 44% of the urinary tract infections are caused by *Proteus mirabilis* [11]. Due to the production of urease by this organism, infection with *Proteus mirabilis* not only develops into cystitis and acute pyelonephritis but also causes stone formation in the bladder and kidneys [12]. This urolithiasis is a hallmark of infection with this organism [13].

The prevalent pathogens of UTIs have been found to be resistant to most chemotherapeutic agents [14], though the antimicrobial susceptibilities of these pathogens are highly predictable. Development of resistance to these antimicrobial agents in UTI cases will therefore affect future treatment and management of the infection with these drugs. Adequate treatment and control of these conditions need a good knowledge of the bacteria species involved and their susceptibility to antimicrobial agents [15]. Majority of the treatments begins or is done completely empirically, the knowledge of the organisms, their epidemiological characteristics and their antibacterial susceptibility is therefore mandatory [16]. Data obtained are essential to optimize the treatment and avoid the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failure [17].

The primary objective of this study was to identification and determination of the prevalence of risk factors associated with the existence of Enterobacteriaceae as the causative pathogen in patients with UTIs admitted to internal medicine departments. We also assessed the susceptibility and resistance of isolated urinary tract bacterial pathogens to commonly used antibiotics.

Materials and Methods

Isolation site

The study was undertaken at Ibb city of Yemen between December 2008 and May 2009. The total study population was 113 patients that chosen from free different hospitals (Alqadree hospital, Alnasr hospital, and Clinical laboratory). Different clinical samples of urine collected from 113 patients suspected of suffering from infectious diseases of the urinary tract were

cultured to isolate the organisms. Demographic data (such as age, sex, in-patient and outpatient status) of the patients was recorded prior to sample collection. There were no ethical matters concerned with this study, as results from routine laboratory diagnosis of clinical samples constituted the data for analysis; no particular identifiable group of patients were involved and their individual identities could not be traced. The urine specimens were examined macroscopically for the colour and turbidity. In addition that the microscopic examination was performed according to procedure of Cheesbrough.(1993) [18] to investigate, Pus cells (W.B.Cs), Bacteria cells, Red blood cells, Casts and crystal. The urine specimens were examined by chemical reagent strip tests for estimation of protein, nitrite and leucocytestrase.

The strip was inserted to the urine specimen, removed a strip, reading the result reaction according to manual. Clean-catch midstream urine was collected from each patient into a sterile test tube container and samples were cultured on cysteine lactose electrolyte deficient agar (CLED), using a calibrated drop delivering 0.002ml of urine. Streaked culture plates were incubated at 37oc overnight. On the next day, the bacterial growth on the respective media was looked, and total colony count was done. A single colony was picked from culture plates with significant bacteriuria (105 colony forming units per ml urine of one or two isolate (s) and was suspended in nutrient broth, and then sub cultured onto blood agar and MacConkey agar and finally incubated at 37oc for further purification. Pure isolates of bacterial pathogen were preliminary characterized by colony morphology, gram-stain, Indole test, Motility test, Oxidase test, Iron utilization test, Citrate utilization test and catalase test. A standard biochemical procedure was used for full identification of gram- positive and gram negative bacteria. Antimicrobial susceptibility testing was performed for bacterial isolates by using agar diffusion method described by Bauer et al., 1966 on Mueller-Hinton agar (oxoide) [19]. After a pure culture was obtained, a loop full of bacteria was taken from a colony and was transferred to 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 a Mc far land 0.5 tubes measured at 500 nm absorbance 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 plates. The inoculate plates were left at room temperature to dry for 3-5 minutes while the Petridis lids was in place. By using sterile forceps, appropriate antimicrobial discs were evenly distributed on the inoculated plates.

Results and Discussion

A total of 113 midstream urine specimens were collected from patient during the period from December 2008 to May 2009 to investigate the prevalence of bacterial rate in UTI patients. A total of 113 specimens UTI, distributed (39/113), (34.5%) males and (74/113), (65.49%) females (Table 1).

In present study revealed that positive specimens for UTI showed positive bacterial growth were 94% (106/113). However, 6% (7/113) showed negative growth (Figure 1).

The findings of this study showed that (70/106) of the total positive growth were Gram negative bacilli predominant

organisms with a ratio (66%) and the gram positive bacteria isolates were (36/106) with a ratio (44%) (Figure. 2).

Over all the gram-negative bacteria isolated found that, 94%, belonged to *Enterobacteriaceae* and 6% *Pseudomonas* (Figure.3).

The most commonly isolated bacteria were *Escherichia coli* 38 (58%), followed by *klebsiella* 14 (21%), *proteus* pps 4 (6%) and others10 (15%) (Figure.4).

As presented in Figure.5, *E.coli* was detected among 14/21 female (67%) and 7/21 male (33%). *Klebsiella* on the other hand was isolated from 6/8 females (75%) and 2/8 males (25%) of the isolates. However, Proteus was the same distribution in male 50% (1/2) and female 50% (1/2).

The ages of patients ranged between 12 to 65 years. The highest urinary tract infection was detected in the age groups 20-40 with percentage 51.3%, which distributed among 16/58male (27.58%) and 42/58 female (72.41%) then followed by age group 41-56 years with percentage of 29.2%, which distributed among 17/33, male (51.51%) and 16/33female (48.48%), whereas the lowest in the urinary tract infection was detected in the age groups 12-19 which composed19.47% which distributed among 6/22, male (27.27%) and 6/22, male (27.27%), (Table1 and 2).

The sensitivity testing was done to Enterobactericeae isolates (58/106) for investigate the sensitivity of Enterobactericeae in specimen of urinary tract infection isolates. The disc diffusion method was employed by used several antibiotics, were used for the sensitivity test Gram negative bacteria. 12 discs ring antibiotic were obtained from Research & Development Oriented Comp. India. These antibiotics include : Amikacin (AK) ,Amoxicillin (AM) , Ampicillin (I) , Cefotaxime (CX) ,Ceftazidime (CZ) ,Ceftriaxone (XO) ,Cefuroxime (CR), Cephalexin (CP) ,Ciprofloxacin (CL) ,Gentamycin (J) ,Ofloxacin (OF). In presnt study shown that the Sensitivity test Ofloxacin (OF), Ciprofloxacin(CL), and Gentamycin (J) were the most effective antibiotics in vitro, against enterobacteriae and the least effective antibiotics in vitro were Ampicillin (I), Cephalexin (CP), and Amoxicillin (AM) (Table5) .In current investigation found that the Ofloxacin (OF) was more sensitivity for (24/41) *E.coli* with a ratio 58.5% , (8/9) *Klebsiella* with a ratio 88.9% and (5/8) Proteus with a ratio 62.5% , Ciprofloxacin (CL) for (21/41) *E.coli* with a ratio 51.2% ,(5/9) *Klebsiella* with a ratio 55.6% and (5/8) *Proteus* with a ratio 62.5%, and Gentamycin (J) for (19/41) *E.coli* with a ratio 46.3% ,(8/9) *Klebsiella* with a ratio 88.9% and (5/8) *Proteus* with a a ratio 62.5% (Figure 6).

However the Ampicilln(I) was more resistant for (18/41) *E.coli* with a ratio 43.9% , (8/9) *Klebsiella* with a ratio 88.9% and (6/8) Proteus with a ratio 75% , (AM) for (15/41) *E.coli* with a ratio 36.6% ,(8/9) *Klebsiella* with a ratio 88.9% and (6/8) Proteus with a ratio 75% ,and (CP) for (11/41) E.coli with a ratio 26.8% ,(7/9) *Klebsiella* with a ratio 77.8% and (5/8) *Proteus* with a ratio 62.5% (Figure 7).

Table.1 Distribution of UTI patients according to age

Age (in year)	NO. of patients	Male		Female	
		NO.	%	NO.	%
< 20	22	6	27.27	16	72.72
20 -40	58	16	27.58	42	72.41
>40	33	17	51.51	16	48.48
Total	113	39	34.5	74	65.49

Fig.1 Percentage of positive growth for U T I specimens

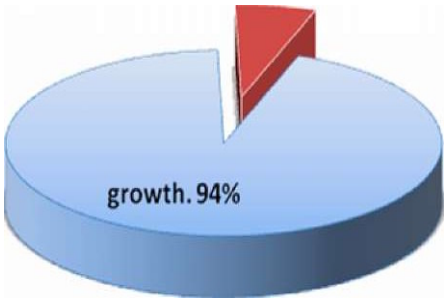


Fig.2 Percentage of Gram positive and Gram negative isolates for UTI

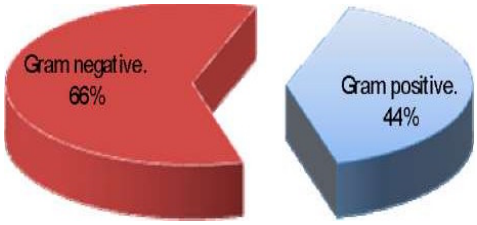


Fig.3 Percentage of isolated Gram negative bacteria

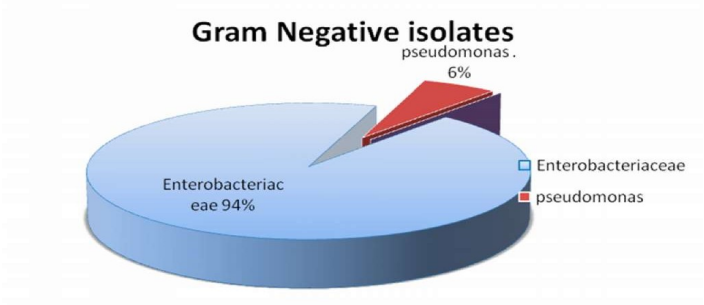


Fig.4 percentage of Enterobacteriaceae for UTI specimen

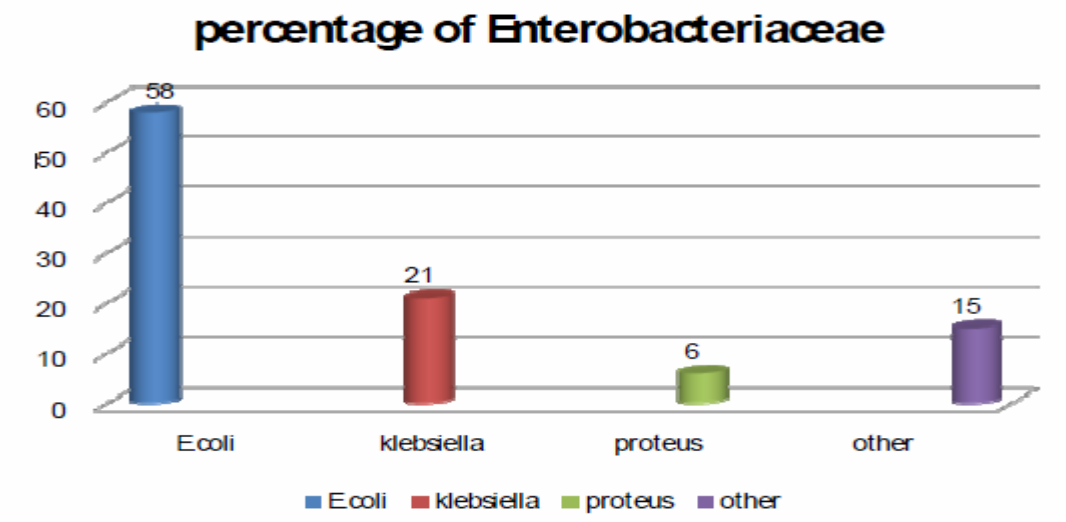


Fig.5 Percentage of Enterobacteriaceae according to age in UTI patient

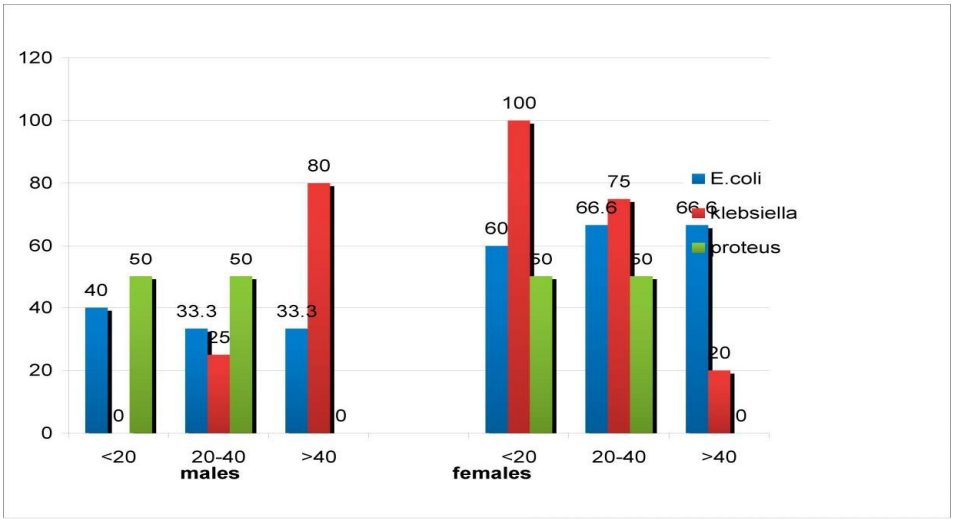


Table.2 Distribution of Enterobacteriaceae isolates with sex of the patients

S.No.	*Bacterial isolates*	Total		Male		Female	
		No.	%	No.	%	No.	%
1	*E. coli*	38	58	13	34.21	25	65.78
2	*Klebsiella*	14	21	6	42.85	8	57.14
3	*Proteus*	4	6	2	50	2	50
4	Other	10	15	1	10	9	90
5	Total	66	100	22	33.3	44	66.7

Fig.6 Percentage of sensitive for antibiotic

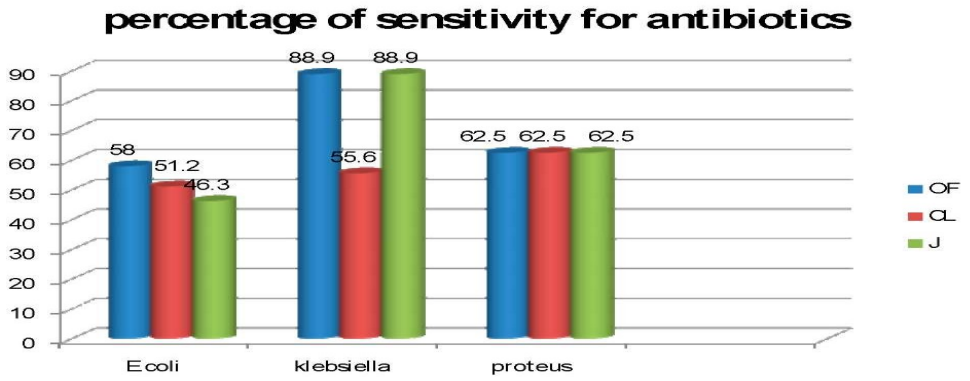


Fig.7 Percentage of resistance for antibiotics

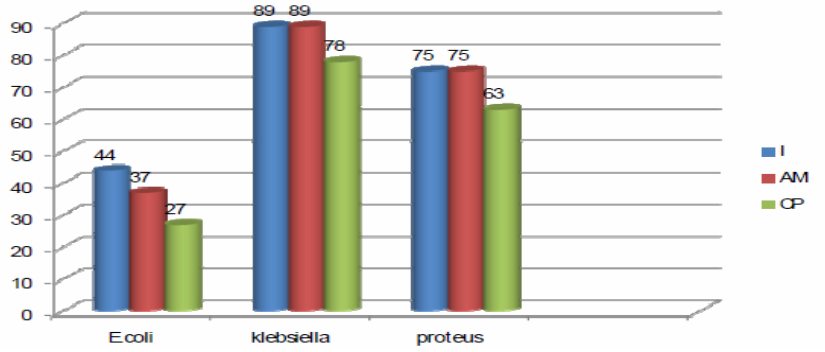


Table.5 The percentages of susceptibility of the different Enterobacteriaceae isolates to the different antibiotics

Ciprofloxacim [CL]	S	21	51.2%	5	55.6%	5	62.5%
	R	3	7.3%	1	11.1%	-	-
Gentamycin [J]	S	19	46.3%	8	88.9%	5	62.5%
	R	5	12.2%	-	-	-	-
Ofloxacin	S	24	58.5%	8	88.9%	5	62.5%
	R	5	12.2%	-	-		
Ampicilin [I]	S	2	4.9%	-	-	-	-
	R	18	43.9 %	8	88.9%	6	75%
Cefotaxme [CX]	S	8	19.5 %	2	22.2%	2	25%
	R	7	17.1%	4	44.4%	3	37.5%
Ceftrazdme [CZ]	S	-	-	-	-	2	25%
	R	8	19.5 %	5	55.6%	3	37.5%
Ceftraxone [XO]	S	9	21.9%	1	11.1%	5	62.5%
	R	3	7.3%	3	33.3%	3	37.5%
Cefuroxime [CR]	S	15	36.6%	_	_	2	25%
	R	6	14.6%	7	77.8%	4	50%
Cephalein [CP]	S	2	4.9%	-	_	2	25%
	R	11	26.8%	7	77.8%	5	62.5%

Bacterial urinary tract infection is one of the serious issues which needed an urgent medical attention in community [20]. The most effective management of UTI patients is the identification of pathogens and selection of effective antimicrobial agent against them [21]. The effective and traditional method for the diagnosis of UTI is plate count method in which >105 bacteria/mL of urine indicates bacteriuria [22, 23]. In present study, gram-negative bacteria were more prevalent (66%) than gram-positive bacteria which constituted 44%. Similar findings have been reported in Tanzania and Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia [24, 25].

In our investigation the *E. coli* was the most predominant bacteria isolated. Similar findings have been reported in Nigeria, Sudan, and Yemen [26, 27, 28] respectively. *E. coli* is considered uropathogenic due to a number of virulence factors specific for colonisation and invasion of the urinary epithelium, such as the P-fimbria and Sfimbria adhesions [29]. Coagulase-negative *Staphylococci* and *S. aureus* were the second and third most predominant pathogen isolated. The findings of this study showed that *E. coli* was the causes of 58% and this finding is in agreement with others finding too [30, 31, 32, 33, 34, 335, 36, 37]. In other study done in Ethiopia on UTI investigation from diabetic patients also showed that E. coli (31.7%) was the most prevalent bacterial isolate from asymptomatic and symptomatic diabetic patients [38]. In The reason of high ratio for *E. coli* is the presence of this bacteria in the feces, thus it cause autoinfection [39]. In addition, after gaining entry to the bladder, E. coli are able to attach to the bladder wall and form a biofilm that resists the body's immune response [40]. *Klebsiella* spp. came in second place and caused 21% followed by *Enterobacter* spp.which was the causative agents of 15%, thus they play important role in urinary tract infection. However, Salvatore et al. [41] claimed that this bacteria together with *Klebsiella, Proteus* and *Pseudomonas a*re uncommon and typically related to abnormalities of the urinary system or urinary catheterization. However, the ratio of Proteus in this study was 6% which was much lower than the that reported by Akram et al. [42] which was (22%).

The ages of patients ranged between 12 - 65 years. The age group 20-40 years were the most urinary tract infection with percentage 51.3%, which distributed among 16/58male (27.58%) and 42/58 female (72.41%) and may therefore be considered as the most prone to *Enterobacter* spp infections (Fuger 5).

The least prone age group to *Enterobacter s*pp infections was 12-20 years which composed 19.47% which distributed among 6/22, male (27.27%) and 6/22, male (27.27%), as shown in table(1 and 3). Increasing resistance against antimicrobial agents is a worldwide problem [43]. This study revealed that there is a higher prevalence rate of resistance against commonly prescribed antibiotics in yemen. A considerable reduction is also found in the activity of nitrofurantoin among the commonly used drugs in treatment of UTI. These findings are supported by other studies done in Kuwait [44] and also in the U.S., southern Europe, Israel, and Bangladesh with up to 50% of E. coli strains being resistant to antibiotics used [45]. The most useful antibiotics in this study were Ofloxacin (OF), Ciprofloxacin(CL), and Gentamycin (J) in 69.8% ,56.4% and 65.9% overall cases

respectively. These drugs are relatively low cost when compared to other antibiotics used. These findings differed from other reports where quinolones are the most effective antimicrobial agent against UTI causing bacteria [46, 47, 48, 49]. The findings have no doubt there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used anti-microbial agents. The data of this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics. However, there was a high prevalence of resistant bacteria to a number of antimicrobials tested in this study. A large number of the isolates were to Ceftrazdme (CZ), Ampicillin (I), Cephalexin (CP), and Amoxicillin (AM). Similar findings have been reported in Iran and Aligarh [50]. This observed resistance to these drugs is a probable indication of earlier exposure of the isolates to these drugs, which may have enhanced resistant development. These drugs are very common due to low cost and often purchased without prescription in different areas.

Total of 113 urine samples, 106 (94%) were positive for urinary tract infection and 6 (6%) were negative. Out of 106 positive growth were 70 (60%) and 36(44%) Gram negative bacilli. The highest urinary tract infection was detected in the age groups 20-40 with percentage 51.3%. *E. coli* was the causes of 58% followed by *Klebsiella* spp. and caused 21% followed by *Enterobacter* spp. which was the causative agents of 15% and *Proteus* in this study was 6%**.** The most useful antibiotics were Ofloxacin (OF), Ciprofloxacin(CL), and Gentamycin (J) in 69.8% ,56.4% and 65.9% overall cases respectively.

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