Prosthetic Joint Infection Caused by *Helcococcus kunzii*

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*Helcococcus kunzii* was isolated by sonication and conventional cultures obtained from a case of infection following total knee prosthesis in an immunocompetent patient. The patient recovered uneventfully. This is the first known case of an *H. kunzii* prosthetic joint infection.

CASE REPORT

A 39-year-old man presented with a severe problem in his right knee. Fifteen years before, at the age of 24, he was diagnosed with osteochondritis and treated by arthrotomy with microfractures. An acute infection developed after surgery and was treated by means of arthroscopic debridement and intravenous antibiotics. The final result was substantial joint rigidity.

Fourteen months ago, the patient was diagnosed with secondary osteoarthritis, due to his functional deficits, and underwent surgery for cemented total knee replacement. The patient reported that his postoperative recovery was torpid and complicated; the wound took some 4 months to heal and required a vacuum-assisted system, while the rigidity forced a closed manipulation under general anesthesia. Following these events, the patient experienced a slow but progressive improvement until 10 months after surgery.

Ten months later, the patient was seen because of the appearance of two tumors in the anteromedial side of the tibial plateau that measured 1 cm in diameter. Repeated intraarticular aspiration and cultures were negative. The tumors were drained and the cultures were negative.

The patient was seen by us 14 months after surgery. Both tumors were covered with dried exudation/suppuration. X-rays showed clear signs of loosening. The C-reactive protein (CRP) level was 1.1 mg/liter, and the erythrocyte sedimentation rate (ESR) was 30 mm/h. A diagnosis of chronic infected total knee arthroplasty was established, and a two-stage exchange procedure was proposed and then accepted by the patient and his family. During surgery, 3 periprosthetic tissue samples were obtained. These samples and the retrieved implant were sent to the microbiology laboratory for culture.

The tissue samples obtained during surgery were homogenized and inoculated in the following culture media: tryptic soy-5% sheep blood agar (TSS), chocolate agar (CHA), Schaedler-5% sheep blood agar (SCS), MacConkey agar (McC), and Sabouraud-chloramphenicol agar slants (SC). Implant samples (femoral, tibial, and knee cap) were processed according to the previously described protocol (8) with the following modifications: samples with 100 ml of sterile phosphate buffer (bioMérieux, Marcy l’Etoile, France) were sonicated in rigid plastic containers following 1 min of vortexing. The sonicate was subsequently centrifuged, and the sediment was resuspended in 10 ml of the same buffer. The sediment was then inoculated in the same media and also in Middlebrook 7H10 agar plates. TSS, CHA, and Middlebrokk 7H10 were incubated at 37°C in a 5% CO2 atmosphere. SCS was incubated in an anaerobic atmosphere at 37°C, and SC slants were incubated at room temperature. All media were incubated for 15 days with the exception of the SCS medium, which was incubated for 4 weeks.

After 3 days of incubation, pinpoint colonies were detected in all samples except the femoral component of the prosthesis. Scanty growth was detected in the tissue samples, and >100,000 CFU/ml was detected in the tibial part and in the polyethylene components of the implant. Colonies were alpha-hemolytic and grew with similar characteristics in TSS, CHA, and SCS media. Gram staining showed Gram-positive cocci arranged in clumps. The organism was catalase and oxidase negative and grew in 6.5% NaCl brain heart infusion broth, and the API 20 STREP system (bioMérieux, Marcy l’Etoile, France) gave the code 4100413, which was identified as doubtful *Aerococcus viridans* in the API database. Susceptibility testing was performed by disk diffusion in Müller-Hinton agar supplemented with 5% sheep blood. The disk diffusion plates were incubated at 36°C in 5% CO2 for 72 h. The criteria for *Streptococcus* were followed for the interpretation of the results (9). The strain was susceptible to ampicillin, cefazolin, clindamycin, ciprofloxacin, vancomycin, trimethoprim-sulfamethoxazole, rifampin, and linezolid and resistant to erythromycin and gentamicin. No induction of resistance was detected between erythromycin and clindamycin. To achieve the identification of the isolate, a 5’-end 16S rRNA gene PCR was performed with universal primers E8F and E533F (http://rdna.ridom.de). The amplicons obtained were subsequently sequenced for identification by the BigDye Terminator method and detected in an ABIPrism 3100 automatic DNA sequencer (Applied Biosystems Inc.). 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 99% sequence similarity with the sequence having a high score in accordance with previously published criteria (7). This search identified the bacterium as *Helcococcus kunzii*, with a 99% similarity to the sequences of *H. kunzii* DQ082898 and DQ082899 (GenBank accession numbers).

Received 22 June 2011 Returned for modification 22 September 2011 Accepted 26 October 2011 Published ahead of print 30 November 2011

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doi:10.1128/JCM.01244-11
The patient was treated empirically with clindamycin and gentamicin. After the results of the cultures were obtained, the therapy was changed to clindamycin and rifampin. After 3 months of therapy, CRP and ESR presented normal values, and the patient underwent a second surgery for prosthesis implantation. During this surgery, several tissue samples were sent for culture, producing negative results. All of this study was performed according to the ethical committee requirements of our institution.

**Helcococcus kunzii** is a Gram-positive coccus that has been isolated as part of the human skin microbiota, especially from the lower extremities (2, 10, 11). It has been isolated from infected wounds (10), blood cultures from a patient with sepsis (16), a breast abscess (1), and an infected sebaceous cyst (13). Other species of this genus have been isolated from animals (*Helcococcus avis* [4]), human wounds (*Helcococcus suiciensis* [3]), and an infected knee prosthesis due to *Helcococcus pyogenica* (12), a species that has not yet been accepted in the international taxonomy of the genus (http://www.bacterio.cict.fr/).

Many infected wounds have been diabetic foot ulcers, and the organism has been recovered as part of a mixed microbiota, including other organisms that are also skin commensals, such as staphylococci. To the best of our knowledge, this is the first case of infection due to *H. kunzii* associated with biomaterials. The fact that this organism is isolated mainly from the lower extremities suggests that the origin of the infection (infected knee prosthesis) may have been contamination occurring in the first surgery, since surgery is the usual pathogenic mechanism of osteoarticular prosthesis infection.

The phenotypical characteristics of *H. kunzii* are similar to those of *Aerococcus* sp., although the former’s ability to grow with 6.5% NaCl, together with its lipophilic growth and capacity for facultative anaerobic growth, allow it to be differentiated from the latter. The characteristic API code also allows us to identify the isolate as *H. kunzii*, since all other species yielded different results in biochemical tests (Table 1). A presumptive identification can be performed for a Gram-positive coci arranged in clusters, with tiny, slightly hemolytic colonies, facultatively anaerobic, and with growth stimulated by 1% horse serum or 0.1% Tween 80. Although species identification can also be performed by phenotypical tests, molecular biology is needed in some cases to perform a detailed identification of the organism (14).

The antimicrobial resistance of this species shows erythromycin resistance as a common characteristic (1). Detection of the ermA gene was reported in one study (16) as the cause of this resistance, although we have not studied this characteristic in our isolate.

In our case, the organism grew after 3 days of incubation. This slow growth could be due to the presence of the bacteria in a prosthesis, which implies the development of a biofilm. This fact also explains the small amount of bacteria recovered from tissues and the high counts obtained after implant sonication. Bacteria from a biofilm are recovered with greater difficulty than planktonic organisms, and prolonged incubation has been recommended for the processing of these samples as a way to increase the sensitivity of the cultures (5, 6, 15). The fact that previous cultures (performed without such prolonged incubation) from this patient were negative lends credibility to this hypothesis.

In conclusion, this organism, like other skin commensals, is able to cause prosthetic infections. Prolonged incubation time, together with the use of a combination of diagnostic procedures, including implant sonication, makes it possible to recover this and possibly other uncommon organisms from these infections, and doing so contributes substantially to the proper management of these patients.

**ACKNOWLEDGMENT**

We thank our translator, Oliver Shaw, for his great work.

**REFERENCES**