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Detection of bacterial DNA in septic arthritis samples using the MYcrobiota platform

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ABSTRACT

Background/Objective

Bacterial septic arthritis is considered a medical emergency that may lead to disability or death. While the majority of these infections are described to be caused by gram-positive bacteria, clinicians should be aware of less common bacterial causes of septic arthritis that are not detected by routine bacterial culture strategies. Therefore, we investigated 23 joint fluid samples that were obtained from 19 patients with suspected bacterial septic arthritis using a culture-free 16S rRNA gene next-generation sequencing (NGS) platform (MYcrobiota) and compared the results to routine diagnostic testing.

Methods

In this cross-sectional descriptive study, all samples were collected over a period of three months in 2017 and processed retrospectively using our previously validated and published MYcrobiota platform without prior knowledge of their culture, PCR or traditional sequencing results.

Results

All joint fluid samples tested were found to be culture-negative and MYcrobiota confirmed the absence of bacterial operational taxonomic units (OTUs) in 13/23 samples. However, MYcrobiota detected the presence of either an *Enterococcus*, *Kingella*, *Parvimonas*, *Prevotella*, *Ruminococcus*, *Turicella*, or *Ureaplasma* OTU in the other 10 samples. Four out of seven OTUs detected by MYcrobiota confirmed the additional diagnostic test results (i.e. blood culture results and molecular test results) that has led to an effective targeted antibiotic treatment for four patients.

Conclusions

The accurate detection of bacterial OTUs using MYcrobiota greatly improves the identification of the aetiology of bacterial septic arthritis compared to routine diagnostic testing.

Bacterial septic arthritis is an inflammation in native (non-prosthetic) joints with an incidence that ranges between 4-29 cases per 100,000 people per year, depending on population variables and pre-existing structural joint abnormalities.[1] Most infections are introduced into the joints as a result of bacteraemia, though the joints may also become infected directly via trauma or an infection around the joint.[2] In routine procedures, the diagnosis of bacterial septic arthritis is confirmed by detection of bacteria in joint fluid samples using culture-based techniques. However, a substantial proportion of joint fluid samples are culture-negative, even from patients with typical signs of septic arthritis, suggesting a role of 'difficult-to-culture' microorganisms (e.g. anaerobic or fastidious bacteria) in such clinical presentations.[1,3] The failure of bacterial culture will delay an effective antimicrobial treatment of the septic arthritis patients who may suffer the destruction of the joint tissue.[4] With this in mind, we investigated to what extent a culture-independent 16S rRNA gene next-generation sequencing (NGS) platform (MYcrobiota) could add to the microbiological diagnosis obtained by culturing of joint fluid samples. For this, we tested 23 joint fluids that were obtained from 19 patients with suspected bacterial septic arthritis using MYcrobiota and compared the results to routine bacterial cultures.

MYcrobiota is a consolidated tool that includes a validated, quantitative micelle PCR/NGS methodology and a dedicated bioinformatics pipeline that was specifically designed for use in clinical diagnostic laboratories.[5] The MYcrobiota platform enables the detection, quantification and characterization of bacterial DNA derived from live, fastidious, and dead bacterial cells present within clinical samples, and takes account of possible bacterial DNA contamination derived from laboratory reagents and/or the laboratory environment. This is achieved by quantifying contaminating 16S rRNA gene copies within negative extraction controls and subtracting these 16S rRNA gene copies from the quantitative microbiota profiles obtained from actual samples.[6] Therefore, MYcrobiota possess a much lower limit of detection compared to (semi-quantitative) conventional 16S rRNA gene NGS methods, which allows the detection of bacterial operational taxonomic units (OTUs) at very low abundances, or alternatively, can reliably confirm the absence of bacterial DNA in culture-negative joint fluids.

The joint fluid samples were collected from the Regional Laboratory of Public Health Kennemerland and processed retrospectively using MYcrobiota, with no prior knowledge of specimen culture results. An acknowledged national ethics committee from the Netherlands (Medisch Ethische Toetsingscommisie Noord-Holland, http://www.metc.nl) approved the study protocol (M015-021) and all experiments were performed in accordance with relevant national guidelines and regulations. The national ethics committee waived the need for participant consent as all data were anonymized and analysed retrospectively under code. The joint fluid samples were cultured directly on a blood-based agar (under aerobic and anaerobic conditions) and chocolate-based agar (under 5% CO_2

conditions), and after thioglycolate enrichment. The left-over specimens were stored at –80°C for subsequent MYcrobiota analysis that was performed as previously described.[5]

Bacterial culture of all joint fluid samples included in the study generated culture-negative results. In contrast, MYcrobiota revealed the presence of bacterial OTUs in 10/23 (43%) joint fluids and confirmed the culture-negative results in the remaining 13 samples. As shown in Table 1, the bacterial OTUs detected using MYcrobiota were classified as *Enterococcus*, *Kingella*, *Parvimonas*, *Prevotella*, *Ruminococcus*, *Turicella*, and *Ureaplasma* and confirmed the clinician's suspicion of bacterial septic arthritis in seven patients that were found to be culture negative. From two patients, we received and processed multiple joint fluid samples that resulted in identical results from each sample. Specifically, four distinct joint fluid samples taken from one patient all tested positive for a *Parvimonas* OTU and two joint fluid samples obtained from a different patient confirmed the absence of bacterial DNA in both of these culture-negative joint fluids.

Table 1. Seven different bacterial OTUs were detected from 23 joint fluid samples using MYcrobiota and confirmed the suspicion of bacterial septic arthritis in 7 patients (including serial samples from individual patients). All joint fluids tested negative using routine bacterial culture techniques.

Bacterial OTUs	Patients (#)	Samples (#)	16S rRNA gene copies/µL	Clinical decision
Enterococcus	1	1	6,600	NA
Kingella	1	1	25	On the basis of additional molecular testing, treatment was started with amoxicillin/clavulanic acid, directed against *Kingella*.
Parvimonas (4/4)	1	4	1,500 – 51,000	NA
Prevotella	1	1	71,000	On the basis of additional blood culture results, treatment was switched from flucloxacillin/gentamycin to intravenous amoxicillin/clavulanic acid and later oral clindamycin, directed against *Prevotella*
Ruminococcus	1	1	192,000	On the basis of additional molecular testing, treatment was switched from vancomycin/rifampin to intravenous penicillin, directed against *Ruminococcus*
Turicella	1	1	50	NA
Ureaplasma	1	1	1,200	On the basis of additional molecular testing, clindamycin was switched to doxycycline, directed against *Ureaplasma*
Negative	12	13	0	Empirical antibiotic treatment was stopped in one case. In the other cases, MYcrobiota data were not available during treatment or no empirical antibiotics were given.

NA: MYcrobiota data were produced in retrospective analysis and were not available during the clinical treatment period.

In all bacterial OTU positive cases, only a single bacterial genus was identified per sample with bacterial loads that ranged from 25 to 192,000 16S rRNA gene copies per microliter of DNA extract used. Importantly, although 16S rRNA gene copies can provide some relevant information on bacterial load (tens of copies versus thousands of copies for example), it should be noted that it is not possible to accurately translate 16S rRNA gene copies into an actual number of bacterial genomes or cells present within a sample. This is because different bacterial species may carry different copy numbers of 16S rRNA genes in their genomes and copy numbers for all bacterial species are not known.

All bacterial genera detected using MYcrobiota, except *Turicella*, have been previously described as unconventional pathogens in bacterial septic arthritis.[7-12] Although *Turicella otitidis* (the only *Turicella* species described) is usually isolated from ear exudates, this bacterium has been previously identified as a cause for bacteraemia in at least two independent cases.[13] Further, the main discrepancies between routine bacterial culture and MYcrobiota results could be explained by a difficulty in culturing anaerobic bacteria e.g. *Parvimonas* spp., *Prevotella* spp., and *Ruminococcus* spp., as well as fastidious bacteria e.g. *Kingella* spp. and *Ureaplasma* spp. Interestingly, some culture-negative joint fluid samples were found to contain DNA from 'easily-culturable' bacteria, including *Enterococcus* spp. and *Turicella* spp.

Although all routine bacterial cultures from all joint fluid samples included in this study generated culture-negative results, additional diagnostic testing – including blood cultures, specific PCRs, and 16S rRNA gene PCR/Sanger sequencing – was performed to confirm the initial culture results. However, these additional diagnostic tests revealed the presence of four different bacterial pathogens (*Kingella*, *Prevotella*, *Ruminococcus* and *Ureaplasma*) in four joint fluid samples, which led to the subscription of an effective targeted antibiotics treatment for four patients with bacterial septic arthritis (Table 1). These patients received antibiotic treatment for six weeks in a routine clinical setting, including routine clinical follow up of treatment outcome that showed clinical and laboratory evidence of successful treatment. The additional diagnostic test results were later confirmed by our retrospective MYcrobiota analysis in all four cases. However, MYcrobiota detected an additional three OTUs (*Enterococcus*, *Parvimonas* and *Turicella*) in three joint fluids that were not identified using additional diagnostic testing. Unfortunately, we were unable to find an explanation for these discrepancies. Nonetheless, these data illustrate that MYcrobiota is an accurate diagnostic platform and its results can be used to start, or switch to, targeted therapeutic strategies for patients presenting with suspected bacterial septic arthritis. Importantly, MYcrobiota provides this clinical relevant information without the dependence on 'traditional' molecular tests that require *a priori* knowledge of the likely pathogen present within the sample (i.e. specific PCRs), or those that can only process monomicrobial samples with a bacterial load that

exceeds the inevitable background DNA contamination derived from the experimental set-up (i.e. 16S rRNA gene Sanger sequencing methods).

Joint infection is generally secondary to haematogenous dissemination of bacteria from other sites within the human body. Interestingly, for two patients, we were able to causally link the bacterial OTU detected by MYcrobiota to recent invasive surgery and dental procedures. The first patient underwent gastrointestinal surgery after which DNA derived from the *Ruminococcus* bacterium – that normally resides within the gastrointestinal tract – was detected within his joint, and in a second patient we identified DNA derived from the odontogenic *Parvimonas* bacterium following a tooth extraction. Taken together, these data further support the need to consider unconventional bacteria as causes of bacterial septic arthritis, especially in those patients who recently underwent surgical procedures of the digestive tract or in the mouth.

In conclusion, obtaining accurate quantitative microbiota profiles using MYcrobiota enables the identification of bacteria present within joint fluid samples that were not identified using routine bacterial culture strategies. Therefore, continued suspicion of bacterial septic arthritis despite culture-negative results should lead clinicians to consider the use of culture-free 16S rRNA gene NGS techniques, such as the MYcrobiota platform.

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