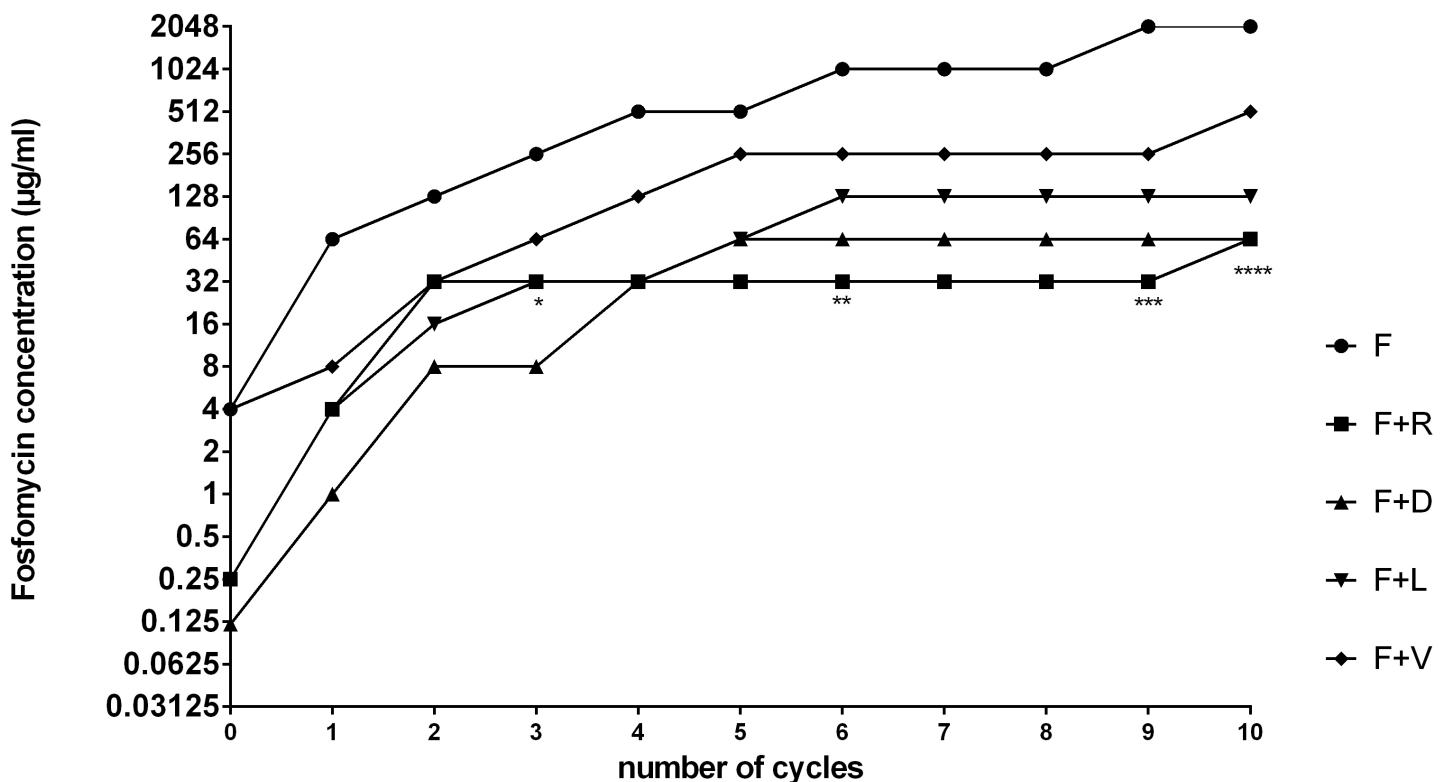


Prevention of in vitro emergence of fosfomycin resistance in methicillin-resistant *Staphylococcus aureus*

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Objectives: Fosfomycin (FOS) is active against *Staphylococcus aureus*, including against biofilms. However, rapid emergence of FOS resistance occurs in vitro. Aim of the study was to evaluate whether emergence of FOS resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) can be prevented by combining FOS with rifampin (RIF), daptomycin (DAP), levofloxacin (LEV) or vancomycin (VAN). **Methods:** A reference MRSA strain (ATCC 43300) was used. The frequency of spontaneous emergence of FOS resistance was tested by incubating 10^9 CFU/ml of MRSA on FOS-containing agar plates for 24 h. For progressive emergence of FOS resistance, bacteria (10^5 CFU/ml) were exposed to two-fold increasing concentrations of FOS, alone and with 0.5 x MIC of RIF, DAP, LEV and VAN for 10 consecutive days. The stability of FOS resistance was evaluated by subculturing FOS-resistant strains on antibiotic-free agar for 5 days. Susceptibility to RIF was determined on day 3, 6, 9 and 10 by gradient strip diffusion test. **Results:** The MICs (in $\mu\text{g/ml}$) were 4 for FOS, 0.008 for RIF, 0.5 for DAP, 0.25 for LEV and 1 for VAN. The mutation rates were $2.23 \text{ CFU} \times 10^{-6}$ (= 4 x MIC) and $9.24 \text{ CFU} \times 10^{-7}$ (= 16 x MIC). FOS resistance emerged rapidly and could not be prevented by the addition of LEV and VAN. The addition of RIF and DAP delayed the increase in the MIC of FOS (Figure). The resistance to FOS remained stable after 5 subcultures in antibiotic-free medium. The MIC of RIF at day 10 was 0.19 $\mu\text{g/ml}$. **Conclusion:** FOS resistance rapidly developed in vitro. Addition of RIF and DAP delayed, but not prevented the emergence of FOS resistance. In contrast, FOS completely prevented the emergence of RIF resistance up to 10 days of progressive exposure to sub-inhibitory RIF concentrations.

*: E-test for Rifampin: 0.006 $\mu\text{g/ml}$ **: E-test for Rifampin: 0.125-0.19 $\mu\text{g/ml}$ ***: E-test for Rifampin: 0.125-0.19 $\mu\text{g/ml}$ ****: E-test for Rifampin: 0.19 $\mu\text{g/ml}$