DETECTION OF VIABLE BUT NON CULTURABLE BACTERIA IN SONICATE FLUID WITH BACTERIOPHAGES AND QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

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OBJECTIVES

The objective of our research was to develop an alternative to conventional microbiological diagnostic procedures, based on specific detection of viable but not culturable (VBNC) bacteria in sonicate fluid, with the use of bacteriophages and quantitative real-time polymerase chain reaction.

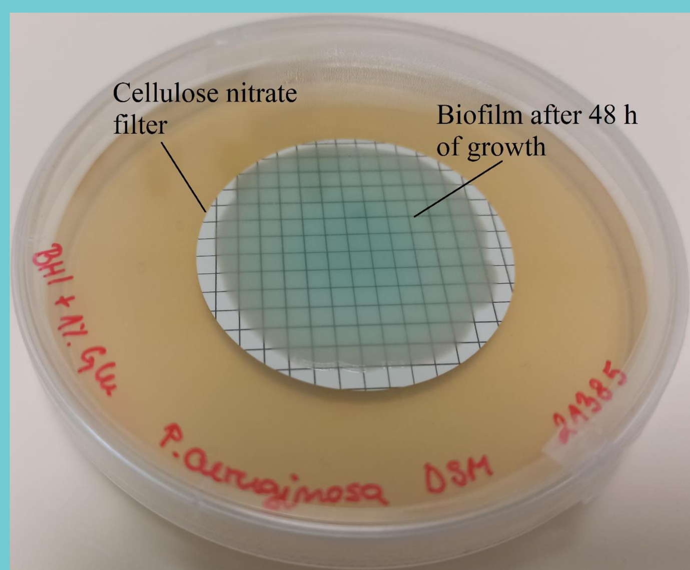

Figure 1: Colony biofilm of P. aeruginosa

INTRODUCTION

Culture negative PJI represent an important clinical issue as conventional culture methods remain negative in about 5 to 45% of otherwise confirmed PJI cases [1]. Bacteria, when exposed to rash environmental conditions, like antibiotics, adopt a survival strategy entering into the VBNC state. Such bacteria show greater resistance to stress, including resistance to antibiotics and form part of bacterial population in biofilms. VBNC bacteria pose a great risk to patients, retaining viability and virulence, thus remaining ready to resuscitate when the environment permits. Their detection is a challenge that urges for a new specific and sensitive method. Bacteriophages are viruses that specifically recognize and infect bacteria as part of their replication cycle. Their use was previously proven as advantageous in detection procedures offering fast and specific targeting and differentiation between live and dead bacteria.

METHODS

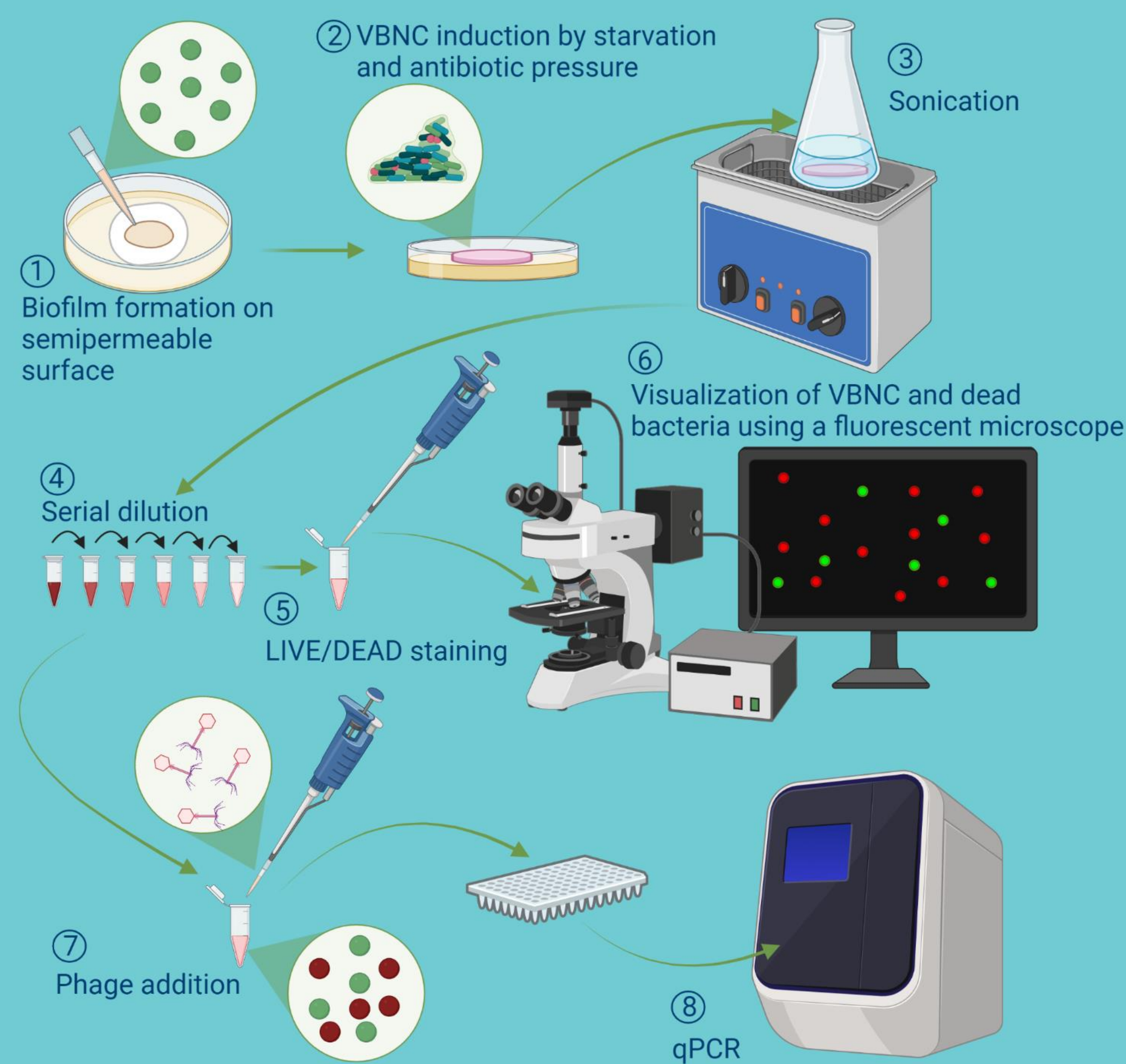

Figure 2: Overview of the experimental workflow*

The VBNC state of *S. aureus* and *P. aeruginosa* were induced *in vitro* by starvation and antibiotic pressure. After confirming the presence of VBNC bacteria, phage K and phage PB-1 were added to bacterial suspensions. Suspensions were assessed for the detection with the method of qPCR with primers specific for each phage DNA. The method monitored the decrease in the quantity of phage DNA as it became unavailable for the reaction if specific bacteria were present in the sample. After optimization, the method was assayed on simulated infected sonicate fluid.

RESULTS

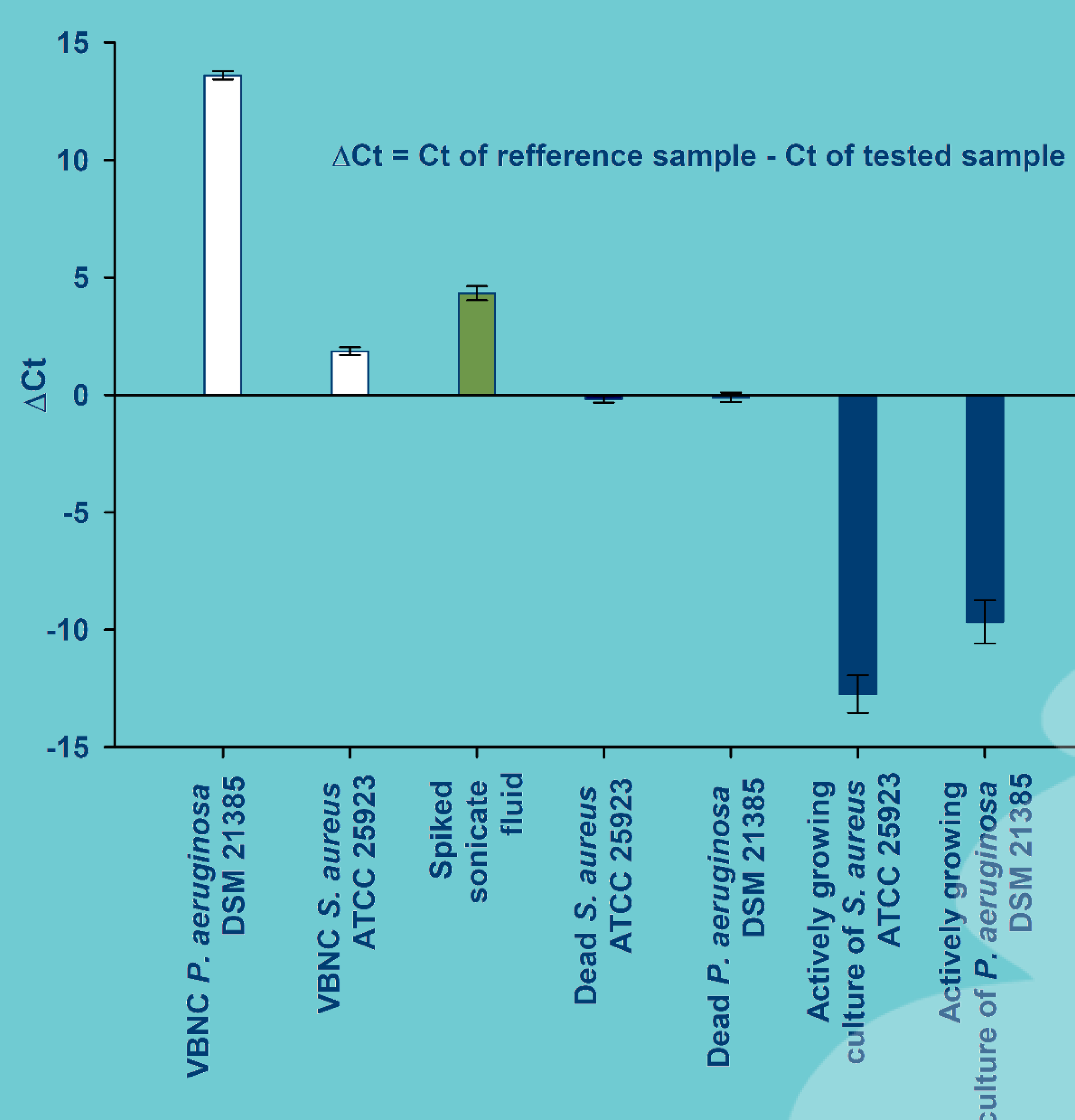

Figure 3: Detection of bacteria in different physiological state with the method of bacteriophage DNA detection with qPCR

The lack of culturability in *S. aureus* was observed after 3 days of starvation and exposure to gentamycin at a 16× MIC, after 5 days where gentamycin 8x MIC was used and after 7 days of exposure to gentamycin at a 4× MIC. The lack of culturability in *P. aeruginosa* was observed after 7 days of starvation and exposure to gentamycin at a 16× MIC, and after 10 days where gentamycin was used at 8x MIC and 22 days for 4× MIC. The presence of VBNC bacteria was confirmed, with 23.5% of bacteria being still alive but not able to grow on conventional culturing media.

CONCLUSIONS AND FUTURE RESEARCH

Detection of VBNC bacteria in sonicate fluid with specific phages and qPCR is rapid, sensitive and specific and allows the detection of only viable bacteria. The implementation of additional phages in the method would allow for the detection of a wider range of bacterial species.

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References

[1] Kalbian, I. *et al.* Culture-negative periprosthetic joint infection: prevalence, aetiology, evaluation, recommendations, and treatment. *Int. Orthop.* **44**, 1255–1261 (2020).

* Created with BioRender.com