

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

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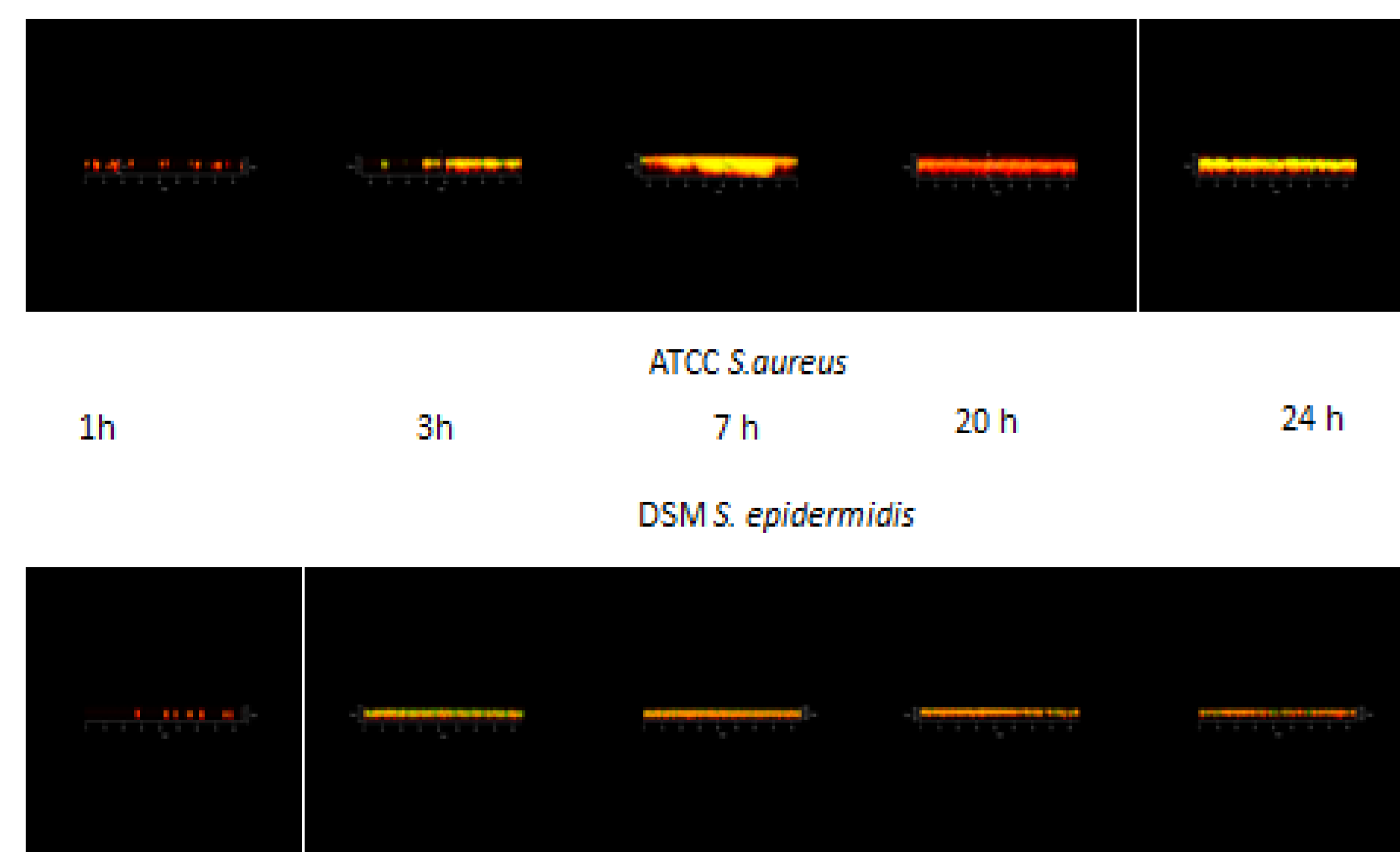
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Objective

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 and 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 (Figure 1 and 2). 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 (Polymer 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

Differential biofilm growth pattern of *S. epidermidis* and *S. aureus* differ in the 24 hour biofilm observation for thickness see Figure 1 and for surface coverage see Figure 2. 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.).

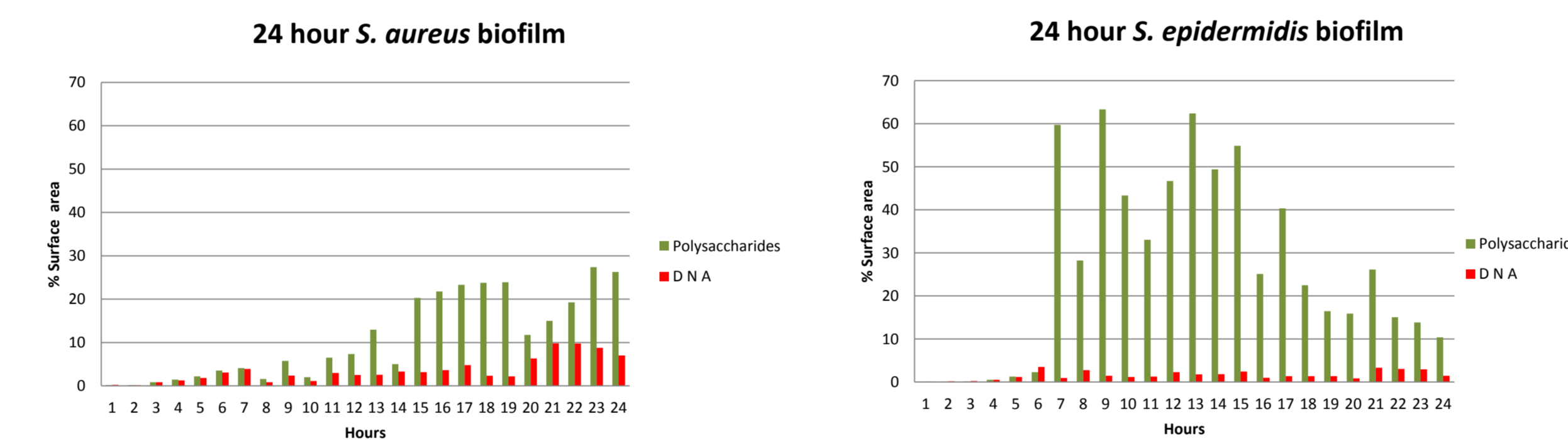


Figure 2: Quantification of surface coverage in % (PI and Con-A staining) comparison of 24 hour biofilm development of reference strains of *S. aureus* and *S. epidermidis*.

Conclusion

S. aureus and *S. epidermidis* exhibit time-dependent growth pattern and 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.

References

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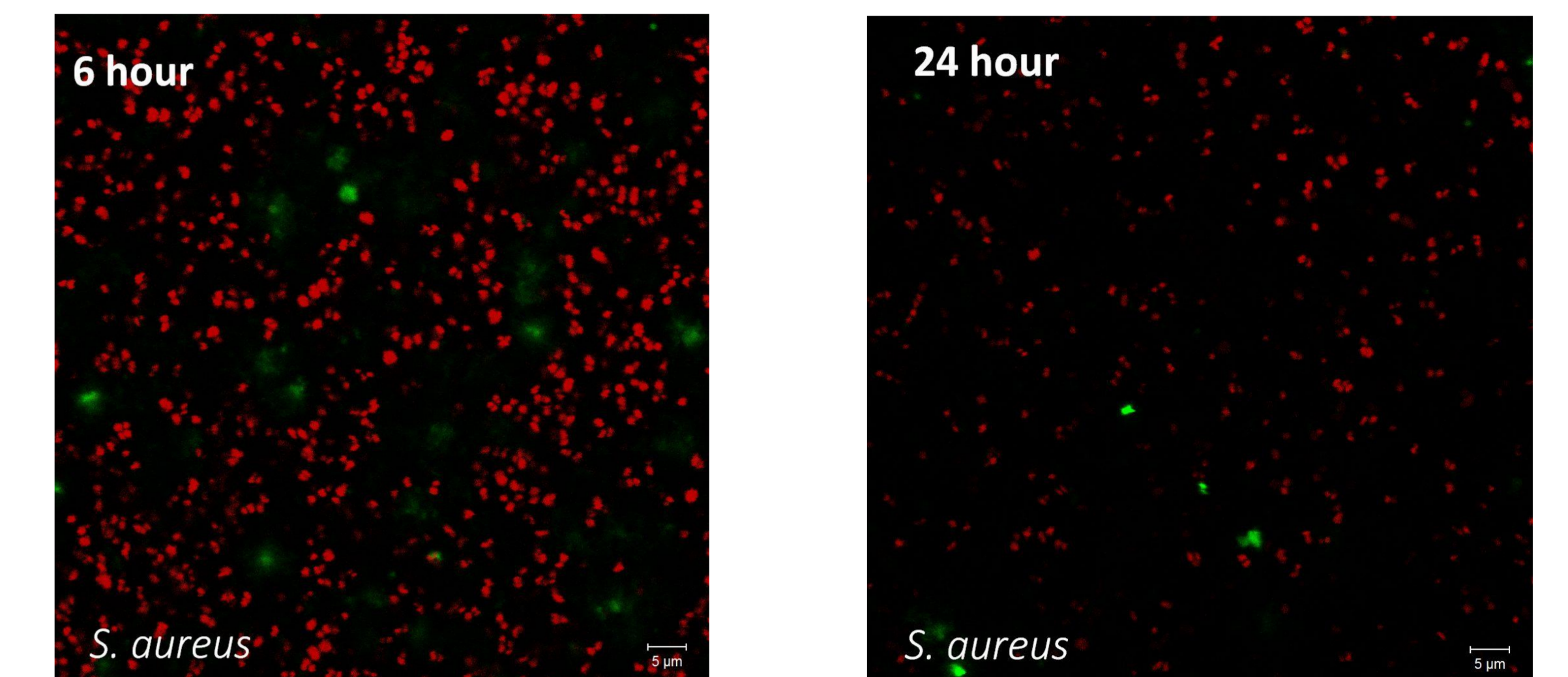


Figure 3: eDNA production: representative pictures of *S. aureus* eDNA production after 6 and 24 hours (TOTO-1 and SYTO-60 staining).

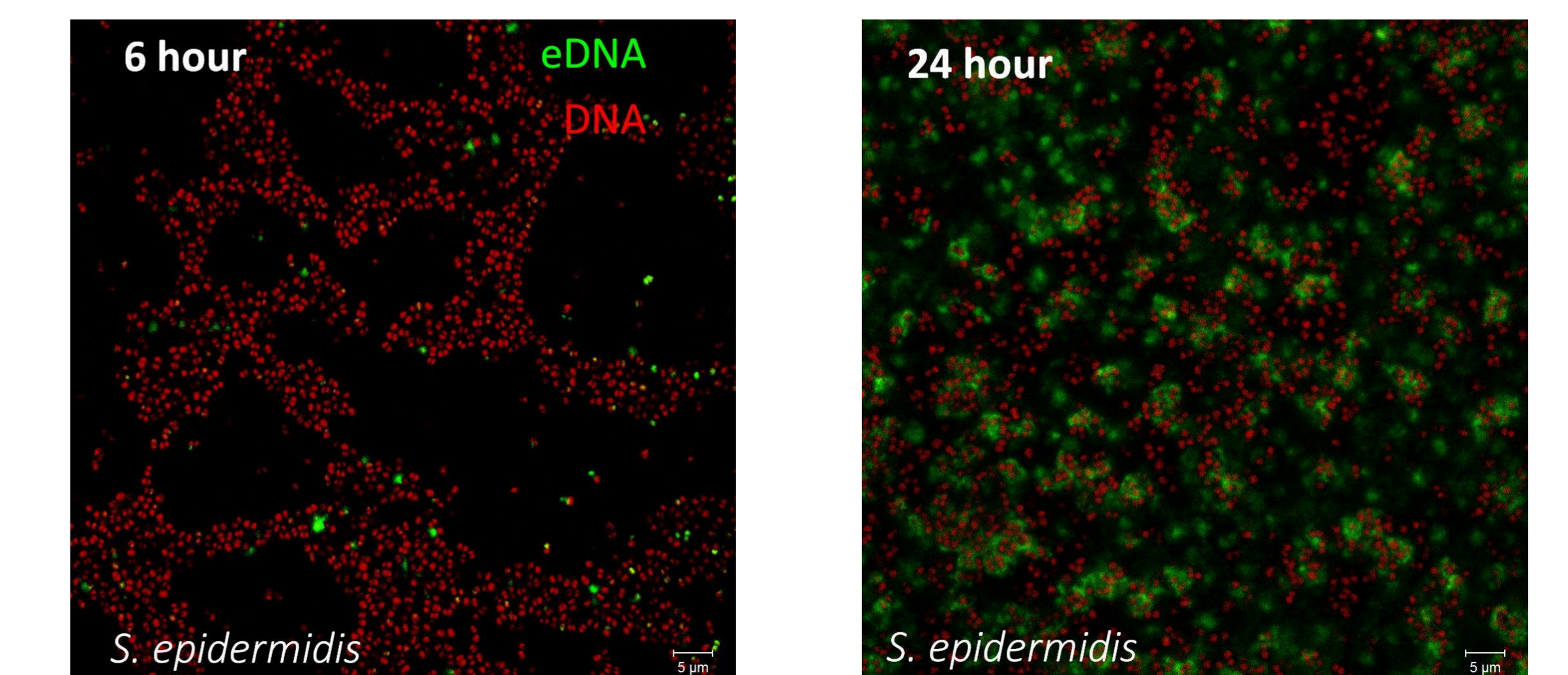
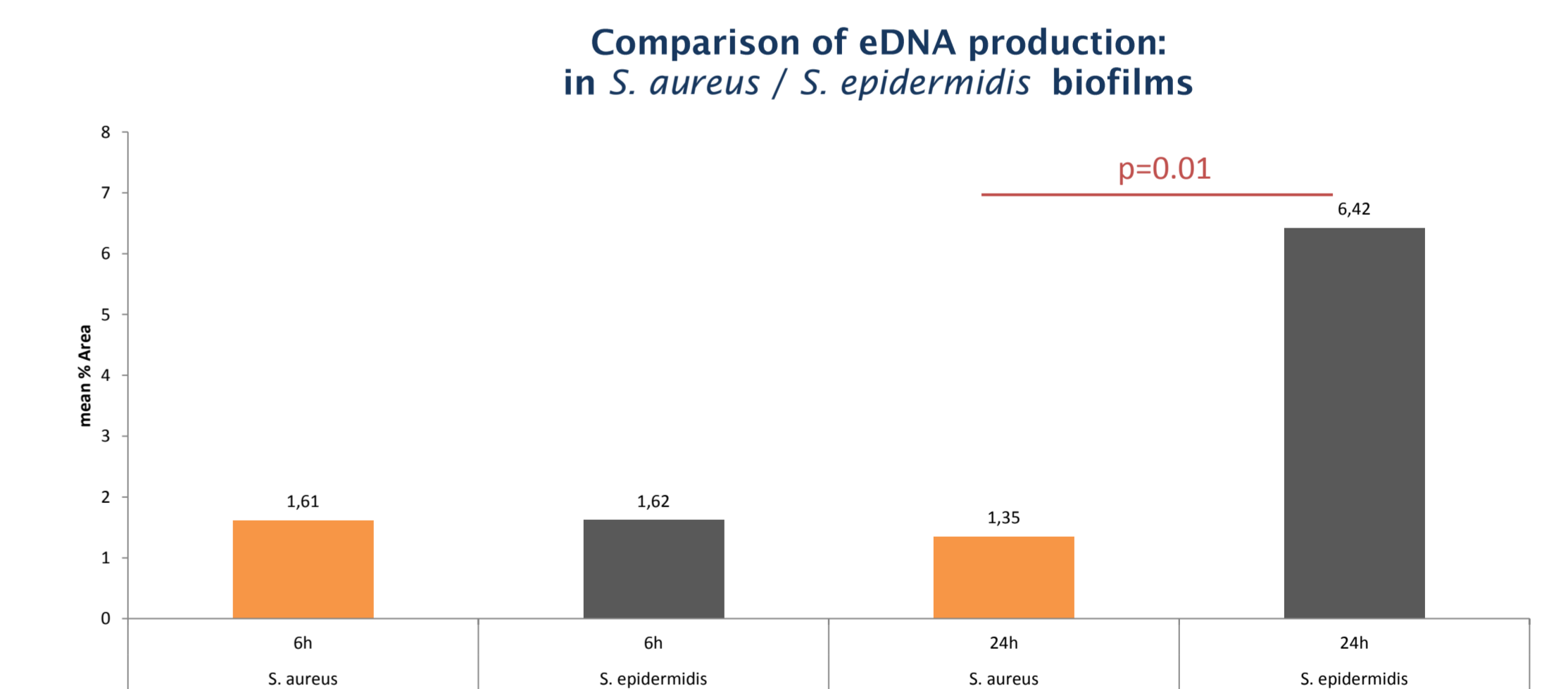


Figure 4: eDNA production: representative pictures of *S. epidermidis* eDNA production after 6 and 24 hours (TOTO-1 and SYTO-60 staining).

Figure 1: Biofilm thickness: comparison of 24 hour biofilm development of reference strains *S. aureus* and *S. epidermidis* CLSM side view; propidium iodide and Concavalin-A staining (1000x magnification)