Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/18834
DOI URL: http://dx.doi.org/10.21474/IJAR01/18834

RESEARCH ARTICLE

A STUDY ON IN VITRO ACTIVITY OF FOSFOMYCIN AGAINST ENTEROBACTERIACAE UROPATHOGEN ALONG WITH MICROBIOLOGICAL PROFILE AND ANTIBIOGRAM OF UROPATHOGENS

Dr. Parimala T.V and Mrs. Swaruparani

Manuscript Info

Manuscript History
Received: 31 March 2024
Final Accepted: 30 April 2024
Published: May 2024

Key words:-
Uropathogen, Enterobacteriaceace, Multi Drug Resistant Organisms, Fosofomycin

Abstract

Background: Urinary tract infection (UTI) is one of the most common infection throughout the world that may affect both the lower and the upper urinary tracts.

Aims & Objective:The present study was done to determine various organisms causing Urinary tract infections and to analyse antimicrobial sensitivity patterns of those isolates.

Materials and Methods: This prospective study was conducted from April 2020 to March 2021at SIMS& RH, Tumkur. All urine samples were received in Microbiology laboratory for routine microbiology investigations were inoculated on Blood agar and MacConkey agar culture media using calibrated wire loop . Urinary isolates with significant bacteriuria were further processed by conventional biochemical tests and antimicrobial susceptibility testing was done as per standard methods.

Results: A total of 697 urine samples were processed in that 324 (46.48%) samples were shown as significant growth of bacteria. Most of the cases seen in (67.28%) female and under age group of 21-30(22.22%).The predominantly Gram-negative bacteria (85.18%) were isolated followed by Gram positive bacteria(10.49%) and Candida spp(4.32%). The Gram negative bacteria showed high sensitivity to Ampicillin sulbactum (88.27%) followed by Amikacin (87.24%). All Gram-positive bacteria isolates is showed sensitivity to Vancomycin (100%).We found that 235(92.88%) Enterobacteriaceace isolates were is susceptible for Fosfomycin**.**

Conclusion:. Antibiotic resistance has become a major clinical problem worldwide and has increased over the years.Hence antibiotic susceptibility testing should be done to diagnose and treat the drug resistant UTI cases. Fosfomycin may be given empirically in patients with uncomplicated urinary tract infection due to Enterobacteriaceace.

Introduction:-

Urinary Tract Infection (UTI) may be symptomatic/asymptomatic . The diagnosis of symptomatic UTI usually straight forward, based on symptoms and signs and support from laboratory data. It can affect lower and upper urinary tract. It may be acquired from community or hospital .Global annual incidence of UTI is 150 million costing about 6 billion dollar per year. Appoximately 10% of human will have a UTI at some times during their live .

Corresponding Author:- Dr. Parimala T.V

Urinary tract infections (UTI) are caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the uroepithelium. Proliferation of bacteria in the urinary tract is the cause of urinary tract infection. Most commonly UTI are caused by Gram negative enteric bacilli like Escherichia coli, Klebsiella spp .Proteus spp and Gram positive organisms like Staphylococcus saprophyticus, Staphylococcus aureus ,Enterococci.

Usually UTI is managed empirically, leading to antimicrobial agents misuse, development of multi-drug resistance among urinary pathogens and failure of empirical therapy. The challenge of antimicrobial resistance , along with the paucity of novel antibiotic , highlight the need to re evaluate older alternatives. These include fosfomycin trometamol(FOF), a stable salt of fosfomycin has a unique structure there is minimal cross resistance with other antibiotics. At present , many multidrug resistant isolates remain susceptible to fosfomycin , even in geographic regions where there has been widespread use of the drug. Also In the last two decades it has been observed that trend of bacterial isolates obtained and their antibiotic sensitivity pattern keeps on changing .Objective of this study was to determine various pathogens causing Urinary tract infections and their antimicrobial sensitivity patterns of those isolates. And also to evaluate invitro activity of Fosfomycin against Enterobacteriaceae Uropathogens.

Material and Methods:-

A prospective study was conducted from April 2020 to March 2021 at SIMS& RH, Tumkur. All urine samples were received in Microbiology laboratory for routine microbiology investigations from both outpatient & inpatients belongs to all age groups & both genders were included.

Sample collection, transport, and storage:

The clean catch mid-stream technique was employed to collect urine samples. For catheterized patient-urine was collected through the draining portal of the urinary catheter using aseptic precaution. The specimens were transferred to the laboratory as quickly as possible, usually within 1 hour after collection and processed as soon as possible. When the processing is delayed more than 1-2 hours, they were stored at $4^{o}C$ up to 4 hrs.

Processing of urine specimen:

Samples were inoculated on Blood agar and MacConkey agar culture media using calibrated wire loop containing 0.001 ml of urine sample and were incubated aerobically at $37^{o}C$ for 18-24 hours. The colony count of 10^5 CFU/ml for mid-stream urine and $>10^3$ CFU/ml in catheterized urine sample was taken as Significant Bacteriuria.

Identification Of The Isolates And Antibiotic Susceptibility Testing:

The identification of the organisms was done by Conventional biochemical tests. Antimicrobial susceptibility testing was done by Kirby Baur disc diffusion method and the interpretation of antibiotic susceptibility was done as per CLSI guidelines.

Quality Control:

E. coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, E. faecalis ATCC 29212 strains were used for quality control of biochemical test and antibiotic sensitivity test.

Statistical Analysis:

Data were analysed using Microsoft Excel 2010 version. Discrete values were expressed as percentages. Descriptive statistics were used to summarize patient characteristics and the prevalence of antimicrobial resistance.

Ethical Consideration:

Ethical approval was obtained from the research and ethical committee constituted by the college of Shridevi Institute of Medical Sciences and Research Hospital, Tumkur. Informed consent was also sought and obtained from the patients. All results were treated with utmost confidentiality.

Results:-

In the present study, a total of 697 urine samples were processed, in that 324 (46.5%) samples were shown as significant growth of bacteria.

Table 1:- Gender wise distribution of uropathogens.

S.no	Gender	Number	Percentage
1	Male	106	32.71%
2	Female	218	67.28%
	Total	324	

Among these 324, 218(67.28%) belonged to female and 106 (32.71%) belonged to male (**Table 1)**.

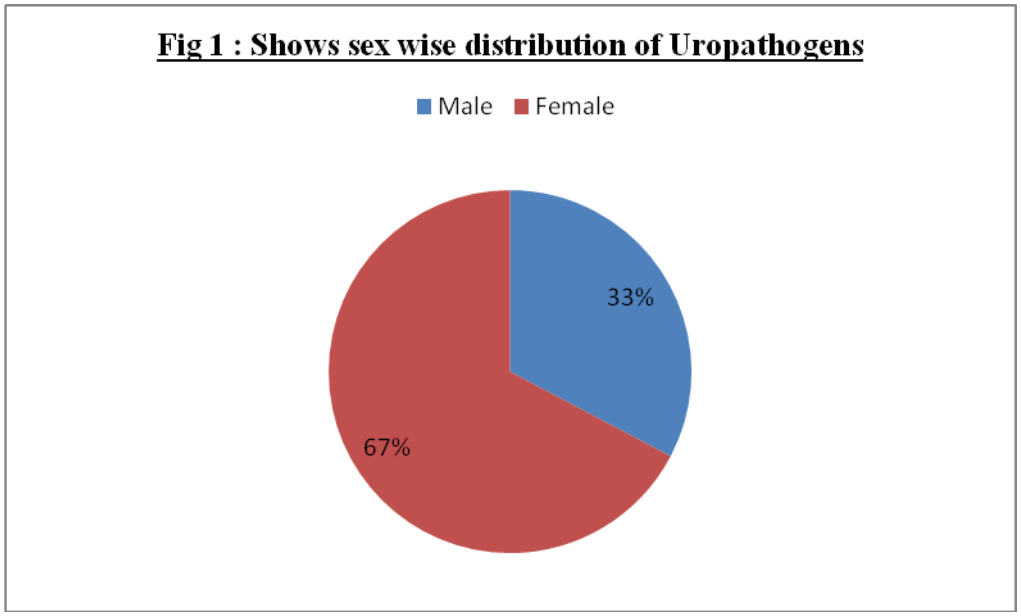

Table 2:- Age wise distribution of uropathogens.

S/no	Age group	Number	Percentage
1	1-10 years	18	05.55%
2	11-20 years	30	09.25%
3	21-30 years	72	22.22%
4	31-40 years	49	15.12%
5	41-50 years	36	11.11%
6	51-60 years	43	13.27%
7	61-70 years	45	13.88%
8	71-80 years	31	09.56%
TOTAL		324	

Most number of cases having Urinary tract infection were fell under age group of 21-30 72 (22.22%), followed by 31-40 age group 49 (15.12%) and then in age group 65-70 45(13.88%) (**Table 2)**.

Table 3:- Pathogens isolated from Urine samples of UTI patients.

Sr.no	ORGANISM	Number of isolates	PERCENTAGE
1	Escherichia coli	158	48.76%
2	Klebsiella pneumoniae	50	15.43%
3	Proteus species	28	8.64%
4	Staphylococcus aureus	24	7.40%
5	Pseudomonas aeruginosa	19	5.86%
6	Candida species	14	4.32%
7	Enterobacter species	11	3.39%
8	Enterococcus species	10	3.08%
9	Citrobacter species	6	1.85%
10	NFGNB	4	1.23%

The most common isolates in this study have been the Gram-negative bacilli which accounts for 276(85.18%) of the total positive isolates. The predominant isolate was the Escherichia coli 158(48.76%) followed by Klebsiella spp 50(15.43%) , Proteus spp 28(8.64%). The main Gram positive organism identified was Staphylococcus aureus 24 (7.40%) and Enterococcus spp 10 (3.08%)**(Table 3)** .

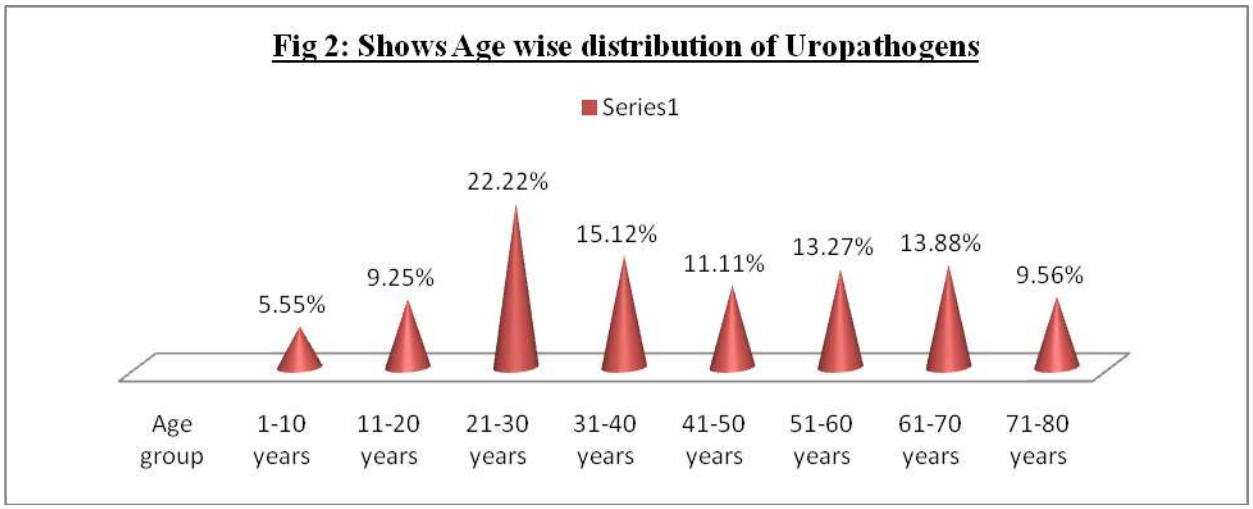

Table 4:- Antibiotic susceptibility pattern of Gram-negative organisms(N=276).

S/NO	ANTIBIOTICS	SENSITIVE %	RESISTANT %
1	Fosfomycin	264(91.03%)	26 (8.96)
2	Ampicillin sulbactum	256(88.27)	34(11.72)
3	Meropenem	250(86.20)	40(13.79)
4	Amikacin	253(87.24)	37 (12.75)
5	Cefuroxime	244(84.13)	46(15.86)
6	Piperacillin	222 (76.55)	68 (23.44)
7	Nitrofurantoin	215(74.13)	75 (25.86)
8	Imipenem	212 (73.10)	78 (26.89)
9	Tetracycline	193 (66.55)	97 (33.44)
11	Cefepime	154 (53.10)	136(46.89)
12	Cot-Trimoxazole	145(50%)	145 (50.0%)
13	Ciprofloxacin	106 (36.55)	184 (63.44)
14	Ceftazidime	122(42.06)	168(57.93)
15	Cefotaxime	81(27.93)	208 (71.72)
17	Nalidixic acid	54 (18.62)	236 (81.37)
18	Ampicillin	0 (0%)	290 (100%)

The Gram negative bacteria showed high sensitivity to Amipicillin sulbactum(88.27%)followed by Amikacin(87.24%) and Meropenem(86.2%). Highly resistant to Ampicillin (100%), followed by Nalidixic acid (81.37%), Cefotaxime (71.72%) and Ciprofloxacin (63.44%)**(Table 4).**

Table 5:- Antibiotic susceptibility pattern of Gram-positive organisms.

S/NO	ANTIBIOTICS	SENSITIVE %	RESISTANT %
1	Vancomycin	34(100%)	0 (0%)
2	Ampicillin	34(100%)	0 (0%)
3	Teicoplanin	30(88.23%)	4 (11.76%)
4	Gentamycin	29 (85.29%)	5(14.70%)
5	Nitrofurantoin	21 (61.76%)	13 (38.23%)
6	Tetracyclin	19(55.88%)	15 (44.11%)
7	Ciprofloxacin	6(17.64%)	28(82.35%)
8	Cefoxitin	6(42.85%)	8(57.14%)
9	Erythromycin	3(08.82%)	31(91.17%)

Note: Cefoxitin disc was used only for staphylococcus isolates.

The Gram-positive bacteria showed high sensitivity to Vancomycin (100%) followed by Amoxycillin (100%) and Teicoplanin (88.23%). Majority of them were resistant to Erythromycin (91.17%) and Ciprofloxacin 28(82.35%) **(Table 5)**.

Table 6:- Susceptibility of Fosfomycin among Enterobacteriaceace uropathogens.

Enterobacteriaceae spp	Fosfomycin susceptibility		
	Sensitive %	Resistant %	
Escherichia coli	147 (93.03%)	11 (6.96%)	158
Klebsiella pneumoniae	43 (86.00%)	7 (14.00%)	50
Proteus species	28 (100%)	0 (0%)	28
Enterobacter species	11 (100%)	0 (0%)	11
Citrobacter species	6(100%)	0 (0%)	6

Among Enterobacteriaceace isolates, Proteus species, Enterobacter species, and Citrobacter species isolates were showed 100 % sensitive to fosfomycin followed by 93.03% of Escherichia coli isolates and 86% Klebsiella spp isolates were sensitive to fosfomycin(**Table 6)**.

Discussion:-

Urinary tract Infection(UTI) are common problem despite age and sex world wide and account for a significant burden of hospital admissions and associated health care expenditure.Periodic surveillance of UTI causing organisms and their antibiotic susceptibility patterns at local level is necessary for effective empirical treatment and crucial in dealing with emerging problems of antibiotic resistance and also provides assistance in management of patients with UTI.

In our study out of 697 samples , 324 (46.48%) showed significant bacteruria which was similar to the study done by Deshkar et al. Some studies reported higher positivity like Vijaya Swetha et al 2014(61.9%) and Maheshwari et al (63.51%) . Our study is higher than Sumanth kumar et al(34.9%) and Mehta et al (36.6%). These variations may be due to differences in the environmental conditions, several host factors, health care practises, standard of living , education and hygiene practices in each geographical area.

In the present study culture positivity was more in females 67.28% than in males 32.71% which correlates with Koripella et al (64.50%) ,Kumar et al (65.1%) and Dnyaneshwari Puroshottam et al (66%), showing a statistically predominance of females with UTI. The elevated incidence of infection among females is related to difference between the male and female genitourinary systems in anatomy and microflora. Highest number of culture positive isolates were from patients between 21-30 yrs of age which is comparable to the studies of Kumar et al[18] and Ghadge et al can be explained by the fact that urinary tract infection are more common in reproductive age group.

Gram negative bacilli(85.18%) contributed more of the total bacterial isolates than gram positive cocci(10.49%) in causing UTI in our study which correlates with Kanaujia et al and Deshkar et al which can be due to several factors like adhesion , pilli, fimbriae and P1 blood group phenotype factors. Escherichia coli was the common organisms causing UTI in this study followed by Klebsiella spp. These results are consistent with previous studies by Naveen Kumar Chaudry et al (52% &16%)[19] and Karishetti et al (56.60% &13%).Among Gram positive organisms Staphylococcus aureus (7.04%) was found to be most common organisms causing UTI which is similar to the results of study by Pardeshi et al while in other studies it was Enterococcus spp followed by Staphylococcus aureus (Karishetti et al).

In our study Gram negative bacilli were highly sensitivity to Ampicillin Sulbactum (88.27 %), Amikacin (87.24%) and Meropenem (86.20%) .They were highly resistant to Nalidixic acid (81.37%) and Ciprofloxacin(63.44%) and Cotimoxazole (50%).Aminoglycosides was still highly sensitive to most of the bacterial isolates most probably due to less use as it was an injectable drug. Resistance to fluroquinolones is on rise as evident from different study done world wide. Fluroquinolones like nalidixic acid & ciprofloxacin and Cotrimoxazole for UTI are more than or equal to 50% of isolates were resistant to it. Studies conducted by Mahajan et al, Raina et al and Mukherjee et al showed similar antibiotic pattern.

All the isolates of Staphylococcus aureus and Enterococcus spp were sensitive to Vancomycin which is similar to the study conducted by Reshmi Gopalakrishnan et al where all of the Staphylococcus isolates and Enterococcus sp were vancomycin sensitive. The prevalence of MRSA in urinary isolates in our study was 57.14% while study from Garg et al was 66.6% Variations in sensitivity may be due to the inappropriate exposure of different localities as to antibiotics which can drive the development of resistance. Lack of guidelines for appropriate use of antimicrobial agents is another factor responsible for spread of resistance.

On studying the antibiotic susceptibility pattern for Fosfomycin , we found that 235(92.88%) is susceptible for Enterobacteriaceace isolates.In other similar studies done by Nanditha Pal et al[25] Fosfomycin was susceptible to 362(93.29%) where as study by Mamoria VP et al found that 526(89%) was susceptible for Enterobacteriaceace isolates.

In this study Fosfomycin resistance against Escherichia coli was 6.96% and Klebsiella spp was 14% Mathew et al[11] in their studies they found 19% of Klebsiella spp and 1% of E.coli urinary bacterial isolates were resistant to fosfomycin. Recent observational studies have demonstrated fosfomycin efficacy in uncomplicated urinary tract infection caused by resistant organisms including non inferiority to carbapenems .In addition , fosfomycin is recommended as a first line treatment for uncomplicated UTIs in the latest EAU guidelines on urological infection. The present results support such guidance.

Conclusion:-

The escalation of drug resistance among the uropathogens poses a global threat. Before prescribing an empirical anti-microbial therapy, an in-depth knowledge of the aetiology, the predisposing factors, the culture positivity and the continued evaluation of the susceptibility patterns of the uropathogens to the traditional as well as the new antimicrobials, is essential to avoid irrational drug usage and to ascertain the optimal prophylactic therapy.

Acknowlegement:-

We would like to thank Mr Kuppala Ramesh , Msc MLT technician for his help in doing this study.

Funding:
No funding.

Conflict of Interest:
None declared.

References:-

1. Bonkat G, Bartoletti R, Bruyère F, Cai T, Geerlings SE, Köves B, et al(2022). EAU guidelines on urological infections. European Association of Urology.

2. Chaudary NK and Murthy SM(2013). Urinary tract Infection:Etiology and antimicrobial resistance with reference to adhesive organelles. Journal of Drug Delivery and Therapeutics. ,3(4),554.
3. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing(2020). 30th Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute.
4. Deshkar DW, Narute JV and Somvanshi, VD (2019). Bacteriological Profile and Antibiogram of Uropathogens- A Retrospective Analysis. Int.J.Curr.Microbiol.App.Sci., 8(08), 2464-2471.
5. Gardner BJ, Stewardson AJ, Abbott IJ and Peleg AY(2019). Nitrofurantoin and Fosfomycin for resistant urinary tract infections:old drugs for emerging problems. Australian Presciber .,42(1):14-19.
6. Garg N, Shukla I, Rizvi M, Ahmed SA, Khatoon A and Khan F(2015). Microbiological Profile and Antibiotic Sensitivity Pattern of Bacterial Isolates Causing Urinary Tract Infection in Intensive Care Unit Patients in a Tertiary Care Hospital in Aligarh Region, India. Int.J.Curr.Microbiol.App.Sci .,Special Issue-1:163-172.
7. Ghadage DP, Muley VA, Sharma J, Bhore AV(2016). Bacteriological profile and antibiogram of urinary tract infections at a tertiary care hospital. National J Lab Med., 5(4):20–24.
8. Kanaujia, R., Kumar, A.,and Bajpai, M(2020). Determination of nitrofurantoin and fosfomycin susceptibility among urinary Escherichia coli isolates. International Journal of Research in Medical Sciences .,8(9): 3274–3276.
9. Karishetti M and Shaik H(2019). Clinicomicrobial assessment of urinary tract infections in a tertiary care hospital. Indian J Heal Sci Biomed Res.,12(1):69.
10. Koripella R, Kanakadurgamba T, Vasanth K and Peddapalli AP(2020).Bacteriological Profile and Antibiogram of Urinary tract infections in a Tertiary care hospital.International Journal of Research and Review .,7(8):2454-2237.
11. Kumar GLSS, Sreedevi S, Prathyusha Y, Balakrishna J and Lavanya V(2020). Bacterial profile and antibiogram of urine culture isolates in a teritiary care center. IP Int J Med Microbiol Trop Dis., 26(1):6-11.
12. Kumar M, Sinha MK, Sharma AK, Prasad A and Verma A(2018). Antibiogram of the prevalent uropathogens isolated at a tertiary care hospital. International Journal of Medical Research Professionals .,4(2)314-17.
13. Mahajan DD and Bulle PA (2016). Microbiological Profile and Antibiogram of Bacterial Isolates Causing Urinary Tract Infection in Tertiary Care Hospital. IOSR Journal of Dental and Medical Sciences.,15(9) : 145-149.
14. Maheshwary D and Saikumar C(2018). Profile of urinary tract infections and resistance patterns in a tertiary care hospital in India. Int.J.Curr.Microbiol.App.Sci., 7(03): 506-512.
15. Mathews PC, Barett LK, Warren S, Stoessor N,Snelling M,Scarborough M et al(2016). Oral fosfomycin for treatment of Urinary tract Infection:a retrospective cohort study.BMC Infectious Diseases.,16:556.
16. Mehta M, Bhardwaj S and Sharma J(2013). Screening of urinary isolates for the prevalence and antimicrobial susceptibility of Enterobacteria other than Escherichia coli. Int J Life Sci Pharma Res.,3:100–104.
17. Mukherjee S, Mishra S and Tiwari S(2020).Aetiological Profile and Antibiogram of Urinary Isolates Causing UTI in Patients Attending a Tertiary Care Hospital of Western Odisha. Journal of Evolution of Medical and Dental Sciences.,9(9):662-667.
18. Pal N and Majhi B(2017).Evaluation of the spectrum of uropathogens, prevalent antimicrobial resistance and prospects of the newbie " Fosfomycin". IOSR-JDMS.,16(9): 54-59.
19. Pardeshi P(2018). Prevalance of urinary tract infections and current scenario of antibiotic susceptibility pattern of bacteria causing UTI. Indian Journal of Microbiology Research., 5(3): 334 – 338.
20. Paula Pietrucha-Dilanchian and Thomas M.Hootan (2017).Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. American Society for Microbiology .Second edition.Chapter 3.41-68.
21. Raina S and Najotra DK(2019). Bacteriological profile and antibiogram of uropathogens from a tertiary care hospital: A two-year retrospective analysis. Int J Curr Microbiol Appl Sci.,8:1206-12.
22. Reshmi Gopalakrishnan and Chandrasekharamurthy BV(2017). Bacteriological profile and antibiogram of uropathogens among antenatal cases in a tertiary care hospital. Indian J microbial Res.,4:333-337.
23. Thattil SJ and Santhosh S(2018). Prevalence of UTI in Different Age Groups in a Tertiary Care Hospital and their Antibiogram. International Journal of Contemporary Medical Research.,5(1):3-6.
24. Tutone M , Johansen TEB , Cai T , Mushtaq S and Livermore DM (2022). Susceptibility and Resistance to Fosfomycin and other antimicrobial agents among pathogens causing lower urinary tract infections: findings of the SURF study. Int J Antimicrob Agents ,59, 106574.
25. Winn WC, Allen SD, Janda WM, Koneman EW, Procop GW, et al(2006). Koneman's color atlas and textbook of diagnostic microbiology . Philadelphia: Lippincott Williams and Wilkins.
26. Vijaya Swetha V, Rao US, Hema Prakash P and Subbarayudu S(2014). Aerobic bacteriological profile of urinary tract infections in a tertiary care hospital. Int.J.Curr.Microbiol.App.Sci.,3(3): 120-125.