In vitro activities of fosfomycin and gentamycin combinations against clinical isolates of Staphylococcus aureus.

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ABSTRACT

Objectives: Daptomycin has proven rapid bactericidal effect against methicillin-resistant and susceptible S. aureus (MRSA, MSSA), however persistent bacteraemia under daptomycin therapy has been reported. Different combinations have been described to improve outcome although controversies still exists.

Methods: Susceptibility testing was performed in duplicate by the E-test method using vancomycin (VAN), levofloxacin (LEV), daptomycin (DAP), Linezolid (LNZ), oxacillin (OX) and imipenem (IMP), gentamycin (GM) and Fosfomycin (FOS). Quality control was performed using *S. aureus* ATCC 29213. Four isolates from our collection recovered form patients with persistent bacteraemia (2 MRSA and 2 MSSA), were evaluated by time kill experiments (TK) against VAN, DAP, LNZ and IMP at 1, 2,4 8x MIC alone and at 1 and 4xMIC in combination with 0,5 and 2x MIC of FOS and GM (MRSA) and against OX, LEV, DAP and VAN at 1, 2,4 8x MIC alone and at 1 and 4xMIC in combination with 0,5 and 2x MIC of FOS and GM (MSSA), using a starting inoculum of 8-9 log CFU/mL. Bactericidal activity was defined as ≥ 3 log10 kill compared to the starting inoculum.

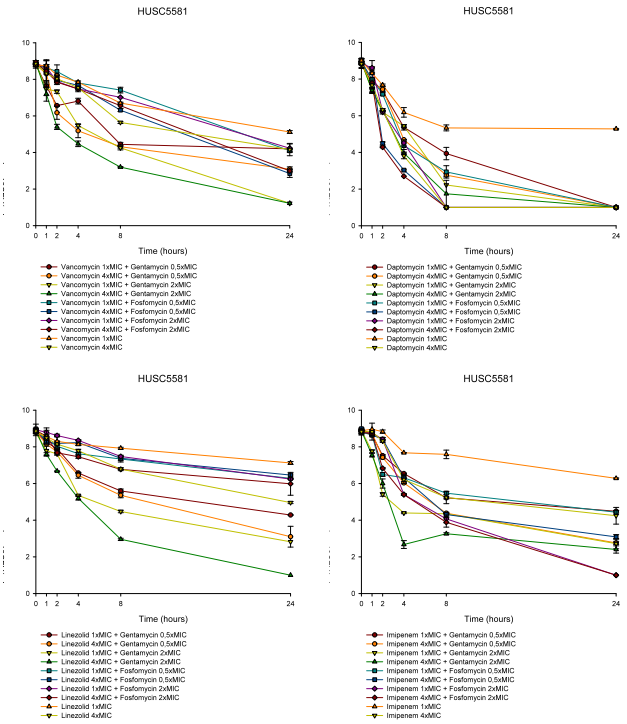
Results: DAP was the most effective drug against all tested isolates, exhibiting cidal activity within 4h at either 4x or 8x the MIC. VAN was cidal at 4 and 8xMIC at 8 hours against MRSA and at 24 h against MSSA. LEV and OX were cidal against MSSA strains at 8 hours. In contrast, LNZ and IMP were bacteriostatic at either 4x and 8x MIC against the MRSA strains. DAP-FOS combination was the most effective against all strains tested being cidal at 4h 3 combinations [1and 4xMIC (DAP) with 2xMIC(FOS) and 4xMIC (DAP) and 0,5xMIC (FOS)]. IMP-FOS combinations ant high doses were also cidal at 8 hours. Finally VAN-FOS combinations were cidal at high doses at 24 hours. GM combinations showed similar pattern being slightly more active than FOS combinations with VAN and LEV. Finally, LNZ combinations performed better than LNZ alone, being LNZ at 4xMIC with either FOS anfdGM at 2xMIC bactericidal at 24 hours.

Conclusions: At 4 and 8xMIC neither FOS nor GM combinations appeared to be better than DAP alone, however at 1 and 2MIC, combination therapy with 2xMIC resulted in a significantly shorter time to achieve cidal activity. OX and VAN combinations with FOS did not resulted in shorter time to cidal activity suggesting a limited role of this combination. Further investigations are needed to better define the therapeutic value of DAP-FOS combinations.

INTRODUCTION

- Increasing resistance of S aureus to almost all groups of currently available antibiotics has lead to a serious problem in the management of infections caused by S aureus, making necessary the study of new therapeutic options.
- Fosfomycin is a natural antibiotic with a rapid bactericidal effect against many pathogens, including resistant strains. Some in vitro studies has shown promising results of fosfomycin combination but few has addressed its efficacy against methicillin-susceptible and methicillin-resistant S aureus (MSSA and MRSA) strains.
- In an effort to understand whether fosfomycin combinations could represent a therapeutic option for staphylococcal infections, we assessed its *in vitro* activity in combination with other anti-staphylococcal agents against a 4clinical isolates of methicilliin-susceptible (MSSA) and resistant *S. aureus* (MRSA) and compared it to those of gentamycin combinations.

Figure 1. Time-kill curves representing the *in vitro* activity of FOS and GM combinations against an MRSA clinical isolate.



METHODS

Antibiotics and Media

- Daptomycin (DAP), vancomycin (VAN), Linezolid (LNZ), Imipenem (IMP), oxacillin (OXA), Levofloxacin (LEV), gentamycin (GM) and Fosfomycin were purchased from commercial sources.
- Mueller Hinton Broth supplemented with 25 mg/L calcium and 12.5 mg/L magnesium (SMHB; Difco Laboratories, Detroit, MI.) was used for all susceptibility testing and time kill experiments. For experiments using oxacillin, SMHB was supplemented with 4% NaCl as recommended by the CLSI guidelines.(7).

Minimum Inhibitory concentrations

- Susceptibility testing were performed in duplicate by E-test. Quality control (QC) was performed using *S. aureus* ATCC 29213.

Time-kill assay (TK)

- Time-Kill assays were performed in duplicate using a starting inoculum of 10^{8-9} CFU/mL. Inoculum in stationary phase of growth was prepared using an appropriate dilution of a 0.5 McFarland. Samples were taken at 0, 1, 2, 4, 8 and 24h.
- Isolates tested in TK included 4 clinical isolates (2 MRSA and 2 MSSA) .
- Regimens evaluated against MRSA included DAP, VAN, LNZ and IMP at 1x and 4x MIC alone and in combination with GM and FOS at 0,5x and 2xMIC. Regimens evaluated against MSSA included DAP, VAN, OXA and LEV at 1x and 4x MIC alone and in combination with GM and FOS at 0,5x and 2xMIC.
- Samples were diluted in normal saline prior to drop plating onto Tryptic Soy Agar plates. Antibiotic carryover was accounted for using vacuum filtration
- Plates were incubated at 35°C for 18h prior to colony counting

RESULTS

- In time-kill experiments, DAP demonstrated rapid concentration-dependent bactericidal activity at 4x and 8x MIC against MSSA and MRSA. $T_{99.9\%}$ was achieved at 8 hours at 8 and 4 x MIC. No other agent displayed similar activity.
- MRSA strains:
  - Combination of DAP at 4xMIC and Fosfomycin at 2xMIC was the most active regimen achieving $T_{99.9\%}$ at 4 hours.
  - IMP-GM and IMP-FOS combination showed sinergistic activity compared to that of IMP alone, however only IMP-FOS combinations achieved the limit of detection at 24 hours against MRSA. In contrast, VAN-GM and LNZ-GM were significantly more active than FOS combinations against MRSA strains (**Fig. 1**)
- MSSA strains
  - DAP and LEV were the most active regimens with $T_{99.9\%}$ at 8 hours (DAP 4x and 8xMIC) and 24 hours (LEV 8xMIC)
  - FOS at 2xMIC combinations displayed additive effect being more active than monotherapy. DAP (1xMIC and 4xMIC) and LEV and OXA at 4xMIC achieved limit of detection at 24 h. VAN and GM 2xMIC combinations achieved limit of detection at 24 h.

CONCLUSIONS

- Daptomycin and Imipenem combinations with Fosfomycin showed potent *in vitro* activity against all MSSA and MRSA isolates.
- Additive effect was demonstrated with all FOS combinations at sub-MIC FOS concentrations.
- Additional *in vitro* and *in vivo* studies to investigate the activity of FOS combinations against organisms with reduced susceptibility to betalactams and glycopeptides are warranted to better define the therapeutic value of FOS combinations in the treatment of multi-drug resistant *S. aureus* infections.

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