#1049 - Posters

Diagnostics Of Staphylococcus Spp. Prosthetic Joint Infections With Bacteriophage K

Orthopaedics / Knee & Lower Leg / Epidemiology, Prevention & Diagnosis

Katja Šuster, Ingrid Milošev, Andrej Cör

Valdoltra Orthopaedic Hospital, Ankaran, Slovenia

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Background
Infection is the most serious cause of prosthetic joint implants loosening with Staphylococcus spp. being the most frequently detected pathogens. Currently, the gold standard for the microbiological diagnosis remains the culturing of preoperative aspirated joint fluid and intraoperative periprosthetic tissue samples. The main drawbacks are the sensitivity of this procedure, most likely due to the formation of biofilm on the implant surface, and the time needed to obtain results. At this point there is still a need for a rapid, specific and sensitive diagnostic method for a fast and accurate diagnosis of prosthetic join infection and the establishment of the right treatment. Lytic bacteriophages are viruses that specifically infect and lyse bacteria within their replication cycle. Their use in detection procedures offers specific targeting, differentiation between live and dead bacteria, avoiding time consuming or laborious steps.
Objectives
The objectives of our research were to develop alternatives to conventional microbiological diagnostic procedures, based on specific detection of live Staphylococcus spp. bacteria in sonicate fluid of infected prosthetic joints, with the use of bacteriophage K.
Study Design & Methods
Bacteriophage K ability to infect and lyse bacteria that can be the cause of prosthetic joint infections was first evaluated. Subsequently, two indirect methods for detecting staphylococci were designed, optimized on bacterial broth cultures and compared. The first method is based on bioluminescence detection after intracellular adenosine-5'-triphosphate release by bacteriophage K mediated lysis, while the second model utilizes quantitative real-time polymerase chain reaction with primers specific for bacteriophage K DNA to monitor its amplification in the presence of staphylococci. To determine suitability of developing models for detecting staphylococci in sonicate fluid of explanted prosthetic joints, simulated clinical tests were done with a sonicate fluid. After additional optimization, 20 sonicate fluid samples with known microbiological status were obtained from patient with implant loosening undergoing surgery and assessed.
Results
Bacteriophage K showed specificity for all tested staphylococci and no specificity for other tested bacteria that can potentially cause prosthetic joint infection. The template DNA extraction step for quantitative real-time polymerase chain reaction was proven unnecessary and thus omitted. Limits of detection for both methods were in the bacterial concentration range of 1000 CFU/mL with the entire detection test taking approximately 4h. The model including quantitative real-time polymerase chain reaction was found to be less labor

intensive and thus simpler compared to the bioluminescence method. Simulated clinical tests results confirmed sonicate fluid as a good sample for both developed models, with no inhibitory effects. In addition, a good correlation between results obtained with bacteriophage K based methods and conventional microbiology results in clinical samples was found.

Conclusions

The developed models for detection of staphylococci within sonicate fluid of infected prosthetic joints using a specific bacteriophage are rapid, sensitive and specific and allow the detection of only live staphylococci. In addition, the implementation of other bacteriophages specific for other bacterial species is possible, improving the current models for the detection of a wider range of pathogens.