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Evaluation of in vitro Susceptibility of Multi Drug Resistant Uropathogenic Escherichia Coli (UPEC) to Fosfomycin: A Comparision of Determination Methods

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Abstract: ***Background and Objective: The increasing incidence of multidrug - resistant (MDR) pathogenic bacteria, both in hospital and community settings, causing Urinary tract infection (UTI) is inducing clinicians to reconsider old antibiotics, such as fosfomycin, to overcome the difficulties posed by these microorganisms. Keeping in the view of above fact, the present study was carried out to know the susceptibility pattern of Fosfomycin against multi drug resistant uropathogenic Escherichia coli (MDR UPEC) by different methods. Materials and Methods: A cross sectional study was conducted for 4 months from july to october 2021 by using conventional methods and pathogenic bacteria were isolated from urine samples followed by antibiotic susceptibility testing MDR UPEC were tested against fosfomycin using the Kirby - Bauer diffusion method and E - test and agar dilution methods according to CLSI recommendations taking agar dilution as gold standard. Results: A total of 652 urine samples were processed, among which 278 number of samples showed significant bacterial growth. From these isolates, 71 (25.5%) were Escherichia coli. Clinical isolates of Escherichia coli showing MDR were tested against fosfomycin using disk diffusion, E - test and agar dilution. Disk diffusion and E - test showed 100% susceptibility to Fosfomycin where as agar dilution test detected 90% susceptibility. Conclusion: Fosfomycin showed very good activity against MDR UPEC. It has the potential to emerge as a promising alternative oral agent for uncomplicated UTI over prolonged period of time.***

Keywords: Fosfomycin, agar dilution, disc diffusion, E – test

1. Introduction

Urinary tract infection (UTI) is defined as a disease caused by microbial invasion of the urinary tract that extends from the renal cortex of the kidney to the urethral meatus. [1] The prevalence of UTI is much more common in women than in men, at a ratio of 8: 1, due to their anatomical and physiological reasons. [2] Most common organisms causing UTI belongs to Enterobacteriaceae family, among which uropathogenic *Escherichia coli* is so far the most common cause of all forms of UTIs is responsible for more than 85% of all UTIs (i. e. community acquired and healthcare - associated UTI and upper and lower UTI). In addition to the members of Enterobacteriaceae, other organisms such as staphylococci, Pseudomonas, Acinetobacter are also increasingly reported. Uncomplicated UTIs typically occur in the healthy adult non - pregnant woman, while complicated UTIs may occur in all sexes and age groups. The severity of UTI depends upon the virulence of bacteria and the host's susceptibility. [3] UTI is the most common bacterial infections that lead patients to seek medical care and frequent indication for antimicrobial use. The increase in rates of antibiotic resistance among Enterobacteriaceae has posed challenges in choosing empiric antibiotic regimens. Incorrect outpatient use of antibiotics can serve as a potentially large breeding ground for antibiotic resistance in the wider community. [4] The increasing multidrug - resistant (MDR) pathogenic bacteria, both in hospital and community settings, causing urinary tract infection (UTI) is inducing clinicians to reconsider old antibiotics, such as fosfomycin.

Fosfomycin is a broad - spectrum antibacterial agent with in vitro activity against both Gram - negative and Gram - positive bacteria, including most species of Enterobacteriaes, staphylococci, and enterococci. [5] Fosfomycin has a unique property that it is involved in inhibiting cell wall synthesis, but in a different way than in the case of β - lactam or glycopeptide antibiotics. It inhibits the MurA enzyme (UDP - N - acetylglucosamine - enolpyruvyltransferase), which is responsible for the synthesis of the pentapeptide mediating the synthesis of peptidoglycan (bacterial cell wall component).

The current study intended to assess the in vitro activity of fosfomycin by three different methods against clinical isolates of *Escherichia coli* to determine the abilities of disk diffusion, E - test and agar dilution, generate equivalent results for fosfomycin - susceptible and especially fosfomycin - resistant isolates.

2. Materials and Methods

A cross sectional study was conducted from july to october 2021 in the Department of Microbiology, MKCG, Berhampur, Odisha.

Collection of urine specimen from people:
652 urine samples were collected from patients who had clinical features suggestive of UTI from the inpatients and outpatients departments. Mid - stream urine was collected in a sterile dry wide necked and leak proof container from the noncatheterized, alert, conscious, adult patients. Surgically collected urine samples and suprapubic aspirates were collected from some patients as indicated. If the patients

were catheterized; then, urine samples were collected from the catheter with proper surgical asepsis with needle and syringe. Then the containers were labeled with the date, the name, time and serial number of the patient. The urine samples were processed immediately after collection as per the standard procedure. [6]

Physical examination of the urine specimen:
The physical parameters of collected urine specimens such as volume, pH, color, appearance was analyzed and recoded.

Microscopical examination of urine specimens:
To examine the urine specimens, microscopically wet preparation was made by centrifuging the sample at 500 - 1000 g for 5 minutes then a drop of sediment was transferred to a slide and covered with glass and finally it was examined under 10x and 40x objective. The crystals, casts and bacterial cells, parasites were recorded. Direct microscopy of the uncentrifuged urine sample was done, and pus cells were noted.

Microbiological analysis of urine specimens:
Then the sample were inoculated using 0.1mm Nichrome urine loop on CLED agar in semi quantitative method and incubated at 37ºC for 18 to 24 hours. On the next day observed for significant growth and isolates were identified using different biochemical lists. *Escherichia coli*, *Staphyloccous aureus*, *Enterococcous spp*., others were isolated and conformed phenotypically.

Antibiotic susceptibility test:
Antibiotic susceptibility testing was done using different antibiotics such as Cotrimoxazole (25µg), Gentamycin (10µg), Piperacillin - tazobactam (100/10µg), Nitrofurantoin (300µg), Ampicillin - Sulbactam (10/10µg) and Fosfomycin (200µg). MDR UPEC (Non susceptibility to atleast one agent in three or more antimicrobial category) samples were futher tested for fosfomycin susceptibilities by the CLSI agar dilution method, and E test. Agar dilution testing used fosfomycin disodium powder and Mueller - Hinton agar (MHA) supplemented with 25 g/ml of glucose - 6 - phosphate (Himedia). MHA was also used with fosfomycin Etest (Himedia).

Fosfomycin (FP) susceptibility:
Susceptibility testing of MDR UPEC against fosfomycin was determined by AD, DD, and E - test methods. Results were interpreted applying CLSI breakpoints/MIC. Using CLSI breakpoints (≤ 64 µg /mL: susceptible, 128 mg/L: intermediate, ≥ 256 mg/L: resistant), MICs results of AD and E - test (Himedia) were interpreted. For DD test, FP (200µg) (Himedia) was used and result was interpreted using CLSI breakpoints (zone diameters ≥ 16mm: susceptible, 13 - 15mm: intermediate and ≤ 12mm: resistance) (CLSI 2020). [7]

Agreement analysis:
Using AD as the reference method, agreement rates were calculated for different methods. Essential agreement (EA) was defined as an E - test MIC equal to or within ± 1 dilution of the AD MIC. Categorical agreement (CA) was met when E - test or DD interpretive criteria agreed (susceptible/intermediate/resistant) with AD results. A minor error (mE) was defined as E - test or DD with a susceptible or resistant result when AD result was intermediate or when E - test or DD results were intermediate, and AD was susceptible or resistant. A major error (ME) occurred when E - test or DD results were resistant, and AD was susceptible and was calculated only for susceptible isolates. Very major errors (VME) occurred when E - test or DD results were susceptible, and AD was resistant and was calculated only for resistant isolates (CLSI 2020).

3. Results and Discussion

Among the 652 urinary samples 279 was bacterial culture positive (Figure: 1), from which 71 *Escherichia coli* were isolated (Figure: 2). Out of 71, males were 27 (38.02%) and females were 44 (61.97%) with male to female ratio of 1: 1.60 with predominance of female patients (Figure 3). These 71 *Escherichia coli* isolates subjected to Disk diffusion and E - test, showed 100% susceptibility to fosfomycin. Rates of antibiotic resistance for UPEC are summarized in Table (Figure 2).

The overall resistance rate for Fosfomycin (200µg), Ampicillin - Sulbactam (10/10µg), Nitrofurantoin (300µg), Piperacillin - tazobactam (100/10µg), Gentamycin (10µg), Cotrimoxazole (25µg) was 100%, 79%, 85.2%, 82%, 57% and 43% respectively. (Table 1) (Figure 6)

Disk diffusion and E - test showed concordance in showing 100% susceptibility to fosfomycin, where as Agar dilution could detect resistant isolates in 9.8% of isolated samples. (Figure 6, 7, 8) Considering AD as a gold standard, more than 90.2% of isolates were observed susceptible to FP. The results of AD, DD, and E - test following CLSI breakpoints had been shown in the graph (Figure 4). The percentage of susceptible and resistant isolates by AD method following CLSI breakpoints was 90.2%, and 9.8%, respectively. (Figure: 5)

Agreement analysis by different methods:
The rates of EA, CA, mE, ME, and VME were assessed against UPEC for DD, E test, AD by applying CLSI breakpoints. (Table 2)

4. Discussion

Escherichia coli being the most common organism in the causation of UTIs, is also an important pathogen which shows multiple drug resistance by various mechanisms. Due to increase in prevalence of drug resistance, the forgotten old drug; fosfomycin is likely to become increasingly called upon for the oral treatment of UTI. This pattern is reflected in our own centre, in which we show that fosfomycin prescriptions have increased over time. A North American study from 1999 reported 94% of 1, 097 isolates of fosfomycin - susceptible *E. coli (*all agar dilution MICs of 64 g/ml) tested within one doubling dilution for agar dilution and E test; the same study compared agar dilution MICs to disk diffusion zone sizes and showed 0.1% mEs and no MEs or VMEs. [8] In a 2018 European study of 775 ESBL - producing *E. coli* the investigators observed 98% categorical agreement between agar dilution and both E test and disk diffusion but unacceptably high rates of VMEs (23.3%, disk

diffusion; 12.9%, E test). The authors of that study concluded that neither disk diffusion nor E test performed satisfactorily due to poor detection of fosfomycin - resistant isolates. [9] A study from Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, found disk diffusion and E test were equivalent to agar dilution, demonstrating categorical agreement of 99% with 1% mEs and no MEs or VMEs when CLSI interpretative criteria were applied. [10] In our study, applying CLSI breakpoints, EA, CA, mE, ME, and VME for E - test, DD and AD we found 92.90%, 91.50%, 2.81%, 0.00% and 9.80% respectively.

5. Limitation

Small amount of sample from a single centre, less time period, even though good activity against MDR uropathogen was observed, study with more population could have been undertaken to validate the result

6. Conclusion

Fosfomycin showed very good activity against UPEC. The MDR UPEC high in vitro susceptibility to fosfomycin, which therefore has the potential to emerge as ***a promising alternative oral agent*** for outpatient therapy of UTI over prolonged time periods. As Agar dilution has been proved to show better detection method for fosfomycin resistant isolates, it should be considered as *standardized technique before reporting the organism as susceptible:* **in order to avoid therapeutic failure.** E test MIC has shown concordance with Agar dilution MIC in this study. **E test can be considered alternative to Agar dilution**, as agar dilution is cumbersome and time consuming.

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Conflict of interest: Nil

Figure: 1

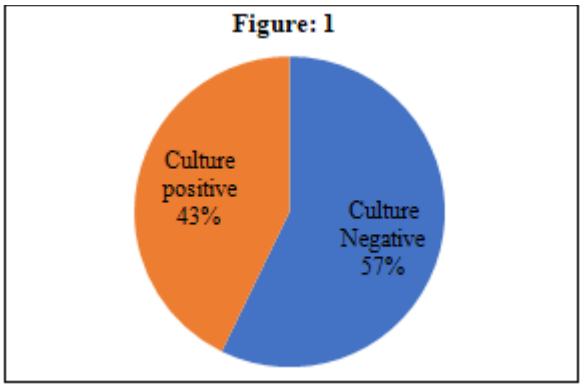
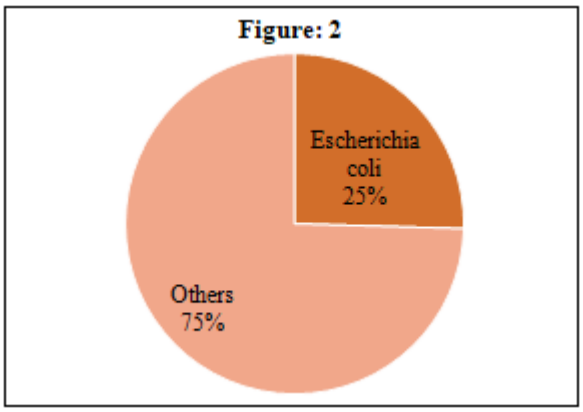


Figure: 2



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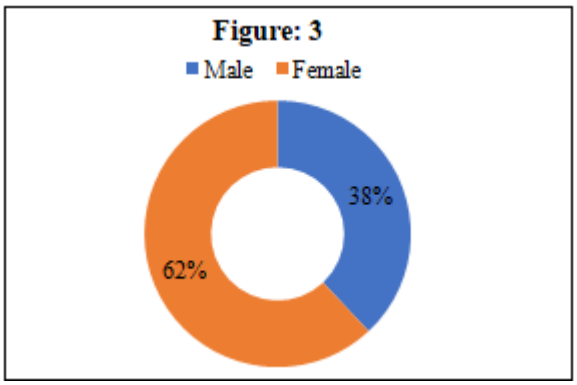

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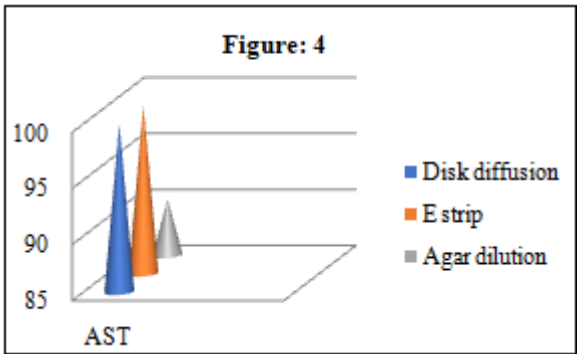

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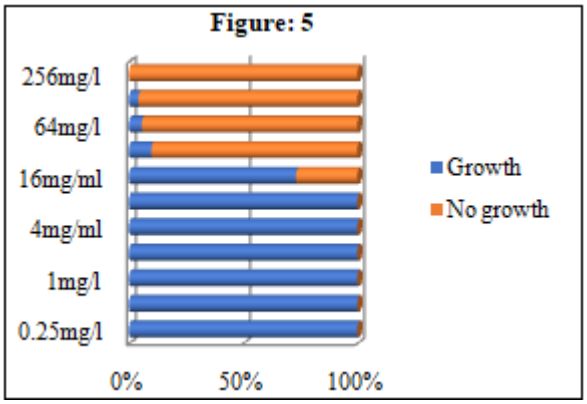

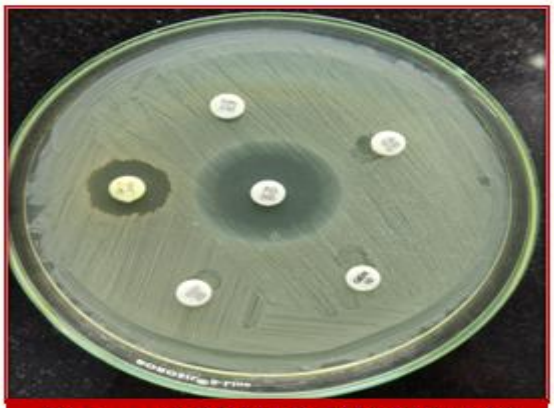

Figure 6 DISC DIFFUSSION

Figure 7 E -STRIP

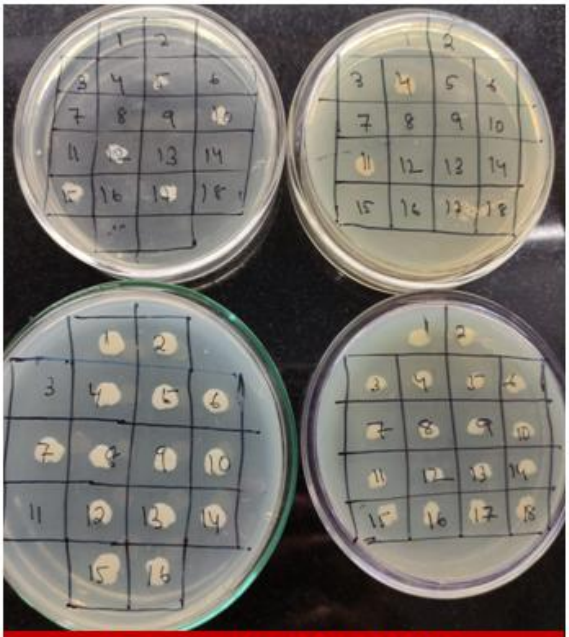

Figure 8 AGAR DILUTION

Table 1

Antibiotics	Sensitivity (%)
Cotrimoxazole (25µg)	43
Gentamycin (10µg)	57
Piperacillin - tazobactam (100/10µg)	82
Nitrofurantoin (300µg	85.2
Ampicillin - Sulbactam (10/10µg)	79
Fosfomycin (200µg)	**100**

Table 2

Agreement analysis	E test
Essential agreement (EA)	92.90%
Categorical agreement (CA)	90.14%
minor error (mE)	4.22%,
major error (ME)	0.00%
Very major errors (VME)	9.80%

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