Investigation of the ability to be internalized in osteoblasts as a pathophysiological mechanism involved in *Staphylococcus* non-*aureus* bone and joint infection

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Abstract

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 *Staphylococcus* non-*aureus* (SNA) in BJIs, the bacterial pathophysiological mechanisms involved have not been studied.

Procedure overview

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* "gentamincin 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.*

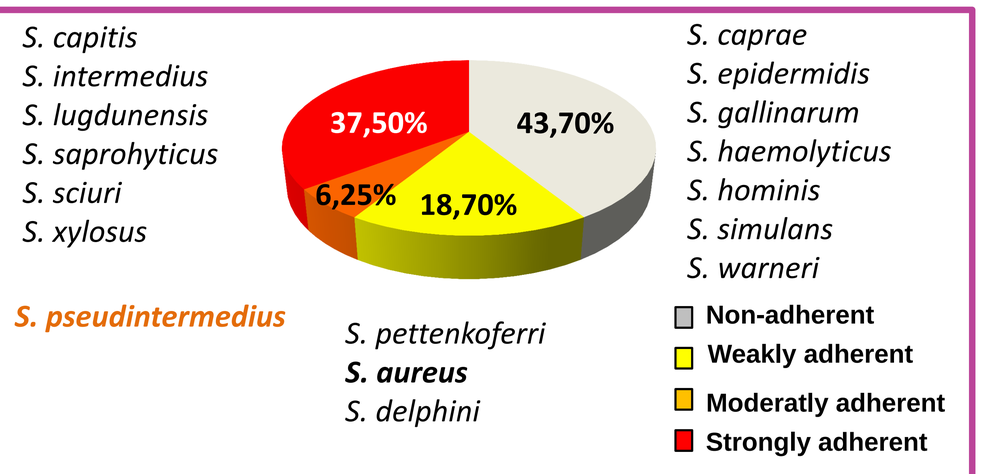
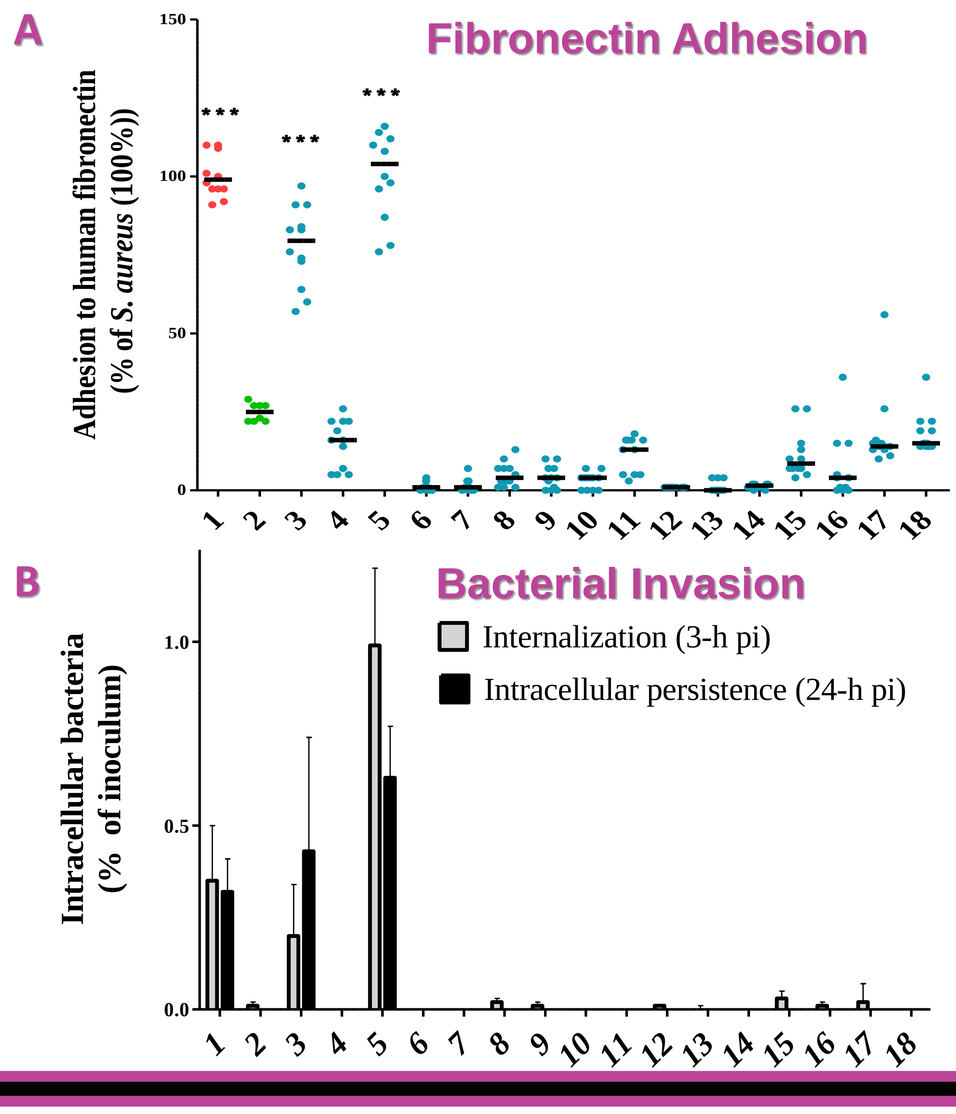
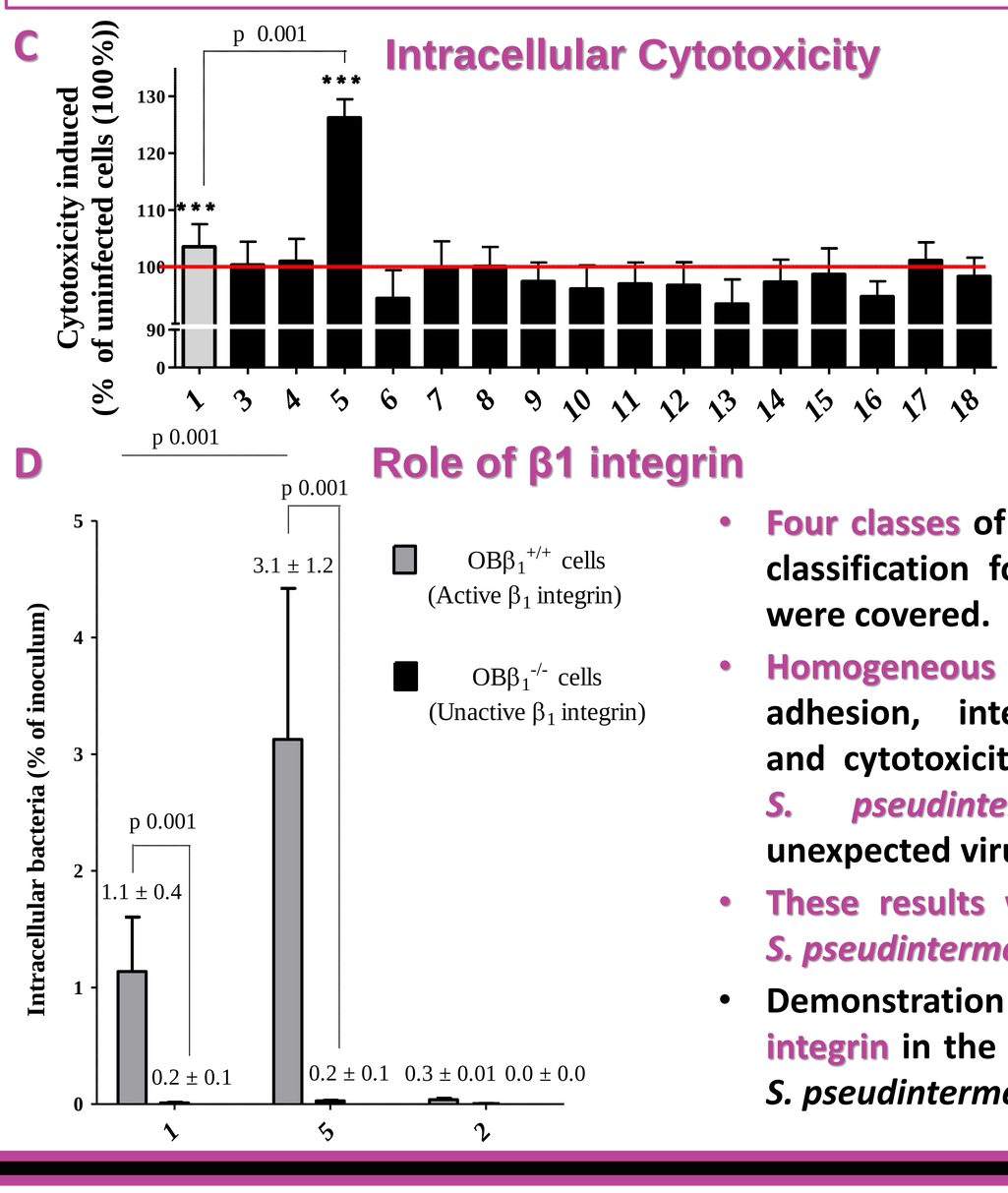


Figure 1 : ***Staphylococcus* non-*aureus* biofilm formation.**
Mature biofilm was evaluated spectrophotometrically after 24h

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 ($OB\beta1^{+/+}$ and $OB\beta1^{-/-}$) with functional and non-functional subunit β1 respectively **(D)**.

1. ***S. aureus***
2. ***S. aureus Δfnb***
3. ***S. delphini***
4. ***S. intermedius***
5. ***S. pseudintermedius***
6. ***S. capitis***
7. ***S. caprae***
8. ***S. epidermidis***
9. ***S. gallinarum***
10. ***S. haemolyticus***
11. ***S. hominis***
12. ***S. lugdunensis***
13. ***S. pettenkoferri***
14. ***S. saprophyticus***
15. ***S. sciuri***
16. ***S. simulans***
17. ***S. warneri***
18. ***S. xylosus***





- **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.***

Conclusion and perspectives

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.