Biolife 2(4):1185-1190
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournal.com
BIOLIFE RESEARCH ARTICLE

EVALUATION OF ANTIBIOTIC RESISTANCE IN *E.COLI* STRAINS FROM UTI CLINICAL ISOLATES

Hazel Leena George[1] and Prasad M.P[2*]

[1]Department of Microbiology, PRIST University, Thanjavur, Tamilnadu, India.
[2]Department of Microbiology, Sangenomics Research Labs, Domlur Layout, Bangalore, India

E-mail: drprasadmp@gmail.com

ABSTRACT

Antibiotic resistance among pathogens causing urinary tract infection (UTI) is increasing at an alarming rate. *E.coli* is found to be the most prevalent among the common bacterial uropathogens isolated. The present study was undertaken to evaluate current antibiotic resistance pattern among the multi drug resistant strains of *Escherichia coli* isolated from positive UTI samples. 342 clean catch midstream urine samples were collected and processed using standard methods on Blood agar, Mac-Conkey agar and EMB agar. A total of 138 urine samples were reported positive for *E.coli* which were further characterized by using Morphological and Biochemical characterization. Antimicrobial susceptibility determined by Kirby Bauer disc diffusion method as per Clinical and Laboratory Standard Institute (CLSI) guideline showed 62 isolates (44%) were multi drug resistant. Out of the 14 clinically prescribed antibiotics tested, high level of resistance was seen to Ciprofloxacin (75%), Gatifloxacin (68%), Ceftazidime (62%), Meropenem (51%) and Imipenem (39%). Nitrofurantoin (20%) showed lowest resistance rate respectively. Due to the high prevalence of drug resistance, regular monitoring of patterns of resistance in different areas is very much necessary. This would prevent the unsuitable consumption of the drugs, helps in implementing judicious measures for control of the infection and also deciding empirical therapies for preventing urinary tract infection.

Keywords: *Escherichia coli*, Antibiotic Drug Resistance, Urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) involves the infection of kidneys, ureters, bladder or urethra by pathogenic invasion of urinary tract, which ultimately leads to an inflammatory response of the urothelium. Urinary tract infections (UTIs) are amongst the most common infections encountered in clinical practices and can occur in both male and female patients of any age having bacterial count as low as 100 colony forming unit (CFU) per milliliters (ml) in urine [1].

Though UTI affects all age groups and both genders, studies show that women are more prone than men due to short urethra, absence of prostatic secretions, pregnancy and easy contamination of urinary tract with faecal flora [2]. Although the etiology is diverse encompassing both Gram positive and negative organisms such as *Staphylococcus saprophyticus, Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus faecalis* [3], *Escherichia coli* is the principal pathogen responsible for both community acquired as well as in the hospital acquired UTI's [4,5] accounting up to 75% to 90% of the total cases.

Antimicrobial chemotherapy advancing remarkably during the 20th century has caused a dramatic change in the treatment of infectious diseases thus conferring huge benefits on human health. However, over the decades in response to the development of antimicrobial agents, microorganisms have evolved to become resistant to various antibiotics through a variety of mechanisms worldwide [6, 7, 8]. Unfortunately the frequency and spectrum of antimicrobial-resistant strains of bacteria that defy not only single but also multiple antibiotics have become increasingly common both in hospital and the community [9]. It has been observed that antibiotic susceptibility of bacterial isolates is not constant, but dynamic and varies with time and environment [10].

Microorganisms are considered multidrug resistance (MDR) when they exhibit resistant to at least three antibiotics [11]**.** MDR bacteria, thus, refers to those which are resistant to a vast range of antibiotics with structural independence (at least to three or more antibiotics). There are many prominent pathogens that are resistant to multiple antibiotic classes. Bacteria can acquire multiple different genes for resistance, making them resistant to multiple families of antibiotic drugs. Such multiple drug resistant strains present the greatest clinical challenge. Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organisms and their associated complications in developing countries [12].

UTIs are very often treated with broad spectrum antibiotics and vary according to the age of the patient, sex, underlying disease, infecting agent and whether there is lower or upper urinary tract involvement. A complicated UTI is more difficult to treat and usually requires more aggressive evaluation, treatment and follow-up [13]. Clinical experience has indicated the presence of numerous cases of antibiotic resistance to common antibiotics by uro-pathogens in both developed and developing countries [14].

E.coli is an important opportunistic pathogen that has shown an increasing antimicrobial resistance to most antibiotics isolated from humans [15, 16]. There are reports of altered pattern of susceptibility to antimicrobial agents marked in uropathogens especially *E.coli* with associated treatment failure [17, 18].

Included in the list of affected antimicrobials are penicillin, cephalosporin, sulpha drugs [19] **a**nd fluoroquinolones [20]. Fluoroquinolone resistant E.coli strains often show resistance to other drugs such as ampicilin, tetracycline, chloramphenicol, trimethoprin, sulphamethoxazole and Gentamycin [21, 22]. And there has been a significant increase in fluoroquinolones resistant *E. coli* in many countries over the last few decades [23]. These resistance patterns are generally associated with genetic mutation and intra or inter species transfer of resistance gene through plasmid [24]
The infections caused by multidrug resistant (MDR) organisms such as UTI due to multi drug resistant (MDR) *E.coli* pose a global public health challenge by impairing the efficacy of antimicrobial agents and resulting in substantial increased morbidity, mortality ultimately have a preposterous effect on health care costs [25, 26] especially in developing countries like India.

Most of UTIs are resulted by E. coli and antibiotics are increasingly used to treat UTIs leading to increased resistance in bacteria in addition to emergence of multi-drug resistant bacteria [27]. Abuse and improper prescribing policy of antibiotics causes remarkable increase of antibiotic resistance pattern among the E. coli isolates from UTI [28].

Studies from various parts of India have shown occurrence of high rates of antimicrobial resistance among *E.coli* causing UTI. The aim of the present study was to determine the prevalence of multiple drug resistance among UTI infections in Women both pregnant and normal.

MATERIALS AND METHODS

The present study seeks to evaluate and identify the MDR patterns of the positive uropathogenic *E.coli* strains from clinical samples. Clinical

samples were collected by clean catch midstream from patients across different hospitals and pathology labs of Bangalore in sterile containers and stored at 4^0 c until further study.

The samples were inoculated on MacConkey agar and Eosine Methylene Blue (EMB) agar plates and then incubated aerobically at 370 C for 24 hours. The identity of *Escherichia coli* was confirmed by a series of Standard morphological and biochemical tests followed by Antibitotic sensitivity tests.

Antibiotic susceptibility pattern of *E.coli* was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test as per Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory). The isolates were tested for common clinically prescribed 14 different antibitotics viz., Ceftriaxone (CTR-50mcg), Sparfloxacin (SPX–5mcg), Amoxyclav (AMC–30mcg), Ticarcillin (TCC–75mcg), Ceftazidime (CAZ – 30mcg), Imipenem (IPM – 10mcg), Nitrofurantoin (NIT- 300mcg), Cefpodoxime (CPD-30mcg), Ceftizoxime (CZX-30mcg), Gatifloxacin (GAT-5mcg), Cefdinin (CDN-5mcg), Cefuroxime (CXM-30mg), Ciprofloxacin (CIP-5mcg), Meropenem (MRP-10mcg).

The criteria for the isolate to be considered as MDR was if it was found resistant to three or more antimicrobials belonging to different classes or groups of antimicrobials.

RESULTS

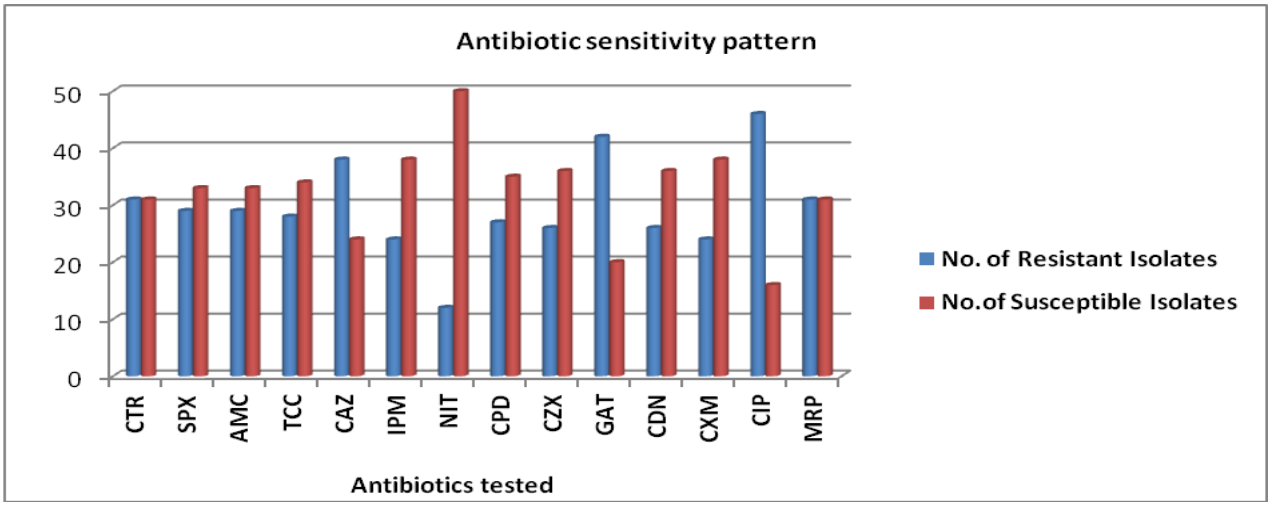
A total of 342 clean catch midstream clinical urine samples were evaluated by various microbiological parameters. *E.coli* was isolated from 138 samples by growing on MacConkey agar and EMB agar plates and were observed for round, entire smooth colonies, moderate in size. The characteristic color of the colony red or pink with precipitation was observed on MacConkey agar plates, and metallic green sheen on EMB agar plates. Several biochemical tests were done for further identification.

Of these 138 *E.coli* positive urine samples 62 isolates (44%) were multi drug resistant obtained by Antibiotic susceptibility pattern by Kirby-Bauer disc diffusion test. 14 clinically prescribed antibiotics were tested, in which high level of resistance was seen to ciprofloxacin (75%), Gatifloxacin (68%), ceftazidime (62%), Meropenem (51%) and Imipenem (39%), Nitrofurantoin (20%) showed resistance rates respectively.

Table-1: Antibiotic sensitivity of the *E.coli* isolates from urine samples

Antibiotics	No. of Resistant Isolates	% of Resistance	No. of Susceptible Isolates
Ceftriaxone - CTR	31	50	31
Sparfloxacin - SPX	29	48	33
Amoxylav - AMC	29	48	33
Ticarcillin - TCC	28	46	34
Ceftazidime - CAZ	38	62	24
Imipenem - IPM	24	39	38
Nitrofurantoin - NIT	12	20	50
Cefpodoxime - CPD	27	45	35
Ceftizoxime- CZX	26	42	36
Gatifloxacin - GAT	42	68	20
Cefdinir –CDN	26	43	36
Cefuroxime - CXM	24	40	38
Ciprofloxacin - CIP	46	75	16
Meropenem - MRP	31	51	31

Figure-1: Graphical representation of Antibiotic sensitivity pattern of the *E.coli* isolates from urine samples



DISCUSSION

Antibiotics have been proved remarkably effective for the control of bacterial infections. Very soon it will be evident that bacterial pathogens are unlikely to surrender unconditionally and some pathogens will soon become resistant to many of the first effective drugs.

Factors which may contribute for antimicrobial resistance are indiscriminate use of antibiotics by healthcare providers, self prescribing and over the counter availability are adding to the high risk factors for high level MDR in microbial pathogens. Other contributing criteria's could also be incorrect diagnosis, over use, miss use and abuse of antibiotics, also seen are use of antibiotics as livestock food additives for growth promotion.

The resistance rates of uropathogenic *E.coli* to various antibiotics have been reported as Beta-lactams (57.4%), Co-trimoxazole (48.5%), Quinolones (74.5%), Gentamicin (58.2%), Amikacin (33.4%), Cefuroxime (56%), Nalidixic acid (77.7%) [29, 30].

The present study provides the information about the antibiotic resistance pattern of MDR *E.coli* isolated from UTI patients. It was found from our study that MDR *E.coli* exhibited resistance to large number of antibiotics and also exhibited wide range of resistance patterns.

Nitrofurantoin (20%), Imipenem (39%) were observed to be the most active against the organism while high resistance were observed against Ciprofloxacin (75%), Gatifloxacin (68%), Ceftazidime (62%), and Meropenem (51%).

With the increasing trend of reported multiple resistance mechanisms in *E.coli* [31] and limited therapeutic options the management of urinary tract infections is likely to become more complicated. Periodic screening, monitoring and evaluating resistance patterns in region specific *E.coli* strains is so important to gain knowledge about the type of urinary pathogens, to determine their antibiotic susceptibility profiles in different communities [32] if any these studies may help the clinicians to choose the right empirical treatment.

Antibiotics resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the prominent public health concern of the 21st century.

There is an urgent need to formulate a policy and put the necessary plan in place to execute a policy targeted at the promotion of rational use of antibiotics as an important element in antibiotic resistant containment. In conclusion a

combination of traditional and innovative prevention and treatment strategies should be deployed to combat the threat of emerging antibiotic resistance among uropathogens.

REFERENCES

1. Akinyemi. K, S Alabi, N Taiwo, E Omonighehin, Antimicrobial susceptibility pattern and plasmid profile of pathogenic bacteria isolated from subjects with urinary tract infections in Lagos, Nigeria. Nig. Qt J Hosp. Med, (1,) 1997, 7-11.
2. Haider G, Zehra N, Munir AA and Haider A. Risk factors of urinary tract infection in pregnancy. JPMA, 2010; 60(3):213-216.
3. Ronald, A., (2003) "The etiology of urinary tract infection: traditional and emerging pathogens". Dis Mon 49: 71-82.
4. Karlowsky JA, Jones ME, Thornsberry C, 2. Critchley I, Kelly LJ, Sahm DF. Prevalence of anti microbial resistance among urinary tract pathogens isolated from female outpatients across the US in 1999. Int J Antimicrob Agents 2001; 18: 121-7.
5. Gorbach SL, Bartlett JG, Balcklow NR. Urinary tract. In: Gorbach SL, Bartlett JG, Balcklow NR, editors. Infectious diseases. Philadelphia: Lippincott Williams & Wilkins Publishers; 2004. p. 861-81.
6. Memon, B.A., 2007. Predominant and common cause of urinary tract infections in Sukkur city. Rawal med. J., 32:99-101.
7. Gupta, K., Hooton, T.M. and Stamm, W.E., 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann. Intern. Med., 135:41-50.
8. Livermore D.M., Bacterial resistance: Origins, epidemiology and impact, Clin. Infect. Dis. 36 (2003) 11–23.
9. Davies. J, Inactivation of antibiotics and the dissemination of resistance genes, Science, 264 (1994) 375–382.
10. Tenover F.C, Development and spread of bacterial resistance to antimicrobial agents: An overview, Clin. Infect. Dis. (Suppl.), 33 (2001) 108–115.
11. Aibinu I E, Ohaegbulam V C, Adenipekun, E, Ogunsola, F. T, Odugbemi, T.O, Mee, B.J, Extended-spectrum Beta-Lactamase Enzymes in Clinical isolates of Enterobacter Species from Lagos, Nigeria. J Clin Microbiol 2003a; 41(5): 2197-2200.
12. Hassan, S.H, J. Trop. Med. Hyg., 88, 243-248 (1995).
13. Santo E, Salvador MM and Marin JM. Multidrug-Resistant Urinary Tract Isolates of Escherichia coli from Ribeirao Preto, Sao Paulo, Brazil. The Brazilian Journal of Infectious Diseases. 2007; 11:575-578.
14. Guyot, A.; Barrett, S.P.; Threlfall, E.J.; Hampton, M.D.; Cheasty, T. Molecular epidemiology of multi-resistant Escherichia coli. J.Hosp. Infect., **1999**, 43, 39-48.
15. Bryan, S Charles, Infectious diseases in primary care (Philadelphia: W.B. Saunders 2002).
16. Gupta K. Addressing antibiotic resistance. Am J Med. 2002; 113 (Suppl 1A):S29–34.
17. Winorkur P.L., D.L. Vanstein, L.J. Hoffman, E.K. Unlentropp, G.V. Doer, Antimicrob. Agents Ch., 45(10), 2716- 2722 (2001).
18. Miranda S., M.G. Davide, J.C. Peter, Microbiology, 150, 1539-1546, (2004).
19. Talan, D. A., K. G. Naber., J. Palou, D. Elkharrat (2004): Extended release ciprofloxacin (cipro XR) for treatment of urinary tract infections. Int. J. Antimicrob. Agents 23, 554-566.
20. Blondeau, J. M. (2004): Current issues in the management of urinary tract infections: extending release ciprofloxacin as a novel treatment options. Drugs 64, 611-628.
21. Flutt, A. C., M. E. Jone, F. J. Schmitz, J. Acar, R. Guptes, J. Verhoef (2000): Antimicrobial susceptibility and frequency of occurrence of clinical blood isolate in Europe from SENTRY antimicrobial surveillance programme 1997 and 1998. Clin. Infect. Dis. 30, 454-460.
22. Sahm, D. F., C. Thornsberry, D. C. Mayfield, M. E. Jones, J. A. Karlowsky (2001): Multidrug resistant urinary tract isolates of Escherichia coli prevalence and patient dermographic in United States in 2000. Antimicrob. Agents Chemother. 45, 1402-1406.

23. Goettsch, W., W. Van Pelt, N. Nagelkerke, M. G. Hendrix, A. G. Buiting, P. L. Petit (2000): Increasing resistance to fluoroquinolones in Escherichia coli from urinary tract infections in the Netherlands. JAntimicrob. Chemother. 46, 223-228.
24. Garau, T., M. Xercavins, M. Rodriguez-Carhalleira, J. R. Gomez-Vera, I. Coll, D. Vidal (1999): Emergence and dissemination of quinolone resistant Escherichia coli in the community. Antimicrob. Agents Chemother. 43, 2736-2741.
25. Komp, L. P., A. Karlsson, D. Hughes (2003): Mutation rate and evolution of fluoroquinolone resistance in Escherichia coli isolates from patient with urinary tract infections. Antimicrob. Agents Chemother. 47, 3222-3232.
26. Van Belkum, A., W. Goessens, C. Vander Schee, N. Lemmens-Dens Toom, M. C. Vos, J. CORNELISSEN (2001): Emergence of ciprofloxacin resistant enterobacteriaea containing multiple gentamycin resistant associated integrons in a Dutch hospital. Emerg. Infect. Dis. 7, 862-871.
27. Hughes M, Datta N. Conjugative plasmid in bacteria of the pre-antibiotic era. Nature. 1983; 302:725-726.
28. D Byarugaba, A view on antimicrobial resistance in developing countries and responsible risk factors, .Int. J Ant Agents, 24, 2004, 105–10**.**
29. Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke- Grauls C, van der Zwaluw K, Huijsdens X and Kluytmans J. Extended Spectrum - Lactamase genes of Escherichia coli in chicken meat and humans, the Netherlands. Emer Infect Dis, 2011; 17(7): 1216-1222.
30. Cohen M.L., Epidemology of Drug Reisistance: Implications for a post Antimicrobial Era. Science 1992; 257: 1050-1055.
31. Yuksel S, Ozturk B, Kavaz A, et al. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infection. Int J Antimicrob Agent 2006; 28(5): 413-6.
32. Kothari A, Sagar V. Antibiotic resistance in pathogens causing. Community- acquired urinary tract infections in India: a multicenter study. J Infect Dev Ctries 2008; 2: 354-8.

*****