ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
September 2015 Vol.:4, Issue:2

Study of Uropathogens and Its Antibiogram at a Tertiary Care Hospital at Coastal Karnataka

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

SHOBHA K.L[a], JESSICA SUSHMA D'SOUZA[a]*, MEENA ALVA[b], ANAND K.M[a], GOWRISH S RAO[a], L.RAMACHANDRA[c]

[a]Department of Microbiology, Melaka Manipal Medical College, Manipal Campus, Manipal University, Manipal.

[b]Department of Microbiology, Kasturba Medical College, Manipal Campus, Manipal University, Manipal

[c]Department of Surgery, Kasturba Medical College, Manipal Campus, Manipal University, Manipal. India.

Submission: 22 August 2015
Accepted: 3 September 2015
Published: 25 September 2015

www.ijppr.humanjournals.com

Keywords: Urinary tract infection, *E coli*, ESBL's, MDR strains

ABSTRACT

Introduction: Urinary tract infection (UTI) continues to be the commonest infection. Untreated cases can go in for complications. Empiric antimicrobial therapy reduces the complications thereby minimizing the cost of treatment. Since resistance rates differ from place to place, knowledge on prevailing patterns of antimicrobial resistance becomes essential. This study was carried out to investigate the etiologic agents of UTI in a tertiary care hospital and its antimicrobial susceptibility pattern. Materials and methods: Midstream urine samples collected from inpatients as well as outpatients in the hospital were inoculated onto the culture media. Strains having significant growth of 10^5 cfu/ml were processed for identification and antimicrobial susceptibility testing as per the CLSI guidelines. Results: The culture plate showed significant growth from 1368 urine samples (53% female and 47% male patients); of which 75% (1026) were from hospitalized patients. UTI was predominant in adults (86.54%) than in pediatrics (13.44%). *E. coli* (59.94%) was found to be the most common uropathogen followed by *Klebsiella* (16.37%). *E. coli* sensitive to Ampicillin and gentamicin was 43.75%. ESBL *E. coli* were 47.43%, MDR strains were 8.82%. Most of Enterococcus species and *Staphylococcus* species were sensitive to Ampicillin. Conclusion: Ampicillin or Gentamicin can be used for empirical treatment of UTI, but ESBL producers and MDR strains should also be considered. Knowledge on the antibiotic susceptibility testing pattern of the uropathogens is vital for proper treatment of UTI.

INTRODUCTION

Urinary tract infection (UTI) continues to be the commonest infection accounting for approximately 40% of all hospital acquired infections.[1] UTI often results in serious complications like secondary bacteremia and sepsis leading to a rise in mortality in addition to bloating hospital charges. It is therefore imperative to treat UTI empirically in relevant situations where indwelling catheter for long duration is inevitable. Empiric antimicrobial therapy reduces the incidence of development of sepsis, and reduces the average hospital stay thereby minimizing the cost of treatment[2]. In recent years, bacterial resistance to different antibiotics has risen leaving the consultants with few therapeutic options. Methicillin- resistant *Staphylococcus aureus* (MRSA), extended spectrum Beta lactamase (ELBS) producing organisms and vancomycin resistant *Enterococci* (VRE) have become the common pathogens. Since the resistance rates to antibiotics differ from place to place, to start the empiric therapy, knowledge on information on prevailing pattern of antimicrobial resistance among common urinary pathogens is must. This study was therefore carried out to investigate the etiologic agents of UTI in a tertiary care hospital and to study their susceptibility pattern to different antimicrobial agents.

MATERIALS AND METHODS

This was a cross sectional study. After the informed consent, midstream urine samples were collected from both inpatients and outpatients, attending or admitted to Kasturba Medical College Hospital, Manipal from January 2012 to June 2012 suspecting UTI in a wide mouthed sterile container. . Urine samples were inoculated onto the blood agar and MacConkey's agar with four quadrant streaking method following semi quantitative culture technique using a standard loop technique[3]. Strains having significant growth of 10^5 cfu/ml of urine on culture plate were processed for the identification of organisms according to Konemann EW *et al*.[3] Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion method using appropriate antibiotics as per the CLSI guidelines.[4] Ecoli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923 were used as controls. Specimens which yielded a single pathogen were processed further, while those which yielded more than one type were considered as contamination. For gram negative bacilli, catalase positive and oxidase negative antibiotic discs like Amikacin (30 µg), Amoxicillin-Clavulanic acid (20/10 µg),

Ampicillin (10 µg), Cefotaxime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Norfloxacin (10 µg) were used. For Gram negative non fermenting bacilli which were catalase positive, oxidase positive antibiotic discs used were Amikacin (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), piperacilin (100 µg), Aztreonam (30 µg), Cefpirome (30 µg), Imipenem (10 µg), Ceftazidime (30 µg) and Piperacillin-Tazaobactum (100/10 µg).

Screening test for ESBL was done according to the criteria recommended by CLSI[5]. An inhibition zone of ≤ 27 mm for cefotaxime and ≤ 22 mm for ceftazidime indicated that the strain probably produced ESBL.

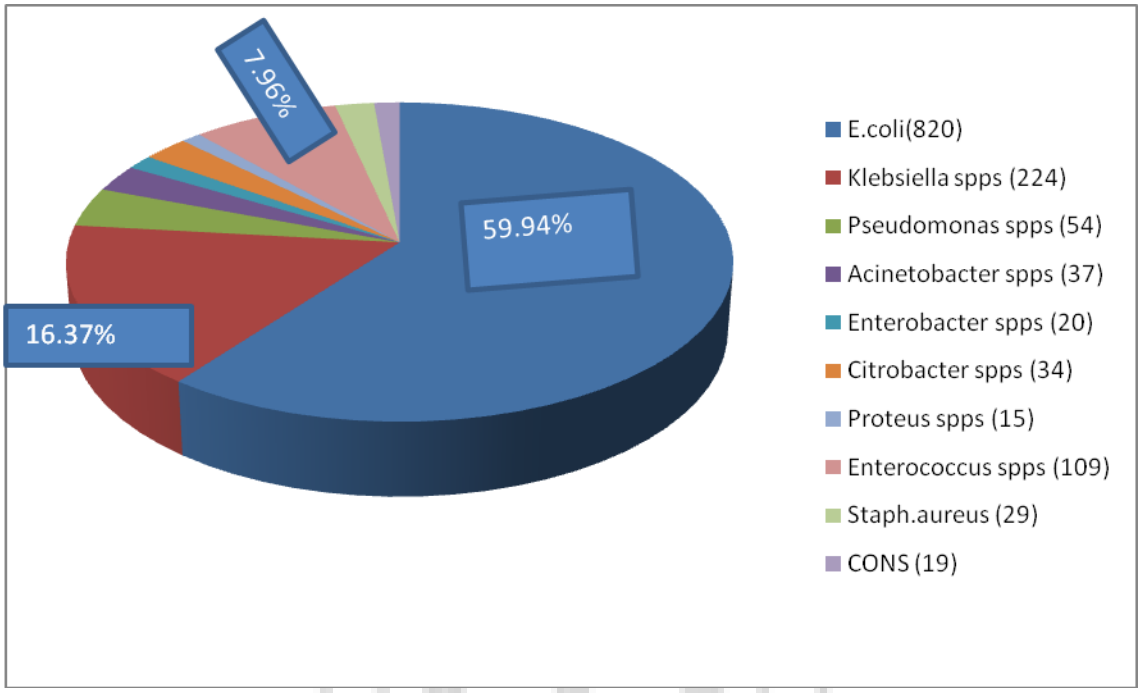
Multidrug resistant strains were tested for colistin susceptibility (10 µg)

Antibacterial susceptibility testing for gram positive cocci included Ampicillin (10 µg), gentamicin (10 µg), gentamicin (120 µg for *Enterococcus*), Norfloxacin (10 µg), Penicillin (10 Units), Linezolid (30 µg), Teicoplanin (30 µg) and Vancomycin (30 µg). *Staphylococcus* species was tested with Cefoxitin (30 µg) for methicillin resistance. All the antibiotic discs were obtained from Hi-media, Mumbai, India.

RESULTS

1368 midstream urine samples had significant growth on blood agar and MacConkey agar. Of these 25% (342) were from outpatient samples and 75% (1026) were from hospitalised patients. Out of the total, 53% (725) were female patients and 47% (643) of them were male patients. UTI was predominantly seen in adults 1184 (86.54%) when compared to pediatric age group 184 (13.44%). Most common uropathogen was found to be *E. coli* 820(59.94%) followed by *Klebsiella* species 224 (16.37%) (Chart: 1). Others included *Candida* species and *Serratia* spp accounting to 7 (0.57%).

Chart 1: Showing different microorganisms isolated



Gram negative bacilli which were catalase positive and oxidase negative and susceptible to Amikacin Amoxicillin-Clavulanic acid, Cefuroxime, Norfloxacin, Ampicillin, Cefotaxime, Cefuroxime and Gentamicin was 43.75% for *E. coli* (Table 1) .

Table 1: Organisms and its antibiotic susceptibility pattern

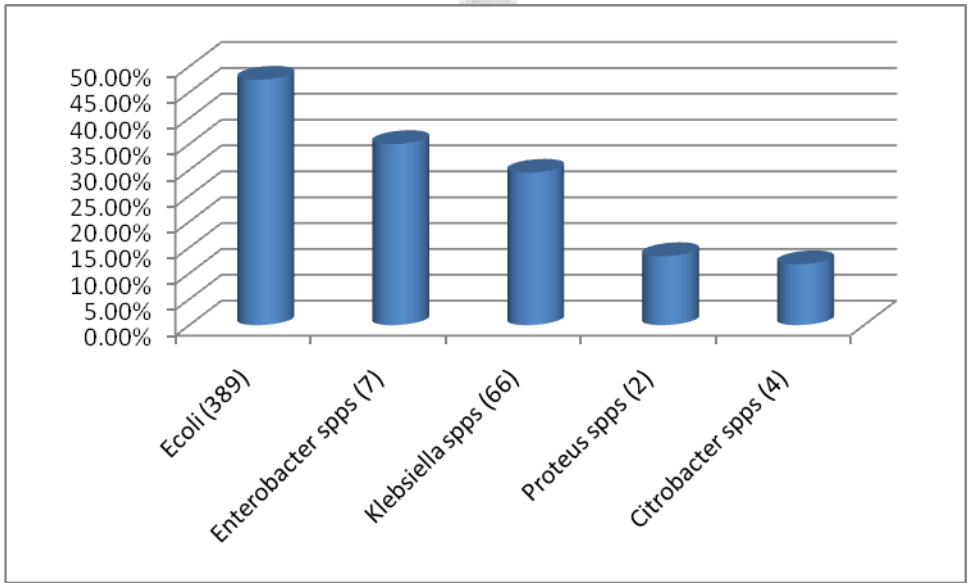
No.	Organisms	Sensitive to (1,2,3,4,5,6,7)*& Percentage
1	*E. coli*	359 (43.75%)
2	*Klebsiella* species	125 (55.81%)
3	*Citrobacter* species	27 (79.42%)
4	*Proteus* species	13 (86.67%)
5	*Enterobacter* species	12 (60%)
6	*Acinetobacter* species	31 (83.79%)

*1=Amikacin (30 µg), 2=Amoxicillin-Clavulanic acid (20/10 µg), 3=Ampicillin (10 µg), 4=Cefotaxime (30 µg), 5= Cefuroxime (30 µg), 6=Gentamicin (10 µg), 7= Norfloxacin (10 µg).

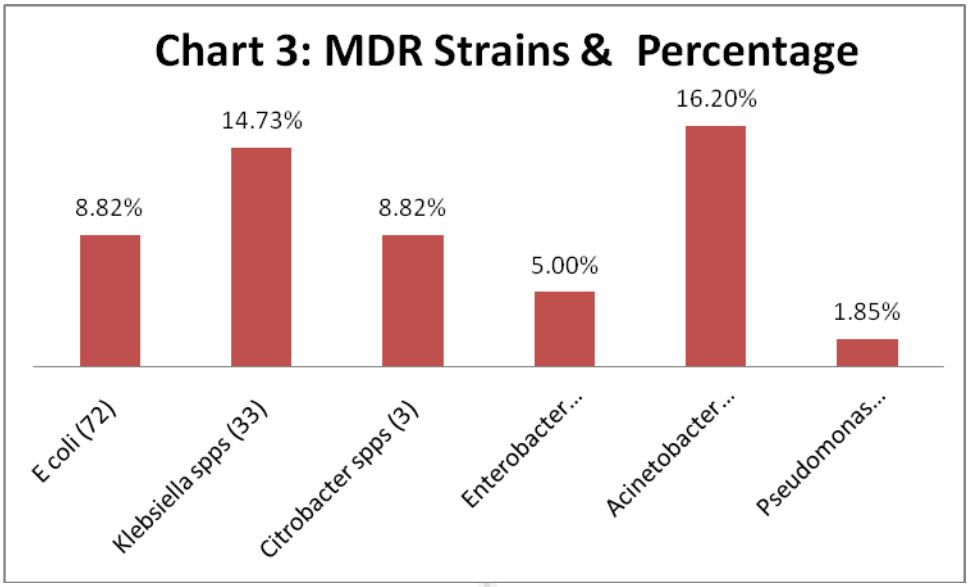
Gram negative bacilli which were catalase positive, oxidase positive and nonfermenters were grouped as *Pseudomonas* species. These organisms sensitive to Amikacin (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), piperacilin (100 µg), Aztreonam (30 µg), Cefpirome (30 µg), Imipenem (10 µg), Ceftazidime (30 µg) and Piperacillin-Tazaobactum (100/10 µg) were 52 strains (95.45%) out of 54 strains.

Gram negative bacilli of Extended Spectrum Beta-Lactamase producers (ESBL) sensitive to Amikacin (30 µg), Gentamicin (10 µg), Norfloxacin (10 µg), imipenem (10 µg) and resistant to Amoxicillin-Clavulanic acid (20/10 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Cefuroxime (30 µg), Cefpirome (30 µg) was 47.43% for *E. coli* (Chart 2).

Chart 2: ESBL producing gram negative bacilli: Numbers and percentage



The gram negative organisms that were multidrug resistant (MDR) bacteria, resistant to Amikacin (30 µg), Norfloxacin (10 µg), Cefpirome (30 µg), Amoxicillin-Clavulanic acid (20/10 µg), Imipenem (10 µg) and the strains were sensitive to Colistin (10 µg) (Chart 3).



Among the gram positive cocci which mainly included *Enterococcus* species and *Staphylococcus* species. Antibacterial susceptibility testing for *Enterococcus* species included Ampicillin (10 µg), gentamicin (120 µg), Norfloxacin (10 µg), Penicillin (10 Units), Linezolid (30 µg), Teicoplanin (30 µg) and Vancomycin (30 µg). *Enterococcus* species had a sensitivity of 38.53% for ampicillin (Table: 2).

Table 2: Antibacterial susceptibility of *Enterococcus* species

Antibiotic disc	**Sensitivity: number and percentage**
Ampicillin (10 µg)	42(38.53%)
gentamicin (120 µg),	39(35.77%)
Norfloxacin (10 µg),	38(35%)
Penicillin (10 Units),	0%
Linezolid (30 µg),	109 (100%)
Teicoplanin (30 µg)	109(100%)
Vancomycin (30 µg).	109 (100%)

Staphylococcus species were classified further as Coagulase positive and Coagulase negative strains. Cefoxitin disc (30 µg) was used to test the methicillin sensitivity .For *Staphylococcus aureus* zone size of ≥20mm was considered sensitive and less than 20 mm was considered as resistant. For Coagulase negative *Staphylococcus* species zone size of ≥25mm was considered sensitive and any zone less than this was considered as resistant4. The antibiotic discs were used to test the sensitivity were Ampicillin (10 µg), gentamicin (10 µg), Norfloxacin (10 µg), Penicillin (10 Units), Linezolid (30 µg), Teicoplanin (30 µg) and Vancomycin (30 µg). Sensitivity to ampicillin in Methicillin sensitive *Staphylococcus aureus* was 65.55% (Table 3).

Table 3: *Staphylococcus* species and their antibacterial susceptibility testing

Antibiotic disc	*Staphylococcus aureus* number and percentage	*Staphylococcus aureus* number and percentage	Coagulase negative *Staphylococcus* species	Coagulase negative *Staphylococcus* species
Cefoxitin (30 µg)	Methicillin sensitive *Staphylococcus aureus* (MSSA) 27(93.10%)	Methicillin resistant *Staphylococcus aureus* (MRSA) 2(6.89%)	Methicillin sensitive Coagulase negative *Staphylococcus* species (MS-CONS) 17(89.4%)	Methicillin resistant Coagulase negative *Staphylococcus* species (MR-CONS) 2(10.34%)
Ampicillin(10 µg)	18(65.55%)	0(0%)	15(88.23%)	0(0%)
Gentamicin (10 µg),	22(81.48%)	0(0%)	16(94.11%)	0(0%)
Norfloxacin(10 µg),	23(85.18)	0(0%)	13(76.47%)	0(0%)
Linezolid (30 µg),	27(100%)	2(100%)	17(100%)	2(100%)
Teicoplanin (30 µg)	27(100%)	2(100%)	17(100%)	2(100%)
Vancomycin (30 µg).	27(100%)	2(100%)	17(100%)	2(100%)

DISCUSSION

Our study showed that the number of patients admitted into various wards and suffering from UTI 75 % (1026) were more than the patients attending the out- patient departments with UTI, 25% (342). Similar studies were also reported by the study conducted by Aruna K et al[6].

We found higher percentage of females suffering from UTI, n= 725 (53%) when compared to males, n=643 (47%). This was in accordance with other studies done in India by Aruna K et al[6] as well as in a study done in Nepal by A Acharya et al[7]. UTI was predominately seen in adults 1184 (86.54%) when compared to paediatric age group 184 (13.44%). Similar results were also published by Colgan R et al[8]. *E. coli* is known to be the important and most prevalent uropathogen according to the study conducted by Sood .S et al[9]and Manjunath GN et al [10]: which was in concordance with our study, however we found in our study , uropathogens like *Klebsiella* species , *Pseudomonas* species , *Citrobacter* species and *Acinetobacter* species to be very less in numbers when compared to their study . Our study showed *Enterococci* to be the predominant Gram positive uropathogen followed by *Staphylococcus*. This study was also opined with the study conducted by Sara M Soto11. Some of the rare organisms like *Serratia* spp and fungi like *Candida* species were also isolated but in very less number, 7 (0.5%). This study was in concordance with the study conducted by KasiMurugan et al[12] and Otajevwo, F. D[13].

Gram negative bacilli which were catalase positive and oxidase negative and susceptible to Amikacin Amoxicillin-Clavulanic acid, Ampicillin, Cefotaxime, Cefuroxime, Gentamicin and Norfloxacin were ranging from 43.75% to 86.67%, but the study done in Southwest Ethiopia by GetenetBeyene[14] showed 100% resistance. *Pseudomonas* species had 95.45% sensitivity to Amikacin, Imipenem, Ceftazidime and Piperacillin-Tazaobactum, whereas study conducted by Syed Mustaq Ahmed et al[15] showed 100% sensitivity .

Gram negative bacilli producing ESBL for *E. coli* was 47.43% and for *Klebsiella* 29.46%. Earlier studies from Shobha et al[16] from the same center showed *E. coli* producing ESBL's were 26.39% and *Klebsiella* species producing ESBL's were 27%. This indicated that the strains producing ESBL's were increased over the years.

Among the multidrug resistant bacteria (MDR), *Acinetobacter* species was the highest (16.20%) followed by *Klebsiella* species (14.73%) and *E. coli*. (8.82%). Similar study conducted by

P. Cornejo-Juárez et al[17]had an overall 39.5% multidrug resistant bacteria, *Acinetobacter* species having highest strains of MDR. All the strains were sensitive to colistin.

Among gram positive bacteria, *Enterococcus* species susceptible to Ampicillin was 38.53% and high level gentamicin was 35.77% and Vancomycin was 100%. This study was in concordance with the study conducted by S. Mohanty et al[18].

Staphylococcus aureus resistant to methicillin was 6.89%, strains susceptible to ampicillin was 65.55%, norfloxacin was 85.18%, gentamicin was 81.48%. Among the Coagulase negative *Staphylococcus* species 10.34% was resistant to Methicillin, 88.23% of strains were susceptible to ampicillin, 94.11% to gentamicin and 76.47% to norfloxacin. Studies done by HadizaHima-Lerible et al [19]showed similar results.

CONCLUSION

E. coli was the predominant uropathogen isolated. Ampicillin or Gentamicin can be the drug of choice for empirical treatment for UTI, but some of the gram negative bacilli causing UTI were ESBL producers and some were MDR strains. Knowledge on the antibiotic susceptibility testing pattern of the uropathogens is vital for proper treatment of UTI because antibiotic susceptibility pattern varies from place to place and changes from time.

CONFLICT OF INTEREST

All authors have none to declare.

REFERENCES

1.Basavaraj CMetri,PJyothi,Basavaraj VPeerapur: Antibiotic resistance in Citrobacterspp isolated from urinary tract infection.Urology Annals Oct-Dec 2013.Vol 5 Issue 4:312-313

2. Larsen EH,Gasser TC, Madsen PO:Antimicrobial prophylaxis in urologic surgery.UrolClin North Am; 1986:13:591-604

3.KonemannEW,AllenSD,Janda WW et al : Koneman's Color Atlas and textbook of diagnostic microbiology 6th edition 2006: LipinCott Williams and Wilkins publications.

4. National Committee for Clinical Laboratory Standards.Methods for diluting antimicrobial susceptibility tests for bacteria that grow aerobically ,5th edition.Approved Standard M7-A5.Wayne,PA:NCCLS,2000

5. Amitha Jain, IndranilRoy,MahendraKGupta,Mala Kumar and S.K Agarwal.Prevalence of ESBL producing gram negative bacteria in septicaemic neonates in tertiary care hospital.J Med Microbiology 2003: 52:421- 425

6. K. Aruna,T. Mobashshera"prevalence of extended spectrum beta-lactamase production among uropathogens in south mumbai and its antibiogrampattern"EXCLI Journal 2012;11:363-372

7. A Acharya, R Gautam and L Subedee "Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal"Nepal Med Coll J 2011; 13(1): 30-33
8. Colgan R, Williams M. Diagnosis and treatment of acute uncomplicated cystitis. *Am Fam Physician*. 2011 Oct 1;84(7):771-6.
9.Sood S, Gupta R. Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. Indian J Community Med Nov 2012;19;37:39-44
10. Manjunath GN , Prakash R , AnnamV , Shetty K"Changing trends in the spectrum of antimicrobial drug resistance pattern ofuropathogens isolated from hospitals and community patients with urinary tractinfections in Tumkur and Bangalore"Int J Biol Med Res. 2011; 2(2): 504 – 507
11. Sara M. Soto, "Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches," Advances in Biology, vol. 2014, Article ID 543974, 13 pages, 2014. doi:10.1155/2014/543974
12.KasiMurugan, Savitha T S. Vasanthi3Retrospective study of antibiotic resistance among uropathogensfromrural teaching hospital, Tamilnadu, India.Asian Pac J Trop Dis 2012; 2(5): 375-380
13. Otajevwo, F. D "Urinary Tract Infection among Symptomatic Outpatients Visiting aTertiary Hospital Based in Midwestern Nigeria"Global Journal of Health Science; Vol. 5, No. 2; 2013
14. GetenetBeyene and WondewosenTsegaye:Bacterial Uropathogens in Urinary Tract Infection and Antibiotic Susceptibility Pattern in Jimma University Specialized Hospital, Southwest Ethiopia: Ethiop J Health Sci. 2011 Jul; 21(2): 141–146.
15.Syed Mustaq Ahmed, Ramakrishna PaiJakribettu, Shaniya koyakutty, Arya B, Shakir VPA:Urinary Tract Infections – An overview on the Prevalence and the Anti-biogram of Gram Negative Uropathogens in A Tertiary Care Centre in North Kerala,India :JCDR:2012 |: September : Volume : 6:| Issue : 7:| Page : 1192 – 1195
16. ShobhaK.L,Ramachandra L, Gowrish Rao, Majumdar S, SugandiP.Rao: Extended Spectrum Beta-Lactamases (ESBL) in Gram Negative Bacilli at a Tertiary care Hospital: JCDR:2009:Feb: Vol:3:,Issue:1: 1307- 1312.
17.P. Cornejo-Juárez, D. Vilar-Compte, C. Pérez-Jiménez, S.A. Ñamendys-Silva, S. Sandoval-Hernández, P. Volkow-Fernández:The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit: International Journal of Infectious diseases 2015:Vol 31: Feb; 31-34
18.S Mohanty, A Kapil, BK Das, B Dhawan"Antimicrobial resistance profile of nosocomial uropathogens in a tertiary care hospital".SMohanty, A Kapil, BK Das, B DhawanIndian J Med Sci 2003;57:148-54
19.HadizaHima-Lerible, Didier Ménard and AntoineTalarmin ."Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in Bangui, Central African Republic"Journal of Antimicrobial Chemotherapy (2003) 51, 192–194.