High-throughput screening identifies bioactive compounds targeting intracellular *Staphylococcus aureus*

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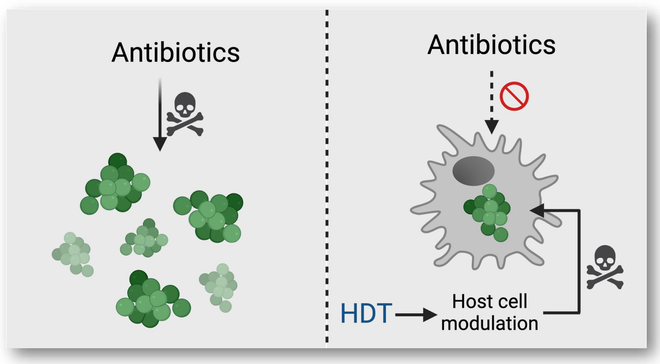
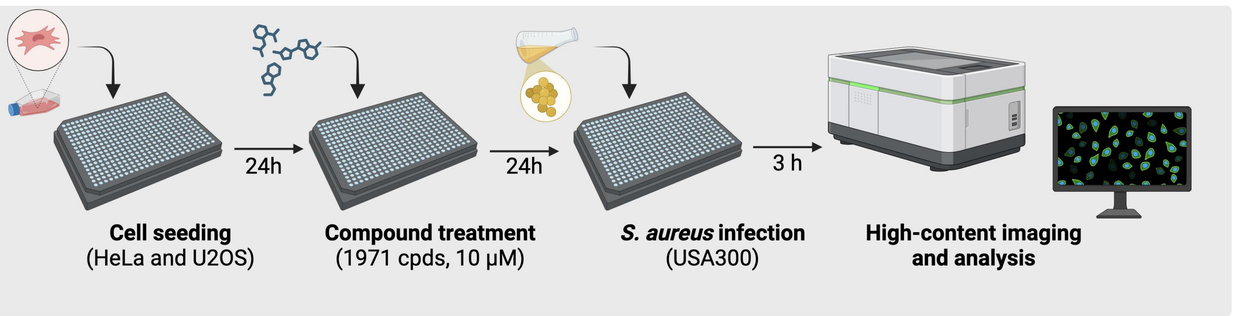
1. Background

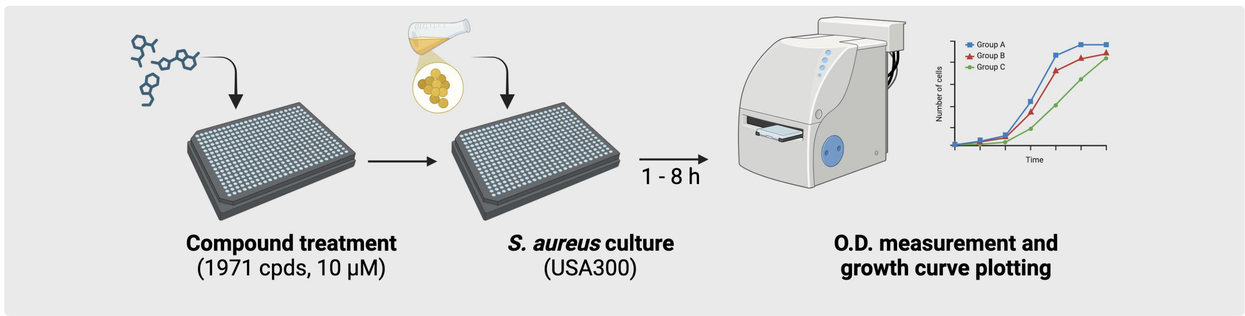
- *S. aureus* is a facultative intracellular pathogen that can **invade** and **replicate** in distinct host cells.
- The intracellular niche is an important reservoir for chronic and recurrent infections, in which bacteria are protected from host defence and antibiotic treatment.
- **Host-directed therapy (HDT)** has emerged as an **alternative** to overcome the limitations of classical treatments, since it is focused on interfering with **host cell mechanisms** required by bacteria for invasion, survival, and replication.



2. Methodology

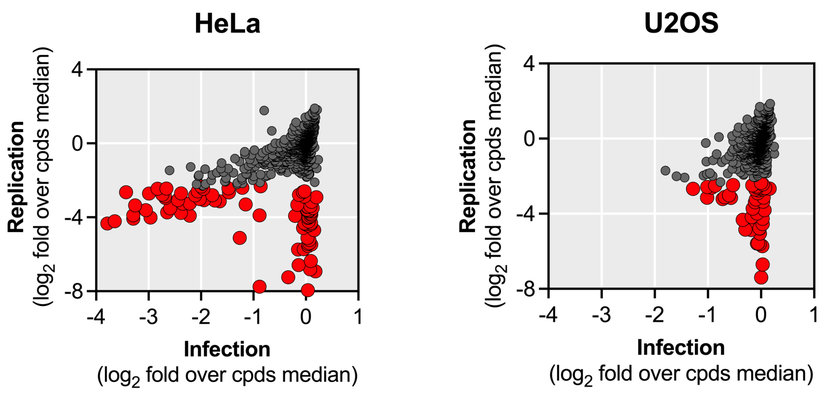
High-throughput screening of bioactive molecules against *S. aureus*





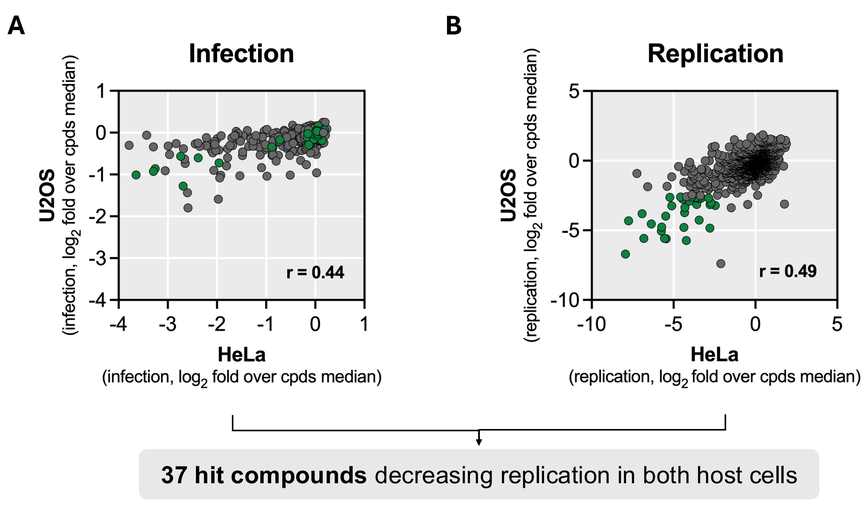
3. Results

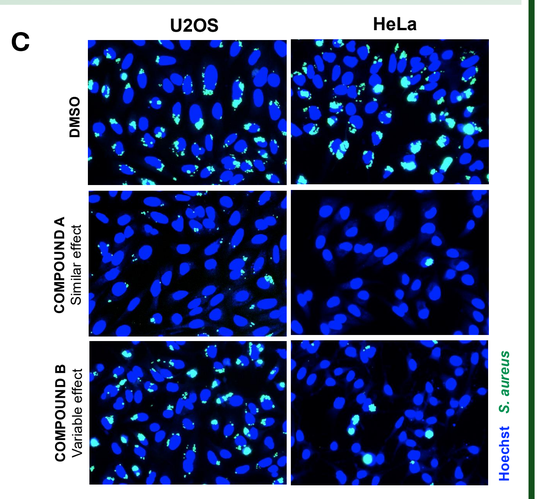
I. Bioactive compounds strongly reduced *S. aureus* infection and intracellular replication



Effect of compounds on *S. aureus* infection and/or intracellular replication in epithelial cells (HeLa – left) and osteoblasts (U2OS – right). Data represent results from two independent experiments and are expressed as log_2 fold over compounds median. Compounds highlighted in red decreased *S. aureus* infection or/and replication by at least 5-fold.

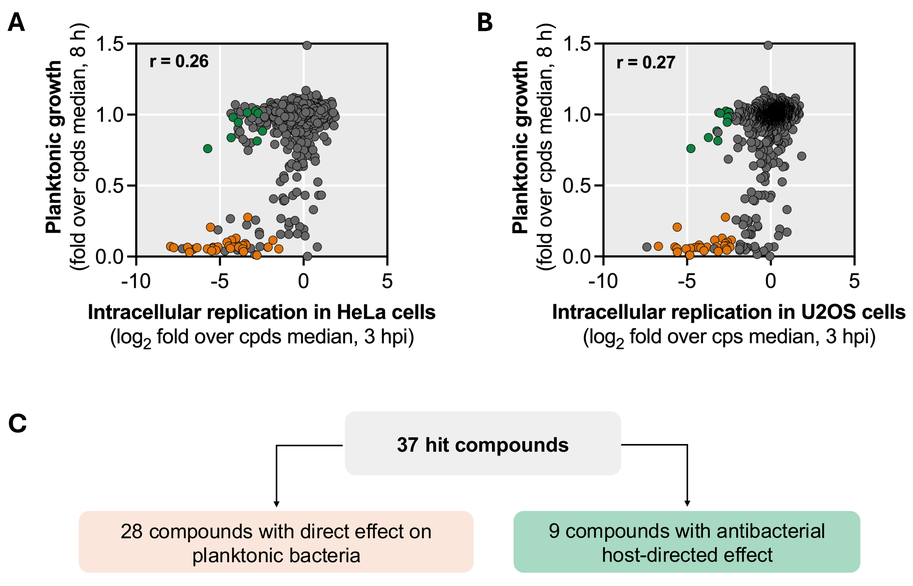
II. A small set of compounds presented similar effect in epithelial cells and osteoblasts





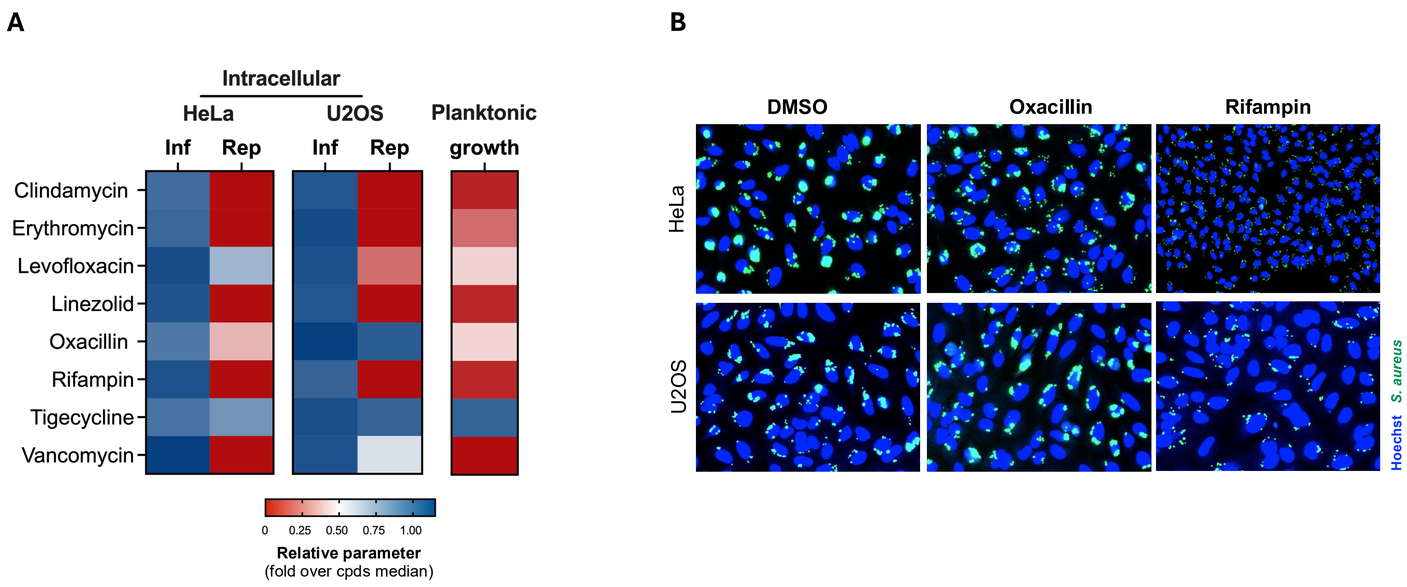
A and B. Comparison of the effect of compounds on *S. aureus* infection (A) and intracellular replication (B) in epithelial cells and osteoblasts. Spearman´s rank correlation coefficients are shown in the bottom right corner of each graph. The 37 hit compounds decreasing *S. aureus* intracellular replication in both host cells (by at least 5-fold) are highlighted in green color. **C.** Representative images of host cells treated with two selected compounds or control and infected with *S. aureus* at 3 hours post-infection.

III. Compounds decreased *S. aureus* intracellular replication without inhibiting planktonic growth



A and B. Comparison of the effect of compounds on bacteria planktonic growth and intracellular replication in HeLa (left) and U2OS cells (right). Spearman´s rank correlation coefficients are shown in the top left corner of each graph. Compounds highlighted in orange inhibited planktonic bacteria growth, whereas compounds in green were selected with a host-directed effect. **C.** Selection of host-directed antimicrobial compounds.

IV. Antibiotics did not promote *S. aureus* clearance and presented different effects on intracellular replication



A. Heatmaps showing the changes in *S. aureus* infection (Inf), replication (Rep), and planktonic growth upon treatment with eight classical antibiotics. **B.** Representative images of host cells treated with two antibiotics (Oxacillin and Rifampin) or control and infected with *S. aureus* at 3 hours post-infection.

4. Final remarks

- By applying a high-throughput screening approach, we identified **37 bioactive compounds** reducing the infection/intracellular replication of *S. aureus* in distinct host cells; of them, **nine presented a host-directed antibacterial effect**.
- Antibiotics classically used to treat infections caused by *S. aureus* were not able to promote bacterial clearance, despite their strong inhibitory effect on bacteria planktonic growth.
- HDT is a potential **alternative** to develop more efficacious treatments for staphylococcal infections.

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