



**ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF UROPATHOGENIC
ESCHERICHIA COLI IN A TERTIARY CARE CENTRE
AT KANCHEPURAM DISTRICT**

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ABSTRACT

Escherichia Coli is the commonest cause of urinary tract infection (UTI) both in the community and hospital settings. Females experience recurrent UTI in the reproductive age group while men in the older age. Drug resistance among *E.coli* strains has now become a major concern as clinicians are left with very minimal therapeutic options.

AIMS AND OBJECTIVES: To isolate and identify the commonest pathogen causing UTI. To perform the anti-biotic susceptibility pattern of the isolated *E.coli*. To screen for Extended Spectrum Beta Lactamase (ESBL) and Amp C Beta Lactamase enzyme production.

MATERIALS AND METHODS: 319 significant urine samples were collected from period of Jan.2011-Jan 2012, in the Department of Microbiology, SRM Medical College Hospital and Research Centre, Kancheepuram district Tamilnadu. The clinical samples were processed and identified as per standard microbiological techniques. Antimicrobial susceptibility was done using Kirby-Bauer disc diffusion method as per CLSI guidelines. ESBL and Amp C production were also identified.

RESULTS: Out of 319 samples 200 (62.6%) were identified to be *E.coli*, the commonest cause of UTI. Females (74%) in the reproductive age group were more affected than males. There was an increase in UTI in male (39.5%) after the age of 45 years. Most of the *E.coli* strains to be found to be resistant to both Beta Lactam and Non Beta Lactam drugs. Out of 200 *E.coli* samples 84 (42%) were ESBL producers, 44(22%) were Amp C producers, 38(19%) were Amp C and ESBL co-producers.

CONCLUSION: Multiple drug resistance among uropathogenic *E.coli* as left clinicians with very few therapeutic options. This study emphasizes on judicious use of antibiotics to prevent both Community and Hospital Acquired Infections.

KEY WORDS: Urinary Tract Infection, Multi-drug Resistance, Beta Lactamase.



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INTRODUCTION

Urinary tract infection is one of the most common infections encountered in medical practice. UTI is a spectrum of disease caused by microbial invasion of the genito-urinary tract that extends from the renal cortex of the kidney to urethral meatus. It is commonly seen in female (20-30%) during the reproductive age group because of the short urethra and close proximity to the anus. While in male it is most common in the older age group because of the prostate hypertrophy encountered. *E.coli* is widely recognized as the most common (50-90 %) etiological agent responsible for uncomplicated UTI. *E.coli* is responsible for both community and acquired infections (Mayfield D, Stamm WE et al; 2001) The primary virulence factor associated with the ability of *E.coli* to cause UTI is the pili which allow uropathogenic strains to adhere to epithelial cells and not be washed out with urine flow (Hanson L A Eriksson B. et al ;1977) .Bacterial resistance among *E.coli* causes treatment failure which leads to serious consequences. AmpC Beta Lactamase are the first bacterial enzyme reported to destroy penicillin (Abraham E.P and Chain.E, et al;1940). These enzymes are group 1 cephalosporinases that confer resistance to many Beta Lactam antibiotics. They are typically associated with multiple antibiotic resistances, leaving few therapeutic options. Mechanism of resistance to Beta Lactam antibiotic is by an enzyme Beta Lactamases which have the ability to attack Extended spectrum of cephalosporins especially against Oxyimino-cephalosporins. Hence these enzymes are called Extended Spectrum of Beta Lactamases (Kliebe et al;1985). These enzymes are clinically important as these can be transferred between various species of Enterobacteriaceae (Bradford P.A ;2001). Prevalence of ESBL and AmpC Beta Lactamase among *E.coli* are now increasing, they are either chromosomal or plasmid mediated (Victor Lorain, 3rd edition). ESBLs producing organisms contain multi drug resistant plasmid hence treatment becomes very difficult (Parul S, Rajni Sharma et al; 2008).

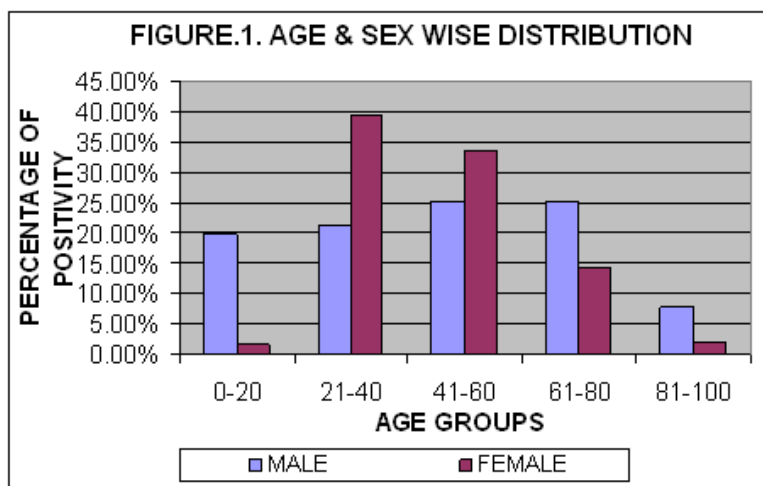
MATERIALS AND METHODS

319 non repetitive, significant urine samples were collected from period of Jan.2011-Jan 2012, for this cross-sectional study in the Department of Microbiology of SRM Medical College Hospital and Research Centre, Kancheepuram District, Tamilnadu. The clinical samples were inoculated into Cysteine Lactose Electrolyte Deficient Agar (CLED) and Blood Agar using standard loop. The plates were incubated at 35 degrees for 18-24 hours. Identification of the organisms was done using standard biochemical tests. Antimicrobial susceptibility test was done using Kirby-Bauer disc diffusion method as per CLSI guidelines. *E.coli* ATCC 25922 strains was used as quality control for anti-microbial susceptibility testing (Khanmeh ZR et al ;2009, Crichton P.B et al; 1999 and CLSI guidelines; 2008). Phenotypic detection of AmpC Beta Lactamase for all the Cefoxitin resistant strains by AmpC Disk Test (S Singhal et al;2005) was done. A Cefoxitin (30 microgram) disc was placed in the center of the lawn culture of ATCC *E. coli* 25922. A plain disc moistened with sterile saline was inoculated with several colonies of the test organism is placed beside the Cefoxitin disc plates were incubated at 35 degrees C for 18-24 hours. Flattening or indentation of the Cefoxitin inhibition zone in the vicinity of the disc is a positive test for AmpC Beta Lactamase production as shown in figure 3. Screening for second and third generation cephalosporin resistance by Kirby Bauer Disk Diffusion method was done to detect ESBL production, for which second generation Cephalosporin disk Cefoxitin 30microgram and third generation Cephalosporin disk Ceftazidime 30 microgram, Ceftriaxone 30 microgram, Cefataxime 30 microgram were procured from HiMedia. Ceftazidime 30 microgram and Clavulanic acid 10 microgram were also procured in powder form. Minimum Inhibitory Concentration by agar dilution method was done on Ceftazidime with and without Clavulanic acid (Figure. 2).

RESULTS AND DISCUSSION

In this study, out of 319 urine samples 200 *E.coli* (62.6%) was found to be the commonest cause of UTI. Females(74%)were more affected than the males (26%).Females in the reproductive age group experienced recurrent UTI.(39.5%) Males had UTI around the age of 40-60 years, as prostrate hypertrophy was a predisposing factor which is similar to the study by Honderlick R et al;2006, Biswas O .et al;2006 as shown in the Fig 1. *E.coli* has widely been implicated in both community and hospital acquired UTI (Shah et al; 2002). This is similar to the other study by Jha and Bapat;2005, Astalze;2005, Kresken and Hefner 2006; Olewe et al ;2008 and Mohammed et al ;2010). Pathogenic isolates of *E.coli* have relatively high potential of developing resistance as studied by (Karlowsky et al ;2004). High resistance of *E.coli* to antimicrobial agents was observed in the study. High level of resistance was seen to both Beta Lactam and Non Beta Lactam drugs was observed in our study as shown in diagram. Out of 200 samples 84 (42%) were ESBL producers, 44(22%) were Amp C producers, 38(19%) were Amp C and ESBL co-producers. A total of 44/200 ESBL isolates were screened phenotypically by MIC agar dilution method. 24/44 (54.5%) were found to be

resistant and 20/44 (45.4%) were found to be sensitive. Mathur et al; and Manchandra et al have reported the prevalence ranging from 55% to 69%. In our study moderate to high resistance to Norfloxacin, Ofloxacin, Gentamicin and Co-Trimoxazole was observed. These isolates were also resistant to Beta Lactam drugs also hence Amikacin and Imepenem remain as the only treatment option. Interestingly the isolates were found sensitive to Nitrofurantoin which is cheaper and less used antibiotic. There was 4.5% resistance to the Carbapenem drug-Imepenem. It was found in our study 128/200 were resistant to the Cephalosporin antibiotics. 38/200(19%) were both AmpC and ESBL co producers. This could be because plasmid mediated AmpC enzymes have been shown to disseminate among Enterobacteriaceae, some times in combination with ESBLs (S.Singhal et al ;2005). Such strains which co-produce AmpC betalactames and ESBL may give false negative tests for the detection of ESBLs (Parul sinha et al; 2008). It was observed from our study that the resistant rates to Cephalosporins among *E.coli* from urine samples are very high. Increasing resistance to other class of antibiotics will make the treatment options difficult. Rational antibiotic use and cycling of antibiotics may help to revert this situation.



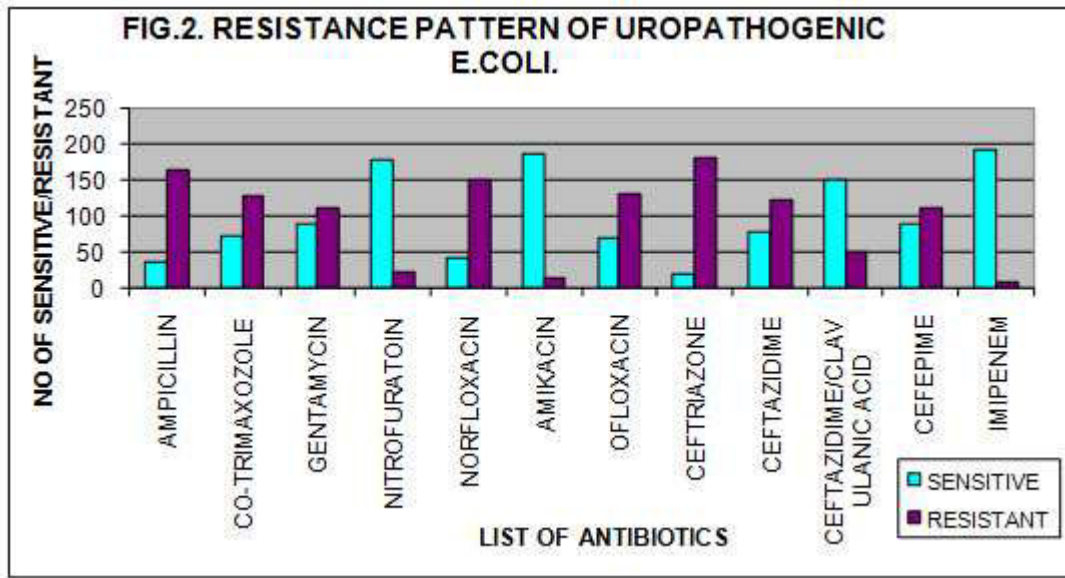
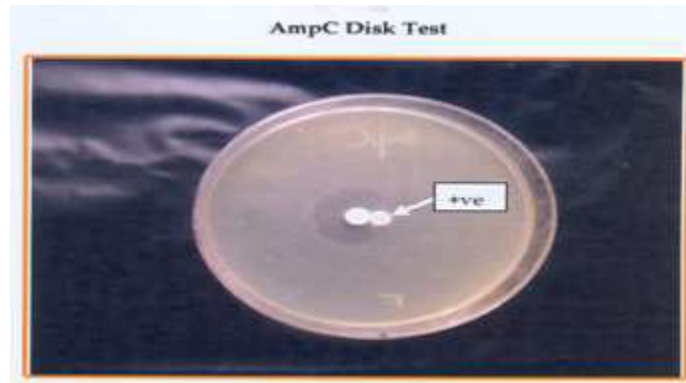


Figure 3
Amp C Disc Test



CONCLUSION

Antimicrobial resistance among uropathogens is increasing worldwide. It is at present a global public health problem. Antibiotic resistant patterns are constantly evolving, hence there is necessity for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empirical therapy. Continuous analysis of antibiotic resistance pattern and understanding the impact of drug resistance act as a guide to initiate treatment. Urine culture and sensitivity is the gold standard in treatment of UTI.

REFERENCES

1. Astal, Z.E, 2005. Increasing Ciprofloxacin resistance among prevalent Urinary tract bacterial isolates in the Gaza strip. Singapore Med. J;46:457-459.
2. Blomgran,R; Zheng and Stendahl, O, 2004. Uropathogenic Escherichia Coli trigger oxygen-dependent apoptosis in human neutrophils through the cooperative effect of type I fimbriae and

- lipopolysaccharide. *Infect. Immuno.* 72: 4570-4578.
3. Ena J, Amador C, Martinez C et al, 1995. Risk factors for acquisition of urinary tract infections caused by ciprofloxacin resistant E.Coli. *Clin. urology*; 153:117-120.
 4. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P, 1998. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clin. Infect. Dis.* 26:341-345.
 5. James AK, Laurie J, Clyde T, Mark EJ, Daniel FS, 2002. Trends in antimicrobial resistance among urinary tract infection isolates from female outpatients in the United States. *Antimicrob Agents Chemother.* 46 (8): 2540-2545.
 6. Jha, N and Bapat S.K, 2005. A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu Valley. *Kathmandu Univ .Med .J*; 3:123-129.
 7. Karlowsky JA, Jones ME, Draghi DC, Thornsbery C, Sahm DF, Volturo GA , 2004. Prevalence of antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann. Clin. Microbiol. Antimicrobio.* 3:7.
 8. Kresken, M and Hafner, D, 2006. Further increase of fluoroquinolone resistance among E.Coli isolates in a central European area. 16 ECC MID , Nice/France, April-4, <http://www.blackwell Publishing.com/eccmid16/abstract.asp?id=50191>
 9. Kurutepe, S; Surucuoglu, C; Sezgin, H; Gazi ,G. Gulay and Ozckaloglu, 2005. Increasing antimicrobial resistance in Escherichia Coli isolates from community acquired urinary tract infections during 1998-2003 in Manisa, Turkey. *Jap. J. infect. dis* 58:159-161.
 10. Olowe OA, Olayeni AB, Eniola KIT and Adeyeba AO, 2003. Aetiological agents of diarrhea in children under 5 years of age in Osogbo. *Afr. J. Clin . I and Exp. Microbiol* 4(3):62-66.
 11. Olowe O.A, Okamlawon B.M, Olowe R.A and Olayemi A.B, 2008. Antimicrobial resistant pattern of Escherichia coli from human clinical samples in Osogbo, south western Nigeria. *African J of Microbio Res*; 2, 08-11.
 12. Goluszko; J. Singhal, C. Carnoy, S. Moseley and B. Hudson, 2004. Interaction of adhesion with collagen type iv is a Critical step in E.Coli renal persistence. *Infect. Immun.* 72:4827-4835.
 13. Shah A A, Hasan F and Hameed A, 2002. Study on the prevalence of enterobacteriaceae in hospital acquired and community acquired infections. *Pakistan J Med Res* 41:1.
 14. Mohammad M, Gharemi E, Mokhayeni H, Pournia Y and Boroun H, 2010. Antimicrobial resistance patterns of E.Coli detected from hospitalized urine culture samples. *Asian J of Bio Sci* ;3(4): 195-201.
 15. Tobih, JE; Taiwo SS, Olowe OA, Olaosun OA, Adejumo SO, 2006. Microbiological profiles of discharging ears in Osogbo, Nigeria. *Trop Doc.* 36 (3): 165-166.
 16. Khameneh ZR, Afshar AT. Antimicrobial susceptibility pattern of urinary tract pathogens. *Saudi J Kidney Dis Transpl* 2009; 20:251-253.
 17. Crichton PB 1999 Enterobacteriaceae: Escherichia, Klebsiella, Proteus and other genera. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds) *Mackie & McCartney Practical Medical Microbiology*, 14th edn. Churchill Livingstone, Ch20, p361-384.
 18. Clinical and Laboratory Standards Institute: Performance standard for antimicrobial susceptibility testing; Eighteenth Informational Supplement. CLSI document M100-S18. Clinical and Laboratory Standards Institute, Wayne, Pa 9th edition. 2008.
 19. Rubin RH, Shapiro ED, Andriole VT, Davis RJ, Stamm WE. 1992. Evaluation of new anti-infective drugs for the treatment of urinary tract infection. *Infectious Disease Society of America and the Food and Drug Administration. Clin Infect Dis* 15:216-227.
 20. Gupta V, Yadav A, Joshi RM. 2002. Antimicrobial resistance pattern in

- uropathogens. *Indian J Med Microbiol* 20: 96-98.
21. Karlowsky JA, Thornsberry C, Jones ME, Sahm DF 2003. Susceptibility of antimicrobial-resistant urinary *Escherichia coli* isolates to fluoroquinolones and nitrofurantoin. *Clin Infect Dis* 36:183-187.
22. Keah SH, Wee EC, Chng KS, Keah KC 2007. Antimicrobial susceptibility of community acquired uropathogens in general practice. *Malaysian Family Physician* 2:64-69.
23. Akram M, Shahid M, Khan AU 2007. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob* 6:4.
24. Biswas D, Gupta P, Prasad R, Singh V, Arya M, Kumar A. 2006. Choice of antibiotic for empirical therapy of acute cystitis in a setting of high antimicrobial resistance. *Indian J Med Sci* 60:53-58.
25. Honderlick P, Cahen P, Gravisse J, Vignon D 2006. Uncomplicated urinary tract infections, what about fosfomycin and nitrofurantoin in 2006. *Pathol Biol* 54:462-466.