P0513
Paper Poster Session
Bone and prosthetic joint infection

Staphylococcus non-aureus and pathophysiological mechanisms of bone and joint infections: interspecies heterogeneity and specific behaviour of the species S. pseudintermedius

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Background: Bone and joint infections (BJI), associated with significant morbidity and mortality, are mainly caused by *Staphylococci*, representing >60% of all BJIs. To date, concerning *S. aureus* two virulence mechanisms have been associated with BJI therapeutic failure, leading to host immune system evasion: i) bacterial internalization in non-professional phagocytic cells; ii) biofilm formation on biotic and abiotic area. However, despite the high prevalence of *Staphylococci* non-*aureus* (SNA) in BJIs, the bacterial pathophysiological mechanisms involved in BJIs caused by SNA have not been studied.

Material/methods: A panel of 16 reference strains, each belonging to a different species of SNA, was compared for different features: i) biofilm formation ability using the standard colorimetric crystal violet staining, ii) adhesion to human fibronectin, a major protein of the extracellular matrix measured by adhesion microplate assay, and iii) capacity of adhesion, internalization, and intracellular persistence (by plate counting), and cytotoxicity (by quantifying lactate dehydrogenase (LDH)) using *in vitro* "gentamincin protection" infection model of human osteoblasts (MG-63 cells). The atypic results concerning internalization obtained with *S. pseudintermedius* reference strain led us to also extend these experiments to 17 clinical isolates of *S. pseudintermedius*. The involvement of the subunit β1 integrin in the invasion process of *S. pseudintermedius* in osteoblasts was evaluated by the use of murine osteoblasts ($\beta1^{+/+}$ and $\beta1^{-/-}$) with functional and non-functional subunit β1 respectively.

Results: A significant heterogeneity in phenotypes between different strains/species SNA has been demonstrated using the three models. The strains studied covered the 4 classes described by the Christensen et al. (1985) classification for the biofilm formation : from non-producer such as *S. hominis* to high producer as *S. lugdunensis*. In addtion to *S. aureus* (100%), only two species were able to adhere to human fibronectin*: S. delphini* (80±7.78%) and *S. pseudintermedius* (104±13.72%, $p<0.05$). Our study also revealed unexpectedly very high capacity of internalization, intracellular persistence

and cytotoxicity for the species *S. pseudintermedius*, even with superior values to those obtained with *S. aureus*. These original results were confirmed with 17 *S. pseudintermedius* clinical isolates. The capacity of internalization in osteoblasts was completely abolished when using murine osteoblasts with defective β1 integrin suggesting the involvement of this integrin in the internalization process of *S. pseudintermedius*, while many studies described this mechanism as specific to *S. aureus*.

Conclusions: Our results suggest that the pathophysiology of SNA infections involves different mechanisms depending on the species, which should lead to adapt the management of patients according to the SNA species. In addition, the results for *S. pseudintermedius* species open new fields of investigation particularly in veterinary medicine where this species is extremely prevalent in dogs pyoderma and associated with purulent necrotic forms that make sense with our data.