Case Report

Septic arthritis due to a *Sneathia* species most closely related to *Sneathia sanguinegens*

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Sneathia sanguinegens is an infrequent bacterium in clinical specimens. We describe a case of right elbow septic arthritis due to a *Sneathia* species most closely related to *S. sanguinegens* in a young immunocompetent woman. *S. sanguinegens* has never been implicated in osteoarticular infections.

Case report

In May 2008, a 19-year-old French woman was admitted to our hospital (Nantes University Hospital) for an increasing pain and swelling in her right elbow. Her past medical history was not significant. She was student in a school for auxiliary nurses. Since November 2007, the patient complained of joint pain, heat and swelling in her right elbow, without any type of traumatic injury.

On examination, she appeared well and afebrile. Joint fluid, aspirated in April 2008, was clear with 2200 leukocytes mm⁻³ (percentage of polymorphonuclear leukocytes unknown). Aerobic cultures from the joint fluid did not show growth of any organisms. The patient showed an inflammatory syndrome with a C-reactive protein concentration of 129 mg l⁻¹. Magnetic resonance imaging did not show synovitis or osteitis. Anti-inflammatory drugs were given to the patient but no clinical improvement was seen. An intra-articular corticosteroid injection significantly reduced pain for up to 6 weeks after injection. A second joint fluid aspiration was carried out in May 2008 because of increasing pain. The joint fluid was purulent, with numerous leukocytes (120 000 leukocytes mm⁻³, 90 % polymorphonuclear leukocytes), but without bacteria being detected by the microscopic examination. All aerobic culture remained negative after 4 days of incubation.

The patient was then hospitalized in the Department of Rheumatology (Nantes University Hospital) at the end of May 2008. The patient presented with a limited and painful elbow flexion. An arthroscopic synovectomy with lavage and debridement was carried out. The aspirated joint fluid and two synovial biopsies were cultured under aerobic and anaerobic conditions. After 6 days of incubation, a Gram-negative bacterium grew only in the anaerobic liquid culture media from the three specimens. Colonies of less

than 1 mm appeared after 4 days of incubation under anaerobic culture conditions on chocolate and blood agar medium plates. Gram staining of the colonies demonstrated filamentous Gram-negative organisms. Conventional identification methods failed to identify this organism. This fastidious isolate was identified by sequencing (1345 bp) of the 16S rRNA gene, after amplification by PCR. The PCR was performed directly from the three positive anaerobic liquid media. The resulting sequences were compared to those in the BIBI (Bio Informatic Bacteria Identification) database (<http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi>) (Fig. 1). The isolate NTS65407 showed 98 % sequence similarity with *Sneathia sanguinegens* (GenBank accession number AJ344093). The 16S rRNA partial sequence has been deposited in GenBank under accession number HM567404.

Antibiotic susceptibility testing, performed by the disc diffusion method, required 4 days of incubation on chocolate agar medium plates under an anaerobic atmosphere before analysis. The organism was found to be susceptible to β -lactams, including imipenem, clindamycin, rifampicin, tetracycline and chloramphenicol, but was found to be resistant to erythromycin, aminoglycosides and fluoroquinolones.

An antibiotic regimen with 1.8 g oral clindamycin daily for 6 weeks was started when the bacterium was identified by 16S rRNA gene sequencing, 2 weeks after the joint fluid was aspirated. After 1 month the patient was discharged from the hospital. In January 2009, she presented with limited elbow extension and flexion. Radiological examination showed degenerative osteoarthritis of the right humero-ulnar joint following arthritis.

Discussion

The genus *Leptotrichia*, based on 16S rRNA gene sequencing, is believed to be one of the six genera along with the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA partial sequence of isolate NTS65407 is HM567404.

Fig. 1. Neighbour-joining tree showing the phylogenetic placement of strain NTS65407 (shown in bold) among the 16S rRNA gene sequences selected from the BIBI database. Bar, number of substitutions per nucleotide position.

genus *Sneathia* in the family *Fusobacteriaceae*, and in the phylum *Fusobacteria*. Since 2001, the so-called '*Leptotrichia sanguinegens*' was shown to be distinct from *Leptotrichia buccalis* and was assigned to a new genus, *Sneathia*, as *S. sanguinegens* sp. nov. (Collins *et al.*, 2001). As seen in the newly Bergey's manual, the genus *Sneathia* was placed in the phylum *Fusobacteria* along with the genus *Leptotrichia* (Brenner *et al.*, 2005).

Like *Leptotrichia*, *Sneathia* species are non-motile, non-spore-forming anaerobic Gram-negative, requiring complex nutrients and incubation under anaerobic conditions. After several days of incubation under an anaerobic atmosphere, very small grey colonies grow on blood agar plates (Eribe & Olsen, 2008). After Gram staining, the organisms appear as filamentous Gram-negative bacteria. In spite of all these conditions, some *S. sanguinegens* remain uncultivable, limiting phenotype-based identification in clinical microbiology laboratories. The use of molecular genetic methods such as 16S rRNA gene sequence analysis has resulted in much improved and more reliable species identification.

The strain isolated here was found to be susceptible to a number of antimicrobial agents such as clindamycin, which has good activity against anaerobic Gram-negative bacteria with excellent oral bioavailability and tissue penetration. Daily doses and duration of treatment were published as clinical practice guidelines for osteoarticular infections (SPILF *et al.*, 2009).

Sneathia species belong to the normal flora of the oral cavity, the gastrointestinal tract and the female genital tract. In all reported cases, bacteria were identified using standard microbiological cultures (Eribe & Olsen, 2008). Identification of *S. sanguinegens* species was obtained by the cloning and sequencing of the 16S rRNA gene. Ten previous cases of *S. sanguinegens* have been reported in the literature, five cases of delivery and post-partum bacteraemia, two cases of neonatal bacteraemia, two cases of bacteraemia in non-obstetric patients, and one amniotic-fluid infection (Collins *et al.*, 2001; Hanff *et al.*, 1995; De Martino *et al.*, 2004).

Several recent reports suggest that *S. sanguinegens* may be an under-recognized reproductive-tract pathogen. Some studies analysed the bacterial flora in amniotic fluid during preterm labour by the identification and sequencing of the 16S rRNA gene (Jacobsson *et al.*, 2003; Gardella *et al.*, 2004; DiGiulio *et al.*, 2008). Gardella *et al.* (2004) identified two *S. sanguinegens* based on bacterial sequences of five culture-negative samples. In the most recent study, 17 bacterial taxa including *S. sanguinegens* were identified from 166 amniotic fluids (DiGiulio *et al.*, 2008). Other studies analysed the bacterial flora in the genital tract of non-pregnant women by using 16S rRNA sequencing (Hebb *et al.*, 2004; Verhelst *et al.*, 2004). Verhelst *et al.* (2004) demonstrated the presence of *S. sanguinegens* in three women with true bacterial vaginosis. Hebb *et al.* (2004) reported the presence of *S. sanguinegens* in three Fallopian-tube specimens

from 45 women with laparoscopically confirmed acute salpingitis.

To the best of our knowledge, this is the first case of arthritis involving *S. sanguinegens* in an immunocompetent patient. Diagnosis was made 7 months after the beginning of clinical signs, as the cultures of two consecutive samples of joint fluid did not show growth of any organisms after 2 days of incubation on solid culture media agar plates under aerobic conditions. In such instances infection with slow-growing anaerobic bacteria like *S. sanguinegens* should be kept in mind. Primary solid culture media can dry out after some days of incubation and anaerobic liquid media can be subcultured on blood agar incubated under anaerobic conditions.

It was remarkable that our young patient did not have any risk factors for developing an infection due to a rarely encountered anaerobic bacterium. One possibility was an iatrogenic infection through the intra-articular corticosteroid injection. A delay in receiving appropriate treatment was probably responsible for the loss of elbow mobility and consistently poor outcome. *S. sanguinegens*, in our case identified from three different perioperative specimens (one articular fluid and two biopsy samples), should be considered as a potential pathogen of acute osteoarticular infections. One limitation is that fastidious organisms may be missed in a routine clinical laboratory. Molecular identification of *S. sanguinegens* directly from positive culture media shortens the delay in starting treatment. The resistance of this organism to fluoroquinolones should be kept in mind, as those antibiotics are currently used in the treatment of osteoarticular infections.

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