Combining Bacteriophage and Vancomycin is More Efficacious in Treating MRSA Aggregates Formed in Human Synovial Fluid Compared to Using Vancomycin or Bacteriophage Alone

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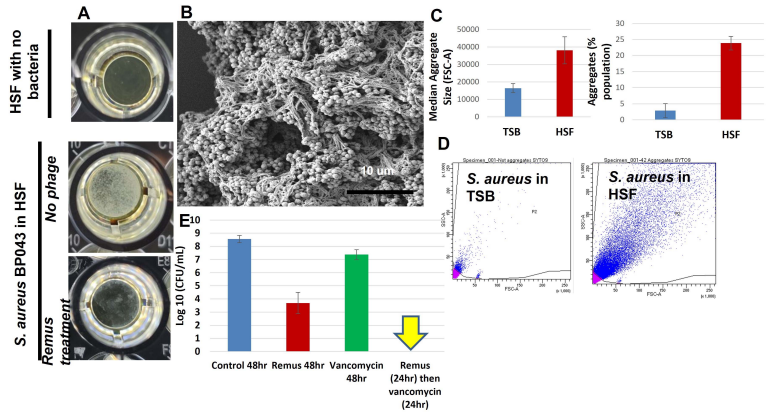
INTRODUCTION: Failure rate of standard treatment for periprosthetic joint infection (PJI) is estimated to be around 40% at two years post revision surgery. A major clinical challenge contributing to treatment failure and antibiotics tolerance is the biofilm formation. Lytic bacteriophages (phages) can target biofilm associated bacteria at localized sites of infection by penetrating and disrupting biofilm matrices. The aim of this study is to test if phage has better antimicrobial effect than vancomycin against *Staphylococcus aureus* biofilm aggregates in human synovial fluid.

METHODS: *S. aureus* BP043 was utilized in this study. This strain is a PJI clinical isolate, methicillin resistant (MRSA) and biofilm-former. Phage Remus, a lytic phage known to infect *S. aureus*, was used. *S. aureus* BP043 was grown in human synovial fluid in 96-well for 24hr. Then the already established *S. aureus* biofilm aggregates were treated with: a) phage Remus at ~ 10^9 PFU/mL for 48hr, b) vancomycin, 500 µg/mL (subminimal biofilm eradication concentration) for 48hr, or c) phage Remus, 24hr followed by vancomycin for another 24hr, at 37°C. Then, aggregates were vortexed vigorously and bacterial survival was assessed by plating on tryptic soy agar plates. Aggregates formation in synovial fluid was assessed using flow cytometer by measuring their size and comparing it to *S. aureus* clumping in Tryptic Soy broth (TSB). The aggregates were also examined by scan electron microscopy (SEM). Each experiment had two technical repetitions and at least two different human synovial fluids were used.

RESULTS: SEM images and flow cytometer data demonstrated the ability of human synovial fluid to significantly influence biofilm formation of *S. aureus* BP043 by promoting aggregation compared to TSB media. Phage Remus resulted in more than 56% reduction in viable *S. aureus* residing in the synovial fluid aggregates, compared to the control aggregates with no treatment (p=0.015). Additionally, Remus is more powerful in breaking BP043 aggregates and eliminating viable bacteria compared to vancomycin (p=0.02). Moreover, combining phage Remus followed by vancomycin is more efficacious in reducing bacterial load than using Remus or vancomycin alone (p=0.023, p<0.001, respectively).

DISCUSSION AND CONCLUSION: We demonstrated synergistic interaction between the phage (Remus) and vancomycin, leading to better clearance of synovial fluid aggregates of the *S. aureus* MRSA isolate. This work is aimed at gathering preclinical evidence for using phage as a new therapeutic avenue to treat PJI.



A: Aggregates formation and distraction in human synovial fluid (HSF) in the presence and absence of phage.
B: SEM image of *S. aureus* aggregates grown in human synovial fluid.
C,D: Flow cytometry shows bigger and more aggregates formation in synovial fluid (HSF) compared to tryptic soy broth (TSB) when looking at the percentage of SYTO9 green positive (P2 gate) bacteria and their median size.
E: Synergistic interaction between phage Remus and vancomycin treatments, leading to the highest biofilm/aggregates reductions observed.