Evidence of adaptive processes of *Staphylococcus aureus* isolates in the course of chronic bone and joint infections in patients


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**Objectives**

Bone and joint infection (BJI) is associated with significant morbidity and mortality, due to high rates of chronicity and relapse (10-20% of cases). To date, three staphylococcal virulence mechanisms have been associated with BJI chronicization and therapeutic failure, leading to host immune system evasion: i) bacterial internalization in non-phagocytic bone cells such as osteoblasts; ii) biofilm formation; and iii) the phenotype switching to small colony variants, characterized by reduced metabolic and hemolytic activities. The present study aimed to compare isolates recovered from initial and recurrent BJI episode from the same patients toward these bacterial adaptive mechanisms.

**Results**

The crystal violet staining method revealed that recurrent strains from patient #2 and #3 formed higher levels of mature biofilm (132±23% and 241±67%, respectively) than initial strains (100%) at 48h (p<0.01 for both). No difference was observed with strains recovered from patient 1.

3 couples of MSSA strains isolated from patients suffering from recurrent or persisting prosthetic joint infection (PJI) at initial diagnosis of PJI and at the time of relapse.

**In vitro infection model**

Osteoblasts

MC3T3 cells

MOI=50

Primary human osteoblasts

MOI=250

Gentamicin 200 μg/mL

2h then 40 μg/mL 7th

H24- H48

H3- H24-H48- H72

Cellular lysis

Adhesion: plate agar counting

Cellular lysis

Cytotoxicity: LDH release (host cell damage)

Internalization & persistence: viable intracellular bacteria

**In vivo infection model**

Lung infection model

10⁶ CFU

Patient 1

Patient 2

Patient 3

Bacterial persistence in primary osteoblasts 48h pi (% of initial strain)

Cytotoxicity 48h post-infection (LDH vs uninfected cells)

Results

- Same capacity of adhesion to and internalization into osteoblasts for initial and recurrent strains
- Recurrent strains persist longer in intracellular compartment of osteoblasts than initial strains
- Osteoblasts infected by recurrent strains secrete less inflammatory cytokines than those infected by initial ones

**Results**

In vivo model of lung infection mimicking acute pneumonia revealed that initial strains caused higher mortality (62%) as soon as 24h post-infection, than initial strains (0%), p<0.01. Furthermore, the bacterial counts in the spleen and the lungs were higher in the initial strain- vs recurrent strain-infected animals (7.65±1.00log10 vs 5.85±3.39log10 CFU/lungs (p<0.05) and 5.27±0.28log10 vs 3.39±1.63log10 CFU/spleen (p<0.05) respectively, 48h post infection) suggesting that *S. aureus* isolates recovered from recurrent infections harbors lower virulence power than those recovered from initial infection.

**Conclusions**

These findings suggests for the first time that *S. aureus* BJI chronicization is associated with an in vivo bacterial adaptation leading to host immune escape, linked with higher intraostoblastic persistence and biofilm formation.