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O1134 Isothermal microcalorimetry detects the presence of persister cells in a *Staphylococcus aureus* biofilm after vancomycin treatment

Maria Eugenia Butini[1], Andrej Trampuz[2], Mariagrazia Di Luca*[3]

[1] Berlin-Brandenburg Centrum for Regenerative Therapies, Charité - University Berlin, Germany, Berlin, Germany, [2] Centrum für Muskuloskeletale Chirurgie, Charité - University Medicine Berlin , Berlin, Germany, [3] Department of Biology - University of Pisa, Pisa, Italy

Background: Persisters constitute a biofilm subpopulation of less-metabolically active cells, phenotypically tolerant to higher concentrations of antibiotics. Due to antibiotic tolerance, persisters are responsible for infection relapse upon treatment discontinuation. By isothermal microcalorimetry (IMC), we evaluated the presence/selection of persisters in a methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm, after treatment with higher concentrations of vancomyin (VAN) and their ability to revert to a normal-growing phenotype. Moreover, the efficacy of daptomycin to kill persisters was also investigated.

Materials/methods: MRSA ATCC 43300 24h-old biofilm was exposed to 1024 µg/ml VAN for 24h. Metabolism-related heat of biofilm-embedded cells during or after VAN -treatment was monitored in real-time by IMC for 24 or 48h, respectively. To evaluate the presence of "persisters" after VAN-treatment, beads were sonicated and detached free-floating bacteria were further challenged with 100xMIC VAN (100µg/ml) in PBS+1%CAMHB. All suspensions were plated for colony counting. The resumption of persister normal growth was analysed by IMC on dislodged VAN-treated cells for 15h in fresh CAMHB, without antibiotic and compared to an untreated control. Anti-persister Activity of 16 µg/ml daptomycin was assessedagainst persister by CFU counting.

Results: In presence of 1024µg/ml VAN, MRSA biofilms produced undetectable heat, suggesting a strong reduction of cell viability and/or cellular metabolism. However, the same samples re-inoculated in fresh medium, showed detectable delayed metabolism-related heat flow, similarly to that generated by persisters. The following exposure to 100xMIC VAN resulted in neither complete killing nor bacterial growth, strongly supporting the hypothesis of a persistent phenotype. the IMC analysis indicated that VAN-treated biofilm cells resumed normal growth with ~3h-delay, compared to the untreated growth control. Daptomycin treatment yielded a complete eradication of persisters isolated after VAN treatment.

Conclusions: High bactericidal concentrations of VAN select for persister cells in MRSA biofilm after 24h-treatment *in vitro*. A staggered treatment vancomycin/daptomycin enables the biofilm eradication. These results support the use in the clinical practice of a therapeutic regimen based on the use of two antibiotics to kill persisters and eradicate MRSA biofilms. IMC represents a suitable technique to detect persisters after an antibiotic treatment and characterize in real-time their reversion to a metabolically-active phenotype.