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Case Report

Falsarthrobacter nasiphocae periprosthetic joint infection

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ABSTRACT

We report the isolation of a rare Gram-positive coccobacillary bacterium from synovial fluids of a patient with periprosthetic joint infection on three occasions over an 8-month period. As routine microbiological methods were not able to identify the isolate definitely, sequence analyses of the bacterial 16S ribosomal RNA gene and whole genome were performed. Analysis of the bacterial 16S ribosomal RNA gene showed the highest similarity (98.1%) with that of *Falsarthrobacter* (previously known as *Arthrobacter*) *nasiphocae*, which was first isolated from the nasal cavities of common seals (*Phoca vitulina*). The genome size of the strain (designated as UM1) is 2.4 Mb. With a high G+C content (70.4 mol%), strain UM1 is phylogenetically most closely related to *F. nasiphocae* based on whole genome analysis. Strain UM1 was susceptible to vancomycin, linezolid, trimethoprim-sulfamethoxazole, doxycycline, and intermediate to penicillin and ciprofloxacin. Ceftriaxone resistance was noted. The patient who was also on hemodialysis for his end stage kidney disease died approximately 3 weeks following implant removal and fusion with an external fixator. This study describes the first isolation of *F. nasiphocae* from human clinical samples. The use of emerging technologies has supported more definitive etiological diagnosis associated with rarely encountered organisms in periprosthetic joint infection.

Introduction

Periprosthetic joint infection (PJI) is a serious complication of joint replacement surgery and the most common revision cause of knee arthroplasty [1–5]. It poses a huge socioeconomic burden to patients and healthcare providers as repeated surgical procedures and extended antimicrobial therapy are often required for management of the infection [1,2]. Despite the increasing incidence of PJI worldwide, the microbiological diagnosis and treatment of PJI remain challenging. Accurate identification of the causative agent of PJI is essential to guide clinicians in choosing the most appropriate management strategy for PJI. Although staphylococci have been recognized as the causative agents in most cases of PJI, infections caused by atypical or unusual pathogens are increasingly being documented in recent years [3–5]. This study describes the first isolation of a rare bacterium from synovial fluid samples of a patient with right leg prosthesis. The phenotypic feature, antibiotic susceptibility and whole genome sequence of the bacterium are presented in this study.

Case presentation

A patient in his late sixties, who has a documented history of end stage renal failure and undergoing renal replacement therapy for more than 10 years, presented to our medical center with severe arthritis of both knees. He underwent sequential total knee replacements in 2018, which were spaced 4 months apart (Supplementary Figure 1). Standard antibiotic prophylaxis with intravenous cefazolin was administered preoperatively. During the index surgery of the right knee, he was admitted to intensive care unit for hemodialysis as he developed hyperkalemia intraoperatively. Eleven months post-surgery, a secondary resurfacing procedure for the right patella was performed as the patient experienced anterior knee pain with poor patella tracking. There were no signs of implant loosening at that point. Prophylactic cefazolin was again given preoperatively. A synovial fluid specimen

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was taken as part of routine procedure and sent for culture in BD BACTEC Plus blood culture bottles. A gram-positive coccobacillary bacterium was cultured from the aerobic bottle only. The isolate was initially assumed to be a contaminant as there was only one positive sample and the organism was atypical. However, the knee pain persisted, and radiographs obtained about 3 months later revealed signs of tibial component loosening. Hence, an arthrocentesis was performed, which isolated the same organism again from the synovial fluid cultured in a BacT/ALERT FA Plus (aerobic) bottle.

The isolate grew as smooth, convex, white to greyish colonies of approximately 1-2 mm in diameter after 24 hours of aerobic incubation on blood agar at 37°C (Supplementary Figure 2). There was no growth on MacConkey agar. Upon staining, Gram-positive coccobacillary/diphtheroid forms were observed. The isolate was nonmotile, catalase-positive, indole and bile esculin-negative. It was identified as *Brevibacterium* and *Arthrobacter* sp. with low discrimination (66.8% and 27.9%, respectively) by the API Coryne system, and *Propionibacterium acnes* by Vitek ANC card (accuracy of 93%). No fermentation of D-glucose, D-ribose, D-xylose, D-mannitol, D-maltose, D-lactose, D-saccharose (sucrose), and glycogen was noted. Strain UM1 exhibited pyrazinamidase, pyroglutamic acid arylamidase, alkaline phosphatase, and gelatin hydrolase reactions but negative for nitrate reductase, β-glucuronidase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase glucosidase (esculin), and urease. The colonial morphology, fermentation, and enzymatic profiles of strain UM1 are highly similar to those reported for *F. nasiphocae* type strain [6,7]. MALDI-TOF MS was unable to identify the isolate due to absence of matching spectral profile.

The identity of strain UM1 was confirmed using 16S ribosomal RNA (rRNA) gene sequencing approach for isolates obtained from each episode of infection [8]. Sequence homology searching was performed using Basic Local Alignment Search Tool program (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequence (1372 bp, Genbank accession no: MT928722) shows the highest sequence similarities with *F. nasiphocae* (98.1%) followed by various *Arthrobacter* species, including *A. methylotrophus, A. aurescens, A. crystallopoietes, A. pascens, A. woluwensis, A nitroguajacolicus, A. oryzae* and *A. luteolus* (ranging from 94.5-95.0%). A phylogenetic tree constructed based on the 16S rRNA gene sequences using neighbor-joining method (Tamura-Nei model) of the MEGA software [9] confirms the placement of strain UM1 at the same branch as *F. nasiphocae* (99% bootstrap value) (Figure 1a). These findings supports the identification of strain UM1 as a genetically closely related species of *F. nasiphocae*, in accordance with the taxonomic threshold (97%) proposed by Stackebrandt & Goebel for delineation of a bacterial strain within a species [10].

Whole genome sequencing of strain UM1 was performed on the Illumina HiSeq PE150 platform (Novogene Bioinformatics Technology Co., Ltd., Beijing, China). The raw reads were pre-processed using Galaxy web platform (https://usegalaxy.org.au/) [11] and assembled using Spades version 3.12.0 [12]. The size of the draft genome (2,389,951bp, N50 = 304,736 bp, PRJNA658776) and high G+C content of 70.4 mol% match closely to that of *F. nasiphocae* DSM 13988 (PRJNA708443). Genome comparison using the Type Strain Genome Server (TYGS, https://tygs.dsmz.de/ [13] demonstrates the taxonomic placement of strain UM1 with *F. nasiphocae* DSM13988 with a high (84%) bootstrap value (Figure 1b). The low G+C content difference (0.93%) between the two bacterial genomes also supports its delineation within the same species (Supplementary Table 1).

There is no prior information on the antibiotic susceptibility profile of *F. nasiphocae*. Antimicrobial susceptibility testing was performed for penicillin, vancomycin, ciprofloxacin, trimethoprim-sulfamethoxazole, doxycycline, linezolid and ceftriaxone using Etest strips according to the manufacturer's instructions (bioMérieux, Marcy l' Étoile, France). The minimum inhibitory concentrations were interpreted based on Clinical and Laboratory Standards Institute interpretive criteria for *Corynebacterium* spp. and related coryneform genera (including *Arthrobacter* species) [14] as there are none specifically for *Falsarthrobacter* species. Strain UM1 was susceptible to vancomycin (1.5 µg/ml), linezolid (0.75 µg/ml), trimethoprim-sulfamethoxazole (0.094 µg/ml), doxycycline (0.125 µg/ml), and intermediate to penicillin (0.5 µg/ml) and ciprofloxacin (1.5 µg/ml). Resistance against ceftriaxone (minimum inhibitory concentrations 3 and >32 µg/ml) was noted for two colony size variants tested in this study.

The patient underwent surgical procedures for right implant removal and fusion using an external fixator in October 2019. At this point, the synovial fluid sent for culture in BD BACTEC Peds Plus/F and BacT/ALERT FA Plus (aerobic) bottles, as well as the femoral intramedullary tissue, grew the same organism again (*F. nasiphocae)*, which was identified by 16S rRNA gene sequencing approach. There was also scanty growth of *Kocuria kristinae, Neisseria* sp., and *Streptococcus sanguinis* cultured from the femoral intramedullary tissue, while tissue from the right knee joint grew *Staphylococcus hominis* after an enrichment culture. Tissue from the intramedullary tibia had no growth. During the admission, however; the patient could not recover from the postoperative stress and succumbed to his illness. He died approximately 3 weeks after the surgery.

Discussion

Falsarthrobacter nasiphocae sp. nov. (DSM13988, CCUG 42953^T), previously classified as a species under the genus *Arthrobacter,* was isolated in mixed cultures with *Corynebacterium phocae, Pseudomonas aeruginosa, Staphylococcus aureus* and coagulase-negative staphylococci, from the nasal swabs taken from two common seals (*Phoca vitulina*) housed in a rehabilitation center [6]. The reclassification of *Arthrobacter nasiphocae* to a novel genus, *Falsarthrobacter,* was proposed as it demonstrated distinct peptidoglycan type (Lys-Ala2-Gly2-3-Ala[Gly]) and quinone system, and is phylogenetically distantly related to the type species of the genus *Arthrobacter* (*A. globiformis*) based on 16S rDNA sequence analysis [7]. However, the disease potentials of *F. nasiphocae* in animals and humans have yet to be reported.

In this study, it is likely that *F. nasiphocae* PJI might have developed on the patient's knee implant for some time before its isolation. We are not certain how this organism was introduced to the knee as opposed to the other more common organisms that cause PJI. It might have gained entry during immediate postoperative period or a hemodialysis session in the intensive care unit. Misdiagnosis can occur as PJI can be quite indolent during early infection. When the infection spreads to the interface between the implant and the bone, it may give rise to the loosening of the implant. The repeat isolation of *F. nasiphocae* (on three occasions) from synovial fluids of our patient over an 8-month period suggests its ability to persist and survive host defense at the site of PJI. This is in line with the pathogenesis of PJI whereby organisms can strive within biofilms, impeding penetration of antibiotics. As a result, higher concentrations of antibiotics are required to eradicate the sessile cells [1]. The source of *F. nasiphocae* PJI remains elusive as it has not been reported either in human samples or the natural environment, except for the nasal cavities of housed common seals [6,7]. It is not certain if the other microorganisms isolated from the specimens collected from the patient's last surgery were true pathogens or contaminants.

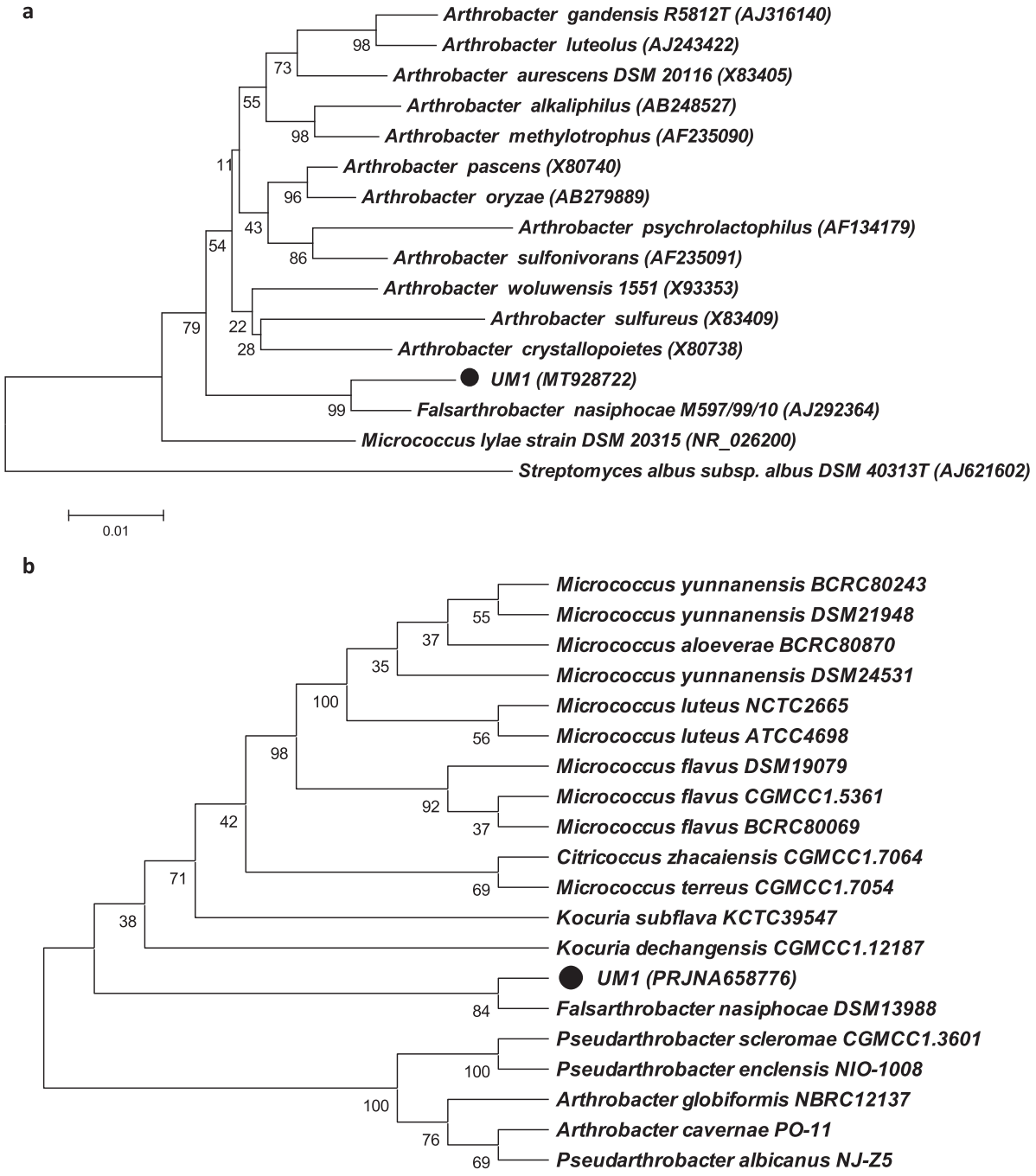

Fig. 1. (a) A dendrogram constructed based on the bacterial 16S ribosomal RNA gene sequence of *F. nasiphocae* strain UM1 (●) with closely related species in the genus *Arthrobacter*. (B) Taxonomic placement of *F. nasiphocae* strain UM1 (●) based on the whole-genome phylogenetic tree generated from the Type Strain Genome Server (https://tygs.dsmz.de/) [13].

With the increased prosthetic joint implantation procedures performed in clinical settings, there has been a growing concern on the emergence of unusual or rare bacterial pathogens associated with PJI. A recent retrospective, single-center study reported the occurrence of rare organisms accounting for approximately 10% of all periprosthetic hip and knee joint infections [3]. Atypical organisms such as *F. nasiphocae* may go undetected in a less well-equipped clinical microbiology laboratory, leading to underreporting and underestimation of the actual incidence, and limiting options for more targeted and effective treatment. In this aspect, the use of emerging technologies is essential to enhance laboratory capability in establishing more definitive etiological diagnoses associated with rarely encountered organisms in PJI.

Declarations of Competing Interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

AMM and **KAA** involved in clinical diagnosis, management of the patient and provided clinical history summary for the

manuscript. **RK, KAJ**, and **RDV** involved in microbiological analysis and antibiotic susceptibility testing verification of the isolate in the MMB Diagnostic laboratory, UMMC. **LJL** and **JC** involved in performing phenotypic tests and 16S rRNA sequencing of the isolate. **TST** performed whole genome analysis and drafted the paper. **RK** and **KAA** critically reviewed the manuscript. All authors approved the final submission. We would like to thank UMMC for permission to conduct this study.

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Ethical approval

No experiments with humans or animals were carried out. Ethical approval for this study has been obtained from Universiti Malaya Medical Ethics Committee (MRECID.NO: 2021629-10287).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.08.025.

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