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## Pathophysiological mechanisms of bone and joint infections of coagulase negative staphylococci: specific behavior of *Staphylococcus lugdunensis*

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**Background:** Associated to significant morbidity and mortality rates, bone and joint infections (BJI) are mainly due to staphylococci representing more than 60% of cases. Concerning *S. aureus*, two mechanisms of virulence have been associated with therapeutic failures: (i) internalization in non-professional phagocytic cells (NPPc), and (ii) biofilm formation. Conversely, little is known about the pathophysiological mechanisms of Coagulase-Negative Staphylococci (CoNS) involved in BJI despite their high prevalence. In this context, we decided to explore them.

**Material/methods:** Clinical strains collected from monomicrobial BJI were studied: *S. epidermidis* (n = 5), *S. haemolyticus* (n = 3), *S. lugdunensis* (n = 7), *S. capitis* (n = 5), *S. caprae* (n = 5), *S. warneri* (n =

5). The internalization capacity of strains in MG-63 osteoblasts was evaluated using an *in-vitro* "Gentamicin protection assay" infection model. In addition, the mature biofilm formation capacity was measured *in vitro* using crystal violet staining test after 24h of incubation. Original data concerning the capacity of biofilm formation of *S. lugdunensis* collected from BJI led us to integrate 10 *S. lugdunensis* strains isolated from nasal carriage as comparators. Finally, the genetic diversity of *S. lugdunensis* isolates was analyzed using MLST (Multilocus sequence typing).

**Results:** The results showed a homogenous behavior within the various CoNS species tested resulting in an inability to be internalized by the osteoblastic cells. No biofilm formation was detected except for isolates belonging to *S. lugdunensis* for which a significant biofilm formation was observed ( $p < 0.001$ ). *S. lugdunensis* isolates collected from nasal carriage have a significantly higher biofilm formation capacity than the clinical strains responsible for BJI ( $p < 0.001$ ). The clustering of *S. lugdunensis* isolates using genetic typing based on MLST demonstrated a correlation between the genotype and the biofilm production. For instance, ST 41 was only found in non-producing isolates and ST27 in moderate- and high-producing isolates.

**Conclusions:** Our study shows that internalization in osteoblasts seems not to be a predominant virulence mechanism in CoNS-related BJIs. A significant production of biofilm was specifically highlighted in *S. lugdunensis* compared to the other *Staphylococcus* species in plastic microplates suggesting a major role of biofilm formation for this species. Nevertheless, because CoNS are specifically involved in the BJIs on materials, these results underline the need of further and wider studies on biofilm formation particularly in the presence of biomaterials.