27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: P026 Biofilms: new developments and anti-biofilm modalities

Category: 9c. Preclinical biofilm studies

23 April 2017, 12:30 - 13:30
P0580

Differential production of extracellular DNA (eDNA) in Staphylococcus aureus and Staphylococcus epidermidis biofilms

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Background: *S. epidermidis* and *S. aureus* are the most frequent pathogens of biofilm-associated implant infections. Recently, the production of eDNA as an employed mechanism of biofilm formation due to antibiotics exposure, has shifted into focus. The aim of this study was to characterize eDNA production in biofilms of *S. aureus* or *S. epidermidis* and their change during the course of biofilm formation.

Material/methods: In a 24-hour confocal microscopy (CLSM) experiment biofilms of *S. aureus* ATCC 25921 and *S. epidermidis* DSM 3269 were visualized hourly. Using propidium iodide (PI) and concavalin-A staining, eDNA and polysaccharides production in biofilms were measured at hours 1, 2, up to 24. Then 24-hour biofilms of 30 clinical isolates of *S. epidermidis* and 30 *S. aureus* were tested for the detection of eDNA production. The fluorescent stain DAPI and a specific extraction method (Polymere Mediated Enrichment, PME) were used to measure eDNA. Additionally, eDNA stained with TOTO1/ SYTO 60 was measured in 6 and 24 hour old biofilms of either species.

Results: The eDNA production quantified by the PME method and by TOTO-1 staining showed significantly higher eDNA production in 24 hours *S. epidermidis* biofilms than in *S. aureus* biofilms (*S. epidermidis*: 6.42 ± 10.60 %; *S. aureus*: 1.35 ± 2.00; $p < 0.01$). The amount of eDNA of *S. epidermidis* biofilms increased at 24 hours compared to 6 hours biofilms (6 hours: 1.71 ± 1.07 %; 24 hours: 6.98 ±

12.62 %; $p < 0.03$). In *S. aureus* the amount of eDNA remained stable from 6 to 24 hours (6 hours: 1. 97 ± 1.51 %; 24 hours: 1.83 ± 2.30 %; n.s.).

Conclusions: *S. aureus* and *S. epidermidis* exhibit time-dependent differential production of eDNA. eDNA may be a future target to inhibit biofilm formation in order to prevent or to improve treatment of biofilm associated infections.