Prosthetic joint infection due to *Lysobacter thermophilus* diagnosed by 16S rRNA gene sequencing

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Abstract

We report the first case of prosthetic joint infection caused by *Lysobacter thermophilus* which was identified by 16S rRNA gene sequencing. Removal of prosthesis followed by antibiotic treatment resulted in good clinical outcome. This case illustrates the use of molecular diagnostics to detect uncommon organisms in suspected prosthetic infections.

Key words: *Lysobacter thermophilus*, prosthetic joint infection, total knee replacement

Introduction

Prosthetic joint infection (PJI) is one of the most serious complications following joint arthroplasty. PJI occurs in 2.18% of patients after primary total hip or knee replacements.[1] As primary arthroplasties are more frequent, it is accompanied by a higher total number of revision surgeries. The most common reason for revision surgeries includes aseptic loosening and infection.

Accurate and prompt diagnosis of PJI is very difficult with traditional microbiological techniques. The major reasons for this are previous antibiotic use, biofilm formed by the strongly adherent bacteria and presence of highly fastidious and non-cultivable, or viable but non-cultivable bacteria. These lacunae of culture method can be overcome by the use of molecular techniques to detect the bacterial DNA from the infected area and prosthesis. Several studies using molecular techniques had already published a greater variety of bacteria that are associated with failed prosthetic joints.[2,3] Using 16S rRNA gene sequencing genus, *Lysobacter* was found to be the predominant genus identified from failed prosthetic hip joints.[3] Thus, by using traditional culture method alone may lead to underestimate the infection rate in total joint arthroplasty.

We report a case of PJI by *Lysobacter thermophilus* from a 74-year-old female patient with a history of right total knee replacement (TKR).

Case Report

A 74-year-old female who had undergone total knee arthroplasty in both her knees 16-years back presented with a 7-month history of pain in the right knee and difficulty for walking. On physical examination, she was found to have a swelling in the right knee with a painful range of motion.

Laboratory data included a blood leucocyte count of 8200/mm³, with 73% polymorphonuclear leucocytes, erythrocyte sedimentation rate (ESR) of 38 mm/h and a C-reactive protein (CRP) level of 3.92 mg/l. Radiographs of the right knee revealed signs of loosening of the prosthesis [Figure 1]. The patient was subsequently posted for implant removal and revision TKR in the same sitting. Preoperatively, joint fluid was aspirated, and Gram-stain demonstrated many white blood cells and no organisms. During surgery, aspirated knee fluid and three periprosthetic tissue samples were obtained which were sent for culture and histopathology.

In the microbiology laboratory, tissue specimens were homogenised under sterile conditions. The homogenised tissue and joint fluid were inoculated in the following culture media: Sheep blood agar (BA), chocolate agar (CHA), MacConkey agar (McC), Robertson’s cooked meat media (RCM) and brain heart infusion (BHI) agar. BA and CHA were incubated at

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37°C in a 5% CO₂ atmosphere. McC, RCM and BHI agar plates were incubated aerobically and anaerobically at 37°C, respectively. All media were incubated for 14 days. Cultures from all the four samples remained sterile after 14 days of incubation. Histopathology report showed fibrocollagenous tissue with infiltration by sheets of histiocytes, foci of dystrophic calcification, giant cells and necrosis.

For molecular diagnosis, the DNA was extracted from the specimens using QIAamp Mini Kit (QIAGEN, Hilden, Germany) and 16S rRNA PCR was performed. The PCR, which was specific for Gram-positive and Gram-negative bacteria showed a band size of 410 bp corresponding to Gram-negative bacteria in all four specimens. The amplicons obtained were subsequently sequenced for identification by using “ABI PRISM® BigDye Terminator Cycle Sequencing Ready Reaction Kit (version 3.1).”

The sequences obtained were compared with those stored in GenBank databases using BLAST software (http://www.ncbi.nlm.nih.gov/blast). Identification to the species level was defined as 98% sequence similarity with the sequence having a high score. This search identified the bacterium as L. thermophilus, with a 98% similarity to the sequences of L. thermophilus JQ746036 (GenBank accession number). A phylogenetic tree was constructed by using MEGA version 6. Bootstrap analysis (1000 resamplings) was used to evaluate the branching order of the neighbour-joining dendrograms [Figure 2]. Following surgery, the patient was treated empirically with rifampin and levofloxacin. After 6 weeks of therapy, CRP and ESR presented normal values and patient was doing well.

The genus Lysobacter within the family Xanthomonadaceae was proposed by Christensen and Cook in 1978.[4] So far, the genus comprises 24 species with validly published names.[5] Species of the genus Lysobacter were mostly isolated from soil and water. Until the past decade, the databases were deficient in the information on Lysobacter spp. which led to the misidentification of them as Stenotrophomonas or Xanthomonas.

L. thermophilus, first isolated from a geothermal soil sample, South-west China in 2012[6] is a thermotolerant Gram-negative and aerobic bacterium. Growth occurs from 37°C to 55°C with an optimum temperature of 50°C. Colonies are convex, circular, smooth, non-transparent and yellow. Catalase and oxidase are positive. In a more recent taxonomic study, it has been proposed to reclassify L. thermophilus as Vulcaniibacterium thermophilum comb. nov. based on polyphasic data.[5]

Till date, there are only three reports of human infections caused by Lysobacter spp. Lysobacter enzymogens was shown to be a dominant component of the microflora of the biofilms formed on the hip prosthesis,[7] demonstrating its role in PJI. Lysobacter type spp. was predominant in the microbial community on the dorsal surface of the tongue.[8] L. enzymogens was recovered from bronchoalveolar lavage fluid of a patient with cystic fibrosis.[9] In all these reports, Lysobacter was identified by 16S rRNA gene sequencing. The reasons for failure to culture Lysobacter from sites in which they have been shown to predominate by molecular techniques may be due to the inhibitory effect of the conventional culture media used in the microbiology laboratories.

To the best of our knowledge, this is the first case
of human infection caused by *L. thermophilus*. Several lines of evidence suggest that *L. thermophilus* isolated from this patient was pathogenic and was responsible for PJI: (i) 16S rDNA PCR was positive for Gram-negative bacteria in all four specimens from a sterile site. (ii) definite evidence of infection was present (iii) there was an absence of other pathogens and (iv) the infection responded to treatment.

This case illustrates the ability of a new species of *Lysobacter* to cause a PJI in an immunocompetent patient. Further research is required to understand the pathogenicity of *Lysobacter* spp., the virulence factors involved in infection and the effect on the human immune system. However, *Lysobacter* type species, including *Stenotrophomonas maltophilia*, *Xylella fastidiosa* and *Xanthomonas axonopodis*, have been recently demonstrated to have the ability to form biofilms readily on various substrates.[9][11] This could explain the ability of *L. thermophilus* to cause an arthroplasty infection in our patient. Considering the increasing use of prosthetic devices and the use of molecular diagnostics one can expect the incidence of *L. thermophilus* associated infections to increase as well.

We could not determine the source of *L. thermophilus* in this case; however, a skin wound could be the gateway for asymptomatic bacteraemia with secondary localisation at the prosthesis.

Since, the antibiotic susceptibility profiles of the bacterial species identified by culture-independent techniques cannot be determined; the optimal antimicrobial treatment of *Lysobacter* infections has not been established. Further accumulation of reports on experience with *L. thermophilus* is needed to help formulate a recommended therapeutic strategy.

**Conclusion**

We report a case of PJI caused by *L. thermophilus*. Removal of prosthesis followed by antibiotic treatment resulted in a good clinical outcome. 16S rRNA gene sequencing may help in the diagnosis of infections caused by this unusual microorganism.

Nucleotide sequence accession number: The partial gene sequence of the isolate has been deposited in the GenBank sequence database under the accession number KP056321.

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**Conflicts of interest**

There are no conflicts of interest.

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