Fosfomycin Synergy In Vitro with Amoxicillin, Daptomycin, and Linezolid against Vancomycin-Resistant Enterococcus faecium from Renal Transplant Patients with Infected Urinary Stents

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Fosfomycin is a potential option for vancomycin-resistant enterococcus (VRE) infections despite limited in vitro and clinical data. In this study, 32 VRE isolates from renal transplant patients with urinary stent infections were susceptible to fosfomycin, daptomycin, and linezolid and resistant to amoxicillin, minocycline, and nitrofurantoin based on their MIC<sub>90</sub> and MIC<sub>50</sub>. Fosfomycin was bacteriostatic at 0.5 to 16× the MIC (32 to 2,048 μg/ml); synergy occurred when fosfomycin was combined with daptomycin (2.8 to 3.9 log<sub>10</sub> CFU/ml kill; P < 0.001) or amoxicillin (2.6 to 3.4; P < 0.05). These combinations may be potent options to treat VRE urinary infections pending investigation of clinical efficacy.

Solid-organ transplant recipients are at increased risk for colonization with vancomycin-resistant enterococcus (VRE) and vulnerable to active infections with this organism (1). At our institution, a 7-French, 16-cm double-J stent is inserted through the ureter into the renal pelvis and then into the bladder at the time of kidney transplantation. Biofilm development often precipitates colonization of the stents and results in sequestered, stationary phase bacteria that further resist the effects of antibiotics.

Fosfomycin, a phosphonic acid derivative initially isolated in 1969 from cultures of Streptomyces species, is an oral therapy for uncomplicated urinary tract infections (UTIs) (2). It is often bactericidal against multidrug-resistant Gram-positive and Gram-negative pathogens, although it has not been well studied against VRE. The antibacterial activity of fosfomycin is achieved by inhibiting the enzyme N-acetylglucosamine (UDP-GlcNAc) enolpyruvyl transferase (MurA), which synthesizes UDP-N-acetylenolpyruvylglucosamine, an essential component in the biosynthesis of peptidoglycan (2). It is highly concentrated in the urine, with peak values of 1,053 to 4,415 μg/ml within 4 h after a single oral 3-g dose (2). Fosfomycin has gained recent interest as a potential therapeutic option for treating infections caused by VRE despite limited efficacy data (3).

The aim of this study was to investigate fosfomycin activity in vitro alone and in combination with other antibiotic treatment options against VRE urine isolates collected from renal transplant patients with urinary stent infections. We present synergistic combinations with fosfomycin that may be useful treatment options for these situations.

(Contents of this work were presented at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2011 [4].)

Thirty-two VRE (Enterococcus faecium) isolates were collected from renal transplant patients with urinary stent infections from 2007 to 2010 at a tertiary medical center. Enterococcus faecalis ATCC 29212 was used as a control strain. The antibiotics evaluated were amoxicillin, fosfomycin, nitrofurantoin, and minocycline, purchased from Sigma-Aldrich (St. Louis, MO), as well as linezolid (Pfizer, NY) and daptomycin (Cubist, Lexington, MA), which were commercially purchased.

Antibiotic activities were determined in calcium-adjusted Muller-Hinton broth (MHB) appropriate for the antibiotics tested (5). For assays involving fosfomycin, MHB was also supplemented with 25 μg/ml glucose-6 phosphate. All biofilm assays were evaluated in tryptic soy broth plus 1% dextrose and supplemented with the appropriate requirements for selected antibiotics (6).

MICs and minimum bactericidal concentrations (MBCs) against planktonic cultures were determined by broth microdilution (5). The MIC and minimum biofilm eradication concentration (MBEC) were determined using a transferable solid-phase pin lid (Nunc) as described by Ceri et al. (7).

Antibiotic activity against four of the clinical isolates as well as ATCC 29212 was analyzed in a 24-h time-kill curve in duplicate with an inoculum of 5 × 10<sup>5</sup> CFU/ml. The standard kill curve method for synergy (8) utilized variable concentrations of the primary antibiotic (fosfomycin, 0.5 to 16× the MIC) in combination with a static subinhibitory concentration (0.5× the MIC) of the secondary antibiotic. Synergy, additive effect, antagonism, and indifference were defined as ≥ 2 log kill, < 2 but > 1 log kill, > 1 log growth, and ≥ 1 log kill, respectively (8). Bactericidal activity was defined as 99.9% kill from the initial inoculum.

Clinical VRE isolates were susceptible to fosfomycin, daptomycin, and linezolid and resistant to amoxicillin, minocycline, and nitrofurantoin based on MIC<sub>90</sub> and MIC<sub>50</sub> results (Table 1). Linezolid and daptomycin were the most active antibiotics, with MIC<sub>90</sub> of 2 and 4 μg/ml, respectively. Only linezolid maintained the same MIC profile when tested against biofilm cultures, while fosfomycin and daptomycin had at least a 2-fold increase in the MIC<sub>90</sub> in biofilm (Table 1).

Fosfomycin was bacteriostatic against VRE in the kill curve at

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Interestingly, low exposures of fosfomycin (0.5 \( \mu g/ml \)) comparable to those achieved in the urine of patients after a single oral dose (9). Importantly, fosfomycin modifies the production of penicillin binding proteins (PBPs) in Staphylococcus aureus and Streptococcus pneumoniae (12). This suggests that the combination of fosfomycin and amoxicillin has potential to overcome beta-lactam resistance caused by PBPs (12). In our study, synergy was observed for fosfomycin in combination with amoxicillin, potentially due to fosfomycin increasing the sensitivity of the bacterial cell envelope to daptomycin.

Aminopenicillins are key agents used in the treatment of Enterococcus spp. infections, although the activities of ampicillin and amoxicillin alone against VRE are poor (9). Fosfomycin modifies the production of penicillin binding proteins (PBPs) in Staphylococcus aureus and Streptococcus pneumoniae (12). This suggests that the combination of fosfomycin and amoxicillin has potential to overcome beta-lactam resistance caused by PBPs (12). In our study, synergy was observed for fosfomycin in combination with amoxicillin, potentially due to fosfomycin increasing the sensitivity of the bacterial cell envelope to daptomycin.

The use of fosfomycin in the treatment of VRE has recently gained renewed interest although few in vitro or clinical data support its use. Of the VRE isolates in our study, 91% were susceptible (MIC, \( \leq 64 \mu g/ml \)) to fosfomycin (5), consistent with the limited prior reports of fosfomycin susceptibility in VRE (3, 9). Fosfomycin exhibited a concentration-dependent effect in the standard kill curve. The antibacterial activity of fosfomycin increased from low to high concentrations but still remained bacteriostatic up to 16-fold higher than the MIC. Although fosfomycin concentrations are high at this concentration, they represent concentrations comparable to those achieved in the urine of patients after a single 3-g oral dose (9). Interestingly, low exposures of fosfomycin (0.5 \( \times \) the MIC) in combination with daptomycin, amoxicillin, or linezolid produced greater bacterial killing than high concentrations of fosfomycin alone (1 to 16 \( \times \) the MIC) and bactericidal killing similar to that induced by high fosfomycin exposures in combination with 0.5 \( \times \) the MICs of secondary antibiotics.

Synergy studies with fosfomycin and daptomycin are limited, but one case report describes successful treatment with this combination for S. aureus endocarditis caused by a daptomycin-non-susceptible strain (10). In our study, fosfomycin plus daptomycin was the most active combination against VRE and resulted in bacterial kill at or close to the detection limit. Based on the ability of fosfomycin to inhibit essential enzymes in the biosynthesis of peptidoglycan (11), we hypothesize that this synergistic effect is largely due to fosfomycin increasing the sensitivity of the bacterial cell envelope to daptomycin.

There was no antagonism detected.

The addition of daptomycin, amoxicillin, or linezolid to fosfomycin in the kill curve increased bacterial killing (Fig. 1, each antibiotic at 0.5 \( \times \) the MIC), while combination with nitrofurantoin or minocycline had no further antimicrobial effects, with \( \pm 1 \) log difference in kill compared to any agent alone (data not shown). The most potent and highly synergistic combination was fosfomycin plus daptomycin, which often achieved bactericidal activity against clinical strains (maximum kill, 2.8 to 3.9 \( \log_{10} \) CFU/ml; \( P < 0.001 \)) but was bacteriostatic in only half of the clinical strains tested. Fosfomycin in combination with linezolid in the kill curve was bacteriostatic and produced a synergistic or additive effect. No antagonism was detected.

The treatment of UTIs caused by VRE in renal transplant patients is often difficult to manage due to host immunosuppression, hardware placement, and lack of optimally studied antibiotic options (14). A recent case series of fosfomycin outcomes in complex urinary tract infections due to multidrug-resistant organisms, including VRE, reported that urinary stents were associated with microbiologic failure in fosfomycin monotherapy (3). In order to effectively treat such infections, early hardware removal and complex antimicrobial therapy are often required (9). Based on

<table>
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<th>Antibiotic</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
<th>MBC(_{50})</th>
<th>MBC(_{90})</th>
<th>MBEC(_{50})</th>
<th>MBEC(_{90})</th>
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<tr>
<td>Amoxicillin</td>
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<td>16–1,024</td>
<td>64 (128)</td>
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<tr>
<td>Daptomycin</td>
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<td>2 (4)</td>
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<tr>
<td>Fosfomycin</td>
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<td>128 (128)</td>
<td>64 (128)</td>
<td>256–512</td>
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<tr>
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<td>2 (2)</td>
<td>16–32</td>
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<tr>
<td>Minocycline</td>
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<td>4–32</td>
<td>8 (32)</td>
<td>4–256</td>
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<td></td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>64 (128)</td>
<td>32–1,024</td>
<td>64 (128)</td>
<td>512–1,024</td>
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</table>

a Susceptibility results in planktonic and biofilm cultures of 32 vancomycin-resistant Enterococcus faecium isolates collected from renal transplant patients with urinary stent infections.

b MBC, minimum bactericidal concentration; MBEC, minimum biofilm eradication concentration.

Concentrations of 0.5 to 16 \( \times \) the MIC were the most active combination against VRE and resulted in bacterial kill at or close to the detection limit. Based on the ability of fosfomycin to inhibit essential enzymes in the biosynthesis of peptidoglycan (11), we hypothesize that this synergistic effect is largely due to fosfomycin increasing the sensitivity of the bacterial cell envelope to daptomycin.

Linezolid has in vitro activity against VRE, with susceptibility rates over 99% (13). The isolates in our study displayed similar susceptibility rates (97%), and importantly linezolid susceptibility in biofilm was maintained (MIC, \( \leq 2 \mu g/ml \)). Fosfomycin combined with linezolid was either synergistic or additive in the kill curve. One previous study with fosfomycin and linezolid suggests potential for this combination therapy when used against Staphylococcus epidermis and Staphylococcus aureus (11). This combination could be an effective oral option for the treatment of VRE UTIs, including those with biofilm development on urinary stents.

The treatment of UTIs caused by VRE in renal transplant patients is often difficult to manage due to host immunosuppression, hardware placement, and lack of optimally studied antibiotic options (14). A recent case series of fosfomycin outcomes in complex urinary tract infections due to multidrug-resistant organisms, including VRE, reported that urinary stents were associated with microbiologic failure in fosfomycin monotherapy (3). In order to effectively treat such infections, early hardware removal and complex antimicrobial therapy are often required (9). Based on

![FIG 1 Change in \( \log_{10} \) CFU/ml after 24-h antibiotic exposures in the kill curve. Data represent antibiotics evaluated alone or in combination at 0.5 \( \times \) the MIC. Fosfomycin (FOS) combined with either daptomycin (DAP) or linezolid (LIN) was either synergistic or additive yet bacteriostatic.](http://aac.asm.org/)

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our findings, combination therapy with fosfomycin and either daptomycin or amoxicillin is highly synergistic and results in enhanced bactericidal activity compared to fosfomycin alone against VRE. Additional modeling of these two combinations using pharmacokinetic concentrations achieved in urine will help to define their potential utilities. These combinations should be further explored for clinical efficacy.

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REFERENCES