29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

P0538 Evaluation of *in vitro* synergistic activity of ciprofloxacin and PYO bacteriophage in eradicating a dual-species biofilm formed by *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*

Tamta Tkhilaishvili*[1], Mariagrazia Di Luca[2], Lei Wang[1], Andrej Trampuz[1]

[1] Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Center for Musculoskeletal Surgery; Berlin, Germany., [2] Department of Biology, University of Pisa; Pisa, Italy

Background: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are pathogens able to colonize surfaces and form together a mixed biofilm. The dual-species biofilms are significantly more resistant to antimicrobials than a monomicrobial community, leading to treatment failure. Due to their rapid bactericidal activity, the self-amplification ability and the biofilm degradative properties, bacteriophages represent a promising alternative to antibiotics in fighting biofilm-related infections. In this study, we investigated the effect of either the simultaneous or staggered application of phages and ciprofloxacin versus *S. aureus/P. aeruginosa* mixed biofilm *in vitro*.

Materials/methods: Ciprofloxacin was tested alone and in combination with Pyo bacteriophage cocktail against dual-species biofilm constituted of *P.aeurginosa* ATCC 27853 and MRSA ATCC 43300 formed on porous glass beads. 24h-old biofilms were treated for 24h with sub-eradicating titers/concentrations of phages and ciprofloxacin (corresponding from 1/4 to 1/512 x MBECbiofilm), respectively, administered simultaneously or in a staggered order at 37°C. Heat flow produced by the viable cells still embedded in the biofilm was measured for 48h by isothermal microcalorimetry. After microcalorimetry experiments, beads were sonicated and plated for colony counting. Scanning electron microscopy was used to evaluate the presence of dual-species biofilm on glass beads.

Results: MBEC of ciprofloxacin when tested alone was >512 µg/ml. Phage cocktail was not effective alone as well. However, when dual-species biofilm was pre-treated with phages at different time points resulted in a high synergistic eradicating effect. The maximum synergistic effect was observed after 6 and 12 hours of antibiotic introduction. The MBEC of ciprofloxacin decreased dramatically from >512 µg/ml to < 8 µg/ml. In addition, scanning electron microscopy analysis did not reveal the presence of any adherent cells on the surface of the glass beads.

Conclusions: MBEC of ciprofloxacin against dual-species biofilm of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was above drug concentrations reachable in clinical practice. The co-administration with bacteriophage strongly reduced the antibiotic doses needed to eradicate biofilm. There is a specific time delay in antibiotic introduction to reach the eradication of mix-species biofilm. These results have implications for optimal combined treatment approaches.