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Roseomonas mucosa Isolated from Bloodstream of the MDS Patient

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Roseomonas **is a recently proposed genus of pink-pigmented, nonfermentative, short gram-negative rod-bacteria which have been recognized as a cause of human infections. We report a case of bacteremia associated with *Roseomonas mucosa* isolated from an patient with a history of nodular goiter and multiple episodes of blood transfusion.**

Key words: *Roseomonas mucosa*, myelodysplastic syndrome, infection.

Roseomonas includes five named species, *Roseomonas 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). The genus *Roseomonas* (Rihs *et al*., 1993) was isolated frequently from environmental samples such as drinking water distribution systems (September *et al*., 2004), oil wells, biological soil crust, agriculture drainage water, as well as from water (Rihs *et al*., 1993). These facts indicate that members of the genus *Roseomonas* could occur widely in nature.

Although the isolates of Roseomonas have been recovered occasionally from various environmental sources. Clinical isolates caused human infections which 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; Bibashi *et al*., 2000; Subudhi et al., 2001,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 bacteraemia caused by R. mucosa, in the patient with Myelodysplastic Syndrome (MDS).

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MATERIALS AND METHODS

Case report

In April 2011, an 80-year-old woman, presented to our hospital Emergency Department with a one-day history of fever, malaise, and decreased activity level, and then was admitted to the Observance Department for suspected infection. The patient had a history of nodular goiter and myelodysplastic syndrome(MDS). In addition to having multiple incidences of respiratory tract infections with fermentation genus

and *Enterococcus* species, the patient has also had multiple episodes of bacteremia in the past 3 years with *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis*, and another unidentified Gram-negative bacillus. In the 2009-2011 period, she had been hospitalized in our hospital Hematology Department for several times due to the infection. After given antibiotics, blood transfusion and supportive treatment, the symptoms improved and she discharged. The patient's last admission to our hospital was 4 months ago for Broviac catheter-related bacteremia with the unidentified Gram-negative bacillus and Severe anemia and infection. For these past infections, the patient was treated with extended-spectrum cephalosporins, gentamicin, and ciprofloxacin. Blood and urine samples were collected for culture, and the patient was empirically treated with vancomycin and ceftriaxone.

RESULTS

Samples of two BactT/Alert aerobic blood culture bottles (bio-Me´rieux, France) were drawn from peripheral venous within a 24-hour period of fever. Bacterial growth was detected in the two aerobic bottles on third day of incubation. Short Gramnegative bacilli were detected by Gram staining, and the organism was subcultured in 5% sheep blood agar, chocolate agar, and MacConkey agar and incubated at 35°C in 5% CO_2. The next day, Growth on the 5% sheep blood agar and chocolate agar revealed the tip size of the flat, transparent colonies, On the third day of culture,

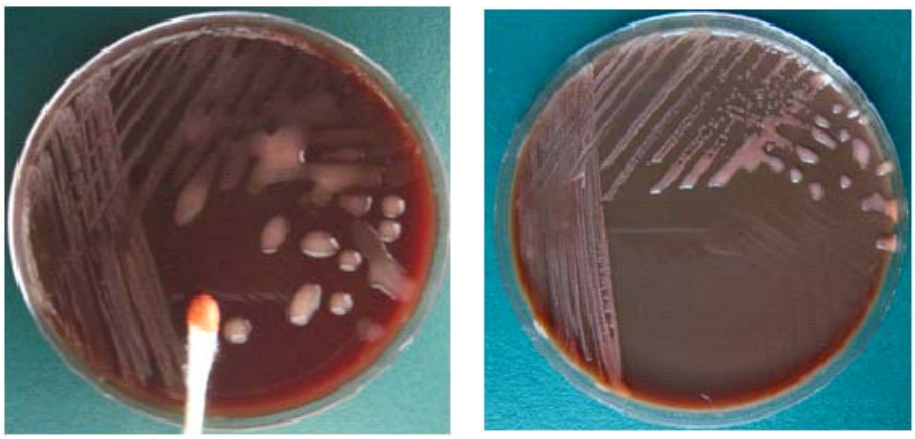

Fig. 1. Pink, mucoid colonies of Roseomonas mucosa on SAB agar(1A) and chocolate agar(1B) on 4-day culture

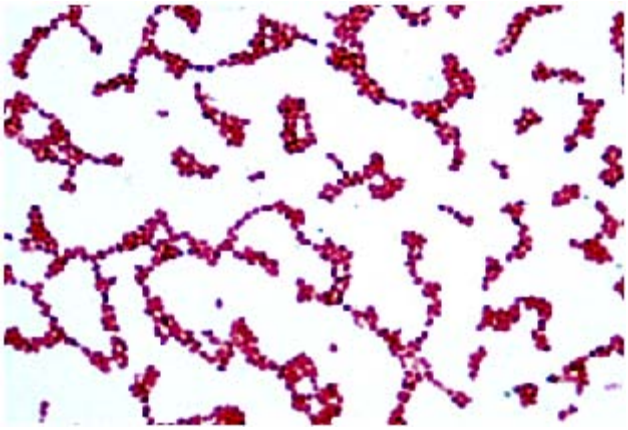

Fig. 2. Roseomonas mucosa: Gram stain of blood culture showing Gram-negative coccoid rods in pairs and small chains (magnification s%1000)

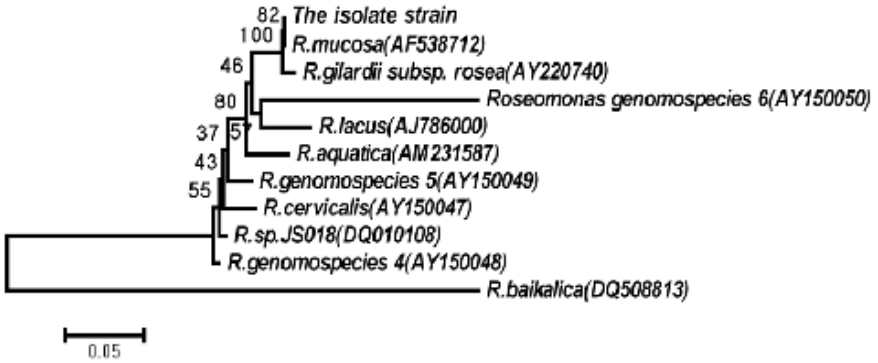

Fig. 3. Phylogenetic tree constructed using the neighbour-joining method based on 16S rRNA gene sequences of the isolate strain and related bacteria. Numbers at nodes indicate bootstrap support based on 1000 resampled datasets

there were slightly pink, mucoid colonies of different sizes growing on the 5% sheep blood agar and chocolate agar (Fig. 1). While no growth was observed on MacConkey agar. Moreover, the colonies had a weak oxidase-positive reaction, a urease-positive reaction and a lack of fluorescence following UV light exposion, and In the light microscope showed a short, Gram-negative, non-vacuolated rod, appearing in pairs or small chains (Fig. 2), which suggested that the organism may belong to *Roseomonas* species. The laboratory workup of the isolate was pursued further, the organism was reported as *Bordetella bronchiseptica* based on identification by a Vitek 2 system (bioMe´rieux), but API 20NE (bioMe´rieux) identified the organism as *Ochrobactrum anthropi*, with biochemical coding 0245045.

To obtain a definitive identification of the isolate, 16S rRNA gene sequencing was performed. Extraction of the bacterial genomic RNA, amplification of the 16S rDNA by real-time polymerase chain reactions, and subsequent sequencing of the amplicons were performed. The region of the 16S rRNA gene was amplified using a set of universal bacterial primers: 5'-AGAGTTTGATCCTGGCTCAG-3'(corresponding to positions 8-27f of *Escherichia coli* J01859) and 5'-TTAAGGATGGTGATGCCGCA-3' (corresponding to positions 1523-1504r of *Escherichia coli* J01859). Sequencing data analysis showed that the isolate strain was phylogenetically related to the genus Roseomonas, with the greatest similarity to *R. mucosa*, followed by *R. gilardii subsp. rosea*. A neighbour-joining tree based on these 16S rRNA gene sequences was constructed, which showed that the isolate strain and other species of the genus Roseomonas(Fig. 3). Thus, the isolate strain was identified as *Roseomonas mucosa* (100% identity by BLAST search).

Antimicrobial susceptibility test of the isolate was performed by the E-test (AB Biodisk, Solna, Sweden) according to Clinical and Laboratory Standards Institute recommended methods (CLSI 2011). Mueller-Hinton agar plates and an inoculum equivalent to that of an 0.5 McFarland standard suspension were used according to manufacturer's guidelines. MICs were read 24 h after inoculation by using the CLSI 2011 interpretative criteria for nonfermentative Gram-negative bacteria. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls. The isolate was susceptible to the following antibiotics (as the MICs shown): amikacin (≤0.5 μg/ml), gentamicin (≤0.5μg/ml), imipenem (≤0.5μg/ml), meropenem (4μg/ml), and tobramycin (1μg/ml). It was resistant to ceftazidime (>32μg/ml), cefoperazone (>32μg/ml), piperacillin-tazobactam (>128/4μg/ml), trimethoprim-sulfamethoxazole (>4/80μg/ml), levoûoxacin (>8μg/ml), and ciproûoxacin (2μg/ml). The patient was treated on 0.25g intravenous drip imipenem 3 times a day. After 5-day course of imipenem, symptoms were improved, and the patient was discharged home.

DISCUSSION

Roseomonas which is a bacterial genus of slow-growing, pink-pigmented, oxidative, and

aerobic Gram-negative coccobacilli is oxidase positive. It derives its name because of artificial media to produce pink pigment. It has been originally isolated from clinical blood, wound secretions, exudate, ulcers, urogenital tract and other clinical specimens for several decades. The genus and its species were ûrst proposed in 1993 to include three named species (*R. gilardii, R. cervicalis, R. fauriae* and three unnamed species(Rihs *et al*., 1993). Subsequent studies found that *Roseomonas spp. was* widespread in air, water, soil substandard environment. The genus currently include: *Roseomonas aerilata, Roseomonas aquatica, Roseomonas frigidaquqe, Roseomonas laucs, Roseomonas ludipueritiae, Roseomonas rosea, Roseomonas stagni, Roseomonas terrae,* and *Roseomonas vinace* 9 microorganisms in environmental *Roseomonas* species isolated, and the other new clinical disease types of *R. mucosa* subspecies rosea were proposed by ((Rihs *et al*., 1993; Han *et al*., 2003).

In China, only a few isolated cases reported so far that *Roseomonas* were internationally recognized and named *Roseomonas laucs* and *Roseomonas vinace* (Jiang *et al*., 2006; Liu *et al.,* 2010; Cao *et al.,* 2011; Zhang *et al.,* 2008) ever, there was no *Roseomonas* isolated from clinical reports in China. It may be relatively new with the *Roseomonas* and the limited correlation detection method. In our case, we reported the first documented case of bloodstream infection due to *Roseomonas mucosa*. The patient was admitted to hospital because of goiter in June 2008, test results showed that the last two years, patients with peripheral blood (PB) trilinear pancytopenia, the morphological analysis of the PB smear showed anisopoikilocytosis of erythrocytes and platelets, along with hypogranularity and nuclear hyposegmentation of granulocytes; no circulating immature cells were found. BM smear showed a marked trilinear dysplasia with 15% blasts, cytogenetic analysis showed a normal karyotype, and diagnosed as Myelodysplastic syndrome (MDS). 2008-2011 due to myelodysplastic syndrome and infection, the patients hospitalized several times, but did not alleviate the anemia. On April 6, 2011, the MDS patient was admitted to emergency Department in hospital again. The major symptoms were fever and malaise. The preliminary blood culture results were reported as *B. bronchiseptica* by the Vitek 2 system, while API 20NE (bioMe´rieux) identified the organism as *Ochrobactrum anthropi*, with biochemical coding as 0245045. But it was later confirmed as *Roseomonas mucosa* on the basis of biochemical testing and 16S rRNA gene sequencing. Since the API 20NE and Vitek 2 system did not identify the Roseomonas species, the clinical isolate from the patient was very unreactive with the biochemical tests on the card, only urease activity was positive, and assimilates glucose (GLU), arabinose (ARA), mannitol (MAN), malate (MLT), and citrate (CIT), so even though the quality of identification was excellent, the initial identification was not believed. Morphology, biochemical tests and 16S rRNA gene sequencing were the key methods of identification for these species. And the result confirmed *R. mucosa* infection. Similar to other presentations of *Roseomonas* infection, as antibiotic treatment of *Roseomonas* can be challenging due to the high risk of resistance (Jiang *et al*., 2006). According to the results of drug sensitivity test, The *R. mucosa* strain isolated from our patient revealed an antimicrobial susceptibility pattern, which was not completely consistent to that of other *Roseomonas spp*. previously reported (Jiang *et al*., 2006). This organism is typically resistant to quinolones. So the patient was treated with imipenem, at the same time, 5-azacytidine (75 mg/m^2) was offered., and the transfusion support was given. several days later, clinical improvement, and the *R. mucosa* isolate was cleared from our patient's bloodstream and the cytopenia gradually improved until near-normalization of PB counts and complete transfusion independence, which were achieved after the third course of 5-azacitidine. To date, although BM dysplastic features persisted; her hemogram is near-normal.

To our knowledge, this is the first report of bacteremia case due to *Roseomonas mucosa* in China in a MDS patient, with initial misidentification as B. bronchiseptica based on results from the Vitek 2 system and as Ochrobactrum anthropi based on results of API 20NE. And it was subsequently corrected via 16S rRNA gene sequencing testing. This organism is an uncommon pathogen in the healthy paediatric population(Marin *et al*., 2001; Dé *et al*., 2004), due to neutropenia and dysfunction easier lead to infectionand and the MDS patient infected

Roseomonas mucosa suggests that may be associated with lower immunity related.

CONCLUSION

Some causative organism should be identified by using advanced diagnostics and genotyping by a microbiology reference laboratory, when it cannot be identified based on Clinical laboratory bacterial biochemical identification method or other routine laboratory bacterial identification technology.

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