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Clinical Study of Extended-Spectrum Beta-Lactamase Producing *E.coli* from Urinary Tract Infections

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ABSTRACT

Urinary tract infections are a major health problem among UTI patient accounting for considerable morbidity and health care costs and the organisms infecting the urinary tract and their antibiotic sensitivity patterns differ from place to place. So this study was designed to determine whether the isolation of various categories like 30-40, 41-50, 51-60, 60-72 years age groups. The study conclude the 41-50 and 51-60 age group was highly affected by bacteria to cause urinary tract infection. The present study highlighted the *E. coli* was found to be the commonest cause of urinary tract infection in UTI patient.72% of urinary tract infection caused by *E. coli* in our study. So I conclude that *E. coli* is predominant bacterial pathogen to cause urinary track infection in UTI patient. The present study indicates that the prevalence rates of urinary tract infection are higher in females than males in all age groups. The sensitivity / resistance ratio of bacterial etiologic agents in this study revealed that the most common bacteria of urinary tract infections were sensitive to *Amikacin, Oflaxin and Ciprofloxin*. However these bacteria were resistant to *Ampicillin, Cefazolin, Ceftazidime, Nalidiric acid.* Antibiotic susceptibility patterns of the various drugs test did not show any significance, showing that the treatment of regimen for urinary tract infection is effective.

Keywords: Urinary tract infections, *E. coli*, Antibiotics.

INTRODUCTION

Urine is one of the most common received specimens in routine microbiological laboratories. Uncomplicated urinary track infections (UTIs) that occur most often in young healthy adult women are easy to treat, often to tend to have a more complicated course to patients with diabetic. Beside organs complications as retinopathy, neuropathy etc. patient with diabetes have an increased risk of infection which tends to be more secures.

Urinary track infections (UTIs) ranks second only after respiratory infections in their incidence in the India. Each year, urinary track infections (UTIs) accounts for about 9.6 million doctor visits. The majority of the cases seen in the doctor's office are in women (30:1), (Female : male ratio). 40% of all women have at least one episode of a UTI at some time in their lives.Upto 20 percentage of young women with acute cystitis develop recurrent urinary track infection (UTIs).Male experience a rapid increase in the incidence urinary track infection (UTIs)

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sometime in their 40s. This is about the time that males are experiencing prostate of land hypertrophy. Women generally don't have many problems with urinary track infection (UTIs) until they become sexually active. The increase frequency of urinary track infections (UTIs) account for considerable morbidity in adult woman. Patients results in several abnormalities of the host defense system that might result in a higher risk of certain infection. The abnormalities include immunologic impairment such as impaired migration, intracellular killer, phagocytosis and chemotaxis is polymorph nuclear leucocytes from diabetic patient. There are more than 7 million uncomplicated urinary track infections (UTIs) per year in United States. Urinary track infections (UTIs) patients with chronical renal insufficiency has attacked little to no attention (1).

Urinary tract infection is a common reason for admission to internal medicine departments. Although guidelines for the empiric treatment of patients with UTI do exis. Hospital colonization by ESBL producing bacteria is usually a complex phenomenon involving different mechanisms, dissemination of several epidemic strains, and dissemination of plasmids and resistant genes. Specific risk factors include prolonged hospital stay, severity of illness, ICU, urinary or arterial catheterization, intubation and mechanical ventilation. ESBLs commonly occur in surgical wards as well as other areas of the hospital and frequently from patients from extended care facilities (2,3,4,5). Since ESBL positive isolates show false susceptibility to expanded spectrum cephalosporins in standard disk diffusion tests, it is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Specific detection methods such as double disk potentiation methods recommended by NCCLS have to be adopted. ESBLs are inhibited by beta lactamase inhibitors like clavulanic acid, sulbactam and tazobactam and this property of specific inhibition can be utilized for the detection and confirmation of ESBLs. Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Symptoms include frequent feeling and/or need to urinate, pain during urination, and cloudy urine. The main causal agent is *E. coli*. Although urine contains a variety of fluids, salts, and waste products, it does not usually have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, they may cause a UTI (6,7,8,9,10). Reflect the current knowledge on the most frequent causative agents and their expected profile of antimicrobial sensitivity. In recent years, Gram-negative bacteria harboring extended beta-lactamase resistance have emerged, particularly but not exclusively *Escherichia coli*. The extended-spectrum beta-lactamases are resistant to many of the antibiotics currently available to treat patients with UTI, including penicillins, cephalosporins and monobactam. An infection with ESBL-producing bacteria is related to a worse clinical course, deferred clinical and microbiological response, longer hospitalizations, higher costs, and higher death toll. Carriage of ESBL-producing Enterobacteriaceae is reported worldwide, particularly in hospital settings. The 2009 International Nosocomial Infection Control Consortium. The rates of resistance to ceftriaxone and ceftazidime as high as 8.1% in those related to *E. coli*. The rate is even higher in intensive care units. The prevalence of such resistant bacteria is still low in the community but is reported to be growing (11).The increasing prevalence of ESBL- producing Escherichia coli prompted our interest to investigate risk factors for ESBL-producing *E. coli* in patients with community-onset urinary tract infections (UTIs). The aim of the present study was to determine the prevalence, type and risk factors for ESBL producing *E. coli* in community-onset UTIs in our area.

MATERIALS AND METHODS

UTIs, colonization by microbes may vary and depends upon the individual and it may also be vary according to time and in diseased condition. The present study was carried out for a period from 2008 to 2009 in the Department of Microbiology, Rajah Muthiah Medical College, Annamalai University. The sources of patient, sample collected the isolation and identification of the different type of Microorganisms was discussed

S. No	Place/ Hospital	No. of Patient
1.	Rajah Muthiah Medical College and Hospital	21
2.	Government Hospital, Kollidam	30
3.	Government Hospital, CDM,	30
4.	Private Nursing Homes around Chidambaram.	33
5.	House visit / Personal	06
6.	Around Chidambaram volunteers	30
	Total	150

Patient Sources

Urinary track infection (UTTs) patient attended out patient Division (OPD) of the RMMC, and Government Hospital and private nursing homes in and around Chidambaram town were included in this study (Table). After getting human ethics clearance, and patient consent the specimen were collected History and Medical detail related to the study were also recorded in the proforma and used in this study.

Age and Sex distribution of the subjects

Both male and females, age ranging from 30 to 73 were included in this study (Both patient and control).

Specimen collection and transportation:

The patients were instructed to collect clean mid-stream specimen as follows:

1. Thoroughly cleans the genitalia with soap and water and urine with a clean drug cloth.
2. Urine sample was collected for avoiding and discarding a initial small amount directly into a sterile wide mouthed. Screw crapped container. Urine transported to lab within one hour at 4°C
3. After collection, the sample was transported to laboratory with in one hour.

Isolation of Microbes

Urine sample taken from patient suffering with urinary track infection were inoculate on nutrient agar plates and incubate at 37°C, for 18-24 hour. Later the numbers of colonies with different colony morphology were counted and interesting colonies which were in large numbers, suspected to the responsible for pathogenic were made into pure culture on Macconkey's agar. The plates were preserved at 4°C and fresh cultures were prepared from them for further experiment.

Media used

- Blood agar (BA)
- Chocolate agar (CHA)
- Macconkey Agar (MA)

Direct microscopic examination

A loopful of well mixed Urine sample without centrifuging placed on a clean glass slid and makes the smear allowed to air drug, heat fixed and stained by gram stain technique.

Colony count on Calibrated loop plates

Multiply the number of colonies by 100, if undilutes urine was used for inoculation, by 1000 of a 1:10 dilution of urine was used by 1000 if 1: 100 dilution of urine was used to plates were poured with Blood agar, Macconkey agar, nutrient agar separately and incubation at 37°C for 18-24 hour aerobically.

Colony morphology

In Blood agar is an enriched in this medium blood is the substances added to a basal medium. *E.coli* was well grown in blood agar plate for *β*. *haemolytic*, spreading, flat secreted edge colonies grown.

In Macconkey agar used to differential lactose fermenting bacteria to non-lactose fermenting bacteria with growth and haemolytic gram negative cell.

In Nutrient agar the colonies and surrounding Medium was changed the greenish blue due to the production of soluble phenazine pigment with grape like or taco like adour.

BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF MICROBES

Identification of Coliform Bacteria

The coliform bacteria which was considered as significant or probably significant was identified by the following conventional tests.

Indole test

Peptone broth was prepared and sterilized and it was dispensed into sterile test tubes and culture was inoculated and incubated. After 24 hrs, a few drops of kovac's reagent were added. Formation of red colour ring at top of the broth indicates positive result and yellow colour indicates negative result.

Methyl red test

MR-VP broth was prepared and distributed into test tubes and it was sterilized. The test cultures was inoculated and incubated. After 24 hrs, a few drops

of methyl red indicator were added. Formation of red colour indicates positive result and yellow colour indicates negative result.

Voges Proskauer test

In a sterilized MR-VP medium, the test cultures was added and incubated. After 24 hrs Barrits reagent A and B was added respectively. Red colour indicates positive result and yellow colour indicates negative result.

Citrate utilization test

Sterile Simmon's citrate agar medium was prepared and poured in test tubes and sterilized. After sterilization, slants were made. The test organisms were streaked with the cultures and incubated at 37°C for 24 hours. After incubation, Colour Change from green to blue indicated positive result. No colour change indicated negative result.

Triple sugar iron agar test

TSI agar slants were prepared and test cultures were streaked along the slants and the tubes are incubated at 37°C for 24 hrs. After 24 hrs the tubes are taken and examine the result.

Nitrate reduction test

Nitrate broth was prepared and dispensed into test tubes. The test tubes were sterilized and one loop full of cultures were inoculated and incubated for 24 hrs. After incubation, few drops of alpha naphthalamine and sulphanilic acid were added. The positive test indicated by red colour formation.

Catalase Test

In a clean glass slide, a drop of bacterial suspension was placed on it. A drop of hydrogen peroxide was added to the culture. Evolution of bubbles indicates positive result. No change, negative result.

Urease Test

Urea agar was prepared and sterilized and poured into test tubes and slants were made. The test culture was streaked with the slants and incubated at 24 hrs. Colour change from yellow to red indicates positive result. No colour change indicates negative result.

Oxidase Test

The cultures were rubbed over the filter paper containing a reagent N-N tetra methyl paraphenylene diamine dihydrochloride. Purple colour indicated positive result.

Sugar fermentation

Nutrient broth was prepared with following sugar such as glucose, sucrose, lactose, maltose and mannitol. All these are prepared with indicator (phenol red). The broth was distributed in test tubes and Durham's tube were introduced and sterilized. The organism were inoculate in sugar tubes and incubate the culture for 24-48 hours at 37°C and Observe the results of sugar fermentation have recorded the colour changes in broth and gas production, yellow colour indicates positive and red colour remains means negative

Starch hydrolysis test

Starch agar medium was prepared and poured in sterile Petri plates. After solidification, the cultures were streaked in the centre of the plates and the plates were inverted and incubated at 37˚C for 24 hrs. After incubation, the plates were flooded with iodine solution for 30 sec. Blue black colour was seen around the streak region indicates positive result. No blue black colour indicates negative result.

Gelatin hydrolysis test

Gelatin medium was prepared and plated in a sterile Petri plates. After solidification, the test bacterial cultures were streaked in centre of plate and the inoculated plated were incubated at 37°C for 24 hrs. After incubation, the hydrolyzing activity was tested by using mercuric chloride solution which was flooded on the gelatin agar surface. Formation of clear zone around the line of streak after the addition of mercuric chloride indicates positive result. No clear zone indicates negative result.

INTERPRETATIONS

(a) For H_2S Production

Black coloration along the streak line or throughout the medium indicated H_2S production. If black colour is not produced, then H_2S is not produced.

(b) Changes in the butt

Yellow colour of the butt indicates acid was produced due to glucose fermentation. Red/Pink colour of the butt indicates that no acid is produced.

If gas bubbles are present or butt is broken - then gas is produced. So the reactions can be

- A = (Yellow)
- AG+ = Acid and Gas
- Alkaline (K) = Pink

(c) Changes in the slant

Yellow colour indicates acid production due to lactose or sucrose or fermentation of both. Pink colour indicates slant is alkaline, no fermentation of lactose or sucrose.

The final reactions can be:

Alkaline Slant/ Alkaline Butt (KK)	No fermentation of any carbohydrates and no H_2S production.
Alkaline Slant / Acid Butt (K/A)	Fermentation of glucose, non-fermentation of lactose and sucrose and no H_2S production.

INOCULUM PREPARATION OR STANDARDIZATION

Few colonice of each isolate were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity test as describe by NCCLS.

DECECTION OF EXTENDED SPECTRUM BETA LACTAMASE

In the screening test for ESBL production was done as part of routine susceptibility testing. Antibiotic disc were placed (cefltazimide 30g, and cefotaxime 30g) for screening the ESBL. Plates with Muller Hinton Agar were prepared and inoculated with the test organism to form of lawn culture. The above disc was incubated at 37°C overnight and senstive and resistant pattern were recorded by reading the zone diameter of the organism. If a zone diameter of < 27 mm for cefotaxime was recorded the strain was considered suspicious for ESBL production.

TEST FOR ESBL PRODUCTION:

a) Double disc test

In the ESBL production test the first step is double disc approximation test, Muller Hinton Agar plated with a depth of 4mm were prepared. With aseptic precaution 2 to 4 pure colonies of bacteria isolates selected and inoculated into 2ml of nutrient broth. Then it was incubation at 37°C for 2-3 hours to get a moderate turbidity equivalent to 0.5 McFarland standards. A sterile swab was dipped into the inoculum and soaked was rotated against the upper inside wall of the tube to remove excess fluid. The entire Muller Hinton Agar was swabbed to form a lawn culture and inoculm was allowed to dry for 15 minutes with lid in place. After that place the antibiotics with the sterile forceps. Cefotaxime was placed in centre of Agar plates.

Giving centre to distance of 15mm, augmenting (Amoxicillin 20g and Claviulanic acid 10g) were placed near ito Cefotaxime. The result were found to give the best result for the detection of ESBL and it was based on twice the radius of the inhibition zone produceds by Cefotaxime by its own. The plates were incubation at 370°C for 16-18 hours. Each plate was examined for enhancement of zone of Cefotaxime disc at the side facing augmentin disc. If the strains ESBL producer, then the zone around Cefotaxime disc was extended towards augmentin disc.

NCCLS CONFIRMATORY TEST:

The sensitivity of standard inoculum of isolates to Cefotaxime, ceftadime, ceftazimide/Claviulanic acid disc was determined on Muller Hinton Agar used in Kirby Bauer Method (12). Muller Hinton Agar plates were swabbed with the test organism having the turbidity to 0.5mm, McFarland standards. Inoculm was allowed to dry for few minutes with lid in place. The antibiotics were placed on the surface of the Agar. The plates were incubation at 37°C for 18-24 hours after which the plates were read. For disc diffusion testing a >5mm increase in zone diameter for either antimicrobial agent tested in combination with Claviulanic acid versus its diameter when tested alone confirmed the presence of ESBLproduction by that organisms. The increase in zone of diameter was due to inhibition of the B-lactamase by clavulanat.

RESULTS

Urinary track infections (UTIs) study was carriedout for a period from 2008 to 2009 in the Department of Microbiology, Rajah Muthiah Medical College, Annamalai University. Totally 150 sample was collected from different location of Chidambaram. The sample was collected from different age groups and

sex distributed (Table -1). 30-40 Age group diabetic patient 6 (female 4, male 2), 41-50 age group diabetic patient 63 (female 34, Male 29), 51-60 age group diabetic patient 60 (female 29, male 31), 60-72 age group diabetic patient 21 (female 9, male 12).Majority of the cases seen in urinary track infection in age (41-50) from women.

List of Organism isolated in this study

In this studies the list of isolates were given in Table – 2, Fig -1. The Bacterial Organisms are isolated in Nutrient, Blood agar, Macconkey agar plate. The non-heamolytic and lactose fermenting bacterial Colonies are formed in plates. These colonies are observed by direct microscopic Examination. All urine samples are collected form diabetic patient and subject to gram stain, procedure and observed under microscope. Gram negative bacteria were pink in colour conducted by biochemical test Catalase test a small portion of the colony was mixed in 3% hydrogen peroxide. Formation of bubbles indicated the presence of catalase in the organism. Oxidase test a small portion of the colony was rubbed onto a filter paper strip dipped with 1% N, N, tetramethyl parapheylene diamine dihydrochloride. Formation of purple colour will indicate the presence of oxidase. But the isolates did not show purple colour within 10 seconds and were considered negative. Indole production test the isolates were inculcated onto 5ml of Tryptone broth, incubated overnight at 37°C. When 0.2 of Kovac's reagent was added, positive reaction was indicated by the formation of a pink ring at the junction of the indole reagent. Methyl red test pure culture of the isolate was inoculated onto 5 ml of Glycose phosephate broth incubated for 48 hours at 37°C. When 0.5 ml of Methyl red reagent was added to the culture, red colour formation was considered to be positive and yellow colour was considered negative. Voges - Prasukauer Test the isolates were incubated onto 5 ml of glucose phosphate broth, incubated for 48 hours at 37°C. When 0.6 ml of 40% Potassium hydroxide (Brarrit's. Reagent A) and 0.2 ml of 5% a – Naphthol (Barrit's Regent B) was added, gently mixed and allowed to stand for 15 minutes, pink colour formation was considered to be positive and if there is no colour change it was considered negative. Citrate Utilization test a drop of 4-6 hour old isolates was inoculated onto Simmons citrate agar slant and incubated for 24 hours at 37°C. A change in colour from green to deep Prussion blue formation was to be positive and no colour change was considered to be negative. Mannitol motility nitrate test the isolates were stabbed onto the mannitol motility nitrate medium and incubated for 24 hours at 37°C. Mannitol fermentation was indicated by change of the colour of the medium to yellow. Motile organisms grown out from the stabbed line throughout the medium. Nitrate reduction was identified by the formation of red colour when 0.5 ml of Nitrate Regent A and 0.5 ml of Nitrate Reagent B was added. Triple sugar iron agar (TSI) test the pure growth of the isolate was taken with a straight wire and inoculated by stabbing down the center of agar butt and carefully withdrawn and then the surface of the slant was streaked and incubated at 37°C for 18-24 hours. Biochemical test are identify bacterial organisms. The different isolates percentage in given below (Plate-1,2,3).

E.coli (60%) is the most prevalent bacterial organisms to cause urinary track infection in diabetic patient, followed by *Staphylococcus sp* (7.3%) *klebsiella sp* (6.6%) and *Pseudomonas sp* (3.3%), 20% of sample are no growth is the medium.

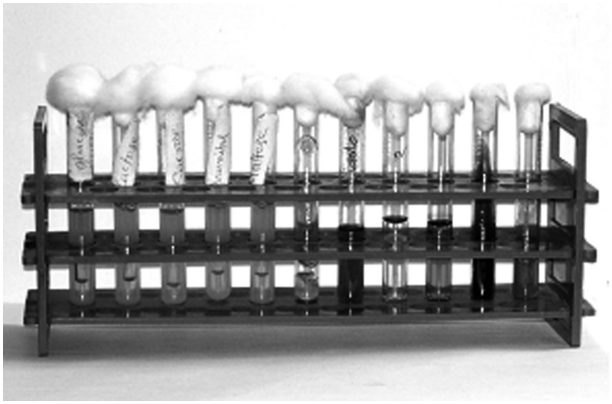


Plate -1 Biochemical test for bacterial isolates

Plate-2 Isolation of *E. coli* from patient sample

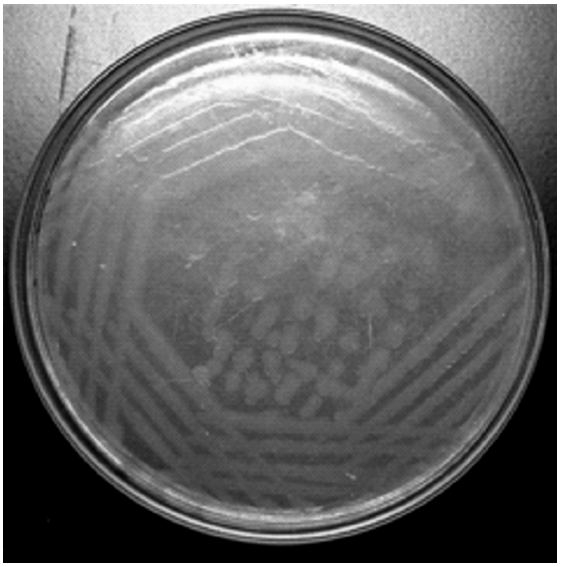


Plate-3 Isolation of Pseudomonas from patient sample

Antibiotic sensitivity pattern results

Sensitivity pattern of *E.coli* strains results are given in Table -3, Fig – 2. *Amikacin* (75%) is more sensitivity to *E.coli* followed by *oflaxin* 50% and *ciprofloxin* 45.8% sensitivity to *E.coli*. The *cefatnine, proflazn, Gentamycin, Cytazidime, Norfloxacin, cytrionone, Ampicillin*, and *Amoxycla* are moderate sensitive to *E.coli* organisms. Nalidixic acid is more resistant antibiotic to *E.coli* organism.

Table – 1 Urine samples collected from the diabetic patients with different age and sex groups

S.No.	Age group	No. of patients	Sex	
			Female	Male
1.	30-40	6	4	2
2.	41-50	63	34	29
3.	51-60	60	29	31
4.	60-72	21	9	12
	Total	150	76	74

Table – 2 Percentage of different Microorganisms isolated from UTI patients

S.No.	Organisms	Percentage (%)
1.	*E.coli*	60.3
2.	*Klebsiella sp*	10.0
3.	*Staphylococcus sp*	6.6
4.	*Pseudomonas sp*	3.3
5.	*Proteus sp*	0.6
5.	No growth	20

Fig : 1 Percentage of different Microorganisms isolated from UTI patients

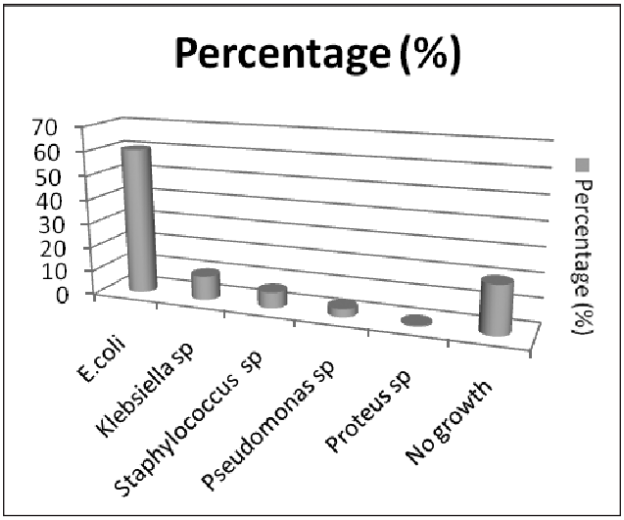
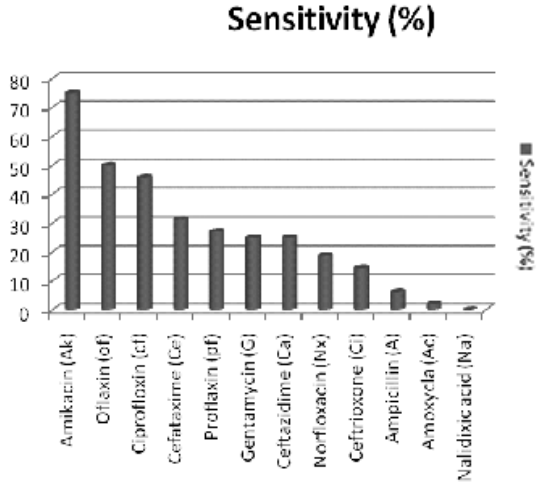


Table 3 Sensitivity pattern of *E. coli* strains isolated from UTI patients

S.No.	Antibiotics	Sensitivity (%)
1.	*Amikacin (Ak)*	75.00
2.	*Oflaxin (of)*	50.00
3.	*Ciprofloxin (cf)*	45.83
4.	*Cefataxime (Ce)*	31.25
5.	*Proflaxin (pf)*	27.08
6.	*Gentamycin (G)*	25.00
7.	*Ceftazidime (Ca)*	25.00
8.	*Norfloxacin (Nx)*	18.75
9.	*Ceftrioxone (Ci)*	14.58
10.	*Ampicillin (A)*	6.20
11.	*Amoxycla (Ac)*	2.08
12.	*Nalidixic acid (Na)*	0.00

Fig : 2 Sensitivity pattern of *E. coli* strains isolated from UTI patients



DISCUSSION

Urine is sterile; it is usually free of bacteria, viruses and fungi but does contain fluids, salts and waste products. An infection occurs when tiny organism, usually bacteria from the digestive track cling to the opening of the urethra and begin to multiply. Then urethra is the tube that carries urine from the bladder to outside the body most infections arise from one type of bacteria *Escherichia coli* which normally lives in the colony. *Escherichia coli* is a large group of facultative anaerobic, non-sporulating gram negative bacterium, it in commonly found in human being and animals. Urinary track infections (UTIs) study was carried out for a period of six months from October 2008 to March 2009. Nearly 150 samples were collected from different locations of Tamilnadu. The sample was collected from different age groups.In the present study, four different categories of diabetic patient urine specimen samples was collected. 41-50 age group of patients recorded high urinary infection in women (34), and men (29). Similarly in earlier (13) reported women even though they generally have anatomically urinary tracts.

The different types microorganisms isolated from diabetic patients urine samples, in this study. Among the different group *E.coli* recorded 41.3% in the sample followed by *Klebsiella sp*, (10.0%) *Staphylococcus sp*, *Pseudomonas sp* and *Proteus sp*. (14) also reported that E. coli (42%), followed by *Staphyloccus aureus*, *Klebsiella sp* etc., from the urine cultures.

The sensitivity pattern of E.coli strains isolates from diabetic patients was studied. Among the twelve antibiotics tested for sensitivity pattern, *Amikacin* (75%) is more sensitivity to *E.coli* followed by *oflaxin* (50.00%), *Ciprofloxin* (45.83%), *Cefataxime* (31.25%), and *proflaxin* (27.08%) Zinnah et al., 2008, in earlier study reported that *Genetamicin, Ciprofloxacin*, *Ampicillin* resistant to *E.coli* isolates from samples of different biological and environment sources.

Resistance of the *E.coli* isolates from different sources to a particular drug was also variables. There is clear evidence of a base of antibiotics due to which emergence of multidrug resistant *E.coli* are continuously increasing day-by-day. Based on the present study it may be concluded that *Amikacin* and *Oflaxin* will be first drugs of choice *Ciprofloxin* and *Cefataxime* will be the second drugs choice to resist the infections caused by *E.coli* in human.

CONCLUSION

The current incidence of antibiotics has reached a serious point when contemplating appropriate therapy for urinary tract infection (UTI). We underline the importance of early recognition of the clinical picture and urologist should implement timely therapy. Hospitalization is protracted in these patients, particularly due to time taken for the antibiotic therapy to achieve remission of the severe infections picture. The correct management of such infections is extremely important for the future, in particular in particular in term of reducing the incidence of new antibiotic resistance patterns.

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