## ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTEMBER 2015 ISBN 1595-689X VOL16 No.3
AJCEM/1520 http://www.ajol.info/journals/ajcem

*AFR. J. CLN. EXPER. MICROBIOL. 16(3): 119-123* http://dx.doi.org/10.4314/ajcem.v16i3.6

# *IN VITRO* ACTIVITY OF FOSFOMYCIN AGAINST UROPATHOGEN MULTI-DRUG RESISTANT (MDR) *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII*

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**ABSTRACT**

**Urinary tract infections caused by multidrug resistant Gram negative bacilli constitute a major global healthcare problem. Fosfomycin is considered the best treatment option for such infections. Urine samples were collected and cultured in a tertiary care hospital (Urology). Identification of these uropathogens and their antibiotic sensitivity screening were performed according to CLSI guidelines. Urine samples (n=436) were selected in which *Ps. aeruginosa* and *Acinetobacter baumannii* were found to be the significant pathogens and treated-exposed to fosfomycin. Sixty six (15%) were identified as *Acinetobacter baumannii, Ps. aeruginosa* n=370(85%). Forty four percent of all *Ps. aeruginosa* were found to be multidrug resistant while 48.5% of the *Acinetobacter baumannii* strains were found multidrug resistant. Polymyxin B was found to be the most effective drug (100%) against all uropathogens and fosfomycin was found effective against 73% of the multidrug resistant *Acinetobacter baumannii* isolates and 70% of the multidrug resistant *Pseudomonas aeruginosa* strains. It may be concluded that antimicrobial activity (*in vitro*) of fosfomycin, especially against MDR uropathogens, is very effective.**

**Keywords: Fosfomycin, Multidrug resistant Gram negative bacilli, Urinary tract infections, *Ps. aeruginosa, Acinetobacter baumannii***

# L'ACTIVITE IN VITRO DE LA FOSFOMYCINE CONTRE UROPATHOGEN MULTI-DRUG RESISTANT (MDR) *PSEUDOMONAS AERUGINOSA* ET *ACINETOBACTER BAUMANNII.*

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**RESUME**

**Les infections des voies urinaires causées par les bacilles de multi résistants Gram négatifs, constituent un problème majeur de sante mondiale. Fosfomycine est considéré comme la meilleure option de traitement pour telles infections. Les échantillons d'urine ont été recueillis et cultives dans un hôpital de soins tertiaires. Identification de ces uropathogènes et programmation de leur sensibilité aux antibiotiques ont été réalisés selon les directives (CLSI). Les échantillons d'urine (n = 436) ont été choisis dans laquelle *Ps. aeruginosa* et *Acinetobacter baumannii* se sont trouves être l'agent pathogène important et traités – exposés a Fosfomycine. Soixante – six (15%) ont été identifies comme *Acinetobacter baumannii, Ps. aeruginosa* = 370 (85%). Quarante – quatre pourcent de tous les *Ps. aeruginosa* se sont trouvés être multi résistants et 48,5% des souches *Acinetobacter baumannii* se sont trouves multi résistants. Polymyxine B a été trouvé d'être le médicament le plus efficace (100%) contre tous les uropathogènes et Fosfomycine a été trouvé efficace contre 73% des isolats de multi résistants *Acinetobacter baumannii* et 70% des souches de multi résistants *Pseudomonas aeruginosa*. On peut conclure que l'activité antimicrobienne (*in vitro*) de Fosfomycine est très efficace, particulièrement contre les uropathogenes MDR.**

**Mots – clés : Fosfomycine, les bacilles de Multi résistants Gram négatifs, les infections des voies urinaires, *Ps. aeruginosa, Acinetobacter baumannii*.**

## INTRODUCTION

Urinary infections (UTIs) due to multi-drug resistant Gram-negative bacilli (MDR-GNB) are an increasing clinical problem worldwide (1, 2). The prevalence of multi-drug resistant (MDR) bacterial species of *Pseudomonas aeruginosa and Acinetobacter baumannii* has increased considerably since the introduction followed by arbitrary use of new generation extended

spectrum antibiotics like third and fourth generation cephalosporins, carbapenems, monobactams, broad and extended spectrum penicillins etc (3). During the last few years these organisms are undergoing genetic modifications and result in highly resistant forms that cause untreatable nosocomial infections and healthcare associated complications (4, 5). These bacterial strains create very serious problems for antibiotic treatment especially in critically ill patients admitted in intensive care units. Fosfomycin can be a potentially useful agent for urinary tract sepsis (caused by MDR-GNB), as many such strains remain susceptible to this decades old drug (6, 7). It is for this reason and along with its soft administration that it has been widely recommended and used for the treatment of uncomplicated urinary tract infections (8). It is a well-tolerated drug and has a broad spectrum of activity (9). The objective of this study is to manifest fosfomycin bioactivity against multi drug resistant *Pseudomonas aeruginosa and Acinetobacter baumannii* strains encountered in urinary tract infections.

## MATERIALS AND METHODS

### Collection sites (anatomical) of the urine samples

Urine samples were collected from patients showing overt symptoms of urinary tract infections (UTIs) in a tertiary care hospital (Urology). A variety of collections were done including Foleys catheter collection, Midstream sample collection, Left and Right Percutaneous nephrostomy (L-PCN and R-PCN) collection and Suprapubic (S/P) collection depending on the patient's condition (10).

### Inoculation of urine samples

All urine samples were inoculated on Cystine lactose electrolyte deficient (CLED) agar medium plates by 1µl calibrated loops (Culti loops). Plates were incubated under aerobic conditions at 37$^{o}$C for 24 hours when colonies were observed for significant count and lactose or non lactose fermentative activity (10).

### Identification of uropathogens

Significant counts (100 colonies) were counted on CLED medium plate i.e. equal to $10^5$cfu/ml. Gram staining was performed as preliminary step. Pathogens were identified by standard biochemical reactions or by automated profile index (API 20 NE) system (bioMerieux) where needed (10, 11). In this study 436 urine samples (Positive for *Ps. aeruginosa and Acinetobacter baumannii)* were selected for fosfomycin bioactivity.

### Antibiotic sensitivity screening and media

Antibiotic sensitivity testing was performed by Kirby-Bauer disc diffusion method on Muller Hinton agar (Oxoid, UK) according to Clinical laboratory standard institute (CLSI) and European committee on antimicrobial susceptibility testing (EUCAST) (12, 13). Amikacin (AK30µg), Ampicillin (AMP10µg), Amoxicillin-clavulanic acid (AMC20/10µg), Aztreonam (ATM30µg), Ceftazidime (CAZ30µg), Cefoperazone-sulbactam (SCF105µg), Cefotaxime (CTX30µg), Ciprofloxacin (CIP5µg), Fosfomycin (FOS300µg), Imipenem (IPM10µg), Nalidixic acid (NA30µg), Nitrofurantoin (F300µg), Pipracillin-tazobactam (TZP100/10µg), Polymyxin B(PB300µg) and Trimethoprim / sulfamethoxazole (SXT1.25/23.75µg) discs were used. All the antibiotic discs were obtained from Oxoid. MacFarland 0.5 suspension of the isolate was made in normal saline that was spread by swab over the Muller Hinton (MH) agar and appropriate discs of the above indicated antibiotics were placed at the 15 mm distance from each other. Quality control strains *E.coli* ATCC25922 *and Pseudomonas aeruginosa* ATCC27853 were used for the standardization of antibiotic sensitivity testing.

## RESULTS

In this study 436 urine samples (Positive for *Ps. aeruginosa* and *Acinetobacter baumannii)* were selected for fosfomycin bioactivity. Out of the isolated bacterial strains, a total of n=66(15%) were identified as *Acinetobacter baumannii* and n=370(85%) as *Ps. aeruginosa.* Antibiogram for *Acinetobacter baumannii* is shown in fig-1 and fig-2 depicts the antibiogram for *Ps. aeruginosa.* Data was interpreted in percent by using Microsoft Office Excel 2007.

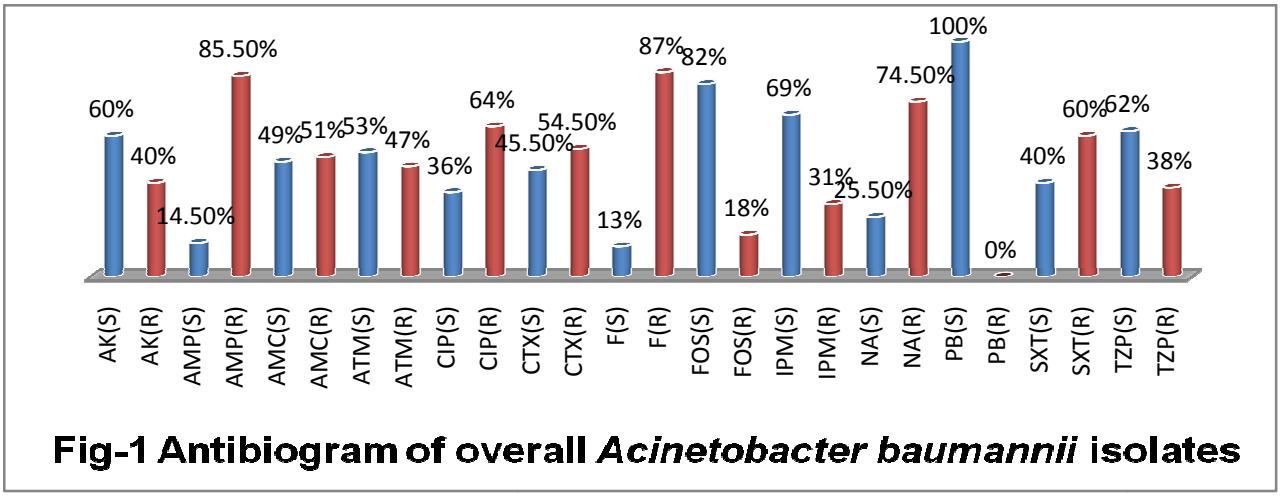


**Fig-1 Antibiogram of overall *Acinetobacter baumannii* isolates**

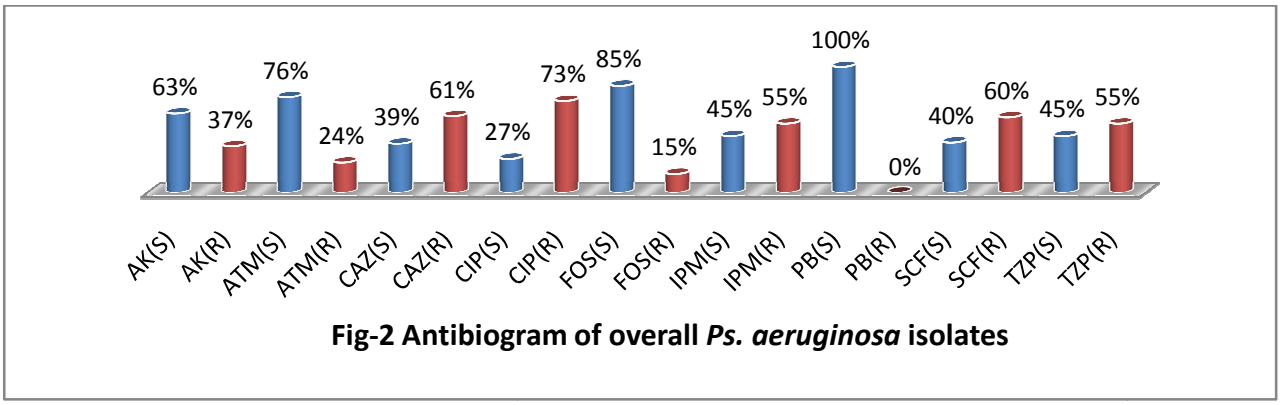


**Fig-2 Antibiogram of overall *Ps. aeruginosa* isolates**

**Key: (S)= Sensitive, (R)= Resistant**

The expansions of all the antibiotics abbreviations are given in materials and methods section. A total of 44% of *Ps.* aeruginosa isolates were found to be multidrug resistant while 48.5% of all *Acinetobacter baumannii* isolates were also found multidrug resistant. Results of antibiogram and bioactivity of fosfomycin against the MDR isolates are presented in table.1.

**TABLE: 1 ANTIBIOTIC SENSITIVITY PATTERNS OF MULTI-DRUG RESISTANT (MDR) *ACINETOBACTER BAUMANNII* AND *PS. AERUGINOSA*.**

| Antibiotics | *Acinetobacter baumannii* (MDR) 48.5% Percentage (%) of resistant strains to individual drug | *Ps. aeruginosa* (MDR) 44% Percentage (%) of resistant strains to individual drug |
|---|---|---|
| Amikacin | 33 | 38 |
| Ampicillin | 0 | - |
| Amoxicillin-clavulanic acid | 11 | - |
| Aztreonam | 14 | 49 |
| Cefotaxime | 8 | - |
| Ceftazidime | - | 2 |
| Ciprofloxacin | 11 | 1 |
| Fosfomycin | 73 | 70 |
| Imipenem | 42 | 6 |
| Nalidixic acid | 8 | - |
| Nitrofurantoin | 5.5 | - |
| Polymyxin B | 100 | 100 |
| Cefoperazone-sulbactam | - | 2 |
| Pipracillin-tazobactam | 28 | 8 |
| Trimethoprim/sulfamethoxazole | 18 | - |

## DISCUSSION

The present study was conducted to evaluate the potential of the older antibiotic (fosfomycin) for the treatment of UTIs, especially against MDR-GNB pathogens. Prevalence of MDR *Acinetobacter baumannii (48.5%)* and *Ps. aeruginosa* (44%) in patients of UTI was observed. These findings are on higher side than the previous reports regarding prevalence of MDR-GNB in Karachi (14) which points to an increase in the drug resistance.

The current study demonstrates the resistance of *Acinetobacter baumannii* and *Ps aeruginosa* to therapeutically important antibiotics. Interestingly, higher frequency of resistance was noticed in *Acinetobacter baumannii* as compared to *Ps. aeruginosa.* Compared to other antibiotics, Polymyxin B (100%), Fosfomycin (82%), Imipenem (69%), Pipracillin-tazobactam (62%) and Amikacin (60%) were found to be effective (bioactive) against all the isolates of *Acinetobacter baumannii* (fig.1). For MDR *Acinetobacter baumannii,* many antibiotics showed a decrease in susceptibility (< 50% sensitive) but interestingly, Polymyxin B and Fosfomycin were found bioactive (100%) and (73%) of these isolates, respectively (table 1). For all the isolates of *Ps. aeruginosa,* most effective antibiotics included*:* Polymyxin B (100%), Aztreonam (76%), Amikacin (63%) and Fosfomycin (85%) respectively (fig.2). For MDR *Ps. aeruginosa,* all antibiotics showed decreased bioactivity except Polymyxin B (100%) and Fosfomycin (70%) which showed more bioactivity against these isolates. Very important antibiotics like Amikacin, Amoxicillin-clavulanic acid, Cefotaxime, Ciprofloxacin, Imipenem, Cefoperazone-sulbactam and Pipracillin-tazobactam were found to show decreased bioactivity against both MDR-GNB types (with some variations).

However, Polymyxin B has come out to be the most effective against both MDR-GNB type of the isolated strains but this antibiotic leaves behind many side-effects as well. So, Fosfomycin should be the better choice for MDR-GNB and it has another merit (can be used orally as well as intravenously). In fact, Fosfomycin has emerged as a promising treatment option. It has rare adverse reactions which may develop in 1-8% of all the patients, the most common ones being diarrhea, nausea, vomiting, skin rashes, heartburn, vaginitis, headache, chills and asthenia (15). Fosfomycin has a low molecular weight with a relatively long half‑life post intake (mean half life-SD, 5.7-2.8 h) and therefore, penetrates various tissues with ease, achieving the minimum inhibitory concentrations needed to inhibit the growth of most of the pathogens (16). Resistance emergence rate is low and most frequently acquired by chromosomal mutations that do not spread easily (17).

In previous studies, around 10% of strains of *Ps. aeruginosa* were found resistant to fosfomycin (18). Current studies on *Ps. aeruginosa* isolates demonstrated similar rates of resistance to fosfomycin *in vitro* (19), and this study correlates with these findings. Polymyxin B and colistin also demonstrated good results against *Ps. aeruginosa* and *Acinetobacter baumannii*. Keeping this in view, further trials can be done for combined therapy (Fosfomycin with colistin or Polymyxin B). Further studies to be based on molecular mode of action of fosfomycin are needed. Fosfomycin appears to be picked as an excellent therapeutic choice for the treatment of both MDR-GNB pathogen types.

## CONCLUSIONS

Fosfomycin is a bactericidal agent that encounters a low level of resistance as compared to other antibiotics. Antimicrobial activity of fosfomycin, especially against MDR uropathogens, makes it an effective and safe drug for the treatment of UTIs caused by Gram‑negative bacteria, especially against the MDR *Acinetobacter baumannii* and *Ps. aeruginosa* for which previous antibiotics have failed to treat the infections.

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