

Actinomyces neuui subsp. *neuui* Associated with Periprosthetic Infection in Total Hip Arthroplasty as Causative Agent[∇]

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***Actinomyces neuui* has until now not been described as a pathogen associated with periprosthetic infection in total joint replacement. The case presented here suggests that *A. neuui* subsp. *neuui* is a causative pathogen. The discussion and review of the literature indicate the impact that detection of *Actinomyces* species could have.**

CASE REPORT

A 78-year-old woman suffering from serious coxarthrosis underwent total hip arthroplasty on her right hip in July 2006 without complications (cement-free acetabular and cemented femoral component). In June 2008, she consulted the orthopedic outpatient department of St. Josef Hospital in Wuppertal, Germany, with clinical signs of local pain in the replaced hip, radiating into the femur. No further relevant symptoms were elicited. A peripheral hematological white blood cell count revealed 11.8×10^9 leukocytes/liter, and C-reactive protein was measured at 3.2 mg/dl. Triple-phase bone scintigraphy with ^{99m}Tc showed an increased uptake of radioisotopes in all of the phases, and in the radiographs, a continuous radiolucent line at the bone-cement interface was visible.

Joint aspiration revealed purulent synovial fluid. Microscopic analysis identified a mass of polymorphonuclear granulocytes, but no microorganisms could be detected. No antimicrobial treatment had been administered 4 weeks prior to aspiration of synovial fluid. The day after admission to the hospital, the patient underwent revision surgery for removal of the prosthesis. Girdlestone arthroplasty was performed with implantation of a spacer made of antibiotic-loaded bone cement. Intraoperatively, several biopsies of periprosthetic tissue were taken for microbiological investigation. Subsequently, postoperative empirical intravenous antibiotic treatment was commenced with cefazolin (2 g three times a day) and rifampin (450 mg twice a day).

Histopathological examination of the tissue samples showed a high degree of infiltration with inflammatory cells without any sign of malignancy.

The specimens, synovial fluid, and intraoperative biopsies were subjected to culture, and both the preoperative joint fluid as well as the intraoperative tissue showed growth of slow-growing gram-positive rods in pure culture. These were identified as *Actinomyces neuui* subsp. *neuui* by their biochemical properties and sequencing of bacterial 16S rRNA.

After 7 days of empirical therapy, the antibiotics were changed to penicillin G (5 million IU four times a day) according to the susceptibility pattern of the pathogen found.

The antimicrobial therapy was continued after procurement of biopsies for microbiological investigation taken at the time of reimplantation of a cemented total hip replacement. Locally, antibiotic-loaded bone cement with 2 g vancomycin, 1 g clindamycin, and 1 g gentamicin per 40 g polymethylmethacrylate bone cement was used for fixation of the prosthesis. The intravenous therapy was discontinued 2 weeks later and replaced by amoxicillin (1 g three times a day) administered orally for another 4 weeks. Since the latest surgery, cultures have revealed no evidence of microorganisms after 2 weeks of incubation.

The postoperative radiological control showed a well-fixed implant, and the patient was discharged from hospital without any sign of local infection 2 weeks after reimplantation. The patient has not been seen for follow-up.

The genus *Actinomyces* contains several anaerobic and aerotolerant gram-positive non-spore-forming organisms with variable morphology. Several species belong to the typical commensals of the oropharyngeal surface.

In 1994, the CDC group 1 and group 1-like coryneform bacteria were reclassified as the genus *Actinomyces* on the basis of 16S rRNA molecular and biochemical analysis.

The new species was proposed by Funke et al. and named *Actinomyces neuui* sp. nov., containing *Actinomyces neuui* subsp. *neuui* for CDC group 1 and *Actinomyces neuui* subsp. *anitratus* for CDC group 1-like coryneform bacteria (4, 5).

The morphology of the cultivated strain provided small whitish colonies with the best growth on blood agar supplemented with 5% sheep blood at 37°C in a 5% CO₂ atmosphere, but the strain could also be isolated from anaerobically incubated plates after 72 h of incubation. Gram staining revealed short gram-positive rods without branching filaments. In initial screening reactions, the presence of catalase and a positive CAMP test were observed. A set of biochemical tests produced the following results: reduction of nitrate (key reaction for differentiation from *Actinomyces neuui* subsp. *anitratus*); no hydrolysis of urea, esculin, and gelatin; activity of α-glucosidase and β-galactosidase, no

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activity of *N*-acetyl- β -glucosaminidase; and acid production from glucose, ribose, xylose, and mannose but no production of acid from maltose, lactose, sucrose, and glycogen. The reactions were generated by the commercial identification kit API Coryne (version 3.0) (BioMérieux, Marcy l'Etoile, France), which had a correspondence of 99% with *Actinomyces neuui* subsp. *neuui* (profile code no. 3410714). The molecular 16S rRNA partial gene sequence analysis showed a similarity of 100% (879/879 nucleotides) compared to the reference sequence of the type strain *Actinomyces neuui* subsp. *neuui* (accession no. Z 33613/GI 1729442 NCBI).

Due to the slow growth, the MICs were determined by the Etest (Oxoid, Basingstoke, Hampshire, United Kingdom) and carried out on Mueller-Hinton agar supplemented with 5% sheep blood with an inoculum corresponding to a McFarland standard of 0.5. The following antimicrobial agents were chosen and revealed low MICs: penicillin G, 0.008 μ g/ml; ampicillin, <0.015 μ g/ml; clindamycin, <0.016 μ g/ml; levofloxacin, 0.5 μ g/ml; vancomycin, 0.25 μ g/ml; and rifampin, 0.003 μ g/ml. The MIC of gentamicin was 1.0 μ g/ml.

Discussion. The literature contains several cases of infection associated with isolates of *Actinomyces neuui* subsp. *neuui*. After the species was renamed in 1994, the majority of isolates were obtained from abscesses and associated with mixed anaerobic flora (6). During recent years, the species was cultured from various clinical specimens and was reported to be the causative organism in infected mammary prosthesis (1), chronic osteomyelitis (13), endophthalmitis (7), infective endocarditis (2), and ventriculoperitoneal shunt infection (15).

In periprosthetic joint infections, coryneform bacteria and *Actinomyces* spp. do not belong to the commonly cultured microorganisms. Many species are regarded as low-virulence bacteria that are part of normal mucocutaneous flora. Therefore, mucocutaneous lesions are probably the main cause of local (dental-oral) and remote infections.

Since implant infections can occur during the entire lifetime of the joint replacement, clinicians are eager to characterize the origin of infection. Depending on the onset of symptoms after primary implantation, prosthetic joint infections are divided into early, delayed, and late infections. Early and delayed infections are mainly regarded as having been acquired during replacement, whereas late infections are predominantly caused by hematogenous seeding (11). As the period of late infection varies up to 1 year, depending on the classification scheme used, it is limited in its significance. In our case, 2 years had passed between primary implantation and revision of the total hip prosthesis, and in any scheme this would be considered as a late infection. According to the physiological oral colonization of coryneform bacteria, oral-pharyngeal lesions are an important risk factor for infections. The patient has had a complete dental prosthesis for many years, so that a dental infection could be excluded as possible source of bacteremia. Other frequent sources like skin, respiratory tract, or urinary tract infection could not be found in the patient's history. Therefore, due to the natural habitat of *Actinomyces* spp., it is difficult to estimate the clinical significance of isolates recovered from human specimens, but in this case it is strengthened by the findings of the same microorganism in multiple specimens from preoperative synovial fluid and intraoperative peripros-

thetic tissue in pure culture, through the presence of inflammation on histopathological examination, and the good clinical response to the high-dose therapy with penicillin G.

A review of the literature indicates that coryneform bacteria (14) and different recognized strains of *Actinomyces* spp. (9), such as *A. naeslundii* (10, 16), *A. israelii* (8, 12, 17), and *A. viscosus* (3), have to be considered as causative agents of periprosthetic joint infections. In one case, it was associated with an apparent focus after dental extraction, and in another, the microorganism was isolated from an intravenous drug user. In all other cases, the origin of infection remained unclear. Nevertheless, these reports underline that coryneform bacteria cultured from synovial fluid or periprosthetic tissue should not simply be regarded as contamination of the specimen, but have to be identified to the species level, because they could be of clinical significance. In five of the six publications mentioned above, reference was made to antibiotic therapy, and the patients were successfully treated either with penicillin, as in our case, or with a cephalosporin. We are reporting on a new case, and to the authors' knowledge, after its renaming in 1994, *Actinomyces neuui* subsp. *neuui* has so far not been described as an etiologic pathogen of prosthetic hip infection.

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