



SHORT COMMUNICATION

Fosphomycin: A ray of hope against multidrug resistant uropathogens

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ABSTRACT

Aim: Urinary Tract Infection (UTI) is one of the common infections in clinical practice. Increasing incidence of Multi Drug Resistant (MDR) uropathogens limited the therapeutic options; thereby prompted the interest in old drugs like Fosfomycin. The current study was undertaken for the comparative evaluation of *in vitro* activity of Fosfomycin and Nitrofurantoin against ESBL producing and carbapenem resistant uropathogens. We also tried to compare the coexistence of resistance of both the drugs with another commonly used oral drug for UTI, i.e. Flouroquinolone.

Methodology and results: A total 101 MDR uropathogens were tested for ESBL production, carbapenem resistance, Fosfomycin susceptibility and Nitrofurantoin susceptibility as per the CLSI guidelines. Fosfomycin susceptibility testing was carried out by disc diffusion test. *Klebsiella pneumoniae* was the commonest MDR uropathogen followed by *Pseudomonas aeruginosa* and *Escherichia coli*. Susceptibility to Fosfomycin among the ESBL producer and carbapenem resistant uropathogen was found uniformly higher (91.8%, 90.1%) in comparison to Nitrofurantoin (27.5%, 21.3%). Coexistence of resistance to Fosfomycin was much less than Nitrofurantoin in presence of resistance to Flouroquinolone.

Conclusion, significance and impact of the study: Fosfomycin showed excellent *in vitro* susceptibility against both ESBL producing and carbapenem resistant MDR uropathogens. Fosfomycin has excellent *in vitro* action of Fosfomycin against ESBL producing and carbapenem resistance uropathogen in comparison to Nitrofurantoin, hence will be useful for the treatment of drug resistant uropathogens.

Keywords: Urinary tract infection, ESBL producer, carbapenem resistance, Fosfomycin, Nitrofurantoin

INTRODUCTION

Urinary Tract Infection (UTI) constitute a large burden of both hospital and community acquired infections. Over 150 million episodes of UTI are reported annually resulting more than six million dollars of expenditure (Sultan *et al.*, 2015). The condition becomes more grave with the ever-increasing incidence of multi drug resistant (MDR) uropathogen isolates. *Escherichia coli* remain the most common etiological agents in both communities acquired and hospital acquired UTI followed by *Klebsella* spp. (Auer *et al.*, 2010, Maheswary *et al.*, 2018). High prevalence of multidrug resistance among these bacteria are observed due to increased production of beta-lactamases and carbapenemases; which further limits the therapeutic option (Lohiya *et al.*, 2015). In the present scenario, older antibiotics like Fosfomycin proved novel and a way for the treatment of these resistant pathogens.

Fosfomycin being one of the oldest drugs; found to have great use against MDR isolates. It is a

phosphoenoil pyruvate derivative produced by *Streptomyces fradiae* (ATCC 21096), *Streptomyces viridochromogenes* (ATCC 21240), and *Streptomyces wedmorensis* (ATCC 21239) (Falgas *et al.*, 2016). It has activity against both Gram-Positive Cocci (GPC) and Gram-Negative-Bacilli (GNB) and has a good oral bioavailability. It is a bactericidal drug which binds to the thiol group of enzyme UDP-N-acetyl glucosamine enolpyruvyl transferase (MurA) that catalyses the synthesis of peptidoglycan of the bacterial cell wall and inhibits cell wall synthesis. There are limited literatures available exhibiting its action against Extended Spectrum of Beta Lactamases (ESBL) producers and Carbapenem Resistant (CR) urinary isolates. Here, we tried to evaluate the *in vitro* antimicrobial activity of Fosfomycin against these uropathogens and also tried to compare its susceptibility with respect to another commonly used oral drug Nitrofurantoin in UTIs.

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MATERIALS AND METHODS

A prospective study was carried out in the Department of Microbiology of a tertiary care health center in north India within a study period of 6 months. A total of 8697 conjugative nonduplicate urine specimens were processed to detect significant bacteriuria. Urine was collected from clinically suspected cases of UTI, transported, and processed in the bacteriology laboratory as per the standard protocol. Culture of the collected specimen was done on Cysteine Lactose Electrolyte Deficient (CLED) media. Specimens with colony count $\geq 10^5$ CFU/mL of urine was considered significant and processed further. Isolated colonies were tested for phenotypic identification using conventional biochemical tests. Antibiotic susceptibility testing by disk diffusion method was carried out on Mueller Hinton Agar (MHA) media using commercial antibiotic disk (HiMedia, Mumbai, India) as per Clinical and Laboratory Standards Institute (CLSI) guideline. The result of the antimicrobial susceptibility testing result was recorded as per CLSI 2017 guidelines (Patel *et al.*, 2017). MDR was defined as the isolate resistant to at least one agent of three or more different classes of antibiotics. All MDR isolates were also further tested for ESBL production, CR as per CLSI 2017 guideline. An isolate resistance to either of the carbapenems (i.e. imipenem, meropenem, ertapenem, or doripenem) disk is defined as CR. All the MDR isolates were also tested for Fosfomycin susceptibility. The zone diameter given in the CLSI 2017 for *E. coli* was considered for all GNBs. The results of antibiotic susceptibility test of isolated organisms were then compared among the ESBL +ve, and carbapenem resistant uropathogens. The primary objective of the study to compare the *in-vitro* action of Fosfomycin and Nitrofurantoin against the ESBL producing and carbapenemase resistance pathogens.

RESULTS

A total of one hundred one MDR uropathogens were included in the study over a period of six months. *K. pneumoniae* was observed to be the commonest among the isolates followed by *Pseudomonas aeruginosa* and *E. coli* (Table 1). The isolates were found mostly resistant to Fluoroquinolones, Cephalosporins, and Aminoglycoside group of antibiotics. The susceptibility to Fosfomycin and Nitrofurantoin among the MDR isolates producing ESBLs and carbapenemases were compared. (Table 2) MDR isolates were observed more susceptible to Fosfomycin than Nitrofurantoin. Isolates producing ESBL and carbapenemases showed greater susceptibility (91.8%, 90.1%) to Fosfomycin in comparison to Nitrofurantoin (27.5%, 21.3%).

On comparison of individual bacterial species, all MDR isolates showed a greater susceptibility for Fosfomycin than that of Nitrofurantoin irrespective of its genus (Table 3). In case of *K. pneumoniae*, susceptibility of Fosfomycin was 90.3% as compared to 13.4% for Nitrofurantoin. Similarly, *E. coli* showed 100%

susceptibility for Fosfomycin as compared to 58.8% for Nitrofurantoin. As per CLSI guideline, Nitrofurantoin breakpoints are available only for family *Enterobacteriaceae*. Moreover, Tribe Proteae, *Pseudomonas* spp. and *Acinetobacter* spp. are intrinsically resistant to Nitrofurantoin. In the present study, all the *P. aeruginosa* MDR isolates found susceptible to Fosfomycin. Similar finding was observed for other GNBs and GPCs. However, the number of isolates other than *E. coli* and *K. pneumoniae* were too less to analyze this result.

Table 1: Incidence of ESBL producers and CR among the MDR uropathogens.

MDR organisms (n=99) (%)	ESBL (n=98)	CR (n=91)
<i>Klebsiella pneumoniae</i> (n=52) (52.5%)	52 (100%)	47 (90.3%)
<i>Pseudomonas aeruginosa</i> (n=19) (19.1%)	18 (94.7%)	19 (100%)
<i>Escherichia coli</i> (n=17) (17.1%)	17 (100%)	14 (82.3%)
<i>Acinetobacter</i> spp. (n=6) (6.06%)	6 (100%)	6 (100%)
<i>Enterobacter</i> spp. (n=2) (2.02%)	2 (100%)	2 (100%)
<i>Proteus mirabilis</i> (n=2) (2.02%)	2 (100%)	2 (100%)
<i>Providentia rettgeri</i> (n=1) (1.01%)	1 (100%)	1 (100%)

*Two isolates of *Enterococcus faecium* were isolated

Table 2: Comparison of antimicrobial susceptibility of isolates positive for ESBL production and carbapenem resistance to Nitrofurantoin and Fosfomycin.

Category	ESBL (n=98)	CR (n=91)
Fosfomycin	90 (91.8%)	82 (90.1%)
	ESBL (n=69)	CR (n=61)
Nitrofurantoin	19 (27.5%)	13 (21.3%)

Table 3: Antimicrobial susceptibility of uropathogens to Nitrofurantoin and Fosfomycin.

Uropathogen	Fosfomycin	Nitrofurantoin
<i>K. pneumoniae</i> (52)	47 (90.38%)	7 (13.46%)
<i>P. aeruginosa</i> (19)	16 (84.2%)	-
<i>E. coli</i> (17)	17 (100%)	10(58.82%)
<i>Acinetobacter</i> spp. (6)	5 (83.33%)	-
<i>Enterobacter</i> spp. (2)	2 (100%)	-
<i>Proteus mirabilis</i> (2)	2 (100%)	-
<i>Providentia rettgeri</i> (1)	1(100%)	-
<i>Enterococcus faecium</i> (2)	2 (100%)	2(100%)

The coexistence of resistance of Fosfomycin and Nitrofurantoin was compared in presence of resistance to other commonly used oral drug for UTI i.e.

Fluoroquinolone among the *E. coli* and *K. pneumoniae* isolates (Table 4). The coexistence of resistance for Fosfomycin was found much lower than Nitrofurantoin in both the groups.

Table 4: Coexistence of the resistance of Fosfomycin and Nitrofurantoin with flouroquinolone.

	<i>E. coli</i> (n=17)	<i>K. pneumoniae</i> (n=52)
	Flouroquinolone (R)	Flouroquinolone (R)
Fosfomycin (R)	00	05 (9.6%)
Nitrofurantoin (R)	07 (41.1%)	45 (86.5%)

DISCUSSION

The present study highlights the *in vitro* action of Fosfomycin against the MDR isolates. We also aimed to compare the action of two oral drugs i.e. Nitrofurantoin and Fosfomycin against the MDR uropathogens.

Total 101 MDR uropathogens were included in the study. GNBs were the predominant among which more than 50% of the MDR isolates were identified as *K. pneumoniae*. The incidence of MDR *P. aeruginosa* and MDR *E. coli* was observed much less in our study. Among the GPC, only two isolates of *Enterococcus faecium* were isolated exhibiting resistance to multiple antibiotics. Most of the MDR isolates were ESBL producers. Gupta *et al.* (2013) had showed 52.6% of ESBL production among the *E. coli* causing UTI. In the present study, all the isolates of *E. coli* and *K. pneumoniae* were ESBL positive. It might be due to the inclusion of only MDR uropathogens were in the study. Secondly, majority of our isolates were obtained from the hospitalized patients, where incidence of resistance is much higher. All MDR isolates of *Pseudomonas* spp. and *Acinetobacter* spp. were also exhibiting CR in comparison to 90.3% and 82.3% among *K. pneumoniae* and *E. coli*, respectively.

In the current study, we used the CLSI break points of Fosfomycin for *E. coli* to analyze the result among all the GNBs. More than 90% isolates producing ESBL and carbapenemases were susceptible to Fosfomycin. Similar result of Fosfomycin sensitivity against ESBL producers was observed in other national and international studies (Sabharwal and Sharma, 2015, Sahni *et al.*, 2013). Although majority of study showed very good activity of Fosfomycin against MDR pathogen, limited studies are available exhibiting its activity against the full spectrum of MDR uropathogen. In the present study, Fosfomycin was found highly active against all spectrums of MDR uropathogens in comparison to Nitrofurantoin. The overall resistance of Fosfomycin was 91% in comparison to 18.8% in Nitrofurantoin.

In the present study, all the isolates of *E. coli* were susceptible to Fosfomycin. CANWARD study showed 94.9% and 96.6% susceptibility of Fosfomycin against beta-lactamase producing and AmpC producing *E. coli* isolates (Karlowsky *et al.*, 2014). *In vitro* susceptibility of

Fosfomycin in our study among *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp. was observed 90%, 84%, and 83%, respectively. Another Indian study by Behera *et al.* (2018) showed 99%, 91.3%, 66% and 0% of susceptibility of MDR urinary isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter* spp., respectively. A recent study showed the *in vitro* action of Fosfomycin against invasive Gram-negative bacilli to be more than 90%. No significant difference in the susceptibility of Fosfomycin was observed for both ESBL producing and ESBL non-producing isolates. (William *et al.*, 2019). Moreover, it's *in vitro* action on the isolates with intrinsic resistance to Nitrofurantoin such as *Proteus* spp. and *Providencia* spp. was found very good. However, use of CLSI *E. coli* breakpoints and less number of isolates may not be appropriate to establish the above fact. Large-scale studies with pharmacokinetic and pharmacodynamic monitoring may require to further evaluate its efficacy against the different bacterial species other than *E. coli*. *Enterococcus* spp. had shown similar result of susceptibility for both Fosfomycin and Nitrofurantoin, but the number of the denominator is too small to comment on this fact.

Currently, the incidence of ESBL producing isolates are increasingly observed in both outdoor and indoor patients. ESBL production not only narrows down the therapeutic option; but also associated with cross-resistance with other antimicrobial agents. Carbapenems, Beta-lactam/beta-lactamase inhibitor and Fosfomycin are few of the important antibiotics used for alternate therapy among the ESBL producers. But in the present study, CR was observed in majority of the ESBL producers. In addition, all the above-mentioned drugs are to be administered parentally except Fosfomycin. Thus, Fosfomycin being an oral drug found very effective against ESBL and CR uropathogens.

In the present study, Fluoroquinolone was observed as one of the most common resistant group of antibiotics. Coexistence of resistance of Fluoroquinolone and Nitrofurantoin was observed in many studies (Patel *et al.*, 2017). In the present study coexistence of resistance of Fosfomycin and Nitrofurantoin with Fluoroquinolone among the common GNBs was compared. Fosfomycin showed 0% and <10% co-resistance with Fluoroquinolone among the *E. coli* and *K. pneumoniae* isolates, respectively. Studies showed relatively low (<5%) co-resistance of Fosfomycin in Fluoroquinolone-resistant or Trimethoprim-sulfamethoxazole-resistant *E. coli* and very high (>50%) for *K. pneumoniae* (Lisemeyer *et al.*, 2016). The likelihood of co-resistance of Nitrofurantoin with Fluoroquinolone in our study was found much higher i.e. 41% and 86.5% for *E. coli* and *K. pneumoniae*, respectively. This study predominantly highlights the susceptibility of Fosfomycin against various ESBL and CR urinary isolates. It also gives an idea of resistance with respect to other oral drug i.e. Nitrofurantoin.

The study has certain limitation in terms of less number of isolates for nil fermenters. As per CLSI guideline, Fosfomycin disc diffusion test remains the standard clinical microbiology practice and FDA approved

drug for treatment of UTI due to *E. coli* only. However, many previous studies have compared and evaluated the susceptibility result of Fosfomycin against various uropathogens. Although, it shows very good susceptibility result, certain areas like breakpoints for GNBs other than *Enterobacteriaceae*, harmonization of EUCAST and CLSI breakpoints, PK/PD studies for determination of its therapeutic efficacy are needed to fill up the gaps in the knowledge.

CONCLUSION

To conclude, the present study was aimed to determine the in-vitro activity of Fosfomycin against the multidrug resistant uropathogen and to compare its action with another potent oral drug used in UTI, i.e. Nitrofurantoin. The study emphasizes excellent in vitro action of Fosfomycin against ESBL producing and carbapenem resistance uropathogen in comparison to Nitrofurantoin. Fosfomycin is a bactericidal drug observed less costly, exhibited low co-resistance, nontoxic and non-allergic. Further clinical studies are warranted to evaluate its therapeutic efficacy among the various uropathogens except *E. coli*.

REFERENCES

- Auer, S., Wojna, A. and Hell, M. (2010). Oral treatment options for ambulatory patients with urinary extended spectrum beta lactamase producing *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **54**, 4006-4008.
- Behera, B., Mohanty, S., Sahu, S. and Praharaj, A. K. (2018). *In vitro* activity of Fosfomycin against multidrug-resistant urinary and nonurinary gram-negative isolates. *Indian Journal of Critical Care Medicine* **22**, 533-536.
- Falgas, M. E., Vouloumanou, E. K., Samonis, G. and Vardakas, K. Z. (2016). Fosfomycin. *Clinical Microbiology Reviews* **29**, 321-347.
- Gupta, V., Rani, H., Singla, N., Kaistha, N. and Chander, J. (2013). Determination of extended-spectrum β -lactamases and AmpC production in uropathogenic isolates of *Escherichia coli* and susceptibility to fosfomycin. *Journal of Laboratory Physicians* **5**, 90-93.
- Karlowsky, J. A., Denisuk, A. J., Lagacé-Wiens, P. R., Adam, H. J., Baxter, M. R., Hoban, D. J. and Zhanel, G. G. (2014). *In vitro* activity of Fosfomycin against *Escherichia coli* isolated from patients with urinary tract infections in Canada as part of the CANWARD surveillance study. *Antimicrobial Agents and Chemotherapy* **58**, 1252-1256.
- Lisemeyer, K., Strymish, J., Weir, S., Berg, G., Brecher, S. and Gupta, K. (2016). Activity of Fosfomycin against extended spectrum- β -lactamases producing uropathogens in patients in the community and hospitalized patients. *Antimicrobial Agents and Chemotherapy* **60**, 1134-1136.
- Lohiya, A., Kant, S., Kapil, A., Gupta, S. K., Misra, P. and Rai, S. K. (2015). Pattern of antibiotic resistance among community derived isolates of *Enterobacteriaceae* using urine sample: A study from Northern India. *Journal of Clinical and Diagnostic Research* **9**, LC15-LC19.
- Maheswary, D. and Saikumar, C. (2018). Profile of urinary tract infections and resistance patterns in a tertiary care hospital in India. *International Journal of Current Microbiology and Applied Sciences* **7**, 506-512.
- Patel, J. B., Weinstein, M. P., Eliopoulos, G. M., Jenkins, S. G., Lewis, J. S., Limbago, B., Mathers, A. J., Mazzulli, T., Patel R., Richter, S. S., Satlin, M., Swenson, J. M., Traczewski, M. M., Turnidge, J. D., and Zimmer, B. L. (2017). Performance Standards for Antimicrobial Susceptibility Testing: M100. 27th Edn. Clinical and Laboratory Standards Institute, USA.
- Sabharwal, E. R. and Sharma, R. (2015). Fosfomycin: An alternative therapy for the treatment of UTI amidst escalating antimicrobial resistance. *Journal of Clinical and Diagnostic Research* **12**, DC06-DC09.
- Sahni, R. D., Balaji, V., Varghese, R., John, J., Tansarli, G. S. and Falagas, M. E. (2013). Evaluation of Fosfomycin activity against uropathogens in a Fosfomycin-naive population in South India: A prospective study. *Future Microbiology* **8**, 675-680.
- Sultan, A., Rizvi, M., Khan, F., Sami, H., Shukla, I., Khan, H. M. (2015). Increasing antimicrobial resistance among uropathogens: Is Fosfomycin the answer? *Urology Annals* **7**, 26-30.
- William, P. C. M., Waichungo, J., Gordon, N. C., Sharland, M., Murunga, S., Kamua, A. and Barkeley, J. A. (2019). The potential of Fosfomycin for multi-drug resistant sepsis: An *in-vitro* activity against invasive paediatric Gram-negative bacteria. *Journal Medical Microbiology* **68**, 711-719.