Case Report

Central line-related bacteraemia due to *Roseomonas mucosa* in a neutropenic patient with acute myeloid leukaemia in Piraeus, Greece

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A case of central venous catheter-related bacteraemia due to *Roseomonas mucosa* in a neutropenic patient with acute myelogenous leukaemia is reported. The patient was successfully treated with amikacin and piperacillin-tazobactam. The clinical isolate was identified as *R. mucosa* by 16S rRNA gene sequencing.

Introduction

Roseomonas is a recently proposed genus of pink-pigmented, oxidative, short Gram-negative rods that includes five named species, *Roseomonas gilardii* (including *Roseomonas gilardii* subsp. *rosea* and *Roseomonas gilardii* subsp. *gilardii*), *Roseomonas cervicalis*, *Roseomonas fauriae*, *Roseomonas mucosa* and *Roseomonas lacus*, and three unnamed *Roseomonas* genomospecies, 4, 5 and 6 (Rihs *et al.*, 1993; Han *et al.*, 2003; Jiang *et al.*, 2006). However, *R. fauriae* and genomospecies 6 have now been placed in the genus *Azospirillum*, which consists of plant growth-promoting bacteria (Cohen *et al.*, 2004).

Although the natural reservoir of *Roseomonas* species remains unknown, isolates have been recovered occasionally from various environmental water sources (Rihs *et al.*, 1993). Clinical isolates have been recovered from blood, wounds, exudates, abscesses, genitourinary sites, chronic ambulatory peritoneal dialysis fluid, corneal scrapings and bone (Nahass *et al.*, 1995; Struthers *et al.*, 1996; Sandoe *et al.*, 1997; Tabin *et al.*, 2001). However, according to Struthers *et al.* (1996), only 60% of isolates are associated with disease. Here, we describe a case of central venous catheter (CVC)-related bacteraemia caused by *R. mucosa*, in a neutropenic patient with acute myelogenous leukaemia.

Case report

A 39-year-old man with a prior history of M-4 acute myelogenous leukaemia was admitted to our hospital for chemotherapy. On day 93, during the third cycle of consolidation therapy (cytarabine, fludarabine), the patient

developed rigors and a temperature of 39.5 °C. He had already been leukopenic ($0.2 \times 10^9 \text{ l}^{-1}$) but had not been receiving any antibiotic prophylaxis. Laboratory investigation revealed a haematocrit of 25%, 8.7 g haemoglobin dl^{-1} ; and a platelet count of $10 \times 10^9 \text{ l}^{-1}$. All other biochemical laboratory tests were unremarkable. Chest X-ray as well as CT-scan of brain, chest and abdomen showed no signs of infection. Urine, stool and sputum cultures were negative. The site of the CVC, which had been placed at the time of admission, appeared abnormal with evidence of inflammation (erythema, swelling, tenderness).

During the episode, a short, Gram-negative, non-vacuolated rod, appearing in pairs or small chains (Fig. 1), was isolated from three blood samples drawn through the CVC (without quantification of colonies). Unfortunately, blood samples through the peripheral vein were not obtained. The CVC was removed and a culture of the Hickman catheter tip again showed the same Gram-negative coccobacillus. The patient was treated with amikacin (1 g per day) and piperacillin-tazobactam (13.5 g per day) for 10 days. The patient became afebrile after the third day of therapy.

Microbiological investigation

Three sets of standard medium blood cultures in a total of six bottles were drawn within 24 h. As mentioned above, the patient was not receiving any antimicrobial agent at the time of collection. Cultures were processed with the BacT/ALERT System (bioMérieux). Bacterial growth was detected after 48 h incubation at 35 °C. Samples from three aerobic bottles grown on blood and chocolate agar formed pink, slightly mucoid colonies that did not autolyse after several days of incubation. The pink pigment, especially on chocolate agar, turned to red after 5 days of incubation. Growth also occurred at room temperature and at 42 °C, but not on

Abbreviation: CVC, central venous catheter.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence determined in this paper is AM161051.

Fig. 1. *Roseomonas mucosa*: Gram stain of blood culture showing Gram-negative coccoid rods in pairs and small chains (magnification $\times 1000$).

MacConkey or anaerobic blood agar. The colonies were catalase-, oxidase- and urease-positive.

The isolate was identified as *Roseomonas* sp. (bionumber 054604, probability 99.9%) by using RapidID NF Plus (Remel Laboratories), whereas the API 20NE (bioMérieux) computer database identified the organism as *Methylobacterium mesophilicum* (formerly named *Pseudomonas mesophilica*) (biotype 0201045, probability 97%). The clinical isolate was further differentiated from *M. mesophilicum* on the basis of Gram-stain morphology (non-vacuolated rod), negative reactions for acid production from methanol and acetate utilization, and the inability to absorb long-wave UV light (Rihs *et al.*, 1993).

In order to determine the primary structure of the 16S rRNA gene of the clinical isolate, chromosomal bacterial DNA was isolated. Chromosomal DNA extraction, PCR and sequencing of the 16S rRNA gene were performed as described by Woo *et al.* (2000). The primers ROSEF1 (5'-ATCCTGGCTCAGAGCGAACG-3') and ROSER1 (5'-CCCTACGGCTACCTTGTACG-3') were used for PCR. The PCR product, 1447 bp in size, was cloned in a pGEM-T plasmid vector (Promega) and sequenced using universal primers at least twice for both strands. The entire procedure was repeated and the PCR product was again sequenced. The nucleotide sequence of the PCR product (GenBank accession no. AM161051) was compared with known 16S rRNA gene sequences by BLAST analysis. Genetic analysis confirmed that the isolate belongs to the genus *Roseomonas*, as the 16S rRNA gene sequence was 93–99.5% identical to those of *Roseomonas* spp. and 84–94% identical with other members of *Methylobacteriaceae*. With regard to the species

level, the highest identity score occurred with the strains '*Candidatus Roseomonas massiliae*' 1461A (GenBank accession no. AF531769) and *R. mucosa* (AF538712). The 16S rRNA gene sequences of both were 99.7% identical to that of the isolate, with four and five bases difference, respectively. In contrast, 99.0% identity occurred with *R. gilardii* subsp. *rosea* (GenBank accession no. AY220740) and *R. gilardii* strain E9464 (AY150051), with 14 and 17 bases difference, respectively; the identity score with *R. cervicalis* and *R. fauriae* was 93%.

Multiple alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program (Thompson *et al.*, 1994) and phylogenetic trees were constructed using neighbour-joining, minimum evolution and parsimony analyses with MEGA version 3 software (Kumar *et al.*, 2004). The isolate clustered with *Roseomonas* spp., as shown in Fig. 2. Bootstrap values of 99, 99 and 90% in the neighbour-joining, minimum evolution and parsimony analyses, respectively, supported the branch separating *Roseomonas* spp. (including the isolate) from their closest relatives, *Craurococcus roseus* and *Paracraurococcus ruber*. More interestingly, the isolate was clearly different from the type strain of *R. gilardii* subsp. *rosea* and *R. gilardii* strain E9464 (data not shown), as well as from '*Candidatus Roseomonas massiliae*', *R. mucosa* and *R. gilardii* ATCC 49956^T with bootstrap values of 100, 100 and 98% in the neighbour-joining, minimum evolution and parsimony analyses, respectively (Fig. 2).

Antimicrobial susceptibility testing of the isolate was carried out by using the panel Neg MIC Type 30 (MicroScan), according to the manufacturer's guidelines. MICs were read after 48 h incubation (Sandoe *et al.*, 1997; Nolan & Waites, 2005) using Clinical Laboratory Standards Institute interpretive criteria for non-fermentative Gram-negative bacteria (Clinical Laboratory Standards Institute, 2006). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls. The isolate was found to be sensitive to ciprofloxacin ($\leq 0.5 \mu\text{g ml}^{-1}$), gentamicin, tobramycin, imipenem and meropenem ($\leq 1 \mu\text{g ml}^{-1}$), levofloxacin and gatifloxacin ($\leq 2 \mu\text{g ml}^{-1}$), tetracycline and amikacin ($\leq 4 \mu\text{g ml}^{-1}$), cefepime ($4 \mu\text{g ml}^{-1}$) and chloramphenicol ($\leq 8 \mu\text{g ml}^{-1}$), and resistant to ceftazidime, aztreonam ($\geq 32 \mu\text{g ml}^{-1}$), cefotaxime ($\geq 64 \mu\text{g ml}^{-1}$), piperacillin-tazobactam ($\geq 128/4 \mu\text{g ml}^{-1}$), ticarcillin-clavulanic acid ($\geq 128/2 \mu\text{g ml}^{-1}$) and trimethoprim-sulfamethoxazole ($\geq 4/76 \mu\text{g ml}^{-1}$). Given that there are no published methodological guidelines or interpretive breakpoints for MICs of antimicrobial agents for *Roseomonas* spp. (Clinical Laboratory Standards Institute, 2006) and conflicting results regarding the sensitivity of *Roseomonas* isolates to β -lactams is not an uncommon finding (Rihs *et al.*, 1993; Lewis *et al.*, 1997; McLean *et al.*, 2005), the MICs of β -lactams were confirmed by using Etest (AB Biodisk). With the exception of cefepime and carbapenems, the isolate was resistant to all β -lactams tested.

Fig. 2. Phylogenetic tree showing the relationship of the isolate (*Roseomonas* sp. GCSP1Z) with related sequences. The tree was constructed by using the neighbour-joining method, based on nearly complete sequences (1413 nt) of 16S rRNA genes. Bootstrap values resulting from 100 replications are given at branch points. Bar, 0.02 nucleotide changes per nucleotide position. *Staphylococcus aureus* was used as an outgroup.

Discussion

Enzymic activities and the ability to utilize various carbohydrates vary among the species and strains of *Roseomonas*. Therefore, definite identification based on acidification of various carbohydrates is difficult, inaccurate and time-consuming. The RapidID NF Plus system identifies *Roseomonas* to the genus level with accuracy (Nolan & Waites, 2005). Conversely, other commercial biochemical systems, such as API 20NE, can misidentify the organism (Schorch *et al.*, 1999; Elshibly *et al.*, 2005). Therefore, a sequence-based typing approach, such as analysis of 16S rRNA genes, offers benefits for the identification of *Roseomonas* to the species level (Nolan & Waites, 2005).

To our knowledge, 78 definite cases of *Roseomonas* bacteraemia have been reported in the literature. *R. gilardii* (39.7%) and *R. mucosa* (29.5%) were the two most common species (Schorch *et al.*, 1999; Han *et al.*, 2003; Dé *et al.*, 2004; Elshibly *et al.*, 2005; McLean *et al.*, 2005). Given that only 37 clinical isolates have been characterized by using molecular methods (Han *et al.*, 2003; Elshibly *et al.*, 2005), the clinical and epidemiological significance of *Roseomonas* species remains relatively unknown. CVC-related bacteraemia is the most common clinical syndrome due to *Roseomonas* species, particularly in people with underlying malignancies or other chronic diseases (Dé *et al.*, 2004). The production of biofilm on foreign materials may play an important role in the virulence of invasive infections due to species with a 'mucoid phenotype' (Elshibly *et al.*, 2005). The outcome of *Roseomonas* CVC-related bacteraemia is favourable, even in patients with neutropenia. Therapy does not usually require removal of an indwelling device (Dé *et al.*, 2004).

The choice of an effective drug for empirical treatment of infections due to *Roseomonas* spp. is sometimes difficult. According to the results of a recent review (Dé *et al.*, 2004), the most active agents against *Roseomonas* species are amikacin and imipenem ($\geq 99\%$ susceptibility), followed by ciprofloxacin (90%) and ticarcillin (83%). Conversely, antibiotics such as third- or fourth-generation cephalosporins are not appropriate for treating infections due to this organism. Susceptibility varies among the different species;

R. mucosa has the highest risk of resistance whereas *R. gilardii* strains are the most susceptible (Han *et al.*, 2003).

The 16S rRNA gene sequence of our isolate was 99.7% identical to that of a strain provisionally named 'Candidatus *Roseomonas massiliae*' and a strain of *R. mucosa* (four and five bases difference, respectively). To date, 'Candidatus *Roseomonas massiliae*' has only been recovered by amoebal co-culture, from the nasal mucosa of a healthy homeless man (Greub *et al.*, 2004). Our clinical isolate had remarkable phenotypic differences from *R. mucosa* strains studied by Han *et al.* (2003). (i) Colonies turned to red after several days of incubation, a phenotypic characteristic observed with strains of *R. gilardii* subsp. *rosea* and *Roseomonas* genomospecies 4. In addition, the colonies were slightly mucoid and did not autolyse after several days of incubation. (ii) API 20NE reaction code matched with two (9%) of the 22 *R. mucosa* strains examined in case weakly positive reactions were excluded. (iii) The isolate was cefepime-sensitive. It is worth noting that the patient discussed here was successfully treated with amikacin and piperacillin-tazobactam, in spite of the *in vitro* resistance of the clinical isolate to piperacillin-tazobactam.

In conclusion, our case underlines the clinical significance of *Roseomonas* species, particularly in the presence of an indwelling device. Differences in susceptibility patterns and virulence among the various species highlight the importance of definite identification of *Roseomonas* isolates. Broad-spectrum antibiotics (carbapenems and possibly quinolones) or combination therapy (including an aminoglycoside) is the first choice for the empirical treatment of *Roseomonas* infections. In contrast, consideration of coverage with a third- or fourth-generation cephalosporin would not be reasonable.

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