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

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Introduction

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.

Procedures overview

- 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).
- Internalization capacity of each strain evaluated in MG-63 osteoblasts using an *in-vitro* "Gentamicin protection assay" infection model.
- Mature biofilm formation capacity measured *in vitro* using crystal violet staining after 24h of incubation.
- Original data concerning the biofilm formation capacity 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

- **Homogeneous behavior** for the **internalization** ability in osteoblasts.
- **Biofilm** formation capacities of ***S. lugdunensis*** species show a high **intra-species variability.**
- ***S. lugdunensis*** isolates collected from **carriage** have a **significantly** higher biofilm formation capacity than the **clinical strains responsible for BJI (p <0.001).**
- **The clustering** of *S. lugdunensis* isolates by **MLST** demonstrates a **correlation between the genotype and the biofilm production.**

Bacterial Invasion

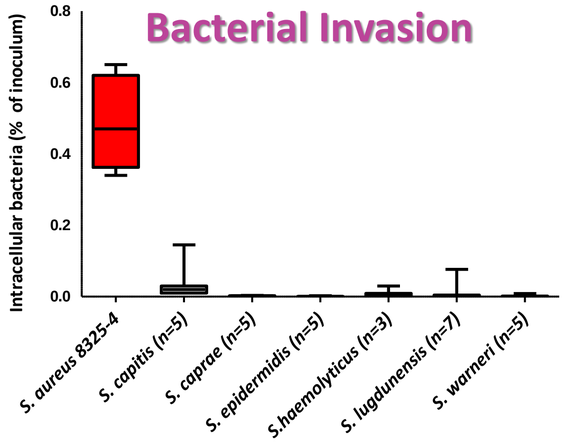

Figure 1 : The invasion of MG-63 cells was assessed by quantifying the viable intracellular bacterial loads at 3h post-infection after gentamicin treatment.

Biofilm

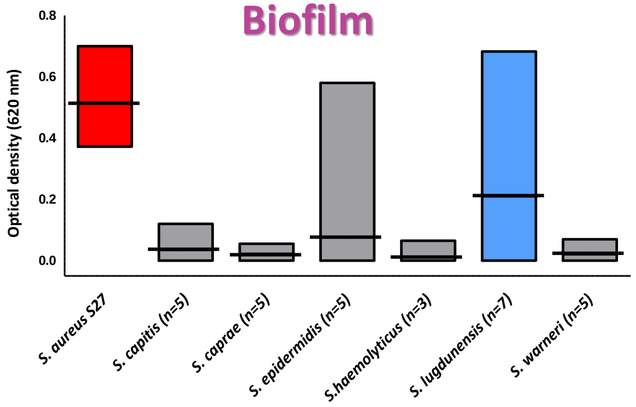

Figure 2 : **CoNS biofilm formation.** Mature biofilm was evaluated spectrophotometrically after 24h.

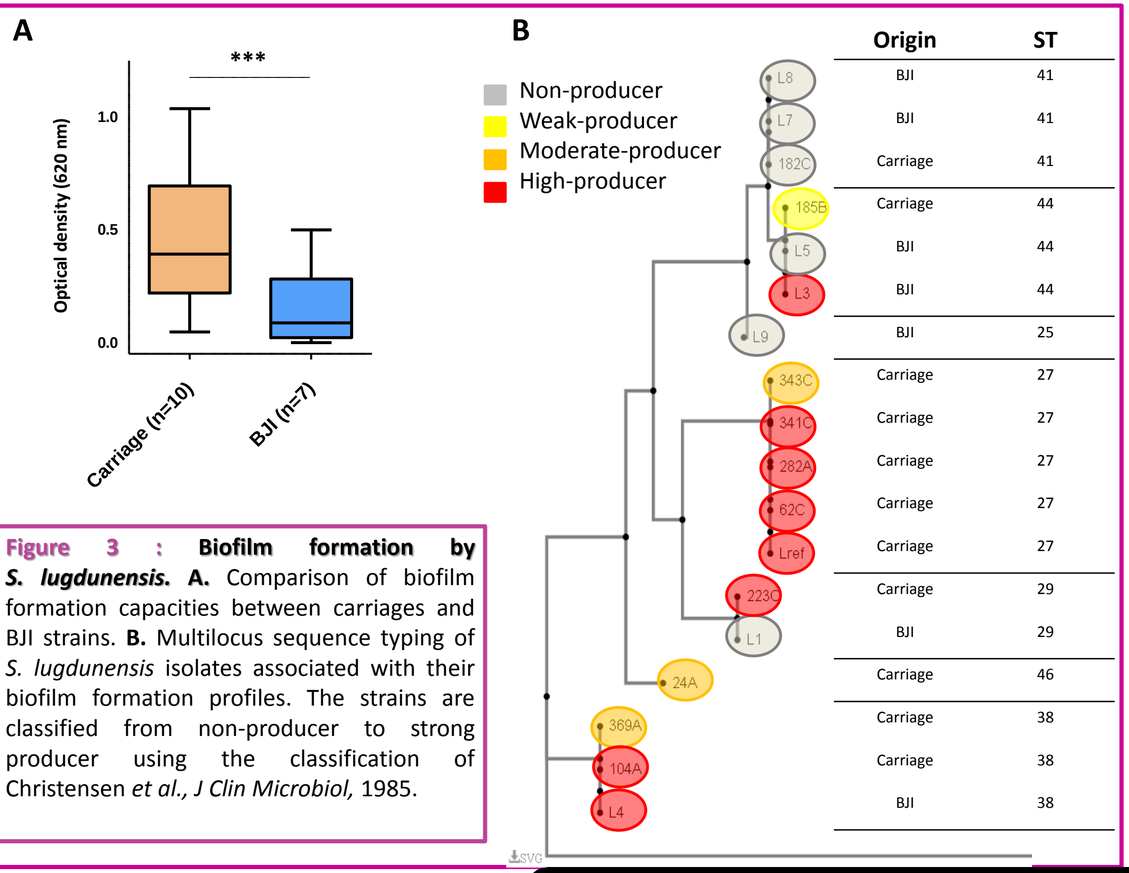

Figure 3 : Biofilm formation by *S. lugdunensis*. A. Comparison of biofilm formation capacities between carriages and BJI strains. **B.** Multilocus sequence typing of *S. lugdunensis* isolates associated with their biofilm formation profiles. The strains are classified from non-producer to strong producer using the classification of Christensen *et al., J Clin Microbiol,* 1985.

Conclusion

Internalization in osteoblasts seems not to be a predominant virulence mechanism in CoNS. Biofilm formation was specifically highlighted in *S. lugdunensis* suggesting a major role in BJI. CoNS are specifically involved in the BJIs on materials, these results underline the need for further and wider studies on biofilm formation particularly in the presence of biomaterials.