Case Report

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Demonstration by PCR and DNA sequencing of *Corynebacterium pseudodiphtheriticum* as a cause of joint infection and isolation of the same organism from a surface swab specimen from the patient

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A case of infectious arthritis following arthroscopy is described. Real-time PCR, using universal bacterial primers targeting the 16S rRNA gene, and subsequent DNA sequencing of the PCR product demonstrated the presence of DNA from *Corynebacterium pseudodiphtheriticum* in the synovial fluid from the affected knee. Culture from a surface swab from the site of purulent discharge from the knee was initially reported as growing normal skin microbiota. Knowing the result of the DNA analysis, the specimen was re-examined and a diphtheroid bacterium was isolated. The DNA sequence of the isolated bacterium was identical to that of the DNA in the joint. The isolated bacterium was tested for susceptibility to relevant antibiotics. Demonstration and identification of bacterial DNA by PCR and gene sequencing may not by itself give information on important characteristics such as susceptibility to antibiotics of the infecting bacterium. The present case illustrates that the results obtained by the method can be used to isolate the relevant bacterium in culture from other sites and thereby characterize it. It furthermore demonstrates that *C. pseudodiphtheriticum* can cause severe arthritis when inoculated into joints.

Introduction

Corynebacterium pseudodiphtheriticum, previously called *Corynebacterium hofmannii*, is a normal inhabitant of the upper respiratory tract that seldom causes disease. It may cause respiratory tract infections (Chiner *et al.*, 1999; Freeman *et al.*, 1994; Manzella *et al.*, 1995; Martaresche *et al.*, 1999) and endocarditis (Morris & Guild, 1991), and on rare occasions has been reported as a cause of other infections (LaRocco *et al.*, 1987; Lockwood & Wilson, 1987; Nathan *et al.*, 1982). The bacterium is rarely implicated in orthopaedic infections. Two cases of osteitis caused by *C. pseudodiphtheriticum* have been described (Roux *et al.*, 2004). The bacterium has also been isolated from one infected prosthetic hip, but the significance of the finding was questioned by the authors (Von Graevenitz *et al.*, 1998). The low number of *C. pseudodiphtheriticum* infections described may be related to a low carriage rate in the population.

PCR using 16S rDNA universal bacterial primers and subsequent DNA sequence analysis of the PCR product is increasingly being used as a method for demonstration and identification of bacteria in clinical samples. The method has,

in particular, proven its value in establishing the cause of infections with negative culture results, especially after antibiotic treatment (Rantakokko-Jalava *et al.*, 2000). A major drawback of identifying infecting bacteria by PCR and DNA sequencing without isolating them in culture is that it does not allow phenotypic characterization such as establishment of antibiotic susceptibility patterns. When live bacteria cannot be recovered from the primary site of infection, it may therefore be worth trying to isolate the bacterium from another sample.

To report *C. pseudodiphtheriticum* as a cause of infectious arthritis and to illustrate the benefit of combining molecular and conventional diagnostic methods we find it relevant to describe a case of knee infection with the bacterium following arthroscopy. The correct bacterial diagnosis and susceptibility pattern was only achieved by comparing a bacterial DNA sequence in the joint fluid with that of a bacterium isolated from a surface swab. The culture of a diphtheroid from the swab was at first erroneously regarded as an insignificant finding.

Case report

A 74-year-old female patient was admitted to hospital with septic arthritis of the right knee. An arthroscopy had been

Abbreviations: t.i.d., Three times daily.

performed 2 weeks earlier due to long-lasting pain. There was haemorrhagic discharge from the anterolateral stab wound incision in the days after the procedure.

The patient had a medical history of being overweight, swellings of the lower legs, muscular rheumatism and arthritis of the knees. Her medication consisted of cortisone [2.5 mg three times a day (t.i.d.)], non-steroid anti-inflammatory drugs (NSAIDs), an analgesic and a diuretic.

On admission purulent discharge from the stab wound was noted, and a swab was obtained for culture. The knee was painful but no swelling or redness was found. An elevated blood leukocyte count of $12 \times 10^9 \text{ l}^{-1}$ (normal range, $3.5\text{--}8.8 \times 10^9$) and a C-reactive protein concentration of 256 mg l^{-1} (normal, < 5) were detected. Intravenous antibiotic treatment (dicloxacillin 1 g t.i.d.) was started. After 2 days a repeat arthroscopy with excision of the fistula and synovectomy was performed. A joint-fluid specimen and a fistula tissue biopsy were obtained. The swab specimen from the time of admission was plated on Danish Blood Agar (SSI diagnostica). After 2 days of culture it was routinely reported as growing normal skin microbiota.

As antibiotics had been administered prior to further specimen collection, culture from the synovial fluid and the tissue biopsy was not attempted. Instead the specimens were examined by PCR. DNA was extracted using the QIAamp DNA Mini Kit according to the manufacturer's recommendations (Qiagen). The primers used for PCR amplification of a 526 bp part of the 16S rRNA gene were BSF-8 (5'-AGAGTTTGATCCTGGCTCAG-3') and BSR-534 (5'-AT TACCGCGGCTGCTGGC-3') (Wilmotte *et al.*, 1993). The Quantitect SYBR Green kit (Qiagen) was used for real-time PCR (50 μl total volume) containing $1 \times$ PCR buffer and 200 μM each primer. Samples (1 μl and 5 μl) were tested in real-time PCR. The amplification profile was 95 °C for 15 min, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. DNA extraction, reagent handling and PCR were carried out in three separate buildings in order to avoid contamination, and adequate negative controls were processed in parallel with the samples.

While PCR of the tissue was negative, a PCR product was obtained from the synovial fluid. Both DNA strands of the amplicon were sequenced using BSF-8 and BSR-534 as sequencing primers. The data obtained were compared to deposited sequences in the NCBI database using the BLAST search engine. The sequences obtained showed 100 % identity (490/490 identical bases) to *C. pseudodiphtheriticum* strain CIP103420T partial 16S rRNA gene, and 99.8 % identity (480/481 identical bases) to *C. pseudodiphtheriticum* strain NCTC11136 16S rRNA gene. The next match of cultured bacteria was *Corynebacterium propinquum* (471/477 identical bases).

The original swab specimen was re-examined bearing in mind the result from the DNA analysis. On re-examination, many colonies of diphtheroids were grown almost in mono-

culture. The DNA sequence was analysed as described above. The sequence obtained from the isolated bacterium was identical to the one from the synovial fluid.

Standard disk diffusion susceptibility testing on SSI resistance plates (SSI diagnostica) showed the isolated bacterium to be susceptible to penicillin, ampicillin, dicloxacillin, cefuroxime and erythromycin. E-test showed a MIC of $0.006 \mu\text{g ml}^{-1}$ for penicillin. The patient was treated with parenteral cefuroxime 1.5 g t.i.d. for 1.5 weeks followed by oral penicillin (1600 mg t.i.d.) for 3 months. The recovery was uneventful and she was discharged after 1 month.

Discussion

DNA base sequence analyses of bacteria are currently expanding the number of bacteria that are recognized as human pathogens. Species previously regarded as non-pathogenic are now recognized as true agents of diseases, while others, previously only associated with certain types of infection, are being accepted as causes of other infectious diseases as well.

Corynebacterium species other than *Corynebacterium diphtheriae*, *Corynebacterium jeikeium* and at times a few more are among the bacteria that are often neglected in the routine clinical microbiology laboratory. The exact infectious potential of these bacteria and their rational antibiotic treatment can only be established by careful species determination and susceptibility examination (Riegel *et al.*, 1996). DNA sequencing of the 16S rRNA gene has recently been used in a polyphasic approach to characterize a large number of rare and recently described *Corynebacterium* species in human infections (Bernard *et al.*, 2002). Sequencing of DNA bases in this or other genes has proven well-suited for identifying diphtheroids to the species level (Khamis *et al.*, 2004; Roux *et al.*, 2004; Tang *et al.*, 2000). Universal bacterial PCR and DNA sequencing has been used in studies of synovial fluid in search of bacterial aetiologies to various types of arthritis in general (Chen *et al.*, 2003; Cuchacovich *et al.*, 2002; Nikkari *et al.*, 1999; van der Heijden *et al.*, 2000; Wilbrink *et al.*, 1998) and to septic arthritis in particular (Jalava *et al.*, 2001; van der Heijden *et al.*, 1999).

In the case presented here, the joint infection by *C. pseudodiphtheriticum* occurred as a complication to an arthroscopy performed 2 weeks before admission to hospital. In this patient immunosuppressive treatment may have contributed to susceptibility to infection. As the patient had already received antibiotics at the time the joint fluid was obtained, PCR for ribosomal bacterial DNA was carried out without attempting culturing first. After *C. pseudodiphtheriticum* had been established as the infecting agent by sequence analysis of the PCR product, efforts were made to isolate the bacterium in culture from another site.

A swab specimen from the day of admittance, before initiation of antibiotic treatment, had resulted in culture of what was routinely reported as 'normal skin microbiota'. The expression 'normal skin microbiota' is used in our routine

laboratory to describe a variety of bacteria from surface swabs, including diphtheroids, which are usually not further identified. Therefore the swab specimen was brought into culture again, with the specific purpose of isolating diphtheroids. One such bacterium, with a DNA sequence identical to the one found in the joint, was isolated and examined for susceptibility to relevant antibiotics. The isolate was susceptible to low concentrations of beta-lactam antibiotics.

The presented case demonstrates that, like most other bacteria belonging to the normal microbiota of the skin or upper respiratory tract, *C. pseudodiphtheriticum* can cause severe infections, such as septic arthritis, when inoculated directly into normally sterile sites. Furthermore, it illustrates that even though demonstration and identification of bacterial DNA does not by itself give information about the antibiotic susceptibility of the infecting organism, it may be possible to isolate the same bacterium in a viable form from other sites for susceptibility testing and other relevant investigations. Combining molecular and conventional techniques for bacterial diagnosis and characterization is a powerful approach for better diagnostic results.

References

- Bernard, K. A., Munro, C., Wiebe, D. & Ongsansoy, E. (2002). Characteristics of rare or recently described *Corynebacterium* species recovered from human clinical material in Canada. *J Clin Microbiol* **40**, 4375–4381.
- Chen, T., Rimpilainen, M., Luukkainen, R., Mottonen, T., Yli-Jama, T., Jalava, J., Vainio, O. & Toivanen, P. (2003). Bacterial components in the synovial tissue of patients with advanced rheumatoid arthritis or osteoarthritis: analysis with gas chromatography-mass spectrometry and pan-bacterial polymerase chain reaction. *Arthritis Rheum* **49**, 328–334.
- Chiner, E., Arriero, J. M., Signes-Costa, J., Marco, J., Corral, J., Gomez-Esparrago, A., Ortiz de la Tabla, V. & Martin, C. (1999). *Corynebacterium pseudodiphtheriticum* pneumonia in an immunocompetent patient. *Monaldi Arch Chest Dis* **54**, 325–327.
- Cuchacovich, R., Japa, S., Huang, W. Q. & 7 other authors (2002). Detection of bacterial DNA in Latin American patients with reactive arthritis by polymerase chain reaction and sequencing analysis. *J Rheumatol* **29**, 1426–1429.
- Freeman, J. D., Smith, H. J., Haines, H. G. & Hellyar, A. G. (1994). Seven patients with respiratory infections due to *Corynebacterium pseudodiphtheriticum*. *Pathology* **26**, 311–314.
- Jalava, J., Skurnik, M., Toivanen, A., Toivanen, P. & Eerola, E. (2001). Bacterial PCR in the diagnosis of joint infection. *Ann Rheum Dis* **60**, 287–289.
- Khamis, A., Raoult, D. & La Scola, B. (2004). *rpoB* gene sequencing for identification of *Corynebacterium* species. *J Clin Microbiol* **42**, 3925–3931.
- LaRocco, M., Robinson, C. & Robinson, A. (1987). *Corynebacterium pseudodiphtheriticum* associated with suppurative lymphadenitis. *Eur J Clin Microbiol* **6**, 79.
- Lockwood, B. M. & Wilson, J. (1987). *Corynebacterium pseudodiphtheriticum* isolation. *Clin Microbiol Newsl* **9**, 5–6.
- Manzella, J. P., Kellogg, J. A. & Parsey, K. S. (1995). *Corynebacterium pseudodiphtheriticum*: a respiratory tract pathogen in adults. *Clin Infect Dis* **20**, 37–40.
- Martaresche, C., Fournier, P. E., Jacomo, V., Gainnier, M., Boussuge, A. & Drancourt, M. (1999). A case of *Corynebacterium pseudodiphtheriticum* nosocomial pneumonia. *Emerg Infect Dis* **5**, 722–723.
- Morris, A. & Guild, I. (1991). Endocarditis due to *Corynebacterium pseudodiphtheriticum*: five case reports, review, and antibiotic susceptibilities of nine strains. *Rev Infect Dis* **13**, 887–892.
- Nathan, A. W., Turner, D. R., Aubrey, C., Cameron, J. S., Williams, D. G., Ogg, C. S. & Bewick, M. (1982). *Corynebacterium hofmannii* infection after renal transplantation. *Clin Nephrol* **17**, 315–318.
- Nikkari, S., Rantakokko, K., Ekman, P. & 8 other authors (1999). *Salmonella*-triggered reactive arthritis: use of polymerase chain reaction, immunocytochemical staining, and gas chromatography-mass spectrometry in the detection of bacterial components from synovial fluid. *Arthritis Rheum* **42**, 84–89.
- Rantakokko-Jalava, K., Nikkari, S., Jalava, J. & 8 other authors (2000). Direct amplification of rRNA genes in diagnosis of bacterial infections. *J Clin Microbiol* **38**, 32–39.
- Riegel, P., Ruimy, R., Christen, R. & Monteil, H. (1996). Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. *Eur J Clin Microbiol Infect Dis* **15**, 657–662.
- Roux, V., Drancourt, M., Stein, A., Riegel, P., Raoult, D. & La Scola, B. (2004). *Corynebacterium* species isolated from bone and joint infections identified by 16S rRNA gene sequence analysis. *J Clin Microbiol* **42**, 2231–2233.
- Tang, Y. W., Von Graevenitz, A., Waddington, M. G., Hopkins, M. K., Smith, D. H., Li, H., Kolbert, C. P., Montgomery, S. O. & Persing, D. H. (2000). Identification of coryneform bacterial isolates by ribosomal DNA sequence analysis. *J Clin Microbiol* **38**, 1676–1678.
- van der Heijden, I., Wilbrink, B., Vije, A. E., Schouls, L. M., Breedveld, F. C. & Tak, P. P. (1999). Detection of bacterial DNA in serial synovial samples obtained during antibiotic treatment from patients with septic arthritis. *Arthritis Rheum* **42**, 2198–2203.
- van der Heijden, I., Wilbrink, B., Tchetverikov, I., Schrijver, I. A., Schouls, L. M., Hazenberg, M. P., Breedveld, F. C. & Tak, P. P. (2000). Presence of bacterial DNA and bacterial peptidoglycans in joints of patients with rheumatoid arthritis and other arthritides. *Arthritis Rheum* **43**, 593–598.
- Von Graevenitz, A., Frommelt, L., Pünter-Streit, V. & Funke, G. (1998). Diversity of coryneforms found in infections following prosthetic joint insertion and open fractures. *Infection* **26**, 36–38.
- Wilbrink, B., van der Heijden, I., Schouls, L. M., van Embden, J. D., Hazes, J. M., Breedveld, F. C. & Tak, P. P. (1998). Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. *Arthritis Rheum* **41**, 535–543.
- Wilmotte, A., Van der Auwera, G. & De Wachter, R. (1993). Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *chlorogloeopsis* HTF ('*mastigocladus laminosus* HTF') strain PCC7518, and phylogenetic analysis. *FEBS Lett* **317**, 96–100.