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The evaluation of antibiotic susceptibility pattern and associated risk factors of UTI in tertiary care hospital of Peshawar

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Abstract: UTIs are majorly caused by species of bacteria in patients of almost all ages. The study was aimed to determine the prevalence rate of uropathogens, its antibiotic susceptibility pattern and associated risk factors. Urine samples were collected from n=470 participants using sterilized containers and were inoculated on culture media. The isolates were identified via gram-staining and biochemical characterization. A total of 43.20% samples were positive. Female contributed the highest prevalence rate, 78.82% as compared to male, 21.18%. The highest prevalence 40.90% was observed in the age-group 31-45, followed by 16-30 with 36.90%. *Escherichia coli* (47.80%) was the most prevalent, followed by *Klebsiella pneumoniae* (18.2%), *Enterococcus faecalis* (12.80%), *Pseudomonas aeruginosa* (10.30%) and *Proteus mirabilis* (7.40%). *Staphylococcus aureus* showed high sensitivity (100%) to amikacin, meropenem, imipenem, fosfomycin, vancomycin, clindamycin and linezolid while in case of *E. faecalis*, vancomycin and linezolid were highly potent. Amikacin and meropenem showed the highest (100%) potency followed by imipenem While Fosfomycin was highly potent to *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* with potency rate 89.97%, 92.31%, 100% and 100% respectively. In the current study, the positivity rate was highly observed in female. *E. coli* and *K. pneumoniae* were found the most ubiquitous for UTI.

Keywords: Urinary tract infections, antibiotic susceptibility testing, risk factors associated with urinary tract infection, bacterial infections, KTH, Peshawar.

INTRODUCTION

Various microbial pathogens particularly bacteria and viruses etc. can cause infection in the urinary tracts of human and other animals is called as urinary tract infections (UTIs)/Urinary tract infections (UTIs), the infections termed as the occurrence of uropathogens such as bacteria, viruses etc. anywhere in the urinary tract (Klein and Hultgren, 2020). The infection is majorly caused by different strains of both gram-positive and gram-negative bacteria which can infect any part of the tract (Santosh and Siddiqui, 2017). The infection when occurs, interferes the normal functions of kidney, ureter, urinary bladder or may target other part of the system (Saleem and Daniel, 2011).

The infection is majorly classified as complicated and uncomplicated UTIs, each of them has correlation with its associated factors such as complicated UTI is associated with factors comprising immunosuppression, pregnancy, urinary retention, renal failure, urinary obstruction, prolong hospitalization and the presence of foreign object like renal transplantation and catheterization required to manage bladder voiding (Levison and Kaye, 2013).Uncomplicated UTI is associated with factors consisting female gender, sexual intercourse, age related changes to the genitourinary tract, prior urinary tract and vaginal infections (Foxman, 2014). If the UTI associated factors are known, then this knowledge can help in prevention and management of UTIs and can prevent the recurrence of UTIs (Nseir *et al*., 2006) because these factors are highly dependent on culture habits (perineal cleaning methods), diaper usage and socioeconomic status of the community set (Sivaraj *et al*., 2015).

On the other hand, antibiotics are the backbone of modern medical practices and have been serving for a long era to treat life-threating infections like UTIs (Carlet *et al*., 2011). A wide range of antimicrobial agents might be recommended to treat UTIs but currently limited antimicrobial agents are suggested due to the emergence of resistance to previously potent antibiotics(Klein and Hultgren, 2020). It is believed that the emergence of resistance in uropathogens can enhance the elevation in

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UTIs. This resistance might be the result of home-medication, misuse and easy approach to antibiotics (Paryani *et al*., 2012). The global research data also claimed that the emergence of resistance in uropathogens makes the treatment difficult against these uropathogens and help in prevailing in the community setup (Khalil *et al*., 2014).

While keeping the above discussion in mind, the present study was designed to find out the causative strains of bacteria, to evaluate the degree of susceptibility pattern of these isolates by Kirby-disc diffusion method and risk factors associated with UTIs to direct the clinicians in the study area towards right antibiotic therapy to prevent UTIs.

MATERIALS AND METHODS

Study area and design

The current research study was designed by department of Microbiology, university of Swabi, Pakistan Health Research Council (PHRC) Peshawar. Sample collection and processing was conducted at PHRC and Khyber Teaching Hospital, Peshawar within six months of duration from November 10th 2020 to May 11th 2021. The patients suspected for UTI and belong to district Peshawar were considered for the study. The patient who satisfied the selection criteria was recruited using simple random sampling method. The patient who failed to give urine, having antibiotic administration history for two weeks, was terminally ill or the female with menstruation or the positive sample other than bacteria was excluded from the current study. Each patient was asked with his/her permission for age, gender, ward and associated factors including catheter, kidney/bladder stone, diabetes mellitus, anatomical disorder of UTI and pregnancy etc.

Sample size determination

Sample size n=470 was calculated using Cochran's formula, $n=Z^2p(1-p)/d^2$. Where, Z is confidence interval which is 1.97, *p* is prevalence, and d is margin of error (Odoki *et al*., 2019).

Sample collection and processing

A convenience-based sample collection was followed in which a total of n=470 midstream urine samples (20-30ml each) were collected both from male and female suspected patients of different wards of KTH including urology ward, gynae ward, medical and surgical ward using sterile container. Each container was closed carefully, labeled properly as name, age, gender, ward and serial number for tracing and was transferred immediately for bacteriological examination to PHRC for further investigation. Each sample was inoculated aseptically on culture media suitable for uropathogens as per microbiology standard and were allowed to incubate at 34±2°C for 24-48 hours. The samples with bacterial growth of CFU>105 were considered as significant growth (Humayun and Iqbal, 2012). In case of congested colonial growth, the culture was purified by sub culturing technique. After colonial morphology, the isolated strains were gram-stained and were examined for the presence of gram-positive and gram-negative bacteria. The isolates were also identified through different biochemical tests (mentioned in table 1).

Antibacterial susceptibility testing

The susceptibility pattern of each isolate was checked via Kirby-Bauer disc diffusion method (Cockerill, 2011) using Mueller Hinton agar (MHA) as prescribed (Ali *et al*., 2020). Prior to test, the inoculum of each isolate was adjusted to 0.5 McFarland index in such that 4-5 similar colonies of pure culture of each isolate were picked up with the help of sterilized wire loop and inoculated into Eppendorf tubes containing nutrient broth. All the tubes were allowed to incubate for almost 7-8 hours at 34±2°C. The medium turned turbid indicated the inoculated culture multiplied. The turbidity of each tube was compared with the standard (0.5 McFarland index). Inoculum from the standard growth of each tube was inoculated on MHA medium with the help of sterilized cotton swab.

Commercially available discs of different antibiotics (Oxoid) were placed with specific distance from each other on the surface of MHA medium (mentioned in Table 6). The plates were allowed to incubate for 18 hours at 34±2°C. On next day, the effectiveness of each antibiotic disc against each pure bacterial isolate was examined by measuring the zone of inhibition in diameter with the help of Vernier caliper. The recorded results of each antibiotic was classified as sensitive and resistance according to the microbiology standard as followed by (Ali *et al*., 2021).

Data analysis

The collected data were analyzed using SPSS version 20.0. The categorical data was cross-tabulated by applying X^2 test (Chi-square test). The bivariate regression was applied on continuous and dichotomous variables while multi logistic regression was applied on multi-chotomous variables. The p-value less than or equal to 0.05 was considered as significant.

Ethical approval

The research study was examined by the institutional bioethical committee of University of Swabi and found in accordance with the ethical principles and policies followed by this university.

RESULTS

A total of n=470 midstream urine samples were collected from indoor-patients of different wards of KTH including gynae, urology, surgical and medical wards with the ages ranging from 17-70 years with the mean age 36.34±10.94

years. 43.20% (n=203/470) of the total collected samples confirmed the growth of different bacteria on suitable culture media. Female participants were more prevalent for UTI than male with frequency n=160/203 (78.82%) and n=43/203 (21.18%) respectively.

While distributing and comparing the positive frequency among the age-groups of patients, high frequency n=83/203 (40.90%) was observed in the age group 31-45 years, followed by age-group16-30 years with frequency rate n=75/203 (36.90%) and the age-group 46-60 years with frequency n=41/203 (20.20%) while no frequency was observed in the age-group 1-15 years fig. 1.

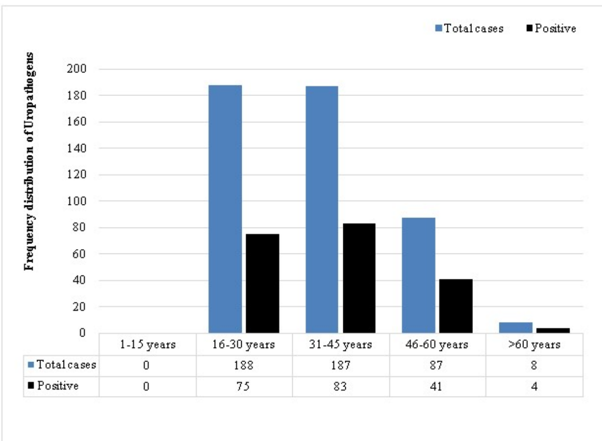

Fig. 1: Age wise frequency distribution of positive cases

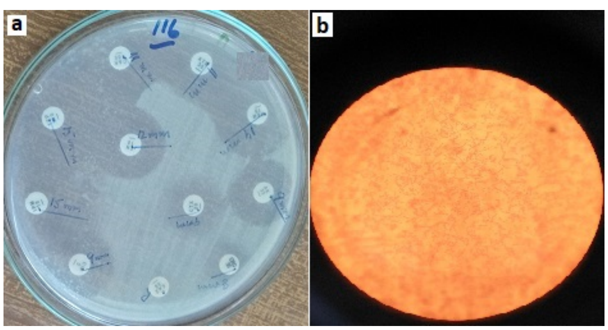

Fig. 2: The gram-reactivity, growth and Zone of inhibitions of *P. aeruginosa*.

While distributing the frequency of uropathogens isolated, the highest bacteriuria was observed in female patients (78.82%) in comparison to male (21.18%). Among the uropathogens, *E. coli* contributed the highest frequency rate, n=97/203 (47.80%), followed by *K. pneumoniae* n=37/203 (18.20%), *E. faecalis* n=26/203 (12.80%), *P. aeruginosa* n=21/203 (10.30%) and *P. mirabilis* n=15/203 (7.40%) whereas *S. aureus* n=7/203 (3.40%) contributed the lowest bacteriuria. Furthermore, each of the isolates were found prevalent in female as compared to male. Gender wise frequency distribution of each uropathogen is given in table 2.

While applying the binary logistic regression analysis on variables, the logistic values obtained were female gender with OR=0.233; 95% CI: 0.142-0.349; $p<0.05$. Gynae ward with OR=0.604; 95% CI: 0.195-1.864; $p>0.05$. Surgical ward with OR=0.564; 95% CI: 0.266-1.195; $p>0.05$. Urology ward with OR=1.488; 95% CI: 0.673-3.291; $p>0.05$. Catheter with OR=0.390; 95% CI: 0.129-1.881; $p>0.05$. Bladder/Kidney stones with OR=0.240; 95% CI: 0.077-0.750; $p<0.05$. Diabetes mellitus with OR=1.075; 95% CI: 0.346-3.336; $p>0.05$. Anatomical disorder of UTIs with OR=0.711; 95% CI:0.207-2.441; $p>0.05$. Pregnancy with OR=0.651; 95% CI: 0.181-2.344; $p>0.05$(table 3).

The isolated bacterial strains exhibited variance in their susceptibility pattern when tested (table 4 and 5). Only two species (*E. faecalis* and *S. aureus*) were confirmed as gram-positive when screened microscopically. Both of the species exhibited various susceptibility pattern to selected antibiotics. *S. aureus* was 100% sensitive to the antibiotics including AK, MEM, IMP, FOS, VA, DA and LZD, followed by CIP, TZP, CAZ and CTX with 28.57% each. Whereas DOX, E and P exhibited no potency. *E. faecalis* was highly sensitive to LZD (100%), followed by VA (92.31%) (table 4).

Only four species of bacteria were confirmed as gram-negative which showed variance in their susceptibility pattern to selected antibiotics. Among the gram-negative species, *E. coli* was highly sensitive to AK and MEM (100% each), followed by TZP, IMP, and FOS (98.97% each). *P. aeruginosa*, *K. pneumoniae* and *P. mirabilis* exhibited almost same result as all the three were sensitive to AK, TZP, MEM and IMP (100% each), followed by FOS (100%, 97.30%, 100% respectively) whereas the rest of antibiotics were highly resistant as mentioned in table 5.

DISCUSSION

As compare to rest of microbes the contribution of bacterial pathogens to cause Urinary tract infections (UTIs) is relatively high (Hossain *et al*., 2020). Different strains of bacteria have been isolated from the patients suffering from UTIs (Asl *et al*., 2017, Gharbi *et al*., 2019). These infections are associated with dozen of risk factors which facilitate the uropathogens to be prevalent easily in the community set and cause the UTIs among the population (Edlin *et al*., 2013). The current study also aimed to investigate different strains of bacteria from the collected urinary samples of UTIs suspected patients, which were associated with different risk factors.

The overall positivity rate of UTIs was found 43.20% (n=203/470). The results indicated that, the prevalence of UTIs caused by bacteria is very predominant in the study area. A previous similar study also found 32.2%

Table 1: Results of microscopy and biochemical tests applied against bacterial isolates.

Test	Results					
Gram-staining	N	N	N	N	P	P
Microscopy	Rod	Rod	Rod	Rod	Cocci	Cocci
Catalase	P	P	P	P	N	N
Oxidase	N	N	N	P	N	N
Coagulase	N	N/A	N/A	N	N/A	P
Indole	P	N	N	N	N	N
H_2S	N	N	P	N	N	N
Citrate test	N	P	P	P	N	P
TSI	Y/Y	Y/Y	Y/R	R/R	Y/R	Y/Y
Pathogen	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*E. faecalis*	*S. aureus*

N-negative, P-positive, Y/Y-yellow/yellow, Y/R-yellow/red, R/R-red/red, N/A-not applied to

Table 2: Gender wise frequency distribution of bacterial isolate

Bacterial isolates		Total n(%)	Male n(%)	Female n(%)	*P-value*
Gram-negative	*Escherichia coli*	97 (47.78)	15 (34.88)	82 (51.25)	0.110
	Klebsiella pneumoniae	37 (18.23)	14 (32.56)	23 (14.38)	
	Proteus mirabilis	15 (7.39)	2 (4.65)	13 (8.13)	
	Pseudomonas aeruginosa	21 (10.34)	5 (11.63)	16 (10.0)	
Gram-positive	*Enterococcus faecalis*	26 (12.81)	5 (11.63)	21 (13.13)	
	Staphylococcus aureus	7 (3.45)	2 (4.65)	5 (3.13)	
Total		203 (100)	43 (21.18)	160 (78.82)	

Table 3: Dichotomous logistic regression analysis of UTI, gender, wards and risk factors

Variable	Level	OR	95% CI	p-value
Gender	Female	0.223	0.142-0.349	0.000
	Male	1		
Wards	Gynae	0.604	0.196-1.864	0.381
	Surgical	0.564	0.266-1.195	0.135
	Urology	1.488	0.673-3.291	0.326
	Others	1		
Associated factors	Catheter	0.390	0.129-1.181	0.096
	Bladder/Kidney stone	0.240	0.077-0.750	0.014
	Diabetes mellitus	1.075	0.346-3.336	0.901
	Anatomical disorder of UTIs	0.711	0.207-2.441	0.588
	Pregnancy	0.651	0.181-2.344	0.512
	Other	1		

OR-Odd ratio, 95% CI-confidence interval

(n=86/267) positivity rate of UTI in suspected patients, which is comparatively lower than recent findings. It may be due to the unhygienic condition in the study area or geographical changes (Odoki *et al*., 2019).

Findings of the current study reported that, the female population was more prevalent for UTIs with prevalence rate 78.81% (n=160/203) in the study area as compared to male population, 21.18% (n=43/203). The main reasons due to which female population is more infected with UTI, may include pregnancy, unhygienic condition in the community set, or other high-risk factors such as the female genitalia is closed to the anus. The previous study links the similar results and findings with the current study. They also found the female population more prevalent than the male population (Hossain *et al*., 2020).

The data reveals that, the uropathogens (Fig. 1) were highly prevalent in the patients with the age group ranging from 31-45 with prevalence rate 40.90% (n=83/203), followed by the patients with age group ranging from 16-30 years with the prevalence rate of 20.20% (n=41/203). The previous study observed the similar findings in their study conducted by Sajed *et al*. (2014) in Lahore. They found that, the patients both male and female with the age group ranging from 16-45 years are highly prevalent with uropathogens. While another study reported the highest prevalence of isolated uropathogens among the patients with age ranging from 20-45 years (Odoki *et al*., 2019). In the current study, the patients with the age less than 15 years have no positivity, which indicated the resistance by this age groupe agianst uropathogens. The similar results with slight diversions

Table 4: Susceptibility pattern of gram-positive isolated strains of bacteria

Antibiotic (code)	*Staphylococcus aureus*		*Enterococcus faecalis*	
	S n(%)	R n(%)	S n(%)	R n(%)
Linezolid (LZD)	7 (100)	0 (0.0)	26 (100)	0 (0.0)
Fosfomycin (FOS)	7 (100)	0 (0.0)	24 (92.31)	2 (7.69)
Doxycycline (DOX)	0 (0.0)	7 (100)	24 (92.31)	2 (7.69)
Ciprofloxacin (CIP)	2 (28.57)	5 (71.43)	2 (7.69)	24 (92.31)
Vancomycin (VA)	7 (100)	0 (0.0)	5 (19.23)	21 (80.77)
Penicillin (P)	0 (0.0)	7 (100)	0 (0.0)	26 (100)
Amikacin (AK)	7 (100)	0 (0.0)	NA	NA
Ceftazidime (CAZ)	2 (28.57)	5 (71.43)	NA	NA
Cefotaxime (CTX)	2 (28.57)	5 (71.43)	NA	NA
Imipenem (IMP)	7 (100)	0 (0.0)	NA	NA
Meropenem (MEM)	7 (100)	0 (0.0)	NA	NA
Clindamycin (DA)	7 (100)	0 (0.0)	NA	NA
Erythromycin (E)	0 (0.0)	7 (100)	NA	NA
Piperacillin/Tazobactam (TZP)	2 (28.57)	5 (71.43)	NA	NA

S-sensitive, R-resistance, NA-not applied to, n-number

Table 5: Susceptibility pattern of gram-negative isolated strains of bacteria

Antibiotic (code)	*P. mirabilis*		*E. coli*		*K. pneumoniae*		*P. aeruginosa*	
	S n(%)	R n(%)	S n(%)	R n(%)	S n(%)	R n(%)	S n(%)	R n(%)
Fosfomycin (FOS)	15 (100)	0 (0.0)	96 (98.97)	1 (1.03)	36 (97.30)	1 (2.70)	21 (100)	0 (0.0)
Piperacillin/ Tazobactam (TZP)	15 (100)	0 (0.0)	96 (98.97)	1 (1.03)	37 (100)	0 (0.0)	6 (28.57)	15 (71.43)
Imipenem (IMP)	15 (100)	0 (0.0)	96 (98.97)	1 (1.03)	37 (100)	0 (0.0)	21 (100)	0 (0.0)
Meropenem (MEM)	15 (100)	0 (0.0)	97 (100)	0 (0.0)	37 (100)	0 (0.0)	21 (100)	0 (0.0)
Amikacin (AK)	15 (100)	0 (0.0)	97 (100)	0 (0.0)	37 (100)	0 (0.0)	21 (100)	0 (0.0)
Ciprofloxacin (CIP)	6 (40.0)	9 (60.0)	6 (6.19)	91 (93.81)	4 (10.81)	33 (89.19)	6 (28.57)	15 (71.43)
Ceftazidime (CAZ)	6 (40.0)	9 (60.0)	4 (4.12)	93 (95.88)	1 (2.70)	36 (97.30)	6 (28.57)	15 (71.43)
Cefotaxime (CTX)	6 (40.0)	9 (60.0)	4 (4.12)	93 (95.88)	1 (2.70)	36 (97.30)	NA	NA
Piperacillin (P)	6 (40.0)	9 (60.0)	0 (0.0)	37 (100)	0 (0.0)	37 (100)	NA	NA
Doxycycline (DOX)	4 (26.66)	11 (73.34)	4 (4.12)	93 (95.88)	2 (4.41)	25 (94.59)	NA	NA

S-sensitive, R-resistance, NA-not applied to, n-number

were documents in another study. They observed few frequent uropathogens in the patients with age less than 15 years (Odoki *et al*., 2019).

The identification of isolated uropathogens were further validated by gram staining and biochemical examination mentioned in table 1 by the techniques previous described (Gharbi *et al*., 2019, Zalewska-Piątek and Piątek, 2019).Some of the confirmed uropathogens identified through the said techniques includes gram positive, *S. aureus* and *E. faecalis*, and gram-negative species including *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* were same as conducted by the previous studies (Asmat *et al*., 2021, Mishra *et al*., 2013).

The current study isolated uropathogens from mid-stream urine samples which causes bacteriuria both in male and female patients including *E. coli*, *Enterococcus faecalis*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *P. mirabilis* species. The previous studies also isolated similar strains of uropathogens. Odoki *et al*. (2019) isolated same bacterial strains including *E. coli*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *P. mirabilis*. Another study Sajed *et al*. (2014) isolated *E. coli*, *Klebsiella spp*., *S. aureus*, *P. mirabilis* and *Pseudomonas* species responsible for urinary tract infections both in male as well as female patients.

Among the uropathogens, *E. coli* was the most prevalent amongst uropathogens with prevalence rate 47.80% (n=97/203) table 1. These findings are agreed by the two previous studies (Odoki *et al*., 2019, Sajed *et al*., 2014). The prevalence of *E. coli* is much higher in female with 51.20% (n=82/160) as compared to male patients with 34.90% (n=15/43). Similar results were documented for female patients in the previous studies (Jia *et al*., 2021, Asmat *et al*., 2021). The high prevalence rate of *E. coli* in female population may be due to the closeness of anus to the vaginal canal or it may be possible due to the adherence and colonization ability of *E. coli* to the urinary tract. *K. pneumoniae* and *E. faecalis* were reported the second and third highest prevalent bacteria respectively. The previous study conducted by Sajed *et al*. (2014) also claimed the species of *Klebsiella* the second highest frequent uropathogens after *E. coli*. Species of *P. aeruginosa* and *Proteus* were reported the lowest frequent uropathogens in the current study. This line of study was also supported by the previous study conducted in Lahore. The research team reported both *P. aeruginosa* and *Proteus* as the lowest prevalent uropathogens among patients (Sajed *et al*., 2014).

The statistical analyses revealed after binary logistic regression that the two variables female gender and Kidney/Bladder stones were highly significant. It means that these variables are highly associated with the UTIs. Similar findings were reported by the previous study conducted in Uganda (Odoki *et al*., 2019). Other selected variables did not shows closed association with the UTIs as given in table 3.

All the isolated strains of bacteria exhibited variance in their susceptibility pattern against the selected antibiotics. table 4 revealed that, in case of gram-positive strains, *S. aureus* was highly sensitive (100%) to most of the selected antibiotics in the current study including AK, MEM, IMP, FOS, VA, DA and LZD while *E. faecalis* was highly sensitive to VA and LZD. Similar studies were conducted in which similar results were recorded for the susceptibility pattern of gram-positive bacterial isolates. Santosh and Siddiqui (2017) found in their study that *S. aureus* 100% sensitive to LZD and VA while other antibiotics showed better potency.

The results also concluded that, AK and MEM were highly potent antibiotics against all gram-negative isolates table 5. Followed by IMP and TZP with same susceptibility pattern 98.97%, 100%, 100% and 100% against *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* respectively. While FOS was highly potent to all gram-negative isolates including *E. coli*, *K. pneumoniae*, *P. mirabilis* and *P. aeruginosa* with potency rate of 89.97%, 92.30%, 100% and 100% respectively. The previous study agreed with the sensitivity results of gram-negative isolates of the current study. They also observed that, the same gram-negative bacterial isolates were highly sensitive to AK, IMP, MEM while it showed better sensitivity against other selected antibiotics (Santosh and Siddiqui, 2017).

CONCLUSION

It was concluded in the current study that 43.20% of collected samples were positive in which female population contributed the most. So, it means that females are at high risk to UTIs in the study area. Furthermore, *E. coli* and *K. pneumoniae* are the major cause of bacteriuria and it was also concluded that the risk factors were highly associated with the UTIs. All the antibiotics showed variance in their potency, so, routine wise UTI diagnosis is highly recommended. If the proper diagnosis was ignored, the bacteriuria may go high and may become resistant to the potent antibiotics in the study area.

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