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O0708 Atypical pathobiome of prosthetic joint infections revealed by hybrid approaches of shotgun metagenomics

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Background: Infection is among the main reasons of prosthetic joint failures along with aseptic failure and mechanical problems. However, around 15-20% of prosthetic joint infections (PJI) are reported to be culture-negative since culture identification is challenging due to antecedent antibiotic administration, unculturable nature or symbiotic growth requirements of the organisms. In this work, metagenomic samples from related cases are studied to gain better insights on the conventionally uncharacterizable pathobiomes causing PJIs.

Materials/methods: Following institutional review board approval two patients diagnosed with PJJ (according to Musculoskeletal Infection Society (MSIS) undergoing revision arthroplasty in January 2018 were prospectively enrolled in this study. Deep-tissue specimens and prosthesis sonication samples were obtained from patients at the time of the surgical procedure. All samples were cultured in both aerobic and anaerobic conditions for prolonged period (10 days). The MolYsis Basic kit, Qiagen Microbiome kit and Qiagen REPLI-g Single Cell Kit were used for microbial DNA isolation and enrichment procedures. Hybrid shotgun metagenomic sequencing was performed using Illumina NextSeq500 and MinION instruments. In order to filter resulting human DNA sequences, all sequencing reads are aligned against hg38 human reference genome by Burrows-Wheeler Aligner. Host filtered reads were assembled to contigs and taxonomic assignments were done using Kaiju metagenomic classifier and Blast. Assignments with low relative abundance filtered out assuming to be contamination or false taxonomic assignments.

Results: All samples were culture negative. According to WGS, in the 1st patient's tissue sample *Rothia spp*., *Streptoccocus spp*, *Cutibacterium acnes*, *Cutibacterium acnes*bacteriophage and unidentified fungus (87% nucleotide identity with Malassezia genus) were detected. In the prosthesis sample of this patient, *Cutibacterium acnes*, *Cutibacterium acnes* bacteriophage were detected. Both the tissue and prosthesis samples of the second patient had *Cutibacterium acnes*, *Cutibacterium acnes* bacteriophage.

Conclusions: Metagenomic shotgun sequencing identified abundant pathogens in culture-negative PJI cases. In the first case we have identified a mix infection that almost equally abundant bacteria and fungi species were existing. Moreover, the coexistence of *C. acnes* and its bacteriophage raises further research questions on non-culturable nature.