Original Research Paper

Volume-10 | Issue-1 | January - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Microbiology

EFFECT OF COMMERCIALLY AVAILABLE GENERIC AND ETHICAL DRUGS AGAINST ISOLATED UROPATHOGENIC *ESCHERICHIA*: A STUDY

Dr. Sandhya Tambekar

Department of Microbiology, Dhote Bandhu Science College, Gondia: 441614

ABSTRACT The resistance pattern of uropathogens is increasing very rapidly because of the unsorted, insufficient and incoherent usage of antibiotics. Urinary tract infection (UTI) is considered as a different type of infection which is caused by the uropathogenic resistant bacteria. This infection is due to the main colonization of normal and opportunistic microflora. The rate of UTI patients is high due to poor hygiene, long time catheterization, uncontrolled sexual intercourse, pregnancy and spermicidal contraception. The present study deal with effect of Generic and Ethical drugs on uropathogenic *Escherichia species* isolated from urine samples. In present study, total 50 samples were collected from five pathology laboratories in Gondia City to isolate Uropathogenic *Escherichia coli*. However, only 44 *Escherichia* spp. were isolated from collected urine samples. On the basis of morphological, cultural and biochemical characteristics, isolates were identified as *Escherichia*. It is evident from the data that 100% *Escherichia* spp. isolated from urine samples were sensitive to both ethical and generic Levofloxacin whereas 88.6% *Escherichia* spp. isolated from urine samples were resistant to both ethical and generic Amoxicillin trihydrate after 24 – 48 hrs incubation. Using generic drug is more economic than ethical drug because it reduces burden of high cost of ethical drugs on patients and lowers their expense on medicine.

KEYWORDS : Urinary tract infections, Drugs, Urine, *Escherichia, Gondia*

INTRODUCTION

Women are highly at risk of developing UTIs because of their short urethra, and certain behavioral factors, which include delay in micturition, sexual activity and the use of diaphragms & spermicides, which promote colonization of the periurethral area with coliform bacteria. Infection in women, most often results from perineal or periurethral bacteria that enter the urethra and ascend into the bladder, often in association with sexual activity, or due to mechanical instrumentation such as catheterization (Litza and Brill, 2010). UTIs are the most common bacterial infections during pregnancy, with pyelonephritis being the most common severe bacterial infections complicating pregnancy.

A generic drug is a drug that is not branded but is similar to a branded or reference listed drug in terms of dosage, administration and performance. It has the same composition as its equivalent brand name drug. A generic drug is identical or bio-equivalent to a brand name drug in dosage form, safety, strength, route of administration, quality, performance characteristics and intended use. Although generic drugs are chemically identical to their branded counterparts, they are typically sold at substantial discounts from the branded price. An ethical drug is prescription drugs which are available to customers/patients only with the prescription of a Doctor. An ethical drug - synonymous to a prescription drug -is a pharmaceutical drug that legally requires a medical prescription to be dispensed. They are essentially opposite to over the counter (OTC) drugs, which can be easily bought from dispensaries without a medical prescription (www.quora.com).

Most commonly, urinary tract infections are caused by uropathogenic *Escherichia coli* (UPEC) (Song and Abraham, 2008). Four main uropathogenic *Escherichia coli* phylogroups (A, B1, B2, and D) have been identified on the basis of the occurrence of genomic Pathogenicity Islands (PAI) and the expression of virulence factors, such as adhesins, toxins, surface polysaccharides, flagella, and iron-acquisition systems (Bien *et al.,* 2012). *E. coli* is the commonest cause of uncomplicated community-acquired UTIs in both outpatient and inpatient settings. Other common uropathogens are *Enterococcus faecalis, Enterobacter* species*, Staphylococcus saprophyticus, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas* species (Watring and Mason, 2008). *E. coli* strains are the leading causes of serious bacterial infections in healthy society and very different antibiotic patterns have been reported based on the source (Ejrnaes *et al.,* 2006). Mobile genetic elements including transposons, plasmids and integrons contribute to lateral transfer of resistance genes in bacteria. *E. coli* can be intrinsically resistant to some special antibiotics and have genes, which are responsible for resistance to some of antibiotics such as aminoglycosides, flouroquinolones and β-lactamas (Blair *et al.,* 2014). According to reports from the USA, Japan, China, India, Saudi Arabia, Brazil, and Nepal, the prevalence of MDR *E. Coli* causing UTIs is increasing (Niranjan and Malini, 2014). The knowledge of the main bacteria usually involved in the UTIs and their antimicrobial susceptibility are necessary for appropriate empirical therapy and prevention of the emergence of antibiotic resistance.

The presence of microbial pathogens in the urinary tract with associated symptoms is called as Urinary tract infection (UTI) which is the second most prevalent bacterial infection after respiratory tract infection in women (Pushpalatha, 2008). UTIs are mostly common among the female population hence about 1% of school-aged girls and 4% of women through child-bearing years are infected with UTIs. As the Incidence of infection in females increase directly with sexual activity and child-bearing condition therefore about 25-30% women between age 20-40 years of come across with UTIs (Wilma, 2002). These infections accounts 8.3 million doctor visits each year and study showed that one in 5 adult women experience a UTI at some stages of life, confirming that it is an exceedingly common worldwide problem (Naber *et al.,* 2008).

From the previous observations, it is clear that uropathogenic *Escherichia coli* are a serious UTI causative agent, which shows resistance against different antimicrobial drugs. Hence, it is necessary to find out which generic and ethical drugs are effective against uropathogenic *Escherichia coli*. This will enable users and medical practitioner to select the effective and affordable drug in the treatment of UTI due to uropathogenic *Escherichia coli*. Therefore, the present study deal with the comparative study between effect of Generic and Ethical drugs against uropathogenic *Escherichia species isolated from urine samples*.

MATERIALS AND METHODS

I. Methods

1) Collection of Samples

Total 50 urine samples were collected from five different pathology laboratories in Gondia city. The samples were collected in pre-autoclaved screw cap containers which were transported in box containing ice pads with the temperature approximately between 4 to 5°C, from site of collection to laboratory. Sample were processed immediately after arriving the laboratory or kept at refrigeration conditions as long as 24 hrs if not possible to process them immediately.

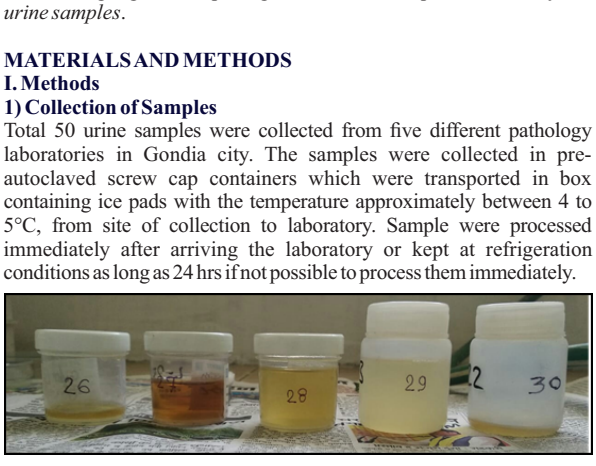


Fig.1: Sample collection and Storage

2) Isolation of Bacteria from Urine Samples

The isolation of bacteria from urine samples collected from pathology

laboratories of Gondia City was carried out with 100mL MacConkey agar media. After attaining the room temperature of collected urine samples, containers were shake vigorously and add 1 to 2 drops of urine sample on solidified MacConkey agar plate aseptically with the help of sterilized dropper. The sample was spread on the surface of MacConkey Agar medium with the help of sterilized spreader and incubated at 37°C for 24 to 36-hr.in incubator. After incubation period, pink colored colonies were picked up and maintained for further process.

3) Identification of Isolated microorganism from Urine Sample

The cultural, morphological and biochemical tests were performed to identify microorganism isolated from collected urine samples.

4) Antibiotics

Following Generic and Ethical drugs are used for observing sensitivity of isolated microorganisms.

a) Generic –

- Amoxicillin and potassium
- Cefixime
- Azithromycin
- Ofloxacin
- Levofloxacin
- Amoxycillin and trihydrate
- Ciprofloxacin
- Cephalexin

Fig.3.1: Generic Drugs

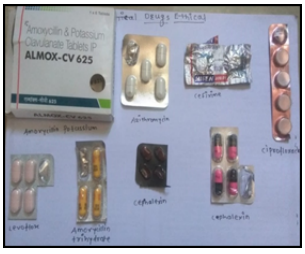


Fig. 3.2: Ethical Drugs

b) Ethical –

- Amoxicillin and potassium
- Cefixime
- Azithromycin
- Ofloxacin
- Amoxicillin and trihydrate
- Ciprofloxacin
- Levoflox
- Cephalexin

5) Preparation of Ethical and Generic Antibiotic Solution

Ethical and Generic antibiotics (Amoxicillin Clavulanate potassium, Cefaxime, Azithromycin, Ofloxacin, Levofloxacin, Amoxicillin trihydrate, Ciprofloxacin, Cephalexin) were purchased from the local medicine market. 200 mg tablet of each antibiotic was dissolved in 10mL sterile distilled water. Shake the solution well to dissolve complete antibiotic powder in distilled water. Keep prepared antibiotic solutions in refrigerator for further use.

Fig. 3.2: Dilution of selected drugs

6) Screening for Antibiotic sensitivity (Diffusion Method)

After autoclaving and cooling at room temperature, test tubes containing nutrient broth were inoculated with *Escherichia spp.* isolated from urine samples collected from different pathology laboratories and tubes were incubated for 18 to 24 hr at 37°C. After solidification of nutrient agar, plates were inoculated with 1mL broth culture of *Escherichia spp.* isolated from urine samples and inoculum added to plates was spread uniformly on surface of nutrient agar medium by using sterile glass spreader and allow settling for 15 min. Total eight well were created aseptically on each Nutrient Agar media plate with the help of sterile borer. Each well was loaded with 50 µl of different antibiotic solutions. Separate plates of same isolate were used for loading generic and ethical antibiotic solutions. Plates inoculated with bacterial culture and loaded with different antibiotic solutions, were incubated at 37°C for 24 and 48 hrs. Incubated plates were observed for clear zone of inhibition due to loaded antibiotics, the diameter of which was measures in millimeters (mm) after 24hr and 48hr incubation. Same procedure was followed to observe generic and ethical antibiotic resistance of all *Escherichia spp.* isolates.

RESULT AND DISCUSSION

In present study, total 50 samples were collected from five pathology laboratories in Gondia City to isolate Uropathogenic *Escherichia coli*. However, only 44 *Escherichia* spp. were isolated from samples collected.

In present study, identification of isolates was done by using morphological, cultural and biochemical characteristics. The isolates were Gram-negative short rods, arranged singly or in pair (Fig. 4.2). They show pink colored, smooth, circular, raised colonies on MacConkey Agar medium (Fig. 4.4). The isolates were Indole and MR positive whereas VP and Citrate negative (Fig 4.1). The isolates were catalase positive (Fig. 4.3). On the basis of these morphological, cultural and biochemical characteristics, isolates were identified as *Escherichia* species.

Fig. 4.1: IMViC Test

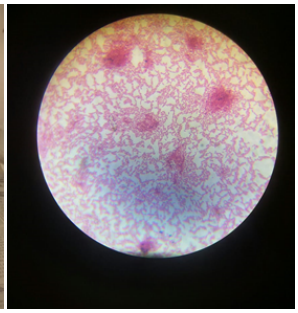


Fig. 4.2: Gram Staining

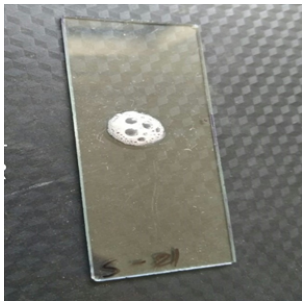


Fig. 4.3: Catalase Test

Fig.4.4: MacConkey agar plate

Sensitivity of isolates to Ethical and Generic Drug after24 hrs

Table 4.2: Comparative assessment of no. of sensitive Escherichia spp. isolated from urine sample to ethical and generic drug after 24 hr incubation

Type of Antibiotic	Ethical		Generic		Z	P	I
	N	%	N	%			
Amoxicillin Potassium	19	43.2	41	93.2	-5.036	0.000	<0.05
Cefixime	42	95.5	39	88.6	1.196	0.2301	NS
Azithromycin	41	93.2	42	95.5	-0.467	0.6384	NS
Ofloxacin	42	95.5	44	100.0	-1.423	0.1556	NS
Amoxicillin Trihydrate	7	15.9	11	25.0	-1.058	0.2891	NS
Ciprofloxacin	44	100.0	41	93.2	1.7599	0.0784	NS
Levofloxacin	44	100.0	44	100.0	-	-	-
Cephalexin	39	88.6	39	88.6	-	-	-

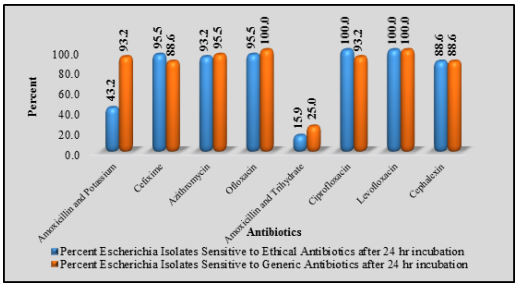
N- No. of *Escherichia* spp. sensitive to ethical and generic drug; Z- Two Proportion Z Value; P- P Value; I – Inference; NS- Non-Significant

Above Table 4.2 illustrates results of comparative assessment of no. of sensitive *Escherichia* spp. isolated from urine sample to ethical and generic drug after 24 hr incubation. It is evident from the data that 100% *Escherichia* spp. isolated from urine samples were sensitive to both ethical and generic Levofloxacin, whereas 88.6% *Escherichia* spp. isolated from urine samples were sensitive to both ethical and generic Cephalexin after 24 hr. incubation.

Furthermore, percentage of *Escherichia* spp. sensitive to ethical drugs Ciprofloxacin, Ofloxacin, Cefixime, Azithromycin, Amoxicillin potassium and Amoxicillin trihydrate was 100%, 95.5%, 95.5%, 93.2%, 43.2% and 15.9% respectively after 24 hr. incubation.

In addition to this, percentage of *Escherichia* spp. sensitive to generic drugs Ofloxacin, Azithromycin, Amoxicillin potassium, Ciprofloxacin, Cefixime and Amoxicillin trihydrate was 100%, 95.5%, 93.2%, 93.2%, 88.6% and 25.0% respectively after 24 hr. incubation.

It is apparent from the result that 100% *Escherichia* spp. isolated from urine sample collected from different pathology laboratories in Gondia City were sensitive to both ethical and generic Levofloxacin drug whereas least percentage of *Escherichia* isolates from urine samples were sensitive to both ethical and generic Amoxicillin trihydrate after 24 hr incubation. Furthermore, there is no significant difference among sensitivity of no. of *Escherichia* spp. isolated from urine sample to ethical and generic drug except to drug Amoxicillin potassium. As compare to ethical Amoxicillin potassium drug significantly ($P<0.05$) high percentage of *Escherichia* spp. isolated from urine sample were sensitive to generic Amoxicillin potassium drug after 24 hr. incubation.



Graph 4.1: Comparative assessment of no. of sensitive Escherichia spp. isolated from urine sample to ethical and generic drug after 24 hr incubation

Sensitivity of isolates to Ethical and Generic Drug after 48 hrs

Table 4.3: Comparative assessment of no. of sensitive Escherichia spp. isolated from urine sample to ethical and generic drug after 48 hr incubation

Type of Antibiotic	Ethical		Generic		Z	P	I
	N	%	N	%			
Amoxicillin Potassium	24	54.5	41	93.2	-4.131	0.000	<0.05
Cefixime	44	100.0	44	100.0	-	-	-
Azithromycin	41	93.2	44	100.0	-1.759	0.0784	NS
Ofloxacin	44	100.0	44	100.0	-	-	-
Amoxicillin Trihydrate	11	25.0	11	25.0	-	-	-
Ciprofloxacin	44	100.0	42	95.5	1.4232	0.1556	NS
Levofloxacin	44	100.0	44	100.0	-	-	-
Cephalexin	42	95.5	40	90.9	0.8571	0.3899	NS

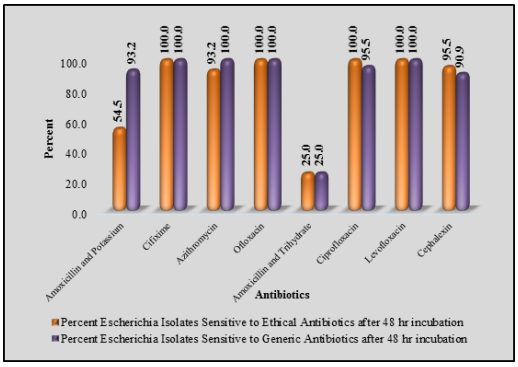
N- No. of *Escherichia* spp. sensitive to ethical and generic drug; Z- Two Proportion Z Value; P- P Value; I – Inference; NS- Non-Significant

Above Table 4.3 demonstrates result of comparative assessment of no. of sensitive *Escherichia* spp. isolated from urine sample to ethical and generic drug after 48 hr incubation. It is evident from the data that 100% *Escherichia* spp. isolated from urine samples were sensitive to both ethical and generic Cefixime, Ofloxacin and Levofloxacin whereas 25.0% *Escherichia* spp. isolated from urine samples were sensitive to both ethical and generic Amoxicillin trihydrate after 48 hr. incubation.

Besides, percentage of *Escherichia* spp. sensitive to ethical drugs Ciprofloxacin, Cephalexin, Azithromycin and Amoxicillin potassium was 100%, 95.5%, 93.2% and 54.5% respectively after 48 hr incubation.

Additionally, percentage of *Escherichia* spp. sensitive to generic drugs Azithromycin, Ciprofloxacin, Amoxicillin potassium and Cephalexin was 100%, 95.5%, 93.2% and 90.9% respectively after 48 hr incubation.

It is apparent from the result that 100% *Escherichia* spp. isolated from urine sample collected from different pathology laboratories in Gondia City were sensitive to both ethical and generic Cefixime, Ofloxacin and Levofloxacin drugs whereas least percentage of *Escherichia* isolates from urine samples were sensitive to both ethical and generic Amoxicillin trihydrate after 48 hr incubation. Furthermore, there is no significant difference among sensitivity of no. of *Escherichia* spp. isolated from urine sample to ethical and generic drug except to drug Amoxicillin potassium. As compare to ethical Amoxicillin potassium drug significantly ($P<0.05$) high percentage of *Escherichia* spp. isolated from urine sample were sensitive to generic Amoxicillin potassium drug after 48 hr. incubation.



Graph 4.2: Comparative assessment of no. of sensitive *Escherichia* spp. isolated from urine sample to ethical and generic drug after 48 hr incubation

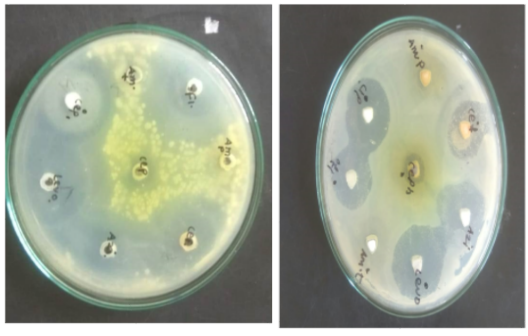


Fig. 4.5: Sensitivity of *Escherichia* spp. against drugs

Present study reveals effect and potentiality of generic and ethical drugs against *Escherichia* spp. isolated from urine samples showed that isolated *Escherichia* spp. are somehow sensitive to all eight (8) ethical and generic drugs. However, no significant difference was evident among sensitivity pattern of *Escherichia* spp. against ethical or generic drug. Only with slight difference, all generic and ethical drugs were effective against isolated *Escherichia* spp. Out of all 8 generic and ethical drugs ethical and generic drug Levofloxacin is found to be most effective against all *Escherichia* whereas ethical drug Amoxicillin clavulanate potassium and generic drug Amoxicillin trihydrate are found to be least effective against isolated *Escherichia* spp.

CONCLUSION AND RECOMMENDATIONS

In present study, total 50 samples were collected from five pathology laboratories from Gondia City to isolate Uropathogenic *Escherichia coli*. However, only 44 *Escherichia* spp. were isolated from collected urine samples. 100% *Escherichia* spp. isolated from urine sample collected from different pathology laboratories in Gondia City were sensitive to both ethical and generic Levofloxacin drug whereas least percentage of *Escherichia* isolates from urine samples were sensitive to both ethical and generic Amoxicillin trihydrate.

Recommendations

The present study revealed that there is no significant difference in the sensitivity of uropathogenic *Escherichia* spp. against ethical and generic drugs. Hence, it is suggested to use generic drug instead of ethical drugs as using generic drug is more economic than ethical drug. This reduces burden of high cost of ethical drugs on patients and lowers their expense on medicine. Medical practitioner should benefit from this study as it provides information pertaining to potent anti uropathogenic *Escherichia* drug. Hence, by prescribing this particular drug they can avoid chances of prolonged recovery of UTI patients.

REFERENCES

1. Bien, J., Sokolova, O. and Bozko, P. (2012). Role of Uropathogenic Escherichia coli Virulence Factors in Development of Urinary Tract Infection and Kidney Damage, International Journal of Nephrology, Article ID 681473, 15 pages
2. Blair, J.M.A, Webber, M.A., Baylay, A.J., David, O., Ogbolu & Laura, J.V. (2014). Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol.; 13:42.
3. Ejrnaes, K., Sandvang, D., Lundgren, B., Ferry, S., Holm, S., Monsen, T., Lundholm R. & Frimodt-Moller, N. (2006) Pulsed-field gel electrophoresis typing of Escherichia coli strains from samples collected before and after Pivmecillinam or placebo treatment of uncomplicated community-acquired urinary tract infection in women. J Clin Microbiol.; 44(5):1776–81.
4. Litza, J.A. and Brill, J.R. (2010). Urinary tract infections. Prim Care; 37(3):491-507.
5. Naber, K.G., Schito, G., Botto, H., Palou, J. and Mazzei T. (2008). Surveillance study in Europe and Brazil on clinical aspects and Antimicrobial Resistance Epidemiology in Females with Cystitis (ARESC): implications for empiric therapy. Eur Urol ; 54(5):1164-75.
6. Niranjan, V. and Malini, A. (2014). Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among in patients, Indian J Med Res., 139(6): 945–948.
7. Pushpalatha, K.S. (2008). Urinary tract infection and management. J Nighting Nursing Times; 4(5):28-32
8. Song, J. and Abraham SN. (2008). Innate and adaptive immune responses in the urinary tract. Eur J Clin Invest ; 38(2):21–28.
9. Watring, N.J. & Mason J.D. (2008). Deciphering dysuria. Emerg Med; 40(9):29-34.
10. Wilma, J.P. (2002). Shafers Medical Surgical Nursing. 7th edition, B.I. Publications: New Delhi p.637-40.