Case Report

Septicaemia due to *Corynebacterium striatum*: molecular confirmation of entry via the skin

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Septicaemia due to *Corynebacterium striatum* occurs infrequently. A case of *C. striatum* septicaemia with a known skin focus is reported in a 69-year-old male with ischaemia, refractory anaemia and treated for thyroid cancer. The characterization and typing of blood and cutaneous isolates was carried out using biochemical and DNA molecular typing methods to analyse the isolates. This is the first reported case with a documented source.

Introduction

Corynebacterium striatum infections are scarcely documented in the English and Spanish literature. The case of septicaemia we describe is the first reported with a known skin focus in which the identity of the strains has been established.

Case report

A 69-year-old man was admitted to the geriatric ward in the Hospital Monte Naranco (Oviedo, Spain) with the diagnosis of chronic ischaemia of the lower extremities (grade IV) and lesions in the first and second toe and the heel of the right foot. The patient had a 10-year history of thyroidectomy and iodine radiotherapy for thyroid carcinoma. Two years ago he had a lung metastasis of the tumour treated with iodine radiotherapy and since then he has presented a refractory anaemia and has undergone periodic blood transfusions.

During hospitalization, in poor general condition he suffered a sudden febrile episode (39.5 °C) and cellulitis in his right lower limb. Laboratory tests revealed anaemia with a red blood cell count of 2.8 million mm⁻³, haemoglobin (g dl⁻¹) 9, an increased erythrocyte sedimentation rate (45 mm for the first hour) and a count of 7.6 × 10³ leukocytes µl⁻¹ with 82 % polymorphonuclear cells.

The chest X-ray showed a consolidation of the left lung with pleural effusion, and nosocomial pneumonia was diagnosed, in this case improving without further interventions.

Blood, urine and skin cultures (from the ischaemic areas) were taken. *C. striatum* was isolated from blood cultures

(three out of four bottles, the growth was in the two different venipunctures) with a previous povidone iodine skin preparation, and isolated from two skin swab cultures (together with *Enterococcus faecalis* in the last one). The Gram stain showed the presence of more than 25 neutrophils under the ×10 objective, comprising three morphotypes and the skin cultures showed a semi-quantitative growth of 4+ for *C. striatum* and 2+ for *E. faecalis*. Treatment with IV amoxicillin/clavulanic acid, 2 g three times a day, was started and continued for 14 days until the resolution of the infection. During this time, the patient required the placement of a femoral bypass and received two transfusions of red cell concentrate.

The strains of *C. striatum*, isolated from blood and skin, were simultaneously identified. Smooth colonies, about 1 mm in diameter and white-cream in colour, grew on blood agar plates. A Gram stain showed Gram-positive short rods. They were catalase-positive and oxidase-negative, fermented glucose and sucrose in 24 h, reduced nitrate, hydrolysed pyrazinamide and tyrosine but did not produce urease or hydrolyse gelatin in the API Coryne (bioMérieux). Both strains and the collection strain CECT 4159 (corresponding to ATCC 6940^T) gave an identical profile of 3100105 *Corynebacterium striatum/amycolatum* in the bioMérieux database (version 2.0) with a score of 98.5 %. To achieve a better definition at the species level, a CAMP test and a test for susceptibility to the vibriostatic agent O/129 (Oxoid) were performed, both being positive, in accordance with other descriptions in which *C. striatum* is distinguished from *C. amycolatum* on the basis of these tests (Funke *et al.*, 1997).

Antibiotic susceptibility was determined in accordance with

previously reported methods (Martínez-Martínez *et al.*, 1995). E-test strips (AB Biodisk) on Mueller–Hinton agar with 5 % sheep blood (Biomedics) were used. The MICs were as follows for both strains: ampicillin, 0.5 µg ml⁻¹; amoxicillin-clavulanic acid, 0.25 µg ml⁻¹; vancomycin, 0.5 µg ml⁻¹; rifampicin, >32 µg ml⁻¹; tetracycline, 1 µg ml⁻¹; erythromycin, >256 µg ml⁻¹; ciprofloxacin, >32 µg ml⁻¹; cephalothin, 10 µg ml⁻¹; and cefotaxime, 2 µg ml⁻¹. There is no approved breakpoint for *Corynebacterium* species and we followed those used for alpha-haemolytic streptococci of the *viridans* group as suggested by the Wider systems manufacturer (Soria).

To further characterize the *C. striatum* isolates (from blood and skin), two variants of the DNA fingerprinting technique, ribotyping and randomly amplified polymorphic DNA (RAPD) analysis were carried out. Genomic DNAs were prepared using 2 × Kirby lytic mix as described previously (Hopwood *et al.*, 1985), and samples of 2 µg were digested with five restriction endonucleases (*EcoRV*, *HincII*, *HindIII*, *PvuII* or *SphI*) according to the manufacturer's instructions (Amersham Biosciences Europe). DNAs from the gels were blotted onto hybridization membranes (Hybond-N Nylon, 0.45 µm from Amersham Biosciences Europe) by capillary blotting (Sambrook *et al.*, 1989). Fragments were hybridized with a DNA fragment carrying an *rrnB* rRNA operon from *Escherichia coli* as a probe. The hybridization was performed using a non-radioactive DNA Labelling and Detection Kit (Roche Molecular Biochemicals) according the manufacturer's recommendations. Three of the five restriction enzymes employed in the analyses (*EcoRV*, *HincII* and *PvuII*) produced good digestion cleavage of DNA, allowing ribotypes to be distinguished (Fig. 1). Use of *HindIII* and *SphI* enzymes resulted in incomplete digestion with the strains in the study. The patterns of the fragments obtained by analysis with the endonucleases were identical in the two isolates, but were different to the profiles of the type strain *C. striatum* ATCC 6940^T.

For RAPD fragments, four primers were used: A (5'-AGCAGCGCCTCA-3') (Martín *et al.*, 1997), S (5'-TCACGATGCA-3') (Martín *et al.*, 1997), OPB17 (5'-AGGGAACGAG-3') (Lin *et al.*, 1996) and M13 forward (5'-GTTTCCAGTCACGAC-3'). PCR amplification was performed in 50 µl reaction mixtures consisting of buffer reaction mix (10 mM Tris/HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl₂; 0.1 % Triton X-100), 200 µM desoxynucleotide triphosphates, 0.45 µM primer, 2 U DyNAzyme II DNA polymerase (Finnzymes) and 20 ng template DNA. Amplification was performed in a DNA thermal cycler (MJ Research). Following an initial denaturation at 95 °C for 5 min, the DNA was amplified during 35 cycles of PCR consisting of denaturation at 95 °C for 1 min, annealing at a specific primer temperature (37 °C for primers S, M13 forward and OPB17; and 50 °C for primer A) for 1 min, and extension at 72 °C for 2 min, except for the last cycle, during which the extension step lasted for 10 min. Fig. 2 shows the bands generated after the amplification with the above-mentioned primers. The close correlation between the analysed strains

Fig. 1. Ribotypes from *C. striatum*. Lanes: 1, skin isolate; 2, blood isolate; 3, ATCC 6940^T; M, lambda DNA cleaved with *PstI*. Molecular sizes are shown on the right.

and the differences with the type strain *C. striatum* ATCC 6940^T can be seen. This pattern was the same for the three isolates in blood cultures, although we show just one of the strains in Figs 1 and 2.

Discussion

C. striatum was first described in 1889 by von Besser (1889) and belongs to the normal flora of the skin of the arms, forehead and cheeks (Cone *et al.*, 1998). *C. striatum* is a non-sporulating, non-acid-fast, pleomorphic Gram-positive rod that is aerobic and facultatively anaerobic. Only 25 cases of infection due to *C. striatum* have been reported, although *C. amycolatum* strains had been misidentified as *C. striatum* in the 1980s and 1990s. Reported cases of *C. striatum* include a variety of different types of infection: pneumonia and empyema (Cowling & Hall, 1993; Martínez-Martínez *et al.*, 1994); CSF-shunt infection (Hoy *et al.*, 1997; Weiss *et al.*, 1996) and endocarditis (Markowitz & Coudrom, 1990; Melero-Bascones *et al.*, 1996); peritonitis (Bhandari *et al.*, 1995); and septic synovitis with arthritis, keratitis, intra-uterine infection, wound infection, breast abscess and osteomyelitis (Cone *et al.*, 1998; Rubinfeld *et al.*, 1989; Peiris *et al.*, 1994; Stone *et al.*, 1997; Fernández-Ayala *et al.*, 2001).

Fig. 2. Analysis of genomic DNA of *C. striatum* by RAPD typing. Lanes: 1, skin isolate; 2, blood isolate; 3, ATCC 6940^T; M, lambda DNA cleaved with *Pst*I.

Furthermore, two outbreaks have been described, with the most likely route of transmission being the hands of health care workers (Brandenburg *et al.*, 1996; Leonard *et al.*, 1994).

Septicaemia with *C. striatum* is a rare event and has been described in only four patients but the route of transmission has not been described and proven in septicaemia or in any other invasive cases. The four cases were: in a 19-year-old man with lymphoblastic lymphoma and an autologous bone marrow transplant (Watkins *et al.*, 1993); in a 26-year-old male intravenous drug abuser with AIDS (Tumbarello *et al.*, 1994); in a 64-year-old neutropenic woman with endometrial adenocarcinoma and central venous catheter (Dall *et al.*, 1989); and in a 74-year-old woman with diabetes mellitus, valvular cardiopathic lesion and hepatic cirrhosis due to hepatitis C (Ezpeleta *et al.*, 1998). Most cases of *C. striatum* infection occurred in either immunocompromised patients or patients whose skin barrier integrity was broken. The presence of bacterial pathogenicity factors in this species remains unknown at present. No sepsis from a cutaneous focus has been previously reported.

The case we have described is, to our knowledge, the first reported in which the source of septicaemia or its invasive infections (in this instance the skin lesions) has been demonstrated by molecular analysis. All patients, including this one, have been cured with the appropriate treatment.

The strains analysed showed the same profile by ribotyping and RAPD analysis. Ribotyping with *Sph*I and *Hind*III has been used previously in this species (Björkroth *et al.*, 1999) and ribotyping with *Pvu*II has been used in *Corynebacterium urealyticum* (Nieto *et al.*, 2000). This study is the first time to our knowledge that *Hinc*II and *Eco*RV have been used. The RAPD typing was previously used in an outbreak as a suitable tool for typing *C. striatum* (Brandenburg *et al.*, 1996). Our results show that the clinical strains belong to the same clonal lineage.

In conclusion, this is the fifth report of septicaemia due to *C.*

striatum described in the literature and the first time that the source of the infection, in this case the skin, has been demonstrated by molecular techniques.

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