Evaluation of the efficacy of an assembly of three bacteriophages compared or associated to antibiotics on *Staphylococcus aureus* embedded in biofilms or internalised in osteoblasts

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### Introduction

*Staphylococcus aureus* is the first causative agent of bone and joint infections (BJI). It is responsible of particularly difficult to treat infections because of its ability to form biofilms and to be internalized and persist inside osteoblastic cells. Recently, phage therapy has emerged as a promising therapy to improve the management of chronic BJI. In the present study, we evaluated the efficacy of an assembly of three bacteriophages previously used in a clinical case report (Ferry 2018) against *S. aureus* in *in vitro* models of osteoblast intracellular infection and biofilm formation.

### Material & Methods

#### Intracellular infection

- **S. aureus** MG63 
  - Human osteoblastic cell line
- Lysostaphin protective assay
- **3h** box
- Assembly of 3 phages (10^7 or 10^9 PFU/mL) and/or antibiotics (vancomycin / rifampicin at bone concentration (6µg/mL))
- **24h** box

#### Mature biofilm

- Counting of intracellular staphylococci
- Counting of intracellular phages
- Counting of viable staphylococci inside biofilm

### "Intracellular" results

1. Intracellular staphylococci
   - Ratio intracellular inoculum 24h / intracellular inoculum 3h
   - LogCFU/mL

2. Intracellular phages
   - non infected cells
   - infected cells
   - Intracellular phage titer PFU/mL

3. Electronic transmission microscopy

4. 4. Vancomycin
   - LogCFU/mL

5. Rifampicin
   - LogCFU/mL

### "Biofilm" results

- No observed intracellular effect of phages on intraosteoelastic *S. aureus* (Fig 1)
- Control of extracellular environment when add vancomycin and phages (Fig 1)
- Presence of intracellular phages next to *S. aureus* inside infected host cell (Fig 2 and Fig 3)
- High anti-biofilm effect of phages and synergy when adding antibiotics and phages (Fig 4 and Fig 5)