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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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Introduction

Bone and joint infections (BJI), associated with significant morbidity and mortality, are mainly caused by *Staphylococci* which represent >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 structures. Despite the high prevalence of *Staphylococci* non-*aureus* (SNA) in BJIs, the bacterial pathophysiological mechanisms involved have not been studied.

Materials And Methods

- Screening approach using a panel of 16 reference strains, belonging to 16 species of SNA, compared for different features:
  - *In vitro* **biofilm** formation by standard colorimetric crystal violet staining after 24h and 48h.
  - Adhesion to human **fibronectin** measured by microplate assay.
  - **Internalization**, and **intracellular persistence** (by plate counting), and **cytotoxicity** (by quantifying lactate dehydrogenase (LDH)) using *in vitro* "gentamicin protection" infection model of human osteoblasts (MG-63 cells).
  - Impact of **β1 integrin** in the invasion process of *S. pseudintermedius* in osteoblasts evaluated by the use of murine osteoblasts (OBβ1$^{+/+}$ and OBβ1$^{-/-}$) with functional and non-functional subunit β1 respectively.
- 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*.

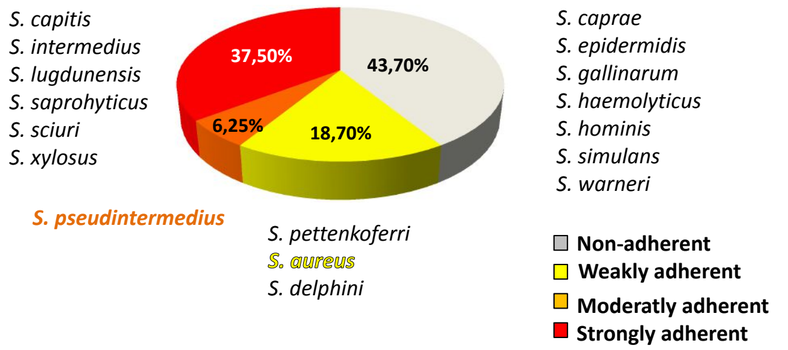
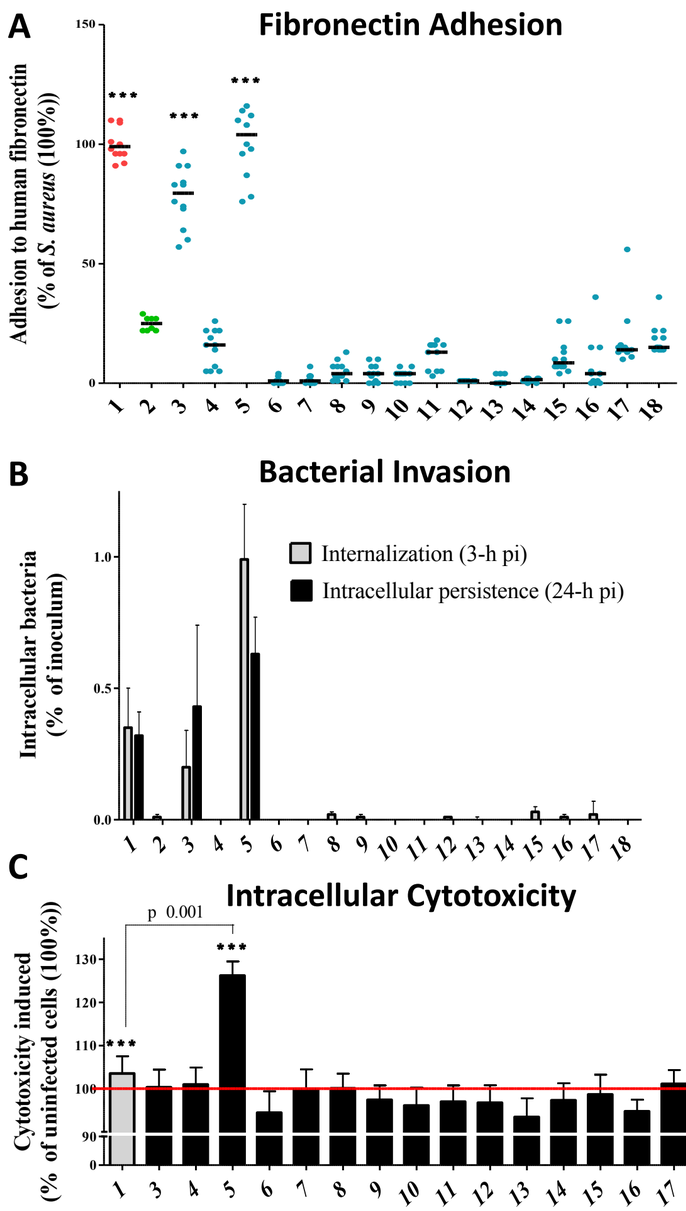


Figure 1 : ***Staphylococcus* non-*aureus* biofilm formation.** Mature biofilm was evaluated spectrophotometrically after 24h incubation. The strains are classified from non-producer to strong producer using the classification of Christensen *et al., J Clin Microbiol,* 1985.



1. *S. aureus* NCTC 8325-4
2. *S. aureus* NCTC 8325-4 *Δfnb*
3. *S. delphini* DSM 20771
4. *S. intermedius* CCM 5739
5. *S. pseudintermedius* LMG 22219
6. *S. capitis* CCM 2734
7. *S. caprae* CCM 3573
8. *S. epidermidis* CCM 2124
9. *S. gallinarum* CCM3572
10. *S. haemolyticus* CCM 2737
11. *S. hominis* DSM 20328
12. *S. lugdunensis* ATCC 43809
13. *S. pettenkoferi* CIP 107711
14. *S. saprophyticus* CCM 883
15. *S. sciuri* ATCC 29062
16. *S. simulans* ATCC 27848
17. *S. warneri* CCM 2730
18. *S. xylosus* ATCC 29971

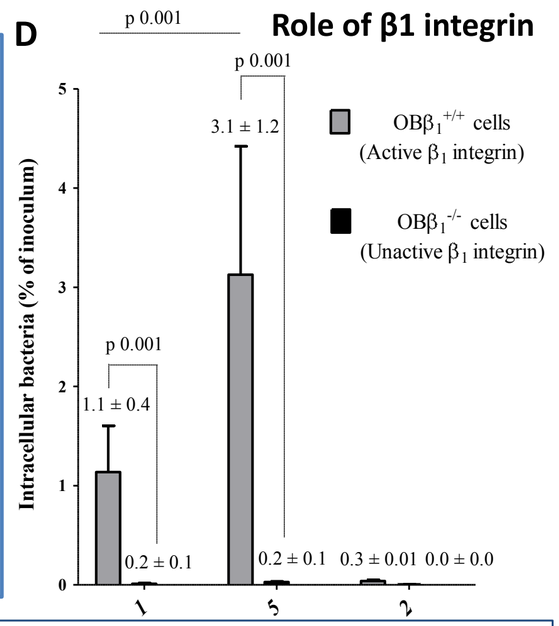


Figure 2 : **Evaluation of *Staphylococcus spp* species to adhere to human fibronectin, to be internalized and to persist in bone cells.** Adhesion of bacteria to fibronectin was assessed spectrophotometrically (OD_{620})(A). The invasion and persistence in MG-63 cells were assessed by quantifying the viable intracellular bacterial loads at 3h and 24h post-infection after gentamicin treatment (B). Quantifications of LDH, reflecting cytotoxicity were performed on culture supernatant at 24h post-infection (C). Determination of the involvement β1 integrin in the *S. pseudintermedius* internalization process was evaluated using murine osteoblasts cell lines (OBβ1$^{+/+}$ and OBβ1$^{-/-}$) with functional and non-functional subunit β1 respectively (D).

Results

- **Four classes** of Christensen *et al.* (1985) classification for the biofilm formation were covered.
- **Homogeneous behavior** for fibronectin adhesion, internalization, persistence and cytotoxicity except for the species ***S. pseudintermedius*** that shows unexpected virulence phenotypes.
- **These results were confirmed with 17 *S. pseudintermedius* clinical isolates.**
- Demonstration of the **involvement of β1 integrin** in the internalization process of *S. pseudintermedius.*

Conclusions

The screening of a large panel of *Staphylococcus* non-*aureus* species, shows that **internalization in osteoblasts does not seem to be a classical pathophysiologic mechanism widespread in SNA species involved in BJI**, except for the species *S. pseudintermedius.* 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 the data presented that show intracellular invasion and high cytotoxicity.