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Prevalence of antibiotic sensitivity pattern of uropathogens in patients of different age-groups from western region of Nepal

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ABSTRACT

UTI, the most common bacterial infection in urinary tract, is a serious health- problem that occurs in millions of people at any age in each year. Its empirical treatment is difficult worldwide. Local susceptibility-pattern of uropathogens is, therefore, important. To determine prevalence of UTI-associated uropathogens and their antibacterial sensitivity-pattern. The midstream urine samples were collected from patients of different age-groups, followed by examination with semi-quantitative culture method and determination of antibacterial sensitivity-patterns using Kirby-Bauer disc diffusion technique. Data were analyzed in MS-Excel, 2007, and chi-square was used to test the significance. Out of 4872 samples, 34 % showed significant growth of pathogens. The patients were from newborn to 80-years-old. Majority of UTI (55.2 %) were from middle-aged patients with 20-49 years-old and 83.9 % organisms were isolated from females. The gram-negative aerobic rods accounted for 83.11 % prevalence and that of gram-positive was 16.88 %. The infections caused by E. coli (44.33 %), Klebsiella spp. (28.23 %), S. saprophyticus (8.32 %), and S. aureus (6.27 %) were prevalent in middle-aged females. These females were markedly associated with pathogens (χ^2=25.14, p<0.001). The drugs such as levofloxacin, ofloxacin, ciprofloxacin, ceftriaxone and cefotaxime were five most sensitive antibiotics. ***Conclusion:*** *Out of 4872 samples, 34 % showed significant growth. Females were markedly associated with pathogens (χ^2=25.14, p<0.001). The infections caused by E. coli, Klebsiella spp., S. saprophyticus and S. aureus were predominant and drugs like levofloxacin, ofloxacin, ciprofloxacin, ceftriaxone and cefotaxime the sensitive antibiotics.*

INTRODUCTION

Urinary tract infection (UTI) is an infection caused by microorganisms anywhere in the region that comprises of kidney, renal pelvis, ureters, urinary bladder, urethra, and adjacent structures including perinephric fascia, prostate, and epididymis. The bacteria from digestive tract climb at opening of urethra and multiply therein to cause UTI [1, 2, 3]. In contrast to males, females are more susceptible to UTI. More susceptibility of UTI among females is due to short length of urethra, absence of prostatic secretion, pregnancy and easy contamination of the tract with faecal flora [4]. The prevalence of UTI is age- and sex-dependents. During first year of life, the prevalence of UTI is less than 2 % in both males and females. The incidence in males lowers after one year of life and until approximately 60-years of age when enlargement of the prostate interferes with emptying of the bladder. UTI is, therefore, predominantly a disease of females. The previous studies have reported that incidence of bacteriuria in girls at five-years is 1 % and increases to 3 % as age advances to 17-years. The 10-12 % prevalence of bacteriuria in older women increases gradually with age. In 20-40 years-old females who had UTI up to 50 % are more prone to re-infection within one year. The association of UTI with sexual intercourse, in addition, contributes to increased incidence because sexual activity raises the chance of bacterial contamination in female urethra. As a result of anatomical and hormonal changes that favour UTI, the incidence of bacteriuria increases during pregnancy. Further these consequences lead to serious infections in both mother and fetus [5].

UTI are important complications of diabetes, renal disease, structural and neurological abnormalities that interfere with urinal flow. UTI is the leading cause of gram-negative sepsis in hospitalized patients and the origin for about half of all nasocomial infections caused by urinary catheters [6]. It is either symptomatic or asymptomatic. Patients

with significant bacteriuria who exhibit symptoms referable to urinary tract are said to have symptomatic bacteriuria. Asymptomatic bacteriuria is a condition characterized by presence of bacteria in two consecutive clear-voided urine samples both yielding positive cultures ($\geq 10^5$ cfu/ml) of identical uropathogen, in patient without classical symptoms. *E. coli* is major etiologic agent causing UTI which accounts for up to 90 % of cases. *P. mirabilis*, *Klebsiella* species, *P. aeruginosa* and *Enterobacter species* are less frequent offenders. Gram-positive organisms are less common in which Group B *Streptococcus*, *S. aureus*, *S. saprophyticus* and *S. haemolyticus* are recognized organisms [7]. Current management of UTI is empirical without the use of a urine culture or susceptibility testing to guide therapy. However, as with many community-based infections, antimicrobial resistance among the pathogens that cause UTI is increasing as a major health-problem in treatment of UTI [8, 9].

MATERIALS AND METHODS

This was a hospital-based retrospective study, conducted at Nepalgunj Medical College and Teaching Hospital, Kohalpur, Nepal, in different age-group patients who attended the hospital during January, 2013 to December, 2014 to determine prevalence of UTI-associated uropathogens and their antibacterial sensitivity-pattern. The mid-stream urine (MSU) samples were collected in sterile containers, processed in accordance with the ethical standards of institutional experimentation and with the Helsinki's Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/17) and analysed using Microsoft Excel software, 2007.

Specimen collection and processing

The inclusion criteria included patients having symptoms referable to UTI that include frequency, urgency, nocturia, dysuria, suprapubic or loin pain with or without fever. Clean catch mid-stream 10-15 ml urine samples were collected using sterile, wide mouthed container with screw cap tops. The appropriately collected urine specimens were processed in the laboratory within 2 hours after collection and specimens, in case of delayed processing after 2-hour, were kept in refrigerator at 4 °C to avoid multiplication of bacteria at room temperature.

Isolation and identification of organisms

All the samples were MSU-specimens and culture was performed by calibrated loop technique delivering 0.001 ml and plated on Cystine lactose electrolyte deficient (CLED), MacConkey agar and sheep blood agar (SBA) media (HiMedia, India). The inoculated plates were aerobically incubated at 37 ^{0}C for 18-24 h and for 48 h in negatives cases. The specimen was considered positive for UTI if a single organism at concentration of $\geq 10^5$ cfu/ml, or 10^4 cfu/ml plus ≥ 5 pus cells/hpf were observed on microscopic examination of urine [10]. The standard reference strains such as *S. aureus* (ATCC25923), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC 27853) were used as control for culture media. The sterility of culture media was checked on regular basis as per the standards of the laboratory protocol. Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram-negative bacteria were identified by standard biochemical tests [5, 11]. Gram-positive microorganisms were identified with corresponding biochemical tests [10].

A standard calibrated sterile wire-loop was used for inoculation of specimens into culture media. It had 4.0 mm internal diameter designed to deliver 0.01 ml. A loopful of well-mixed samples was inoculated on CLED, MacConkey and blood agar plates.

The streak method was used to uniformly spread urine onto agar surface before aerobically incubating the plates at 37 ^{0}C for 18-24 h. The CLED agar was used because of consistent results and growth of both gram-negative and gram-positive bacterial pathogens with prevention of swarming of *Proteus species* [10]. The plates were then examined for bacterial growth macroscopically after 18-24 h incubation. The bacterial colonies were counted and multiplied by 100 to estimate the number of bacteria per ml urine. A significant bacterial count was taken as any count equal to or in excess of 10000 cfu/ml [12, 13, 14].

Microorganisms were identified with the standard procedures using biochemical tests including gram stain, triple-sugar-iron agar, indole-production, methyl-red, Voges-Proskauer-citrate-utilisation, catalase-production, oxidase-reaction, urease, coagulase, and motility tests [15].

Antibiotic susceptibility testing

Antibacterial susceptibility of isolates was tested by Kirby- Bauer disk diffusion [16]. For gram-negative and gram-positive bacteria, following discs were tested with their respective concentration on Mueller-Hinton agar (HiMedia). Amikacin (AK; 30 µg), ampicillin (AMP; 10 µg), gentamicin (GEN; 10 µg), ciprofloxacin (CIP; 5 µg), nitrofurantoin (NIT; 30 µg), nalidixic acid (NA; 30 µg), norflixacin (NX; 10 µg), amoxyclav (AMC; 20/10 µg), chloramphenical (C; 30 µg), ceftriaxone (CTR; 30 µg), tetracycline (TE; 30 µg), cotrimoxazole

(Trimethoprim/sulphamethoxazole, COT; 1.25/23.75 µg), cefotaxime (CTX; 30 µg) and levofloxacin (LE; 5 µg) [17].

Morphologically identical four to six bacterial colonies from overnight culture plate were picked with standard wire-loop, and emulsified into 5 ml sterile saline in test tube. The saline was stirred with loop to uniformly mix the colony. Turbidity was adjusted to match the standard McFarland 0.5, Biomerieux®. Suspension was inoculated using sterile swab onto Mueller-Hinton agar and antimicrobial impregnated discs were placed on agar surface at minimum distance of 25 mm from each other, and the plates were incubated at 37 ^{0}C aerobically for 18-24 h. The diameter of inhibited zones in each disc were measured using a ruler and compared against the zone-diameter interpretative standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS), 2003 [16, 18]. Results were reported as sensitive, intermediate or resistant for each antibiotics used.

Data Analysis

Data obtained were checked appropriately followed by their entry and analysis using Microsoft Excel Software, 2007. Frequencies, cross-tables and graphs were obtained as appropriate and the chi-square test of association was employed to test the significance.

RESULTS

The study revealed that 34 % of total (4872) MSU samples had significant growth of pathogens. The patients were from the stage of newborn to 80-years old. More cases of UTI were recorded among young and middle-aged patients (20-49 years, 55.18 %). Paediatric patients (newborn to 19 years) comprised of 17.25 % and elderly (50-80 years) constituted 27.56 % of the total number positive culture.

The existence of organisms was 83.59 % from women and 16.1 % from men in total 1658 significant isolates. Gram-negative aerobic rods accounted for 83.11 % while gram-positive cocci were apparent in remaining 16.89 % of total isolated pathogens. While observing by age and sex, prevalence was higher among middle-aged female patients (49.91 %) and lower among young male patients (3.92 %; Table-1).

In all ages, the prevalence of uropathogens revealed that *E. coli* (44.33 %) *Klebsiella spp.* (28.23 %), *S. saprophyticus* (8.32 %) and *S. aureus* (6.27 %) infection were more prevalent. The frequency and distribution of different microorganism are summarised in Table-2 with the test of association between male and female patients by uropathogens. There was significant association of pathogens by sex. Female patients were significantly associated with pathogens (χ^2=25.14, df=7 and p<0.001; Table-2).

Table-1. Distribution of isolated uropathogens in various age- and sex-based groups. Out of 1658 significant isolates, 83.9 and 16.1 % microorganisms were isolated from females and males, respectively. Among these, gram-negative rods were extensive (83.11 %) and gram-positive cocci were less (16.89 %). Altogether, prevalence was higher in middle-aged females (49.91 %) and lower in young males (3.92 %)

Isolated uropathogens		NB-19 years		20-49 years		50-80 years	
		Male	Female	Male	Female	Male	Female
E. coli	N	48	80	35	354	62	156
	%	2.9	7.23	3.3	21.35	7.3	9.4
Klebsiella spp.	N	12	68	26	229	35	98
	%	0.72	4.1	1.57	13.8	2.11	5.9
Proteus spp.	N	3	16	8	48	5	18
	%	0.18	0.96	0.48	2.89	0.3	1.08
Pseudomonas spp.	N	0	10	3	29	4	19
	%	0	0.6	0.18	1.75	0.24	1.145
S. saprophyticus	N	0	20	5	92	3	18
	%	0	1.2	0.3	5.55	0.18	1.08
S. aureus	N	2	18	8	48	6	22
	%	0.12	1.08	0.48	2.89	0.36	1.32
Streptococcus spp	N	0	7	1	22	0	8
	%	0	0.42	0.06	1.32	0	0.48
Enterobacter spp	N	0	2	1	6	0	3
	%	0	0.12	0.06	0.36	0	0.18
Total	**N**	**65**	**221**	**87**	**828**	**115**	**342**
	%	**3.92**	**15.71**	**6.43**	**49.91**	**10.49**	**20.58**

Table-2: Test of association between male and female patients by uropathogens. Females were significantly associated with pathogens

Isolated Uropathogens	Male		Female		Test of significance
	N	%	N	%	
E. coli	145	19.7	590	80.3	χ^2=25.14 df=7 p<0.001
Klebsiella spp.	73	15.6	395	84.4	
Proteus spp.	16	16.3	82	83.7	
Pseudomonas spp.	7	10.8	58	89.2	
S. saprophyticus	8	5.8	130	94.2	
S. aureus	16	15.4	88	84.6	
Streptococcus spp.	1	2.6	37	97.4	
Enterobacter spp.	1	8.3	11	91.7	

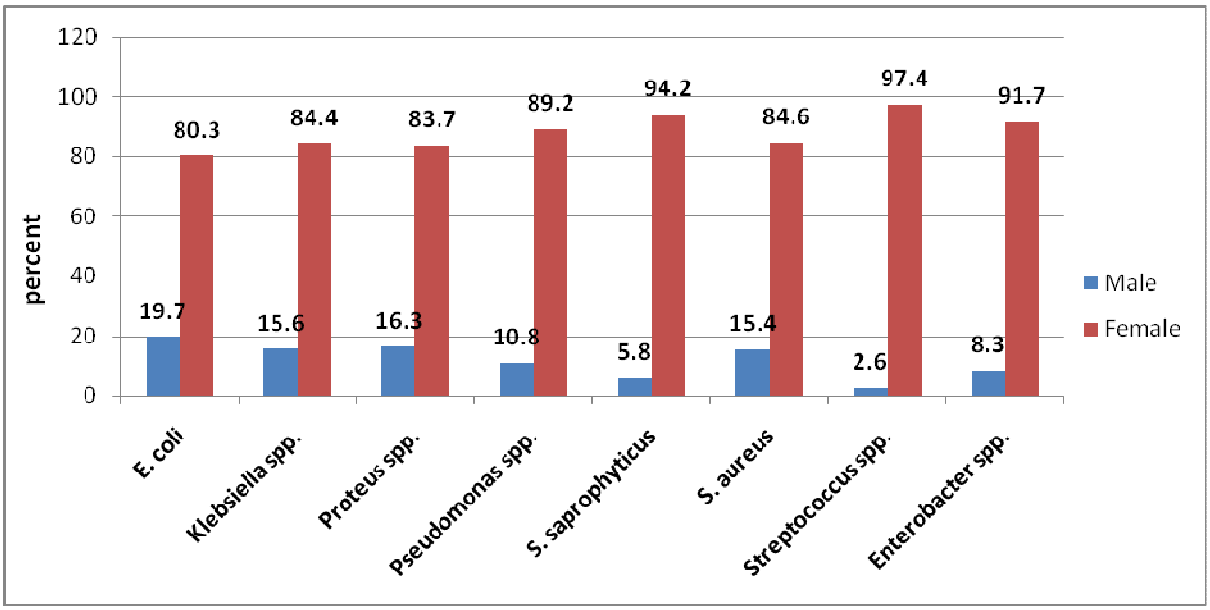

Figure-1. Percent distribution of uropathogens based on sex

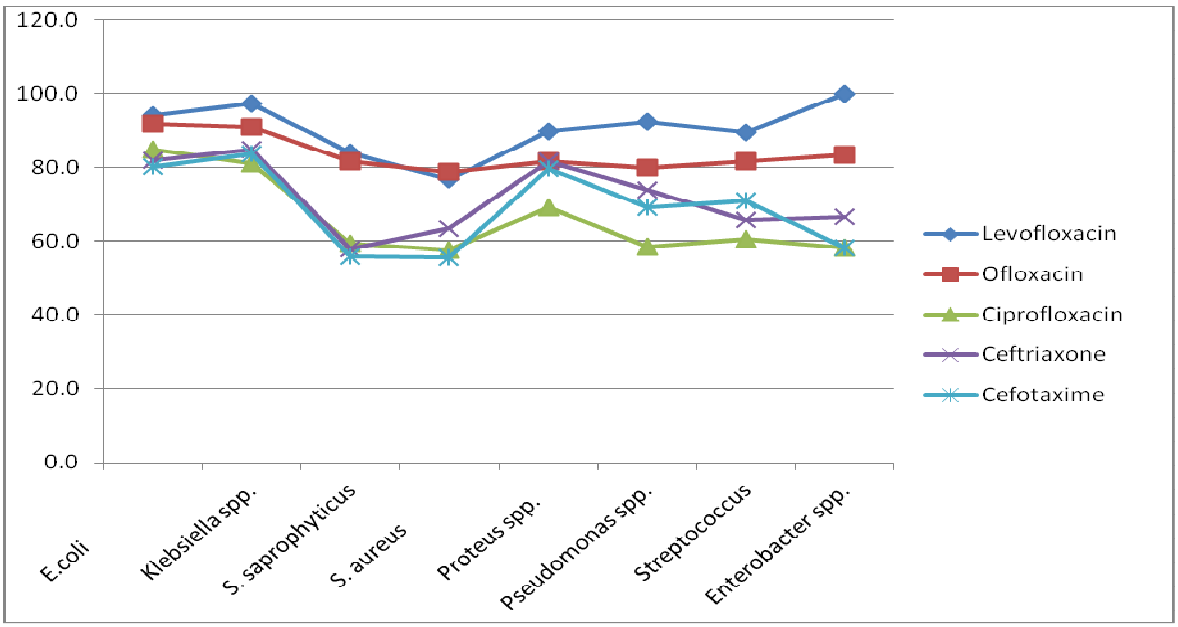

Figure-2. Drug sensitivity by top five antibiotics

Table-3 is representative to overall antibiotic sensitivity pattern of bacterial isolates. Levofloxacin, ofloxacin and ciprofloxacin were found most sensitive antibiotics for various uro-pathogens. Other higher sensitive antibiotics were ceftriaxone, cefotaxime and chloramphenicol.

Levofloxacin was sensitive as 97.2 %, 94 %, 92.3 %, 89.8 % and 89.4 % for the pathogens such as Klebsiella Spp., E. coli, Pseudomonas spp., Proteus Spp. and Streptococcus Spp., respectively. Similarly, Ofloxacin was responsive as 91.8 % and 91.0 % sensitive for E. coli and *Klebsiella spp*. Interestingly, we observed that the drugs ofloxacin showed an equivalent sensitivity i.e., 82 % against both S. saprophyticus and Proteus Spp. By contrast, ciprofloxacin was 85 % sensitive for E. coli and 81% sensitive for *Klebsiella*.

Table-3. Percentage distribution of drug sensitivity by pathogens. Levofloxacin, ofloxacin and ciprofloxacin were most sensitive antibiotics in isolated uropathogens. Ceftriaxone, cefotaxime and chloramphenicol were other higher sensitive antibiotics.

Isolated uropathogens (No) Drug sensitivity		*E.coli* (N- 735)	*Klebsiella spp.* (No- 468)	*S. saprophyticus* (No-138)	*S. aureus* (No-104)	*Proteus spp.* (No-98)	*Pseudomonas spp.* (No-65)	*Streptococcus Spp.* (No- 38)	*Enterobacter spp.* (No-12)
Levofloxacin	N	691	455	116	80	88	60	34	12
	%	94.0	97.2	84.0	76.9	89.8	92.3	89.4	100
Ofloxacin	N	675	426	113	82	80	52	31	10
	%	91.8	91.0	81.8	78.8	81.6	80.0	81.6	83.3
Ciprofloxacin	N	625	380	82	60	68	38	23	7
	%	85.0	81.2	59.4	57.6	69.3	58.5	60.5	58.3
Ceftriaxone	N	603	398	80	66	80	48	25	8
	%	82	85	58	63.4	81.6	73.8	65.8	66.6
Cefotaxime	N	590	392	77	58	78	45	27	7
	%	80.3	83.76	55.8	55.7	79.6	69.2	71	58.3
Chloramphenical	N	588	410	86	68	80	44	26	8
	%	80	87.6	62.3	65.3	81.6	67.7	68.4	66.6
Nitrofurantoin	N	530	360	82	60	68	32	18	7
	%	72.1	76.9	59.4	57.6	69.3	49.2	47.3	58.3
Gentamicin	N	456	351	90	69	70	48	26	6
	%	62	75	65.2	66.3	71.4	73.8	68.4	50
Ampicillin	N	338	190	75	58	28	12	18	3
	%	46	40.5	54.3	55.7	28.5	18.46	47.3	25
Nalidixic acid	N	160	106	18	15	18	3	5	0
	%	21.76	22.6	13	14.4	18.36	4.6	13.1	0
Tetracycline	N	160	90	50	40	15	4	15	1
	%	21.76	19.2	36.2	38.5	15.3	6.1	39.5	8.3
Amoxycillin/ Clavulanic acid	N	40	28	35	46	4	2	16	0
	%	5.4	5.98	33.6	44.2	5.4	3	42.1	0
Co-Trimoxazole	N	22	6	32	19	3	0	5	0
	%	3	1.28	30.7	18.2	3.06	0	13.1	0

DISCUSSION

In MSU-samples (*n=4872*), 34 % showed significant growth of pathogens in the patients from new-born stage to 80-years-old. More cases of UTI were recorded among middle-aged patients (20-49 years; 55.18 %). Paediatric patients (newborn to 19-years) and elderly (50-80 years) constituted 17.24 % and 27.56 %, respectively, of the total number positive culture; proportionately higher organisms were isolated from females (83.9 %). Among 1658 significant isolates, gram-negative aerobic rods had accounted for 83.11 %. While observing by age and sex, the prevalence was higher in middle-aged female patients (49.91 %) compared to young male patients (3.92 %; Table-1).

The infection caused by uropathogens such as *E. coli*, *Klebsiella spp.*, *S. saprophyticus* and *S. aureus* were 44. 3, 28.23, 8.32 and 6.27 %, respectively, suggesting that these pathogens were more prevalent in patients of all ages. Female patients were significantly associated with pathogens (χ^2=25.14, df=7 and p<0.001). Levofloxacin, ofloxacin and ciprofloxacin were most sensitive antibiotics for several uropathogens. Other higher sensitive antibiotics were ceftriaxone, cefotaxime, chloramphenicol.

The hospital-based study, conducted by Chedi *et al.*, in patients with positive urine culture (*n=123*) had shown the results that has been much similar to the observation made by us in present study. In above stydy, *E. coli* was the most encountered uropathogen accounting 39.8, *Proteus spp.* 26 and *Klebsiella spp.* 21.1 %, while *Pseudomonas spp.* was the least that accounted for 0.8 % prevalence. Females (especially at child-bearing-age) had higher frequency of UTI (54.5 %) compared to male counterparts (45.5 %). However, the isolated pathogens showed more sensitivity as 31.3 and 40.6 % to cephalosphorin and flouroquinolones, respectively, and 13.6 % to penicillins (13.6%) in their study which slightly contradicted our present study [19].

Farid J *et al*., conducted cross-sectional study in 200 symptomatic patients during 1st May, 2009 to 31st May, 2012 at Ayub Teaching Hospital, Abbotabad and found more prevalence among females, increased frequency and dysuria

were observed in all patients. *E. coli*, *Klebseilla spp.*, *Staphylococcus spp.* and *Pseudomonas spp.* were more common pathogens. These findings were supportive to the observations that we made in present study [20].

Ahmad S collected clean voided MSU-samples from suspected patients (*n=2190*) during April, 2007 to March, 2009. Among these specimens, 27 % showed significant growth upon culture and approximately 84.1 % patients with UTI were females. Most of them belonged to 21-30 years-old. *E. coli* was most predominant isolate, 53.8 %, followed by *Klebsiella pneumoniae* 22.4 % and *Pseudomonas aeruginosa* 7.6 %. Interestingly, all isolates were fully sensitive to ofloxacin and more than 94 % were sensitive to cefuroxime. This report supports the current study in many dimensions. However, drug sensitivity was in slight contradictory [21].

Arjunan *et al*., collected urine samples from 105 patients, isolated uropathogens and found more than 50 % prevalence of UTI. Women and men especially in age-group 20-29 years-old had higher incidence. *E. coli* was predominant followed by *Citrobacter spp.* and *P. aeruginosa* and the drugs such as norfloxacin, ciprofloxacin and gentamisin were more sensitive. These findings were in accordance with the results obtained by us in many parameters except drug sensitivity [22].

Moreover, other studies had been supportive to current findings. However, certain divergence was observed in sensitivity of the antibiotics against pathogens. The present study has especially focused on prevalence of uropathogens by broad age-group and sex. However, prevalence among detailed age-group by sex and other socio-demographic variables has not been covered herein.

CONCLUSION

In total 4872 MSU-samples, 34 % showed significant growth of pathogens. More cases of UTI were recorded among middle-aged patients (20-49 years; 55.18 %), proportionately higher organisms were isolated from females (83.9 %). In all ages, the infection caused by pathogens such as *E. coli*, *Klebsiella spp*., *S. saprophyticus* and *S. aureus* were more prevalent. Further, female patients were significantly associated with pathogens (χ^2=25.14, df=7 and p<0.001). Levofloxacin, ofloxacin and ciprofloxacin were most sensitive antibiotics for several uropathogens.

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