CURRENT PATTERN OF ANTIMICROBIAL SUSCEPTIBILITY OF MOST COMMONLY ISOLATED ENTEROBACTERIACEAE AGAINST FOSFOMYCIN USING DISC DIFFUSION METHOD

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: ABSTRACT:

Fosfomycin (Phosphomycin) a new antimicrobial substance was first introduced in 1969. It is characterized as bactericidal agent with a wide spectrum of antimicrobial activity, both to Gram-negative and Gram-positive bacteria. It was used for many years as a highly effective antimicrobial drug especially for the treatment of urinary tract infections (UTIs). But currently the evolution and spread of various mechanisms of antimicrobial drug resistance among common family, Enterobacteriaceae is increasing. This results into narrowing of the available therapeutic options, which is of increasing global concern. The objective of this study was to evaluate the current pattern of fosfomycin resistance in Enterobacteriaceae using Disc Diffusion method. Total of 100 Enterobacteriaceae spp. were isolated from different specimen received in Pathology department of Tertiary Care Hospital. All the specimen were subjected to gram staining and culture. The samples were inoculated on Blood and MacConkey agar. Urine samples were cultured on CLED media. Enterobacteriaceae were identified based on their colony morphology, gram staining and biochemical tests (Triple sugar iron agar, Sulphur indole motility agar, Citrate agar) as per standard protocols. Antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion method as per CLSI 2021 guidelines. Susceptibility patterns of fosfomycin against Enterobacteriaceae were observed and results were reported. Most of the gram negative Enterobacteriaceae were resistant to commonly used antibiotics. A total of 100 Enterobacteriaceae isolates were studied, including Escherichia coli (56%), Klebsiella oxytoca (16%), Klebsiella pneumoniae (10%), Serratia spp. (8%), Proteus spp. (5%) and others (5%). Antimicrobial susceptibility rates were highest for fosfomycin (92.8%) Of the 100 isolates, 69% Enterobacteriaceae isolates were fosfomycin sensitive and 31% were fosfomycin resistant.

: INTRODUCTION:

Enterobacteriaceae is the most diverse group of medically important gram-negative bacilli that cause a variety of human infections, including septicaemia, urinary tract infections, wound and gastrointestinal infections. As rapidly as novel antimicrobial agents are introduced, Enterobacteriaceae can develop resistance to antibiotics. (1) Nowadays, a significant increase in antimicrobial resistance is observed. During the past decades, rates of multi drug resistant (MDR) Enterobacteriaceae clinical isolates, mainly Klebsialla pneumonia, Klebsiella oxytoca, Escherechia coli, Proteus species, Enterobacter spp have increased considerably restricting effective antimicrobial treatment. (2)

Fosfomycin is a natural, forgotten antibiotic, known for nearly four decades, broad spectrum and a bactericidal antibacterial agent that inhibits cell wall synthesis in bacteria by inactivating the UDP-*N*-acetyl-glucosamine-3-*o*-enolpyruvyltransferase. (3,4) There are a few studies on the *in vitro* activity of fosfomycin against commonly encountered bacteria, except for *Escherichia coli* and Enterococcus faecalis. Multidrug resistance in Enterobacteriaceae is an ever-increasing problem worldwide. Of particular concern is the spread of carbapenemases, because these β-lactamases mediate resistance to all or almost all β-lactam antibiotics. In addition, strains carrying carbapenemases very often harbor resistance mechanisms against several unrelated antibiotics. Three groups of carbapenemases have been described in Enterobacteriaceae namely, Ambler class A carbapenemases, like KPC, metallo β lactamases, like VIM, GIM, or NDM, and class D carbapenemases, like OXA-48. (5)

Fosfomycin was discovered five decades ago and has been approved for the treatment of uncomplicated urinary tract infections (UTI) since early 1970. Fosfomycin presents good activity against Gram negative bacteria such as *Haemophilus influenza* and most enterobacteria *including* Citrobacter *spp.*, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Serratia marcescens and Shigella spp. Fosfomycin has a bactericidal effect by inhibiting the initial step in the biosynthesis of peptidoglycan. It acts as a phosphoenol pyruvate (PEP) analogue and binds the essential enzyme MurA (UDP-*N*-acetylglucosamine enolpyruvyl transferase) leading to bacterial cell lysis and death. Fosfomycin has also shown good activity for penetrating the interior of biofilms of Gram-negative bacteria, both in mono therapy and in combined therapy, showing excellent eradication activity. Multidrug resistance is the most important problem in antibiotic resistance due to the difficulty in treating multidrug resistant microorganisms. (6,7).

Recently, plasmid-mediated mechanisms of fosfomycin resistance have also been described, which involve the expression of enzymes. Antibiotic treatment of Urinary Tract Infections (UTI) is becoming increasingly difficult to treat due to emergence of multi-drug resistant (ESBLs, AmpC, CRE) uropathogens. Fosfomycin is an old antibiotic that has evoked renewed interest with unique properties of not sharing any structural similarity and lack of cross-resistance with other

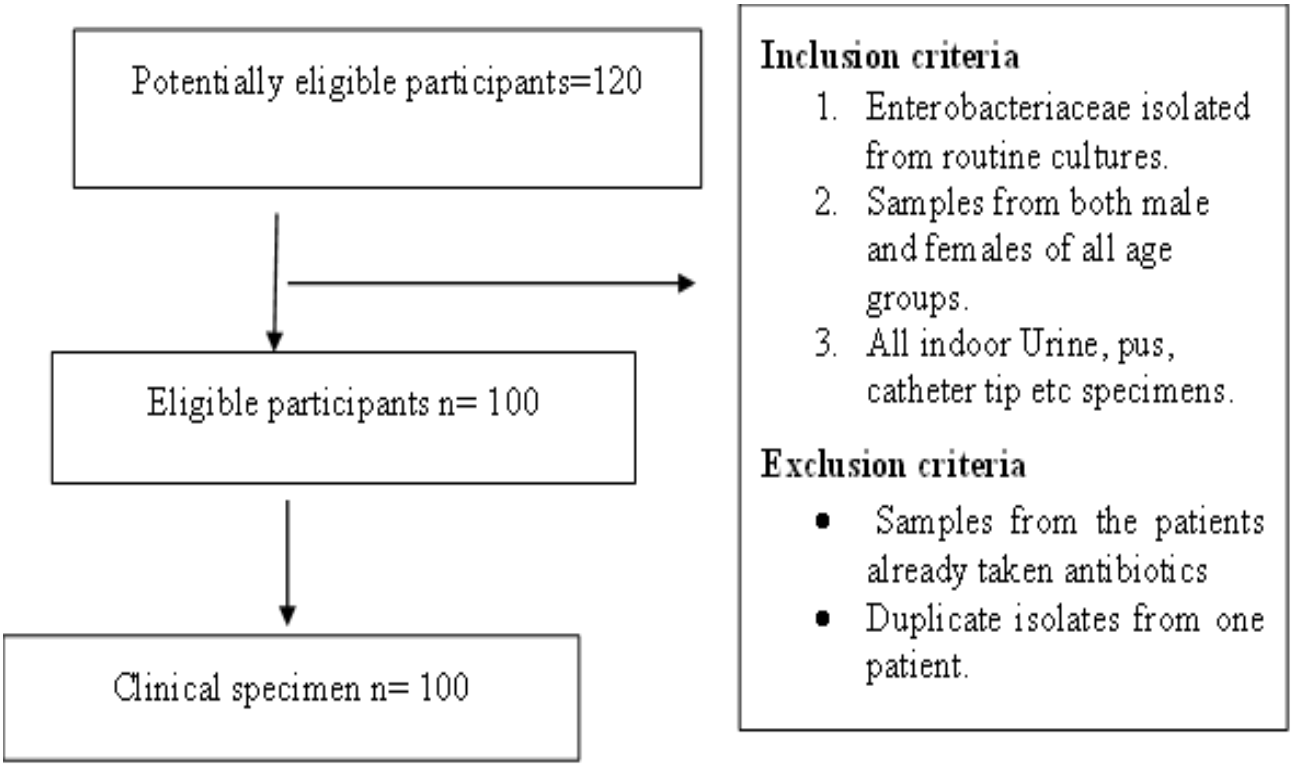
antimicrobial agents. Our aim is to evaluate in vitro activity of Fosfomycin against urinary tract Enterobacteriaceae. (8)

Fosfomycin is not extensively used against MDR infections. However, these imply that it is still regarded as salvage treatment for infections or treatment for breakthrough infections in patients already receiving anti-XDR treatment. (9,10)

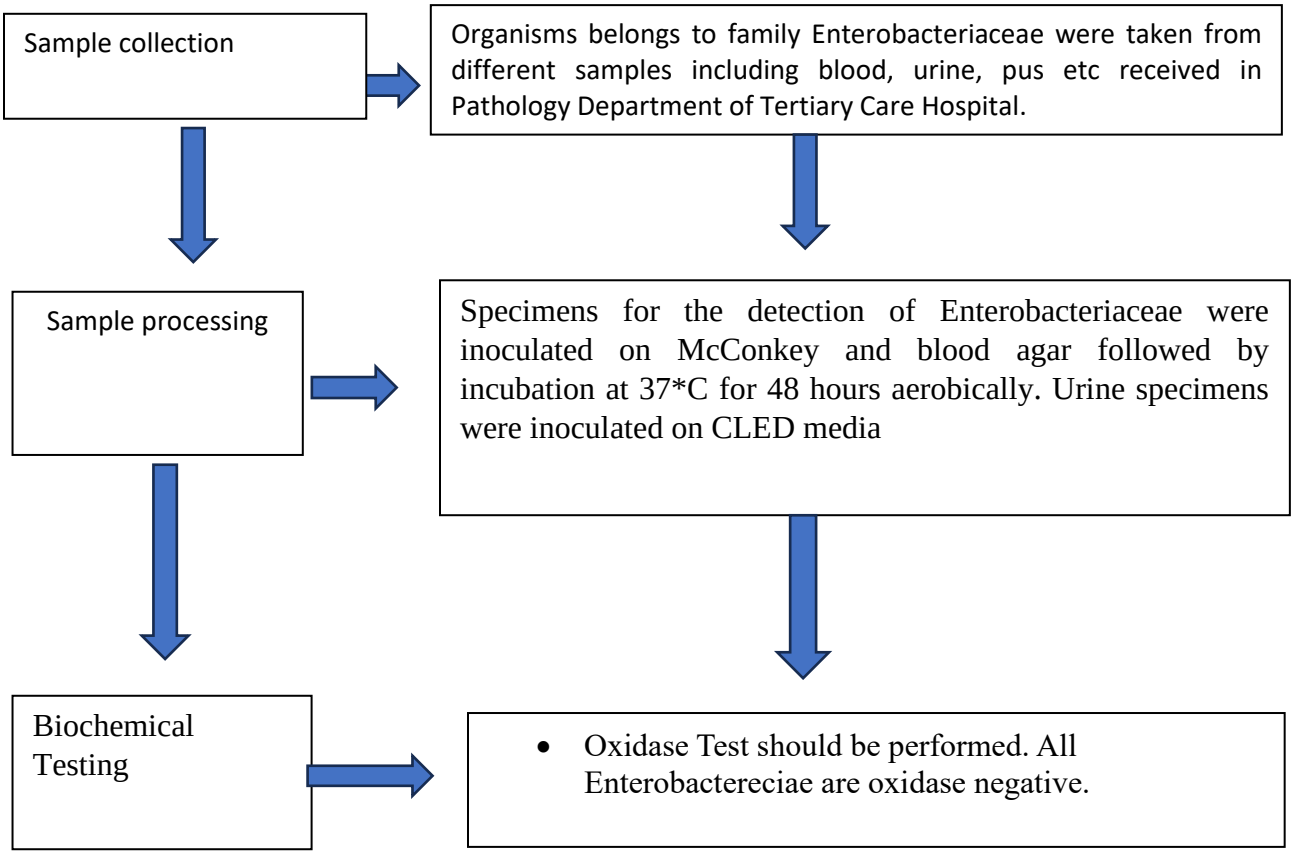
In Pakistan, the rates have been increasing steadily, data from our hospital showed increase in carbapenem resistant *Escherichia coli, Enterobacter spp. and Klebsiella pneumoniae* in recent years. Intravenous (IV) fosfomycin was prospectively evaluated for the treatment of carbapenem resistant *K. pneumoniae* wherein all patients had bacteriological clearance with no adverse reactions. (11,12)

: Material and Methods:

A total of 143 samples were included which was calculated by the WHO sample size calculator. All these samples were taken from Microbiology laboratory of Tertiary Care Hospital, Rawalpindi. The ethical committee of university gave their approval to this study. Patient informed consent was obtained before any data was gathered. The Cross-sectional descriptive study is performed on this sample population. The Klebsiella species isolated from all clinical samples were included and clinical samples taken from all age groups were included in this study. Microorganisms other than Klebsiella were excluded.



: Methodology:



ANTIBIOTIC SUSCEPTIBILITY TESTING:

- Colony suspension of each isolate was made equivalent to a 0.5 Mc Farland turbidity standard.
- Each of the isolates were inoculated on Muller Hinton agar.
- Isolates adjusted to the 0.5 McFarland Standard and incubated at 37 C for 16-18 hours.
- Overnight incubation at 37 C was done in an ambient incubator.
- Fosfomycin resistance Zone size: <=24
- Fosfomycin sensitive zone size: >=24

: Results:

A total of 100 Enterobacteriaceae isolates were studied, including Escherichia coli (56%), Klebsiella oxytoca (16%), Klebsiella pneumoniae (10%), Serratia spp. (8%), Proteus spp. (5%) and others (5%). Antimicrobial susceptibility rates were highest for fosfomycin (92.8%) Of the 100 isolates, 69% Enterobacteriaceae isolates were fosfomycin sensitive and 31% were fosfomycin resistant.

Resistance and Sensitivity Percentage			
Enterobacteriaceae Isolated	**Total**	**Fosfomycin Sensitive**	**Fosfomycin Resistant**
E. coli	**56%**	**36%**	**20%**
Klebsiella oxytoca	**16%**	**12%**	**4%**
Klebsiella pneumonia	**10%**	**8%**	**2%**
Serratia spp.	**8%**	**5%**	**3%**
Proteus spp.	**5%**	**4%**	**1%**
Others	**5%**	**4%**	**1%**
Total	**100%**	**69%**	**31%**

Table 1: This table shows the resistance and sensitivity of Enterobacteriaceae Isolated. According to the result of this study 69% Enterobacteriacease isolate are Fosfomycin sensitive and 31% are Fosfomycin resistant.

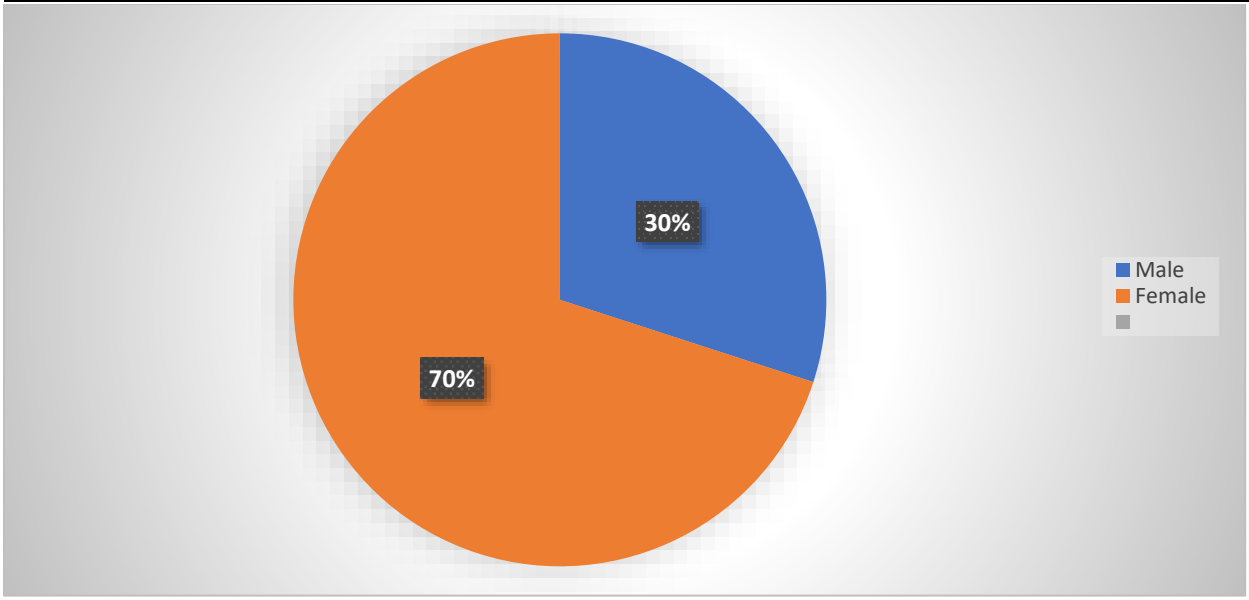


Figure 1: Fosfomycin resistance among Male and Female. The Fosfomycin resistance is most prevalent in female that counts 70% and in males its resistance counts are 30%.

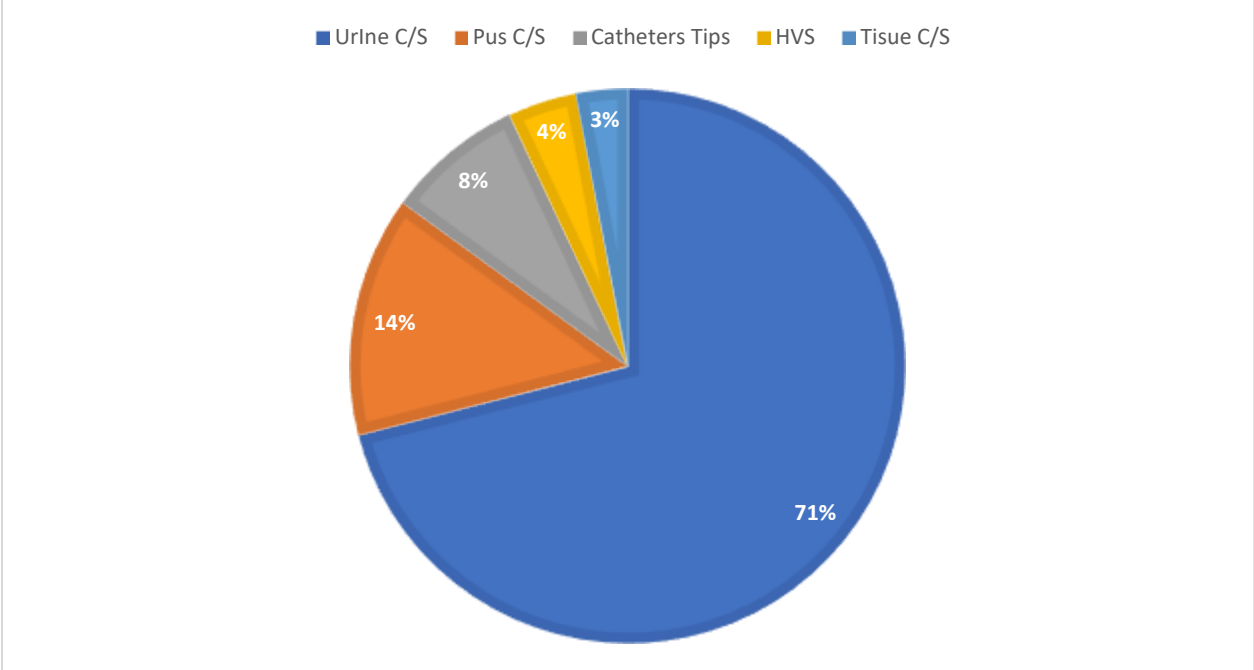


Figure 2: Enterobacteriaceae spp isolated from different clinical specimen. 71 % Enterobacteriaceae spp isolated from Urine C/S,14% from Pus C/S, 8% From Catheters tips ,4% from HVS and 3% from Tissue C/s.

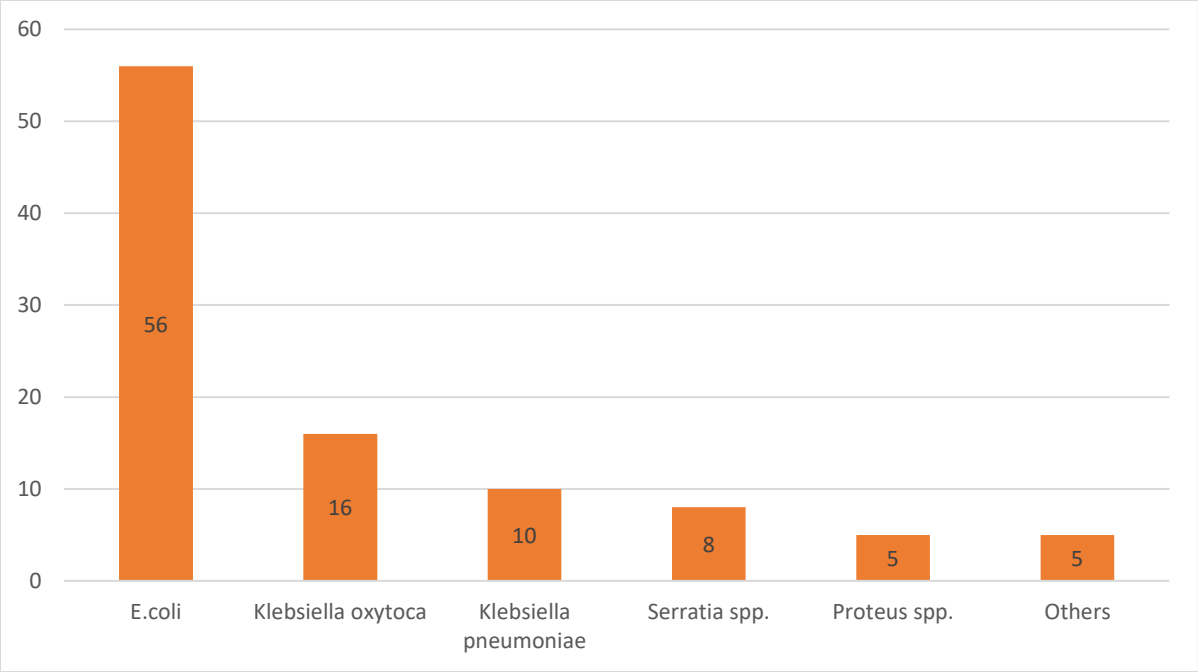


Figure 3: Total Enterobacteriaceae Isolated from clinical specimen. A total of 100 Enterobacteriaceae isolates were studied, including Escherichia coli (56%), Klebsiella oxytoca (16%), Klebsiella pneumoniae (10%), Serratia spp. (8%), Proteus spp. (5%) and others (5%).

: Discussion:

This study concludes that fosfomycin showed substantial antimicrobial activity against selected Enterobacterial isolates. The antimicrobial activity of fosfomycin did not appear to be considerably influenced by the pattern of resistance of the studied isolates (either MDR or XDR) or the expression of specific resistance phenotypes (serine carbapenemases, MBLs or ESBLs).

The increasing incidence of urinary tract infection caused by Gram negative bacteria with multiple drug resistance is well described worldwide and also by the European Antimicrobial Resistance Surveillance Network (EARS-Net), where this phenomenon has been reported in *E. coli* and *K. pneumoniae* isolates. But according to some published studies, the increasing use of fosfomycin worldwide has led to increased levels of resistance in isolates. For example, in a Spanish study an increase of fosfomycin resistance was detected in *Escherichia coli* isolates from 22% in 2013 to 60% in 2017. Moreover, a study performed in Netherland in 2018 showed that Fosfomycin presented activity against 95.9% of *E. coli* and 87.6% of *K. pneumoniae* isolates out of 775 E. coli and 225 Klebsiella species which means that fosfomycin which was previously thought as ideal for treatment of infections caused by Enterobacteriaceae has developed resistance in recent years. A study performed in Clinical Microbiology, Department of Pathology and laboratory medicine at Agha Khan University Hospital, Karachi, Pakistan, consisting of total 251 Enterobacteriaceae showed that the resistance rate of Enterobacteriaceae against fosfomycin as follows E.coli 36.04% (98/251) followed by Klebsiella pneumonia 31% (78/251), Raoultella spp. 15.5% (Raoultella

terrigena and Raoultella ornithinolytica) (39/251), Enterobacter spp. 7.5% (19/251), Klebsiella oxytoca 5.1% (13/251), and Citrobacter spp. 1.1% (3/251). Similarly, a small-scale study performed in 2018 in UK in which twenty-four Enterobactereciae strains including E. coli, Klebsiella pneumonia and Enterobacter cloacae showed that more than half of all the isolates had a detectable resistant subpopulation: four out of nine *E. coli* isolates (0.002%–3.7%), six out of eight *K. pneumoniae* (0.0006%–0.001%), and four out of seven *E. cloacae* isolates (0.003%–2.5%). In our study, the resistance rate observed in Enterobactereciae was Escherichia coli (56%), Klebsiella oxytoca (16%), Klebsiella pneumoniae (10%), Serratia spp. (8%), Proteus spp. (5%) and others (5%).

Several studies have noted good activity of fosfomycin against Enterobacteriaceae producing ESBLs or characterised as MDR. However, relatively few studies have evaluated the antimicrobial activity of fosfomycin against Enterobacteriaceae isolates with extensive drug resistance (including carbapenem resistance), providing favourable findings regarding the potential value of fosfomycin in this regard.

A considerable proportion of the multidrug resistant *Enterobacteriaceae* with diverse resistance mechanisms, including carbapenemases production, tested susceptible to fosfomycin using disk diffusion method.

: Conclusion:

This study shows that Fosfomycin has substantial in vitro antimicrobial activity against a common Enterobacteriaceae isolates (mainly E. coli). It is concluded that fosfomycin can be very effective in treating uncomplicated UTIs. Emergence of fosfomycin resistant strains of Enterobacteriaceae, can be a major problem in coming years.

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