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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 = 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

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 37ºC 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 10⁵ 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 (CLP5µg), 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.
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*.

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

The current study demonstrates the resistance of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to therapeutically important antibiotics. Interestingly, higher frequency of resistance was noticed in *Acinetobacter baumannii* as compared to *Pseudomonas 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 *Pseudomonas 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 *Pseudomonas 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 *Pseudomonas aeruginosa* for which previous antibiotics have failed to treat the infections.

REFERENCES


