Case Report

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Molecular identification of *Exiguobacterium acetylicum* as the aetiological agent of bacteraemia

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A case of catheter-related bacteraemia caused by *Exiguobacterium acetylicum* is reported in an elderly patient. The availability of sequence-based methods facilitated rapid identification and expanded the spectrum of diseases attributed to coryneform bacteria and specifically to the genus *Exiguobacterium*.

Case report

In early 2005, a 92-year-old woman with a past medical history of hypertension, hyperuricaemia and Alzheimer's disease was admitted to hospital with urinary retention, leukocyturia and leukocytosis. On admission, her body temperature was 37.4 °C, she was haemodynamically stable, physical examination disclosed a systolic murmur 2/6 at the lower left sternal border and no other significant abnormalities were detected. Intravenous cefuroxime treatment was initiated for a presumed urinary tract infection.

Urine and blood cultures failed to grow any organism and no source of infection was noted on repeated examinations or imaging of the chest and abdomen. On the tenth day, a fever of 38.4 °C accompanied by tachycardia and leukocyturia appeared. Urine and blood cultures were obtained and antimicrobial therapy was changed to ertapenem after urinary culture grew *Klebsiella pneumoniae* harbouring an extended-spectrum β -lactamase, with rapid resolution of fever.

She remained afebrile for 4 days, after which fever re-emerged with a temperature reaching 38.6 °C. Three sets of blood samples were inoculated in aerobic and anaerobic blood culture vials (BacT/Alert 3D; bioMérieux), a peripherally placed intravenous line was removed and antibiotic treatment was withheld awaiting culture results in a haemodynamically stable patient. Two aerobic bottles from two sets of blood cultures obtained through separate needle puncture sites were positive. Orange colonies of a Gram-positive bacillus that fermented glucose and sucrose and was oxidase- and catalase-positive were grown. Reactions for indole, raffinose, citrate, maltose, urea and aesculin were negative.

The organism was sensitive to penicillins, cephalosporins, aminoglycosides and quinolones. The patient was given

cefuroxime according to the antimicrobial susceptibility of the isolate, and by the next day she was afebrile and remained so throughout the duration of her hospital stay.

The bacterium could not be identified using API Coryne (bioMérieux) and was submitted for identification based on the nucleic acid sequence of the 16S rRNA gene. DNA was extracted from the bacterium using the QIAamp DNA Blood Mini kit (QIAGEN). After amplification with universal bacterial primers (Harmsen *et al.*, 2002), amplicons were purified and directly sequenced. Public databases were then scrutinized for sequences with homology to the resulting nucleotide sequences using BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast/>). The nucleotide sequence of the amplified fragment identified the organism as *Exiguobacterium acetylicum* (99 % identity of 506 nucleotides).

Discussion

First described by Collins *et al.* (1983), the genus *Exiguobacterium* belongs to the expanding group of coryneform bacteria, which encompasses aerobically growing, asporogenous, non-partially acid-fast, irregularly shaped Gram-positive rods.

Case reports attributing disease states to coryneform bacteria are sometimes incorrect for several reasons including: (i) the inability to rely entirely on the databases of commercial identification systems, due to the limited number of taxa that they cover; (ii) significant changes in the taxonomy of coryneform bacteria; and (iii) the distinction between colonization and infection has not been made in many cases (Coyle & Lipsky, 1990). Gavin *et al.* (1992) observed that the API Coryne system identified about 84 % of 177 strains tested to the correct species with no additional tests.

Identification of coryneform bacteria to the species level should be attempted whenever they grow in pure culture from clinical specimens or when they represent the predominant organisms in normally sterile samples, in

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order to identify new species and to ascribe a pathogenic role to species previously regarded as saprophytes (von Graevenitz *et al.*, 1994).

The analysis of cellular fatty acid (CFA) patterns is an extremely useful method for identification of coryneform bacteria. However, it is time-consuming, depends on incubation conditions and is not available to many laboratories (von Graevenitz *et al.*, 1991; Bernard *et al.*, 1991). Molecular genetic techniques for species identification of *Corynebacterium* strains greatly increased the ability to identify coryneform bacteria to a species level and allowed the identification of new potentially pathogenic members (Loubinoux *et al.*, 2005).

The genus *Exiguobacterium* originally described in 1983 contained *Exiguobacterium aurantiacum* as its only species. In 1994, Farrow *et al.* (1994) included the former species *Brevibacterium acetylicum* in the genus *Exiguobacterium*. There are significant differences between the two genera, namely: colonies of *E. acetylicum* exhibit a golden-yellow pigment and the organism is oxidase-positive and facultatively anaerobic with a fermentative carbohydrate metabolism, fermenting D-mannose and cellobiose; *Brevibacterium* colonies are whitish-grey later turning yellow, and it is obligately aerobic with variable oxidative carbohydrate metabolism. Both *Exiguobacterium* species are motile by virtue of peritrichous flagella and have a unique CFA pattern. Several other members have been reported and can be differentiated by fatty acid composition (Hollis & Weaver, 1981; López-Cortés *et al.*, 2006).

The Centers for Disease Control has reported a number of *Exiguobacterium* strains from various clinical sources (e.g. skin, wounds, cerebrospinal fluid) (Funke *et al.*, 1997), but case reports are few, and to our knowledge bacteraemia has not been documented. The source of bacteraemia in this case was not documented but urinary or respiratory sources were ruled out in the presence of normal urinalysis and lung imaging. The bacteraemia followed a hospital stay that included several antibiotic courses to which the organism was susceptible, suggesting nosocomial acquisition. The most likely source is catheter-related, supported by the rapid response to removal of the intravenous line and treatment.

This case report documents the role of *Exiguobacterium* in causing hospital-acquired infection. The availability of

sequence-based methods facilitates rapid, less cumbersome identification and has expanded the spectrum of diseases attributed to coryneform bacteria.

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