Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

Study of Fosfomycin Susceptibility in Enterobacteriaceae Isolated from Urine Samples

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Abstract

Introduction:

Urinary tract infections (UTIs) are among the most common bacterial infections affecting individuals of all age groups in both community and hospital settings. Enterobacteriaceae, particularly Escherichia coli and Klebsiella species, are the leading causative agents. The rising prevalence of multidrug-resistant uropathogens has limited the effectiveness of commonly used antibiotics. Fosfomycin, a phosphonic acid derivative with a unique mechanism of action inhibiting bacterial cell wall synthesis, has re-emerged as a potential therapeutic option for UTIs due to its broad-spectrum activity and oral availability.

Objective:

To determine the fosfomycin susceptibility pattern among Enterobacteriaceae isolates obtained from urine samples in a tertiary care hospital.

Methods:

This study was conducted in the Department of Microbiology at a tertiary care hospital over a one-year period. A total of 11,763 urine samples were processed, of which 2,773 showed significant bacterial growth. Among these, 150 uropathogenic Enterobacteriaceae isolates were randomly selected. Organisms were identified using standard microbiological techniques and the VITEK 2 system. Fosfomycin susceptibility was assessed by Kirby–Bauer disc diffusion method according to CLSI guidelines. Minimum inhibitory concentration (MIC) was determined by E-test for selected isolates.

Results:

Escherichia coli (79.4%) was the predominant isolate, followed by Klebsiella species (18%), Proteus species (1.3%), and Citrobacter species (1.3%). All isolates demonstrated susceptibility to fosfomycin by disc diffusion. MIC values for tested isolates were within the sensitive range (<64 µg/ml).

Conclusion:

Fosfomycin exhibited excellent in vitro activity against uropathogenic Enterobacteriaceae. It may serve as an effective oral treatment option for UTIs, particularly in the era of increasing antimicrobial resistance.

Keywords: Urinary tract infection, Enterobacteriaceae, Fosfomycin, Antimicrobial susceptibility, MIC, Disc diffusion.

1. Introduction

Urinary tract infection(UTI) is one of the most frequent infections among hospital acquired or community acquired infections.[1] urinary tract infection is defined as the presence of multiplying microorganisms in the urinary tract.[2] *E. coli* is the most common pathogen among the Gram negative bacteria capable of causing complicated and uncomplicated urinary tract infections. Most of the uncomplicated UTI's are caused by *E. coli* accounting to 90% of community acquired and approximately 50% of nosocomial UTI's. The origin of these strains in patients is most often from their own intestinal flora.[3]

Other genera of Enterobacteriaceae, such as *Klebsiella*, *Enterobacter, Proteus* and *Serratia*, which were found as normal inhabitants of the large intestine, include organisms that are primarily opportunistic and often nosocomial pathogens. They all frequently colonize hospitalized patients, especially in association with antibiotic treatment, indwelling catheters or invasive procedures, causing extra intestinal infection such as those of the urinary tract.[4]

Many different antimicrobial agents are available for the treatment of UTI. Commonly used drugs for the treatment of UTI are Co-trimoxazole, Trimethoprim, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Cephalosporins and semi-synthetic Penicillins with or without inhibitors and Fosfomycin. The study of etiology of UTI's and their resistance patterns may help the clinician to choose the right empirical treatment.[5]

Fosfomycin is a phosphonic acid bactericidal agent, which is known for nearly 4 decades and particularly useful for urinary tract pathogens.[6] It is an oral drug and found to be effective against several Gram negative and Gram positive aerobic bacteria[7] and also against ESBL and Carbapenemase producing Enterobacteriaceae isolates.[1]

Fosfomycin has a unique mechanism of antimicrobial action that involves the inhibition of UDP-N-acetyl glucosamine enol pyruvyl transferase [MurA], an enzyme that catalyses the first step in bacterial cell wall synthesis within the cell.[2] Fosfomycin inhibits the N-acetyl glucosamine enol pyruvyl transferase, which catalyzes the conversion of UDP-N-acetylglucosamine to UDP-N-acetylmuramic acid.[3] Fosfomycin is an phosphoenol pyruvate analogue that inhibits MurA by alkylating an active site of cystiene residue.[2,12,7] Fosfomycin can enter the bacterial cell only by active transport. Two transport systems are known to exist, the L-α-glycerophosphate system and hexose monophosphate route. [3]

Fosfomycin resistance is mainly due to chromosomal mutations, decreased drug uptake and mutations in the gene coding for MurA.[3] Recently, plasmid mediated mechanisms to Fosfomycin resistance have also been described, which involve expression of enzymes capable of modifying fosfomycin by adding glutathione, L-cysteine or H_2O.[3]

Hence, this study was taken up to know the Fosfomycin susceptibility among Enterobacteriaceae isolates isolated from urine samples.

AIM:

- To Study Fosfomycin Susceptibility in Enterobacteriaceae isolated from Urine Samples.

OBJECTIVES:

- To study the etiology of UTI in patients attending JSS hospital.
- To determine the susceptibility of urinary enterobacteriaceae isolates to fosfomycin by disc diffusion.
- To detect the MIC value for fosfomycin by E-test method.

Review of Literature

Urinary Tract Infection may be defined as a condition in which bacteria are established and multiplying within the urinary tract.[8] Although the infection and resultant symptoms may be localized, the presence of bacteria in urine places the entire urinary system at risk of invasion by bacteria.[8]

E. coli are one of the most prevalent pathogens among Gram-Negative bacteria, capable of causing complicated and uncomplicated UTI's. According to Foxman, Uropathogenic *E. coli* (UPEC) are the primary cause of community-acquired urinary tract infections (UTI's) (70%-95%) and a large portion of nosocomial UTI's (50%). Mulvey et al and Bower et al have reported that, UPEC strains act as an opportunistic intracellular pathogens which colonizes the bladder of the urinary tract, causing cystitis and also ascend through the ureters into the kidneys, causing pyelonephritis. Uropathogenic *E. coli* forms intracellular bacterial communities with many biofilm like properties within the bladder epithelium[8].

Escherichia coli, the most prevalent facultative Gram-Negative bacillus in the human faecal flora, usually inhabit the colon as an innocuous commensal (Eisenstein et al., 1987). Urinary tract infections (UTIs) are the most common form of the extraintestinal pathogenic *E. coli* (ExPEC) infections and *E. coli* is the most common cause of UTIs[8].

Types of UTIs. UTIs are generally classified as:

- Uncomplicated or complicated, depending on the factors that trigger the infections[9]
- Primary or recurrent, depending on whether the infection is occurring for the first time or is a repeat event

Uncomplicated Urinary Tract Infections (UTIs):

Uncomplicated UTIs are due to a bacterial infection, most often *E. coli*. They affect women much more often than men[10].

Cystitis, or bladder infection, is the most common urinary tract infection. It occurs in the lower urinary tract (the bladder and urethra) and nearly always in women[11]. In most cases, the infection is brief and acute and only the surface of the bladder is infected. Deeper layers of the bladder may be harmed if the infection becomes persistent, or chronic, or if the urinary tract is structurally abnormal.

Pyelonephritis(Kidney Infection), sometimes the infection spreads to the upper tract (the ureters and kidneys). This is called pyelonephritis, or more commonly, a kidney infection.

Complicated Urinary Tract Infections:

Complicated infections, which occur in men and women of any age, are also caused by bacteria but they tend to be more severe, more difficult to treat, and recurrent. They are often the result of:

- Some anatomical or structural abnormality that impairs the ability of the urinary tract to clear out urine and therefore bacteria.
- Catheter use in the hospital setting or chronic indwelling catheter in the outpatient setting,
- Bladder and kidney dysfunction, or kidney transplant (especially in the first 3 months after transplant).

Recurrences can occur in patients with complicated UTI if the underlying structural or anatomical abnormalities are not corrected.

Recurrent Urinary Tract Infections:

Most women who have had an uncomplicated UTI have occasional recurrences[12,13]. About 25 - 50% of these women can expect another infection within a year of the previous one. Between 3 - 5% of women have ongoing, recurrent urinary tract infections, which follow the resolution of a previous treated or untreated episode.

Recurrence is often categorized as either **Reinfection or Relapse**:

• **Reinfection:** Most cases of recurring UTIs are reinfections. A reinfection occurs several weeks after antibiotic treatment has cleared up the initial episode and can be caused by the same bacterial strain that caused the original episode or a different one. The infecting organism is usually introduced through faecal bacteria and moves up through the urinary tract.

• **Relapse:** Relapse is the less common form of recurrent urinary tract infection. It is diagnosed when a UTI recurs within 2 weeks of treatment of the first episode and is due to treatment failure. Relapse usually occurs in kidney infection (pyelonephritis) or is associated with obstructions such as kidney stones, structural abnormalities or, in men, chronic prostatitis.

Causes:

- The uropathogenic causing UTI vary by clinical syndrome but are usually enteric gram negative rods that have migrated to the urinary tract. The susceptibility pattern of these organisms vary by clinical syndrome and by geography.
- In acute uncomplicated cystitis, the etiologic agents are highly predictable: *E.coli; Staphylococcus saprophyticus, Klebsiella, Proteus, Enterococcus and Citrobacter species* along with other organisms.
- The spectrum of agents causing uncomplicated pyelonephritis is similar, with *E.coli* predominating. In complicated UTI, *E.coli* remains the predominant organism, but other aerobic negative rods such as *Pseudomonas aeruginosa* and *klebsiella, Proteus, Citrobacter, Acinetobacter* and *Morganella species.*
- In community acquired infection, the increased prevalence of uropathogenic producing ESBL has left few oral options for therapy. Since resistance rates vary by local geographic region, with individual patient characteristics, and over time, it is important to use current and local data when choosing a treatment regimen.[14]

Enterobacteriaceae are ubiquitous organisms that are found worldwide in soil, water, vegetation and they are part of the normal flora of the gastrointestinal tract of most animals, including humans.

Members of the family Enterobacteriaceae show many common properties; they are all Gram negative, non-spore forming bacilli, and they are all relatively small (2-3micrometer X 0.4-0.6micrometer). They are either motile by peritrichous flagella or non-motile. Some of them are encapsulated. Enterobacteriaceae grow rapidly on ordinary laboratory media under aerobic and anaerobic conditions. All species utilize glucose fermentatively, often with the formation of either acid or acid & gas. They are oxidase negative and with only a few exceptions, catalase positive. They reduce nitrates to nitrites.

Escherichia coli:

E coli is a component of the normal intestinal flora of humans.

Escherichia coli comes under the family Enterobacteriaceae, genus Escherichiae, which is Gram -negative, straight rod measuring 1-3 to 0.4-0.7 micrometer (µm), arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be non-motile. Capsules and fimbriae are found in some strains. Spores are not formed[14].

It is an aerobic and facultative anaerobe and grows on ordinary culture medium at optimum temperature of 37°C (temperature range 10-40°C) in 18-24 hours. Colonies of some strains show beta haemolysis on blood agar. On MacConkey's medium, colonies are pink due to lactose fermentation (LF or lactose fermenter colonies).

Cell Wall Composition:

The outer layers of *E. coli* consist of the outer membrane with phospholipids, lipid A and proteins, from which protrude the polysaccharide (LPS) chains, overlayed by capsular polysaccharides (CP) (Jann and Jann 1987).

Pathogenicity and Virulence Factors:

E. coli is a commensal in the human intestinal tract[15], when enters into unnatural sites, it can cause a variety of infections, e.g., UTIs, sepsis, pyelonephritis etc[16]. Certain serotypes of *E. coli* were consistently associated with uropathogenicity and were designated as Uropathogenic *E. coli* (UPEC)[17].

Virulence factors of *E. coli* are of two main types: those produced on the surface of the cell and those produced within the cell and then exported to the site of action. Those on the surface include different sorts of fimbriae that have a role in adhesion to the surface of host cells but may also have additional roles such as tissue invasion, biofilm formation or cytokine induction. Whereas other type have anti host cell activities and enable the bacteria to grow in an environment of iron restriction.

Cell surface hydrophobicity- high hydrophobicity of the bacterial cell surface promotes their adherence to various surfaces like mucosal epithelial cells. It is responsible for recurrent UTI.

Klebsiella:

Klebsiella species are usually found as commensals in human intestines and as saprophytes in soil.

K.pneumoniae has three subspecies:1.*K.pneumoniae* subspecies *pneumoniae* 2. *K.pneumoniae* subspecies *ozaenae* 3.*K.pneumoniae* subspecies *rhinoscleromatis*.

K.pneumoniae is most pathogenic among all other subspecies. *K.pneumoniae* is responsible for severe lobar pneumonia, urinary infections, meningitis, septicimia, and pyogenic infections[18].

Citobactereae

Citrobacter species are mostly environmental contaminants isolated from water, soil, food and feces of man and animals. They are motile, lactose fermenters like *E.coli*, but differ from the latter in being citrate positive and lysine decarboxylase negative.

They occasionally cause urinary tract, gallbladder and middle ear infections and neonatal meningitis[18].

Enterobacter

Enterobacter species are similar to *klebsiella* in most biochemical reactions but differ from the latter in being motile and ornithine decarboxylase positive.

E.aerogenes and *E.cloacae* are the most commonly isolated species from the clinical specimens.

They are opportunistic pathogens, implicated in infected wounds and urinary tract infections and occasionally septicemia and meningitis[18].

Serratia

The characteristic property of serratia is production of a red non diffusible pigment called prodigiosin, which is formed optimally at 30^0C.

S.marcescens is being increasingly reported in various nosocomial infections such as meningitis, endocarditis septicemia, urinary tract infections, respiratory and wound infections[18].

Proteus

Proteus species show pleomorphism, i.e. they vary in size.

P.mirabilis and *P.vulgaris* are the most commonly encountrered species.

They are frequently present on the moist areas of the skin, intestine of humans and animals.

They are opportunistic pathogens, commonly associated with urinary, wound and soft tissue infections and septicemia[18].

FOSFOMYCIN

Fosfomycin is a phosphonic acid bactericidal agent, which is known for nearly 4 decades and particularly useful for urinary tract pathogens.[1] It is an oral drug and found to be effective against several Gram negative and Gram positive aerobic bacteria[12] and also against ESBL and Carbapenemase producing enterobacteriaceae isolates.[1]

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Chromosomal mutations can influence the function of fosfomycin membrane transport systems, resulting in low intracellular levels of the drug. Two transport systems for the uptake of fosfomycin into cells have been described in *E.coli*, involving the glycerol-3-phosphate transporter and a hexose phosphate transporter respectively. The former transporter is constitutively expressed while the latter is inducible, mainly in the presence of glucose-6-phosphate. Mutations affecting either of these transporter can result in mutation.[3]

Resistance to fosfomycin can often be associated with the presence of enzymes that inactivate the antibiotic. Three main mechanisms of this type have been described in pathogenic bacteria specifically, fos A encodes a glutathione S transferase, fos B encodes L cysteine thiol transferase and fos X encodes an epoxide hydrolase.[3]

REVIEW OF ARTICLES

- Silva JO *et al* (2015), have carried out work on Successful treatment of lower urinary tract infections with oral fosfomycin. They have reported three patients with lower urinary tract infections caused by Klebsiella pneumoniae carbapenemase (KPC)-producing Klebsiella pneumoniae who were successfully treated with a seven-day course of oral fosfomycin monotherapy.[19]

- Rodrigo Batista Souzaa *et al* (2015), have carried out work on Bacterial sensitivity to fosfomycin in pregnant women with urinary infection. In their study, 134 samples were selected. The age of the subjects ranged from 15 to 40 years (mean 26.7). *E. coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were the most commonly identi- fied species. In 89% of cases, the microorganisms were sensitive to fosfomycin. *E. coli* and *S. aureus* were the main species of bacteria responsible for urinary tract infections in women in the study area. The most prevalent microorganisms in pregnant women with urinary tract infection were susceptible to fosfomycin[5].

- *In vitro* efficacy of Fosfomycin against clinical strains was carried out by Karadag A *et al.*, (2014). Fosfomycin is an alternative drug for treatment of uncomplicated urinary tract infections. All the MRSA (n=40), MRCoNS (n=40), and VR E. faecium (n=62) isolates were susceptible to fosfomycin. The fosfomycin susceptibility rates for E. coli, K. pneumoniae, and Enterobacter spp. were 97.5% (39 of 40), 97.3% (36 of 37), and 86.9% (20 of 23), respectively. One (2.7%) isolate of K. pneumoniae and three (13.1%) isolates of Enterobacter spp. showed intermediate susceptibility to fosfomycin. Resistance to fosfomycin was detected in only one (2.5%) isolate of E. coli[20].

- Abdul Rouf Khawaja *et al.*,(2015), have carried out work on Fosfomycin tromethamine antibiotic of choice in the female patient: A multicenter study. A total of 400 patients were included in the study. Group A (200 patients) with asymptomatic bacteriuria in pregnancy and Group B (200 Patients) with symptomatic lower urinary tract infections and with any day care endourological procedures were enrolled in their study. It has an advantage of oral single /multiple doses, higher eradication rate of bacteria after 48 hours, excellent tolerability and safety in pregnancy and other female age groups. We recommend Fosfomycin Trometamol as the drug of choice particularly in patients with poor drug compliance and for minor day care endourological procedures[21].

- R. Daza *et al.*,(2001), have carried out work on Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. A total of 13,774 samples were analysed using an automatic system for the detection of bacterial ATP (Coral, USA). Of these samples, 49% were reported to be positive and uncontaminated; bacteria most frequently isolated were E. coli (47%), Proteus mirabilis (7%), *E. faecalis* (6%) and *K. pneumoniae* (5%). The susceptibility studies showed 37% *E. coli* strains resistant to amoxycillin+clavulanate 33% to cotrimoxazole and 22% to ciprofloxacin. Seven strains of *E. coli* produced ESBL. Thirteen per cent of strains were resistant to cefuroxime but only (1%) to fosfomycin. Resistance to nitrofurantoin in *K. pneumoniae* was 38%. *P. mirabilis* showed 52% resistance to cotrimoxazole and 13% *S. aureus*, were methicillin-resistant. *E. faecalis* did not show any special resistance to normal medication. Fosfomycin continued to show high activity against Gram-negative bacilli[22].

- Matthew E Falagas *et al.*,(2010), have carried out work on Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review. They evaluated the evidence on fosfomycin as a treatment option for infections caused by members of the family Enterobacteriaceae with advanced resistance to antimicrobial drugs, including producers of extended-spectrum β-lactamase (ESBL). 11 of the 17 studies reported that at least 90% of the isolates were susceptible to fosfomycin. Using a provisional minimum inhibitory concentration susceptibility breakpoint of 64 mg/L or less, 1604 (96·8%) of 1657 *E. coli* isolates producing *ESBL* were susceptible to fosfomycin. Similarly, 608 (81·3%) of 748 *K.* pneumoniae isolates producing ESBL were susceptible to fosfomycin[3].

- Ya Li *et al.*,(2015), have carried out work on Antimicrobial Susceptibility and Molecular Mechanisms of Fosfomycin Resistance in Clinical *E. coli* Isolates. 1109 non-duplicate clinical E. coli strains isolated from urine, sputum, blood and pus samples in 20 widely dispersed tertiary hospitals, followed by determination of minimum inhibitory concentrations of fosfomycin. In their study, 7.8% (86/1109) E. coli strains were fosfomycin non-susceptible. The majority of isolates were susceptible to fosfomycin, showing that the drug is still viable in clinical applications[23].

- M.de.cueto *et al.*,(2005) carried out work on in vitro activity of fosfomycin against ESBL producing E.coli and klebsiella pneumoniae. This study shows the agar dilution, broth microdilution and disc diffusion methods were compared to determine the in vitro susceptibility of 428 ESBL producing E.coli and klebsiella pneumoniae to fosfomycin. Fosfomycin showed very high activity against all ESBL producing strains excellent agreement between the three susceptibility method was found for E.coli, whereas marked discrepancies were observed for K.pneumoniae.[24]

- Gupta *et al*.,(2013), have carried out work on Determination of ESBL and AmpC production in uropathogenic isolates of E.coli and susceptibility to fosfomycin. A number of 150 E.coli were isolated in their study. ESBL detection was done by double disc synergy and CLSI method. In AmpC positive isolates ESBLs was detected by modifying CLSI method using boronic acid. Antimicrobial susceptibility pattern was determined following CLSI guidelines. Fosfomycin susceptibility was determined by disc diffusion and E-test method. ESBLs production was seen in 52.6% isolates and AmpC production was seen in 8% isolates. ESBLs positive isolates were found to be more drug resistant thanESBLs negative isolates. All the strains were found to be fosfomycin sensitive.[7]

- Chinglanlu *et al*.,(2011), have carried out work on Antimicrobial susceptibility of commonly encountered bacterial isolates to fosfomycin as determined by agar dilution and disc diffusion method. This study shows the antimicrobial activity of fosfomycin against 960 strains of commonly encountered bacteria associated with UTI. E.coli was uniformly susceptible to fosfomycin, as were most strains of K.pneumoniae and Enterobacter cloacae. New tentative zone diameter criteria for K.pneumoniae, E.cloacae, S.aureus and E.faecium were able to set, providing some interim laboratory guidance for disc diffusion until further breakpoint evaluations are undertaken by CLSI and EUCAST.[25]

- Oteo *et al*.,(2010),have carried out work on parallel increase in community use of fosfomycin and resistance to fosfomycin. ESBL producing E.coli. A total of 231 ESBL-EC were collected.

The overall rate of fosfomycin resistance was 9.1% but varied according to ESBL type. Fosfomycin resistance increased in these isolates from 4.4% (2005) to 11.4% (2009)[26].

- Kaase *et al*.,(2014), have carried out work on The study of fosfomycin susceptibility in carbapenem resistant enterobacteriaceae. A total sample of 107 carbapenem-non susceptible enterobacteriaceae isolates of which 80 produced various types of carbapenemase. 78% of the strains had fosfomycin MICs of ≤32mg/liter and were considered to be susceptible according to the current EUCAST break point[6].

- Drosos E. Karageorgopoulos *et al.*,(2011) have carried out work on Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens In their review, they evaluate the available published evidence regarding the mechanisms and the frequency of in vitro mutational resistance to fosfomycin in Gram-negative pathogens. The development of resistance appears to be more frequent both in vitro and in clinical studies for Pseudomonas aeruginosa in comparison with Escherichia coli, whereas relevant data for other Enterobacteriaceae are relatively scarce. The urinary tract seems to provide a favourable environment for the use of fosfomycin with a low associated likelihood for the emergence of resistance, owing to high drug concentrations and acidic pH[3].

- L. V. Perdigão-Neto *et al.*,(2014) have carried out work on Susceptibility of Multiresistant Gram-Negative Bacteria to Fosfomycin and Performance of Different Susceptibility Testing Methods. In their study susceptibility methods using 94 multi-resistant clinical isolates. With agar dilution (AD), susceptibilities were 81%, 7%, 96%, and 100% (CLSI) and 0%, 0%, 96%, and 30% (EUCAST), respectively, for Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp. Categorical agreement between Etest and AD for Enterobacteriaceae and A. baumannii was >80%. Disk diffusion was adequate only for Enterobacter. CLSI criteria for urine may be adequate for systemic infections[27].

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, JSS Hospital, Mysore from Feb 2015 to Feb 2016.

SAMPLE COLLECTION : Clean catch, mid-stream urine samples were collected in a sterile, wide-necked, leak-proof container from clinically suspected cases of UTI.

INCLUSION CRITERIA : Enterobacteriaceae isolated from the urine specimens were included in the study.

EXCLUSION CRITERIA : Organisms other than Enterobacteriaceae isolated from the urine specimens were excluded from the study.

LABORATORY PROCEDURES : All the urine samples received were processed as follows:

1) MACROSCOPY: The urine samples thus obtained were examined for its color, pH and whether it was clear or turbid.

2) **WET MOUNT:** One loopful of uncentrifuged urine was taken on the clean, grease-free glass-slide and covered with cover slip and observed under low power & high power objective for inflammatory cells (considered significant if more than 5-6 pus cell/HPF); RBC and organisms.

3) CULTURE : STANDARD LOOP METHOD[28]

PRINCIPLE : An inoculating loop of standard dimensions is used to take up a small, fixed and known volume of mixed uncentrifuged urine and spread over a plate of agar culture medium. The plate is incubated, the number of colonies are counted and used to calculate the number of viable bacteria per ml of urine.

PROCEDURE : The calibrated loop that holds a fixed volume of 0.001 ml was used. Before inoculation, the calibrated loop was inserted vertically into the urine and was mixed thoroughly. The loop carrying 0.001 ml of mixed uncentrifuged urine was touched to the center of the Urichrome agar plate (UCA) and from which the inoculum was spread in a line across the diameter of the plate and then loop was drawn across the entire plate, crossing the first inoculum streak numerous times to produce isolated colonies. The plates were incubated at 37°C for 24-48 hrs. After incubation, the colonies on UCA were counted.

INTERPRETATION : The presence of 100 or more colonies was considered as significant bacteriuria.

4) IDENTIFICATION : Organisms belongs to the Enterobacteriaceae family was identified using standard microbiological techniques.

5) The Enterobacteriaceae organisms thus isolated were **screened for Fosfomycin Susceptibility by Disc diffusion and MIC by E-test.**

6) VITEK 2 COMPACT SYSTEM :

Principle: Vitek 2 is an automated microbiology system utilizing growth based technology. They accommodate colorimetric reagent cards that are incubated and interpreted automatically. AST cards are based on broth micro dilution MIC technique.

Procedure:

- Vitek identification cards and AST cards were brought to room temperature.
- For each organism, 2 tubes were arranged in a rack
- First tube was labeled with patient number for ID and second tube for AST.
- 5ml of vitek saline was pipetted out for both tubes.
- Single bacterial colony was emulsified in the first tube.
- Then the tube was mixed well using vortex mixture for 1min.
- The turbidity was checked using the densicheck and it should be exactly 0.5 Mc.farland.
- 145µl above suspension was transferred to second tube containing 5ml saline for AST.
- The second tube was also mixed well using a vortex mixture for a minute.
- ID card was placed in the first tube test and AST card in second tube.
- The rack was loaded into filler and start Fill was pressed.
- The patient ID was saved

- The report was interpreted after complete 24 hours.

7) FOSFOMYCIN DISC DIFFUSION BY KIRBY BAUER METHOD[50,51]

PRINCIPLE

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical. This area of no growth around the disc is known as a "ZONE OF INHIBITION".

PROCEDURE :-

- All the isolates were tested for Fosfomycin susceptibility by modified Kirby-Bauer disc diffusion method, according to Clinical and Laboratory Standards Institute guidelines (CLSI).
- Overnight culture of isolate grown at 37°C on MA plates was inoculated into 5ml BHIB and incubated at 35°C for 2-4 hrs and turbidity matched with 0.5 McFarland.
- The sterile cotton swab was immersed into the broth and removed the excess of inoculum by pressing the cotton swabs into the sides of the test tube.
- Lawn culture of inoculum was done on MHA plate, Fosfomycin disc was placed and incubated overnight at 37°C.
- After incubation, the zone of inhibition was measured using a millimeter scale around each antimicrobial disc on the undersurface of the plate and interpreted as per CLSI guidelines.

Interpretation:

ZONE OF INHIBITION	INTERPRETATIVE
≥16µg/ml	SENSITIVE
13-15µg/ml	INTERMEDIATE
≤12µg/ml	RESISTANT

8) Fosfomycin MIC by E-strip method:

All isolates which are resistant to fosfomycin by disc diffusion will be tested for MIC value by E-test[Biomereiux, India] with fosfomycin gradient concentrations ranging from 0.04µgm/ml to 1,024µgm/ml.

Procedure:

- Overnight culture of isolate grown at 37°C on MA plates was inoculated into 5ml BHI broth and incubated at 35°C for 2-4 hrs and turbidity was matched with 0.5 McFarland standards.
- The sterile cotton swab was immersed into the broth and removed the excess of inoculum by pressing the cotton swabs onto the sides of the test tube.
- Lawn culture of inoculum was done on MHA plate.
- After drying, Fosfomycin impregnated E-strip were placed on the inoculated surface and gently pressed.
- Each strip contains a gradient antibiotic and is labeled with a scale of minimal inhibitory concentration values.
- After overnight incubation, an elliptical zone of inhibition was produced.
- The point at which the elliptical zone sides meet at the strip was taken as MIC. The markings at the junction are noted and the concentration was recorded as MIC for that drug.

Interpretation:

MIC VALUE	INTERPRETATIVE
< 64µg/ml	SENSITIVE
128µg/ml	INTERMEDIATE
> 256µg/ml	RESISTANT

RESULTS

The present study was carried out in the Dept. of Microbiology, J.S.S. Hospital from Feb 2015 to Feb 2016 to detect the Fosfomycin susceptibility among Uropathogenic isolates.

11763 various clinical samples were collected from out-patients as well as inpatients, from different clinical departments of JSS Hospital. Among 11,763 samples 2,773 were culture positive for bacterial growth.

TABLE 1 : CLINICAL SAMPLES WITH SIGNIFICANT BACTERIAL GROWTH:

Number of clinical samples collected	Number of clinical samples with bacterial growth	Number of clinical samples with no bacterial growth
11,763	2,773(23.6%)	8,990(76.4%)

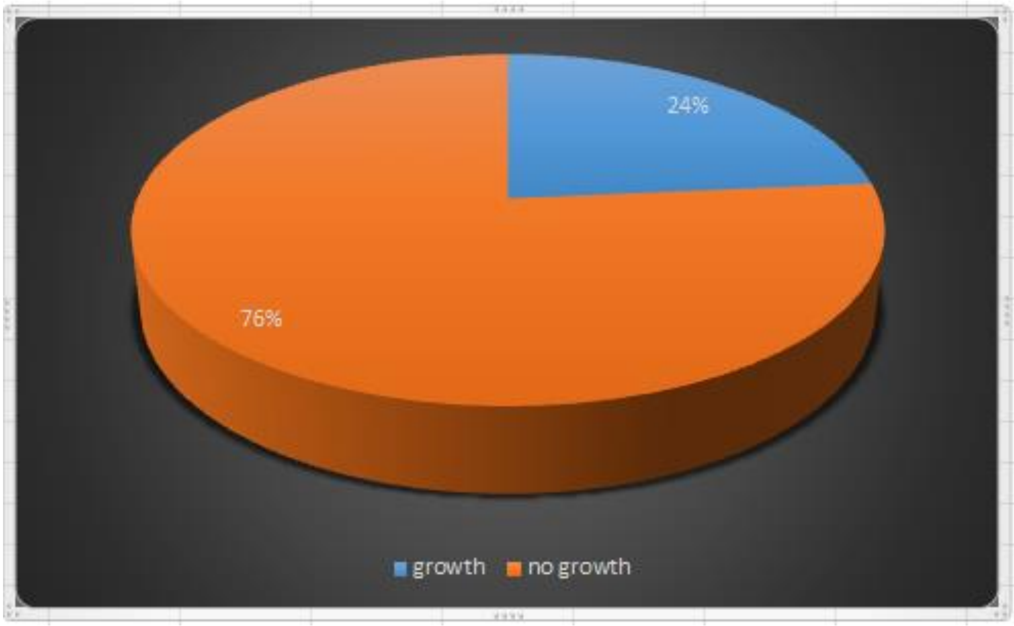


TABLE 2 : Gender distribution of clinical samples with bacterial growth:

GENDER	SAMPLES	PERCENTAGE
MALES	1,424	51.4%
FEMALES	1,349	48.6%
TOTAL	2,773	100%

Among the 2,773 samples that yielded growth, 1,424 clinical samples were from males and 1,349 were from females.

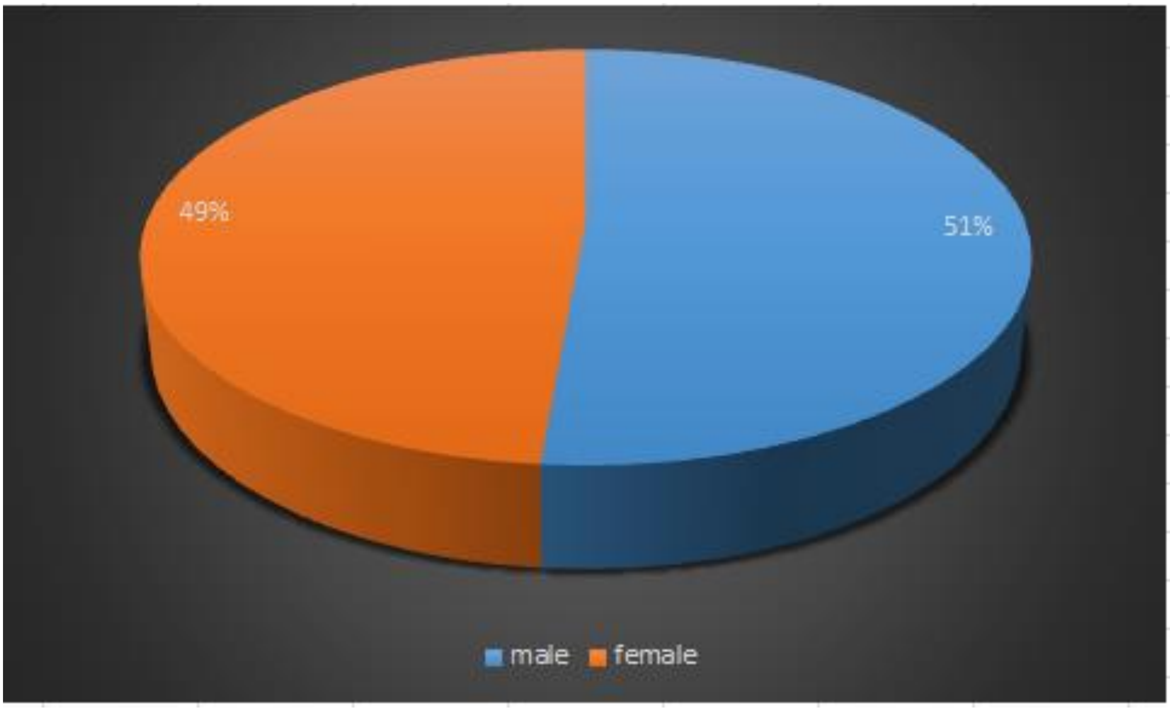
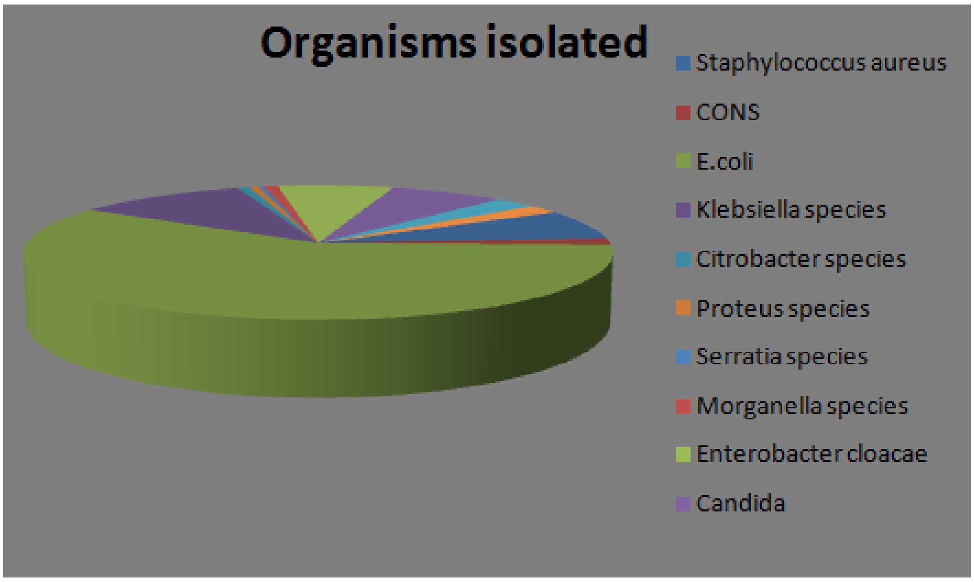


TABLE 3 : Organisms isolated from urine samples

Organisms	Number (%)
Staphylococcus aureus	192(6.91%)
CONS	42(1.5%)
E.coli	1,581(57%)
Klebsiella species	291(10.5%)
Citrobacter species	21(0.75%)
Proteus species	15(0.54%)
Serratia species	9(0.32%)
Morganella species	24(0.9%)
Enterobacter cloacae	195(7.03%)
Candida	195(7.03%)
Pseudomonas	72(6.91%)
Enterococci	55(2.0%)
TOTAL	2,773(100%)

The most common isolated organism from the urine samples was *E.coli* (57%) followed by *Klebsiella species* (10.5%), *Enterobacter cloacae*(10.5%), *Candida*(10.5%) , *Staphylococcus species* (6.91%) *and Pseudomonas* (6.91%).



Out of 2,773 samples, 150 samples of Uropathogenic Enterobacteriaceae were randomnly selected and **screened for Fosfomycin Susceptibility by Disc diffusion and MIC by E-test.**

TABLE 4 : Gender distribu3tion of clinical samples with bacterial growth

Total number of Uropathogenic Enterobacteriaceae isolates – 150

GENDER	SAMPLES	PERCENTAGE
MALES	77	51.3%
FEMALES	73	48.7%
TOTAL	150	100%

Among 150 Uropathogenic Enterobacteriaceae isolates, 77 clinical samples were from males and 73 samples were from females.

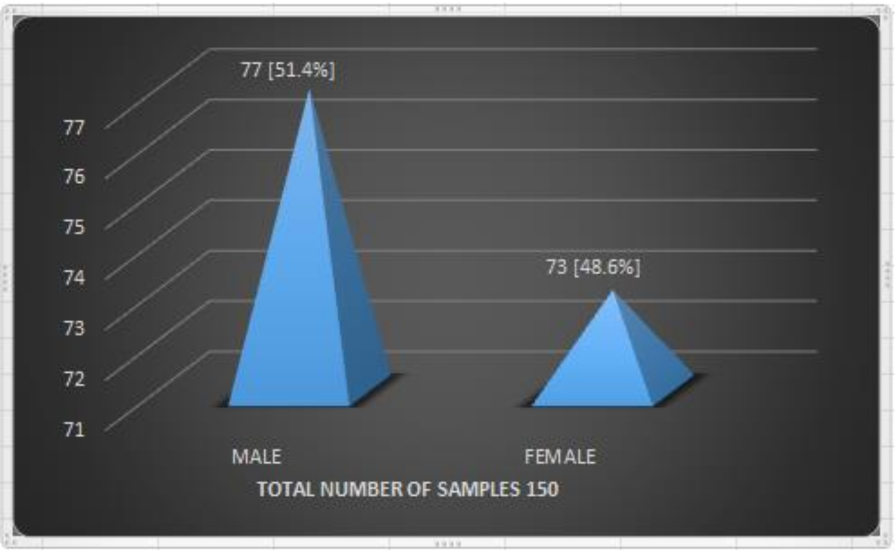


TABLE 5 : Age group distribution of clinical samples

AGE	MALE(%)	FEMALE(%)	TOTAL(%)
<10	11(14.3%)	11(15%)	**22(14.6%)**
11-20	2(2.6%)	6(8.2%)	**8(5.4%)**
21-30	5(6.5%)	11(15.%)	**16(10.6%)**
31-40	2(2.6%)	5(6.8%)	**7(4.6%)**
41-50	8(10.4%)	8(10%)	**16(10.6%)**
51-60	19(25.9%)	13(17.8%)	**32(21.4%)**
61-70	16(20.8%)	10(13.6%)	**26(17.4%)**
>71	13(16.9%)	10(13.6%)	**23(15.4%)**
TOTAL	**77**	**73**	**150**

Majority of the urine samples included in this study were from patients in the age group of <10 years and 51-60 years followed by 61-70 years.

TABLE 6 : Ward wise distribution of Uropathogenic Enterobacteriaceae isolates

WARDS	TOTAL NUMBER
Medicine ward	22
Emergency ward	2
ICU	11
Paediatrics	16
Urology	19
Nephrology	7
OPD	56
Others	11
TOTAL	**150**

Majority of Uropathogenic Enterobacteriaceae isolates were isolated from OPD patients followed by Medicine wards, Urology wards, Paediatrics wards and ICU wards (which include NICU, RICU, ICCU, SICU, PICU, MICU).

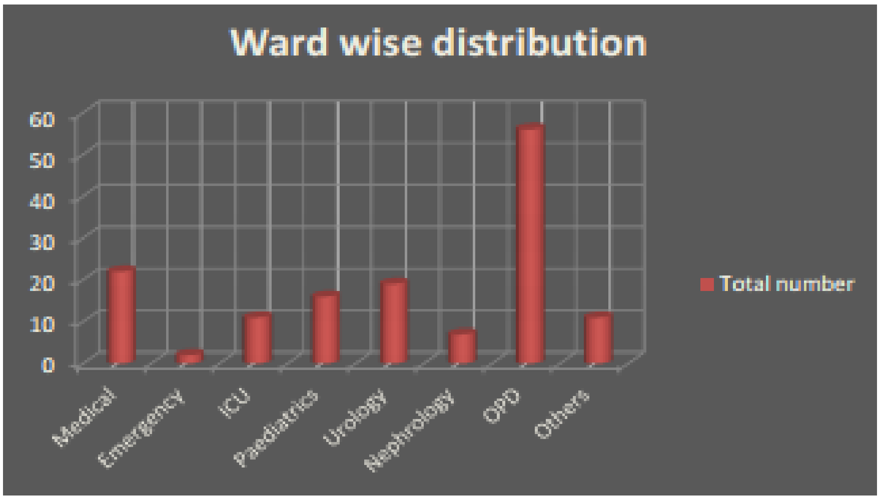


TABLE 7 : Uropathogenic Enterobacteriaceae isolates

Organisms	Number (%)
E.coli	119(79.4%)
Klebsiella pneumonia	27(18%)
Citrobacter	2(1.3%)

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Proteus	2(1.3%)
TOTAL	**150(100%)**

In this, *E.coli* (78.4%) was the most frequently isolated organism, followed by *Klebsiella sps* (18%) ,*Proteus sps*(1.3%) and *Citrobacter sps* (1.3%)

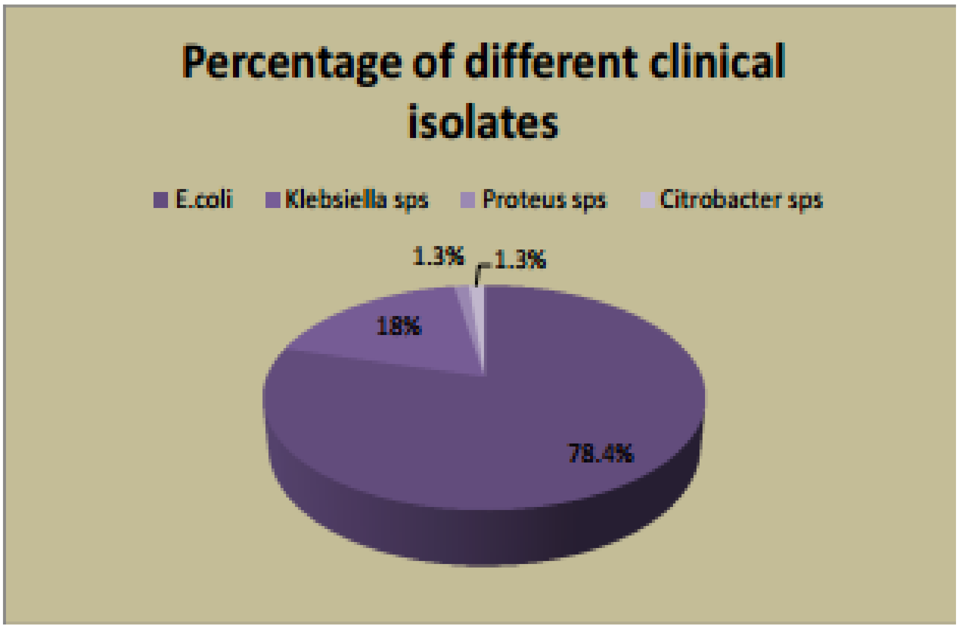

TABLE 8 : ANTIBIOGRAM

DRUGS	*E.coli (n=119)*			*Klebsiella spp (n=27)*			*Proteus spp(n=2)*			*Citrobacter spp(n=2)*		
	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)
Ampicillin	16 (13.45)	1(0.84)	102(85.7)	3(11.11)	1(3.7)	23(85.1)	-	-	-	-	-	-
Amoxyclav	42(35.3)	40(33.6)	37(31.09)	9(33.3)	4(14.8)	14(51.8)	2(100)	-	-	2(100)	-	-
Piperacillin/tazobactam	84(70.5)	8(6.7)	27(22.6)	15(55.5)	4(14.8)	8(29.6)	2(100)	-	-	2(100)	-	-
Cefuroxime	23(19.3)	6(5)	90(75.6)	6(22.2)	1(3.7)	20(74)	1(50)	-	1(50)	1(50)	1(50)	-
Cefuroxime-axetil	23(19.3)	6(5)	90(75.6)	6(22.2)	1(3.7)	20(74)	-	1(50)	1(50)	-	-	2(100

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Ceftriaxone	28(23.5)	-	91(76.4)	9(33.3)	-	18(66.6)	2(100)	-	-	2(100)	-	-
Cefaperazonesulbactam	93(78.1)	5(4.2)	21(17.6)	19(70.3)	-	8(29.6)	2(100)	-	-	2(100)	-	-
Cefepime	54(45.3)	23(19.3)	42(35.3)	16(59.2)	1(3.7)	10(37)	2(100)	-	-	2(100)	-	-
Ertapenem	94(78.9)	1(0.8)	14(11.76)	17(62.9)	-	10(37)	2(100)	-	-	2(100)	-	-
Imipenem	104(87.3)	-	15(12.6)	19(70.3)	-	8(29.6)`	2(100)	-	-	2(100)	-	-
Meropenem	105(88.2)	-	14(11.7)	19(70.3)	-	8(29.6)	2(100)	-	-	2(100)	-	
Amikacin	110(92.4)	-	9(7.5)	20(740	2(7.4)	5(18.5)	2(100)	-	-	2(100)	-	-
Gentamicin	61(51.2)	-	58(48.7)	18(66.6)	-	9(33.3)	2(100)	-	-	2(100)		
Nalidixic acid	6(5)	-	113(94.9)	10(37)	-	17(62.9)	1(50)	-	1(50)	1(50)	-	1(50)
Ciprofloxacin	27(22.6)	-	92(77.3)	11(40.7)	4(14.8)	12(44.4)	1(50)	-	1(50)	1(50)	-	1(50)
Tigecycline	116(97.4)	-	3(2.5)	22(81.4)	2(7.4)	3(11.1)	2(100)	-	-	2(100)	-	-
Nitrofurantoin	92(77.3)	17(14.2)	10(8.4)	5(18.5)	9(33.3)	13(4.81)	2(100)	-	-	1(50)	1(50)	-
Colistin	119(100)	-	-	23(85.1)	-	4(14.8)	-	-	2(100)	2(100)	-	-
Co- trimoxazole	50(42.0)	-	69(57.9)	14(51.8)	-	13(48.1)	2(100)	-	-	2(100)	-	-

Majority of the *E.coli* and *Klebsiella* isolates were found to be susceptible to Tigecycline, Colistin followed by Carbapenems.

TABLE 9 : Sensitivity pattern of organisms isolated from urine samples to Fosfomycin by disc diffusion.

Zone of inhibition	***E.coli***	***Klebsiella species***	***Proteus species***	***Citrobacter species***
<20mm	1	5	-	-
21-30mm	62	14	2	-
>31mm	56	8	-	2

All isolates were found to be sensitivity to Fosfomycin by disc diffusion as per CLSI criteria. However, *E.coli* isolates were found to show bigger zone of inhibition compared to *Klebsiella* and *Proteus* species.

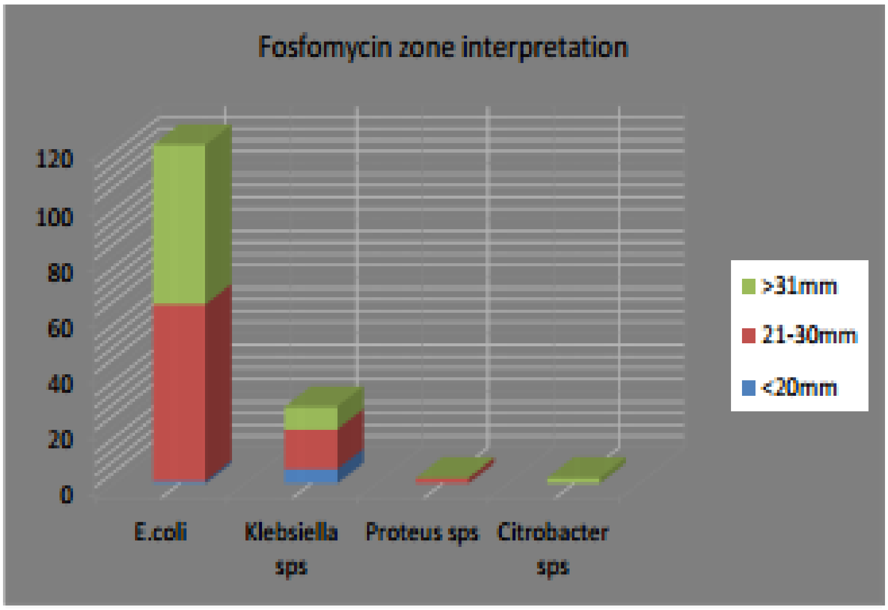
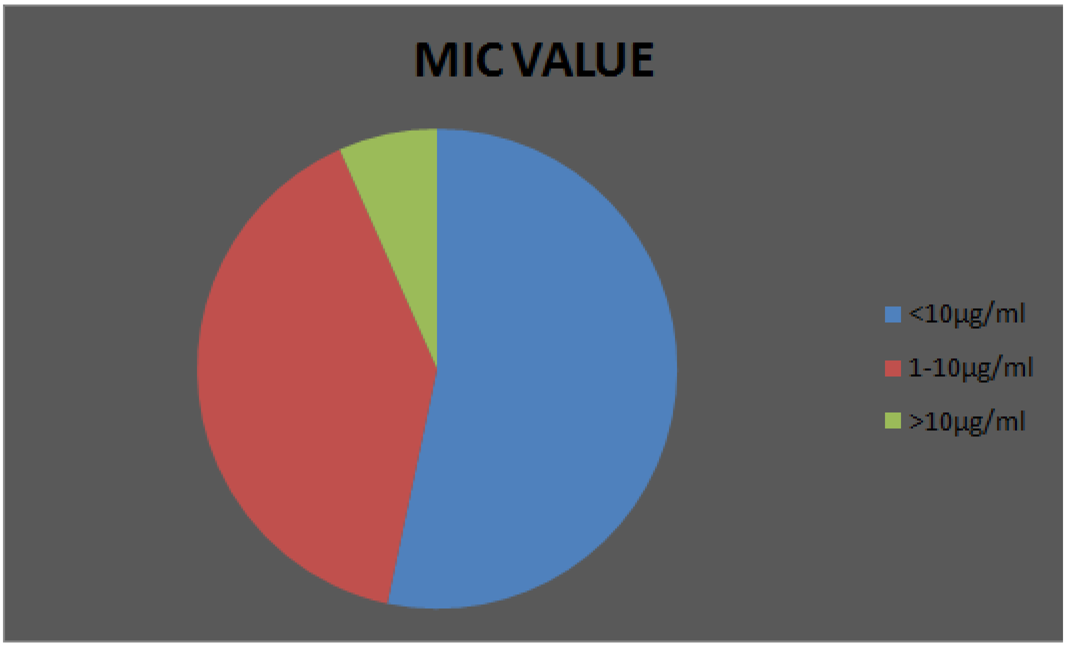


TABLE 10 : MIC value by E-Strip test.

Among 150 sample of Uropathogenic Enterobacteriaceae, 30 samples were randomly selected to know the MIC value by E-Strip test.

MIC value	**NUMBER**
<1 µg/ml	**16**
1-10µg/ml	**12**
>10µg/ml	**2**

All study isolates showed MIC value in the sensitivity range(<64µg/ml) as per CLSI criteria.in our study, we observed 16 isolates to have an MIC value of <1µg/ml, 12 isolates with MIC value in the range of 1-10µg/ml. But 2 isolates had an MIC value of >10µg/ml.



DISCUSSION

UTI's are common cause of morbidity and affects person of all age groups including young women, children and the elderly. At least 10 to 20% of women experience an acute symptomatic UTI at some point during their lives. The severity of the infection depends both on the virulence of the infecting bacteria and on the susceptibility of the host.[27]

E. coli is the most common pathogen among the Gram Negative bacteria capable of causing complicated and uncomplicated urinary tract infections[1]. Most of the uncomplicated UTI's are caused by *E. coli* accounting to 90% of community acquired and approximately 50% of nosocomial UTI's. Other genera of Enterobacteriaceae, such as *Klebsiella*, *Enterobacter*, *Proteus* and *Serratia*, which were found as normal inhabitants of the large intestine, include organisms that are primarily opportunistic and often nosocomial pathogens[4].

Fosfomycin, derived from phosphonic acid is chemically unrelated to other antimicrobials and used to treat community acquired urinary tract infections. Fosfomycin has attracted renewed interest for the treatment of lower urinary tract and even systemic infections caused by Gram-negative pathogens with resistance to traditionally used agents. The main concern regarding the clinical utility of Fosfomycin refers to the potential for the emergence of resistance during therapy.(E.karageo) With the spread of multiresistance, Fosfomycin is a potential option.[27]

In the present study, the frequency of UTI was almost equal in males [51.3%] and females [48.7%] but Gupta *et al*[7], have reported that frequency of UTI was greater in females 98[65.3%] as compared to males 52 [34.7%]. The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having short urethra, presence of normal flora in vagina, menstrual cycle and pregnancy and in males UTIs is due to prostatitis, pyelonephritis, cystitis, urethritis.

In our study,150 clinical urinary isolates were included. Among them 22(14.66%) samples were from medical ward,2 (1.33%) samples were from emergency ward, 11(7.33%) samples were from ICU's, 16(10.66%) samples were from pediatrics, 19(12.66%) samples were from urology, 7(4.66%) samples were from nephrology, 56 (37.33%) samples were from OPD and 11(7.33%) samples were from remaining wards. Similar samples distribution was observed in a study by Sofia Maraki *et.al* [28]were total of 578 clinical urine samples were included. Out of 578 isolates 207 (35.8%) isolates were from adult outpatients, 167 (28.8%) from patients hospitalized in medical wards, 74 (12.8%) from adult patients hospitalized in surgical wards, 17 (2.9%) from intensive care unit adult patients, 9 (1.5%) from adult patients in renal replacement therapy clinics, and 14 (2.4%) from patients from areas other than the above-mentioned hospital units. 90 (15.5%) of the isolates were from pediatric patients.

Our study indicates that *E.coli* (79.3%) is the most common cause of UTI, which is similar to the results obtained by K.Hryniewicz *et al*[30], *E.coli* (73%).

In our study majority of the *E.coli* and *Klebsiella* isolates were found to be susceptible to Piperacillin/tazobactam, Carbapenems (82-86%)which is similar to the study carried out by Gupta *et al*[7], and their study also reports susceptibility to Carbapenems(88%) for both ESBL positive and ESBL negative strains.

In our study all *E.coli*(100%) isolates were found to be susceptible to Fosfomycin by both disc diffusion and E test which correlates with the result carried out by Gupta *et* al[7], who also reports 100% susceptibility to fosfomycin among *E.coli* ESBL positive and ESBL negative strains.

In our study out of 150 isolates, 27 isolates were *Klebsiella* (18%). All Klebsiella isolates (100%) were susceptible to Fosfomycin. In the study carried out by Andrea *et* al[29]. 87% of *Klebsiella* isolates were sensitive to Fosfomycin by Agar dilution, Disc diffusion and Etest and in the study carried by Perdigao *et al*[27] 96% of *Klebsiella* were susceptibile to Fosfomycin.

Even though in our study, 100% Fosfomycin sensitivity was observed in *E.coli* than *Klebsiella,* the study by Yang Hyun Cho *et al*[1] have reported that higher Fosfomycin sensitivity was observed in *E.coli* (94.9%) compared to *Klebsiella* (61.7%) .

In our study, *E.coli* strains had significantly lower Fosfomycin MICs than *Klebsiella* strains which correlates the study carried out by Martin kaase *et al*[6]. The high MICs value of *Klebsiella* shows impending development of Resistance in future.

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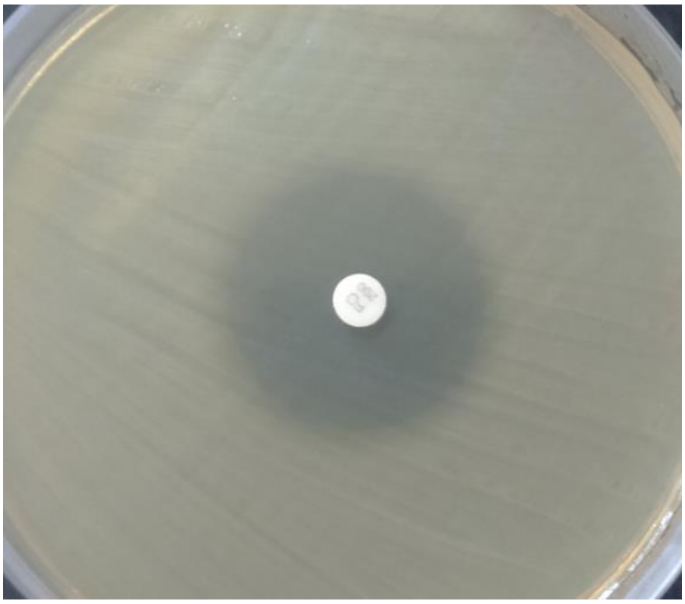
ILLUSTRATIONS

Fig 1: Uropathogenic Enterobacteriaceae isolates showing Fosfomycin sensitivity on Muller Hinton Agar plate by Disc diffusion method.



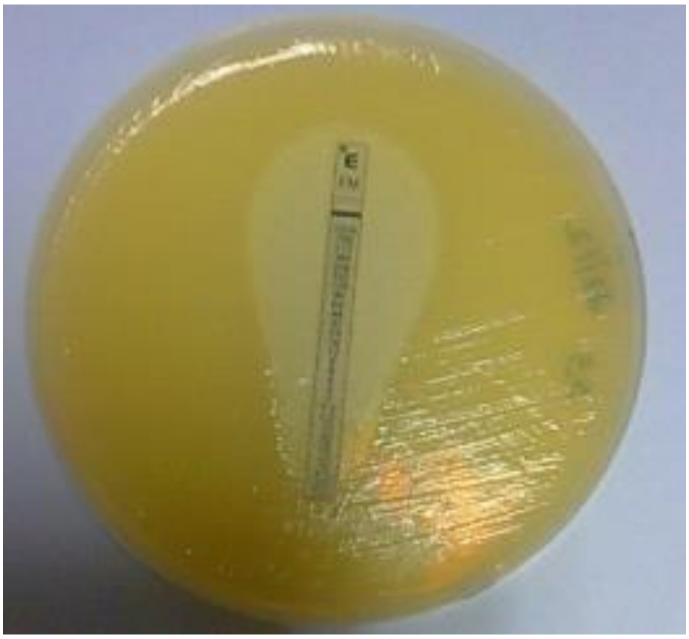
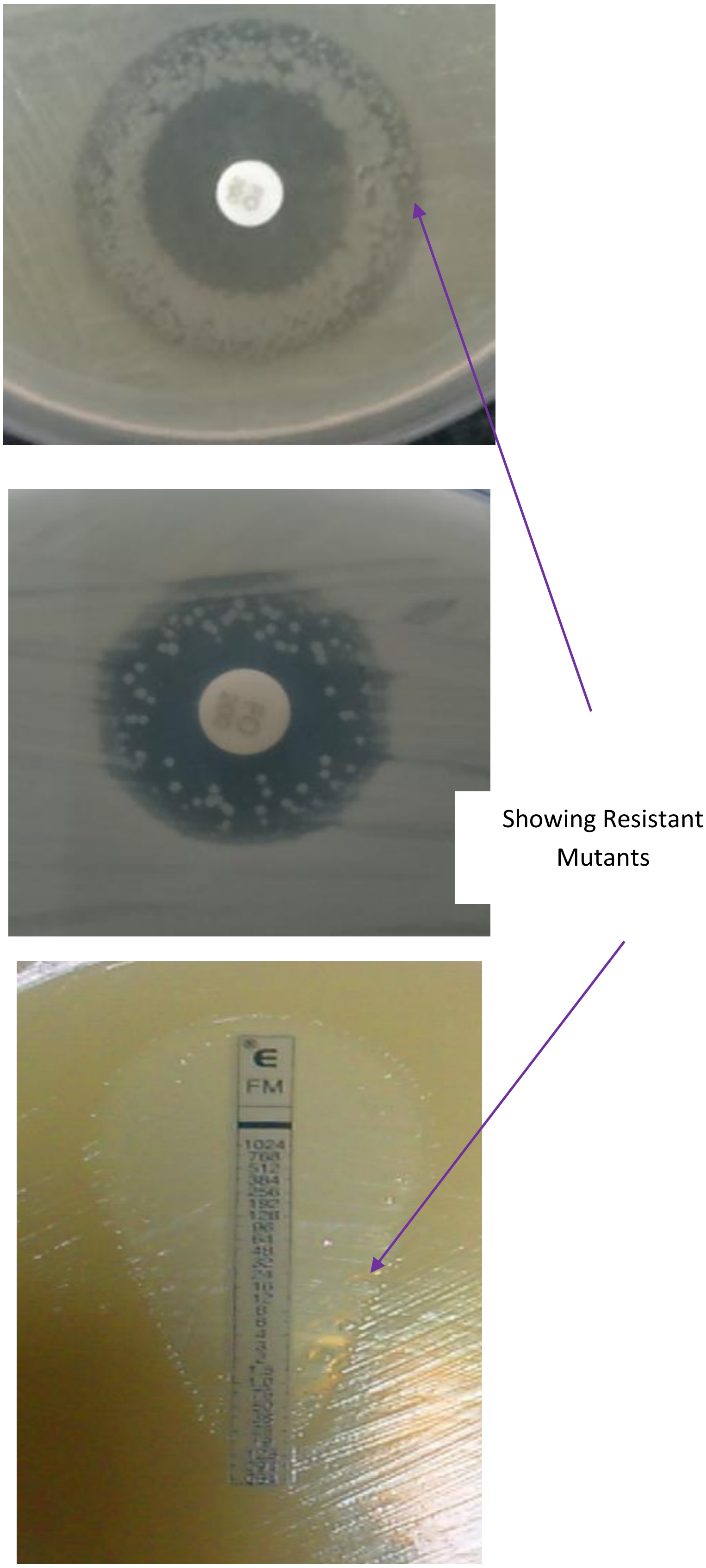


Fig 2: Some Uropathogenic Enterobacteriaceae isolates showing Resistant Mutants by Disc diffusion and E-Strip.


Showing Resistant Mutants
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SUMMARY

- ✓ Urinary Tract Infection is one of the most common infections in health care setting
- ✓ 11763 urine samples were collected from out-patients as well as inpatients, from different clinical departments of JSS Hospital.
- ✓ Among 11,763 samples 2,773 were culture positive for bacterial growth.
- ✓ Among the 2,773 samples that yielded growth, 1,424 clinical samples were from males and 1,349 were from females.
- ✓ The most common isolated organisms isolated from the urine samples was *E.coli* (57%) followed by *Klebsiella species* (10.5%), *Enterobacter cloacae*(10.5%), *Candida*(10.5%) , *Staphylococcus species* (6.91%) *and Pseudomonas* (6.91%).
- ✓ Out of 2,773 samples, 150 samples, which yielded growth of Uropathogenic Enterobacteriaceae were randomnly selected and screened for Fosfomycin Susceptibility by Disc diffusion and MIC by E-test.
- ✓ Majority of the urine samples included in this study were from patients in the age group of <10 years and 51-60 years followed by 61-70 years.
- ✓ Majority of Uropathogenic Enterobacteriaceae isolates were isolated from OPD patients followed by Medicine wards, Urology wards, Paediatrics wards and ICU wards.
- ✓ In this, *E.coli* (78.4%) was the most frequently isolated organism, followed by *Klebsiella sps* (18%) ,*Proteus sps*(1.3%) and *Citrobacter sps* (1.3%)
- ✓ Majority of the *E.coli* and *Klebsiella* isolates were found to be susceptible to Tigecycline, Colistin followed by Carbapenems.
- ✓ All isolates were found to be sensitive to Fosfomycin by disc diffusion as per CLSI criteria.
- ✓ All study isolates showed MIC value in the sensitive range (<64µg/ml) as per CLSI criteria.
- ✓ Fosfomycin may be a potential treatment option for UTI caused by Gram Negative bacteria.

CONCLUSION

The available evidence shows that Fosfomycin has a high level of antimicrobial activity against Enterobacteriaceae isolates. Fosfomycin might be a valuable treatment option for community acquired UTI caused by these pathogens. UTI's is not as low as one might except. Clinicians should be aware of existing data and treat patients according to the susceptibility patterns.

In our study all isolates were found to be sensitivity to Fosfomycin. Fosfomycin resistance in this Geographical area is not yet seen.

Taking into consideration the low cost of Fosfomycin antibiotic along with the possibility of oral administration, it seems to be preferable treatment option against UTI's in countries with limited financial resources. Being aware of the local antimicrobial susceptibility of Enterobacteriaceae uropathogens makes it more possible for clinicians to prescribe an appropriate empirical antibiotic treatment. Therefore, patients would experience fewer treatment failure and health care costs would decrease. Therefore, Fosfomycin could be an alternative treatment option for UTI's.

BIBLIOGRAPHY

1. Yang Hyun Cho, Seung II Jung, Ho Suck Chung, Ho Sung Yu. 2015. Antimicrobial susceptibilities of ESBL producing *E.coli* and *Klebsiella pneumonia* in health care associated UTI:focus on susceptibility to Fosfomycin. 47:1059-1066.
2. Matthew E Falagas, Antonia C Kastoris, Anastasios M Kapaskelis, Drosos E Karageorgopoulos. 2010. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review. **the lancet infection**. 10:43-50.
3. Drosos E Karageorgopoulos, Rui Wang, Xu- hong Yu and Matthew E Falagas. Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-Negative pathogens. **J Antimicrob Chemother.**
4. Text book of Microbiology.Lippincott's illustrated reviews. Third edition.
5. Rodrigo Batista Souzaa, Daisson José Trevisol a,b, Fabiana Schuelter-Trevisol a,b,. 2015. Bacterial sensitivity to fosfomycin in pregnant women with urinary infection. **The Brazilian Journal of INFECTIOUS DISEASES.** 19(3):319–323.
6. Martin Kaase, Florian Szabados, Agnes Anders, Soren G. Gatermann.2014. Fosfomycin susceptibility in carbapenem-resistant enterobacteriaceae. **Department of medical microbiology, Ruhr-university Bochum.**
7. Varsha Gupta, Hena Rani, NidhiSingla, NeelamKaistha, JagdishChander.2013. Determination of ESBL and AmpC production in uropathogenic isolates of E.coli and susceptibility to fosfomycin. **J Lab Physicians.5:90-3.**
8. Ponnusamy Poovendran, Nagappan Ramanathan and Nagarajan Prabhu. 2014. *In Vitro* Evaluation of Antibiotic Susceptibility Pattern of Extented Spectrum β Lactamase (ESBL) Producing Uropathogenic *Escherichia Coli* (UPEC) and their correlation with Biofilm formation. **International Journal of Recent Scientific Research**. 5(10): 1764-1769.
9. Azzarone G, Liewehr S and O'Connor K. 2007. Cystitis. **Pediatr Rev**. 28(12): 474-476.
10. Colgan R and Williams M. 2011. Diagnosis and treatment of acute uncomplicated cystitis. **Am Fam Physician**. 84(7): 771-776.
11. Colgan R, Nicolle L.E, McGlone A and Hooton T.M. 2006. Asymptomatic bacteriuria in adults. **Am Fam Physician**. 74(6): 985-990.
12. Foster R.T. 2008. Uncomplicated urinary tract infections in women. **Obstet Gynecol Clin North Am**. 35: 235-248.

13. Hooton T.M. 2012. Uncomplicated urinary tract infection. **N Engl J Med**. 367(2):185.

14. Text book Of Internal Medicine. Harison's.6th edition.

15. Asima Banu, Jyoti S Kabbin and Mridu Anand. 2011. Extraintestinal Infections due to *Escherichia Coli*: An Emerging Issue. **Journal of Clinical and Diagnostic Research**. 5(3): 486-490.

16. Seema Mittal, Madhu Sharma and Uma Chaudhary. 2014. Study of virulence factors of uropathogenic *E. coli* and its antibiotic susceptibility pattern. **Indian Journal of Pathology and Microbiology**. 57(1): 61-64.

17. Prabhat Ranjan K, Neelima Ranjan, Arindam Chakraborty and Arora DR. 2010. An Approach to Uropathogenic *Escherichia coli* in Urinary Tract Infections. **Journal of Laboratory Physicians**. 2(2): 70-73.

18. Text book of Microbiology. Apurba Sankar Sastry.

19. Oliveira da Silva, Maria Cecilia Zorat Yu, André Doi, Maria Rita Elmor de Araujo, Pedro Aurélio Mathiasi Neto and Guilherme Henrique Campos Furtado. 2015. Successful treatment of lower urinary tract infections with oral fosfomycin: a report of three cases. **Revista da Sociedade Brasileira de Medicina Tropical**. 48(3): 358-360.

20. Adil Karadağ, Yeliz Tanrıverdi Çaycı, Kemal Bilgin, Murat Günaydın, Cafer Eroğlu. 2014. In vitro efficacy of fosfomycin against clinical strains. **Journal of Microbiology and Infectious Diseases**. 4(2): 55-58.

21. Abdul Rouf Khawaja, Farzana Bashir Khan, Tanveer Iqbal Dar, Arif Hameed Bhat, Mohd Saleem Wani, Baldev Singh Wazir. 2015. Fosfomycin tromethamine. Antibiotic of choice in the female patient: A multicenter study. **Central European Journal of Urology**. 68: 371-375.

22. Rosa Daza, Jose´ Gutie´rrez , Gonzalo Pie´drola. 2001. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. **International Journal of Antimicrobial Agents** 211–215.

23. Ya Li , Bo Zheng, Yun Li , Sainan Zhu , Feng Xue , Jian Liu. 2015. Antimicrobial Susceptibility and Molecular Mechanisms of Fosfomycin Resistance in Clinical Escherichia coli Isolates. **PLOS ONE | DOI:10.1371/journal.pone.0135269.**

24. M.de.cueto *et al*.,(2005) carried out work on in vitro activity of fosfomycin against ESBL producing E.coli and klebsiella pneumoniae.

25. Ching-lan Lu, Chia-Ying Liu, Yu-Tsung Huang, Chua-Hsing Liao, Lee-JeneTeng, John D. Turnidge and Po-Rel Hsueh.2011. Antimicrobial susceptibility of commonly encountered bacterial isolates to fosfomycin as determined by the agar dilution and disc diffusion methods.

26. Oteo *et al*.,(2010),have carried out work on parallel increase in community use of fosfomycin and resistance to fosfomycin. ESBL producing E.coli.

27. L. V. Perdigão-Neto,M. S. Oliveira, C. F. Rizek,b C. M. D. M. Carrilho, S. F. Costa,b A. S. Levina, .2014. Susceptibility of Multiresistant Gram-Negative Bacteria to Fosfomycin and Performance of Different Susceptibility Testing Methods. Antimicrobial Agents and Chemotherapy 58 :3. 1763–1767

28. Sofia Maraki, George Samonis, Petros I. Rafailidis, Evridiki K. Vouloumanou, Emmanuel Mavromanolakis, and Matthew E. Falagas.(2009). Susceptibility of Urinary Tract Bacteria to Fosfomycin **ANTIMICROBIAL AGENTS AND CHEMOTHERAPY:** p. 4508–4510.

29. Andrea Endimiani, Gopi Patel, ,Kristine M Hujer, Mahesh Swaminathan and Robert A Bonomo. (2010). In Vitro Activity Of Fosfomycin against K_pKPC isolates, including those Non susceptible to Tgc or Col. **ANTIMICROBIAL AGENTS AND CHEMOTHERAPY.** P 526-529.

30. Katarzyna Hryniewicz, Katarzyna Szczypa, Agnieszka Sulikowska, Krzysztof Jankowski, Katarzyna Betlejewska and Waleria Hryniewicz.(2001). Antibiotic susceptibility of bacterial strains isolated from UTIs . **Journal of Antimicrobial Chemotherapy.** 773-780.

31. Alhambra, J. A. Cuadros, J. Cacho, J. L. Gómez-Garcés and J. I. Alós. 2004. In vitro susceptibility of recent antibiotic-resistant urinary pathogens to ertapenem and 12 other antibiotics. **Journal of Antimicrobial Chemotherapy 53, 1090–1094.**

32. M. De cueto, L. Lopez, J.R. Hernandez, C. Morillo and A. Pascual: Invitro Activity OfFosfomycin against ESBL producing E.coli and Klebsiella pneumonia. **Department of Microbiology hospital'p** G.Fraser, Barrie P.Marmion and Anthony Simmons editors. In **Mackie and McCartney Practical Medical Microbiology.**

33. Mackie and Mc cartney, PRACTICAL MEDICAL MICROBIOLOGY. 14th edition.

34. Giannoula S. Tansarli, a Stavros Athanasiou, Matthew E. Falagas . (2013).Evaluation of Antimicrobial Susceptibility of Enterobacteriaceae Causing Urinary Tract Infections in Africa. Antimicrobial Agents and Chemotherapy p. 3628 –3639

Advanced International Journal for Research (AIJFR)
E-ISSN: 3048-7641 ● Website: www.aijfr.com ● Email: editor@aijfr.com

ANNEXURE

STUDY OF FOSFOMYCIN SUSCEPTIBILITY IN ENTEROBACTERIACEA ISOLATED FROM URINE SAMPLES

PROFORMA

01. LAB.NO: 02.NAME:

03.AGE/SEX: 03.WARD:

04. IP.NO/ RQ NO: 05.REG.NO:

05. DIAGNOSIS:

06. CHIEF COMPLAINTS:

07. PAST HISTORY:

Catheterization

DM

08. EXAMINATION Findings:

09. IAGNOSIS:

10. INVESTIGATIONS:

11. TREATMENT:

LAB PROCESSING

- MICROSCOPY:
- UCA:

Amp	Amc	Pit	Cxm	Cefax	Ctr	Cfs	Cpm	Ert	Imp
Ak	Gen	Na	Cip	Tgc	Nit	Cl	Cot	Cz	Ctx
Nx	Mrp								

- Sensitivity to Fosfomycin by Disc diffusion method

Zone diameter:

Interpretation:

- Sensitivity to Fosfomycin by E-test method

MIC value:

Interpretation: