In vitro activity of antibiotics against biofilms produced by MRSA SCC*mec* IV isolates from hospitals in Rio de Janeiro

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Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens. Biofilm formation is common in medical devices, such as catheters and prostheses, and allows the pathogen to resist to the host immune response and antimicrobials. To quantify the biofilm formation ability and to evaluate the *in vitro* activity of different antimicrobial agents, alone or in combination, against prevalent MRSA SCC*mec* IV isolates from hospitals in Rio de Janeiro. The *in vitro* biofilm production ability was assessed for 43 clinical isolates, including 14 of sequence type (ST) 5, 9 ST1, 4 ST30 and 16 from other ST's, according to Stepanovic *et al* (2000). The biofilm susceptibility to gentamicin (Gen), linezolid (Lin), rifampicin (Rif) and vancomycin (Van) was determined for 6 isolates from three prevalent lineages (USA400/ST1, USA800/ST5, USA1100/ST30). After biofilm formation (24 h), the isolates were exposed to concentrations from 0.25 to 64μg/mL of each antimicrobial agent, applied alone or in combination. Before and after antibiotic treatment, biofilms were characterized in terms of total biofilm mass (by crystal violet) and number of viable cells (CFU/cm^2). The biofilm production was considered positive for 8 (57%) USA800/ST5 isolates (6 weak and 2 moderate), a single isolate USA400/ST1 was considered weak producer, and 2 USA1100/ST30 isolates were weak biofilm producers. After biofilm exposure to concentrations equal to or greater than 4 μg/ml of Rif or Lin, a biomass reduction of 50 % was observed. For Gen or Van, it was verified a reduction of about 45 % but only after exposure to concentrations equal to or greater than 16μg/mL. The synergism experiments showed improved biofilm mass reduction (55 %) using antimicrobials in concentrations lower than those mentioned above (Gen16μg/mL + $Rif_{2\mu g/mL}$, $Lin_{2\mu g/mL}$ + $Rif_{2\mu g/mL}$, $Rif_{2\mu g/mL}$ + $Van_{4\mu g/mL}$, or $Lin_{2\mu g/mL}$ + $Van_{4\mu g/mL}$). These latter results were equivalent to those obtained with high concentrations of the drugs used alone. Regarding biofilm cell viability, it was verified reductions of up to 1 log to $Van_{64\mu g/mL}$ or $Rif_{64\mu g/mL}$, or 2 logs to $Gen_{64\mu g/mL}$ or $Lin_{64\mu g/mL}$ and between 2 and 3 logs in the synergisms of $Lin_{2\mu g/mL}$ + $Rif_{2\mu g/mL}$, $Rif_{2\mu g/mL}$ + $Van_{4\mu g/mL}$ or $Gen_{16\mu g/mL}$ + $Rif_{2\mu g/mL}$). Data demonstrated that antibiotic synergisms involving $Lin_{2\mu g/mL}$ + $Rif_{2\mu g/mL}$ and $Rif_{2\mu g/mL}$ + $Van_{4\mu g/mL}$ appear to be good therapy choices, since both produced greater reductions in biomass and number of cells in staphylococcal biofilms.