#1169 - Posters

Detection Of Viable But Non Culturable Bacteria In Sonicate Fluid With Bacteriophages And Quantitative Real-Time Polymerase Chain Reaction

Orthopaedics / Musculoskeletal Infections / Epidemiology, Prevention & Diagnosis

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Background
Culture negative prosthetic joint infection (PJI) represent an important clinical issue as conventional culture methods remain negative in about 5 to 45 % of otherwise confirmed PJI cases. Bacteria, when exposed to rash environmental conditions, like exposure to antibiotics, adopt a survival strategy entering into the viable but not culturable (VBNC) state. VBNC bacteria represent a part of the bacterial population in biofilms on medical devices and pose a great risk to patients, retaining viability and virulence, thus remaining ready to resuscitate from this state when the environment permits. The detection of VBNC bacteria is a challenge that urges for a new specific and sensitive method to quickly detect non culturable bacteria. Bacteriophages are viruses that specifically recognize and infect. Their use was previously shown to be advantageous in detection procedures offering fast and specific targeting and differentiation between live and dead bacteria.

Objectives
The objective of our research was to develop an alternative to conventional microbiological diagnostic procedures, based on specific detection of VBNC bacteria in sonicate fluid, with the use of bacteriophages and quantitative real-time polymerase chain reaction (qPCR).

Study Design & Methods
The VBNC state of *S. aureus* ATCC 25923 and *P. aeruginosa* DSM 21385 were induced *in vitro* by starvation on low-nutrient medium and by antibiotic pressure. Bacteria were confirmed as non-culturable when no growth was observed after 7 days of incubation on nutrient media. Bacterial viability was confirmed with the LIVE/DEAD ® BacLight™ Bacterial Viability Kit. After confirming the presence of VBNC bacteria, phage K (ATCC 19685-B1) and phage PB-1 (DSM 21516) were added to bacterial suspensions and assessed with the qPCR method. The method monitored the decrease in the quantity of phage DNA. After the optimization of the method on bacterial culture suspensions, the method was assayed on simulated infected sonicate fluid.

Results
The lack of culturability in *S. aureus* and *P. aeruginosa* was observed after 10 days of starvation and exposure to gentamycin at an 8× minimal inhibitory concentration. The presence of VBNC bacteria was confirmed, with 23.5 % of bacteria being still alive but not able to grow on conventional culturing media. Heat-killed bacteria were confirmed dead by the same method. During the qPCR assay, a delay in phage DNA multiplication was observed in samples where phages were added to VBNC bacteria compared to samples, where phages were added to heat-killed bacteria or sterile samples and confirm that the detection of VBNC bacteria with the method was successful based on the adsorption of added phages on present VBNC bacteria. Results also show, that the method does not detect dead bacteria. Simulated clinical test results on spiked sonicate fluid confirmed the optimized protocol to be successful for the detection of VBNC bacteria in clinal samples.

Conclusions

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.