

RESEARCH NOTE

ROSEOMONAS GILARDII SUBSP ROSEA, A PINK BACTERIUM ASSOCIATED WITH BACTEREMIA: THE FIRST CASE IN THAILAND

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Abstract. *Roseomonas* is a pink-pigmented, non-fermentative, gram-negative coccobacillus bacterium. Human infections caused by *Roseomonas* are very rare. We report the first case of bacteremia associated with *Roseomonas gilardii* subsp *rosea* in Thailand. The bacterium was isolated from blood culture and identified by cellular morphology, characteristics of colonies on blood agar, extensive biochemical tests and 16S ribosomal DNA sequencing.

INTRODUCTION

Roseomonas is a genus of gram-negative bacterium that had previously been designated as "pink-pigmented coccobacillus" groups I through IV (Wallace *et al*, 1990; Rihs *et al*, 1993; Lewis *et al*, 1997) by the Special Bacteriology Reference Laboratory of the Centers for Disease Control and Prevention (CDC, Atlanta, USA). On the basis of biochemical reactions and DNA relatedness of pink coccobacillus isolates, *Roseomonas* contains six species: *R. gilardii*, *R. cervicalis*, *R. fauriae*, and unnamed genomospecies 4, 5 and 6 (Rihs *et al*, 1993). Of these six, *R. gilardii* is most frequently related to human infections. *Roseomonas* has been shown in recent years to be uncommon, yet potentially of clinical importance as an opportunistic pathogen that

can cause disseminated infections, particularly in debilitated hosts. It is most commonly isolated from the blood, in association with clinical signs of sepsis. Previous studies reported that in 88% of *Roseomonas* case reports, the organism was isolated from the blood. For other sites of infection, respiratory problems occurred in 16% of cases, wound infection or bone disease occurred in 8%, peritonitis in 4%, enteritis and/or abdominal pain in 8%, kidney transplant infection in 4%, and eye infection in 4% (Rihs *et al*, 1993; Struthers *et al*, 1996; Lewis *et al*, 1997; Sandoe *et al*, 1997). Nosocomial ventriculitis due to *R. gilardii* has also been reported (Nolan and Waites, 2005).

From previous reports (Euzeby, 2003; Han *et al*, 2003), the bacteriologic characterization of 36 *Roseomonas* strains isolated from blood revealed some strains showing significant differences phenotypically and genotypically from *R. gilardii*. Therefore, two novel species were proposed. First, *R. gilardii* subsp *rosea* was proposed for strains that have minor differences from *R. gilardii* and display

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obvious pink to red colonies. Secondly, *R. mucosa* subsp *nov* has been proposed as a new species of *Roseomonas* to indicate its prominent mucoid, almost runny colonies.

We report a case of *R. gilardii* subsp *rosea* associated with bacteremia, the first case in Thailand. This article aims to increase recognition of this rare pathogen.

MATERIALS AND METHODS

Case history

An 80-year old man was referred to Siriraj Hospital (2,400 beds), Faculty of Medicine, Mahidol University, suffering from high fever and altered consciousness. Two months prior to this admission, he was admitted to a local government hospital because of peptic ulcer perforation. After surgery, he developed hospital-acquired pneumonia and a tracheostomy tube was needed. The infection was treated with intravenous ceftriaxone and responded well. Four days before his admission to Siriraj Hospital, still admitted in the local hospital, he developed a high grade fever and altered consciousness. Septicemia was diagnosed when he was referred to Siriraj Hospital. On admission, his temperature was 40°C. Chest x-ray revealed normal lungs. Hemoculture was collected to identify the pathogenic bacteria using standard microbiological techniques (Thomson and Miller; 2003). His white blood cell count was 18,400 cell/mm³, with 83% neutrophils. Imipenem was given and he responded well to treatment.

Microbiology

The patient's blood was inoculated into conventional hemoculture broth at a ratio of 1:5. After 6 hours of incubation, bacteria responsible for the clinically significant infections were present in numbers large enough to recover by blind subculture. The latter was performed by aseptically removing a few drops of well-mixed media and spreading it onto

blood agar. The plate was incubated in 5% CO₂ at 35°C for 3 days. Culture-negative bottles were reincubated for 7 days. Each bottle was examined daily during incubation for evidence of growth, indicated by turbidity, hemolysis or the presence of small colonies. *Roseomonas* was isolated and identified based on cellular morphology; characteristics of colonies on blood agar, chocolate agar, Mueller Hinton agar, Sabouraud dextrose agar; and various biochemical tests (Wallace *et al*, 1990; Rihs *et al*, 1993; Struthers *et al*, 1996; Lewis *et al*, 1997; Sungkanuparph *et al*, 2000; Han *et al*, 2003).

Sequencing of 16S rDNA and phylogenetic tree analysis

We attempted to confirm the identification of this isolate, designated as strain CMU_SI by molecular technique. Extraction of the bacterial genomic DNA, amplification of the 16S rDNA by polymerase chain reaction, and subsequent sequencing of the amplicons were performed as described previously (Edwards *et al*, 1989; Eden *et al*, 1991; Weisburg *et al*, 1991; Kolbert *et al*, 2004). This strain was amplified and sequenced, and the sequence data of 693-base pairs was compared to the GenBank for best matches. The phylogenetic tree was constructed using the Clustal X program and Phylip 3.66 software package.

Other tests

The commercial identification system used for non-fermentative bacteria, the API 20 NE (bioMerieux, France), was tested for this strain. Antimicrobial susceptibility testing was performed on Mueller Hinton agar plates by the disk diffusion method (CLSI, 2006). The 19 antimicrobial agents used in this study were: ampicillin/amoxicillin, amoxicillin + clavulanate, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, sulbactam/sulfooperazone, tazobactam/piperacillin, amikacin, gentamicin, netilmicin, ciprofloxacin,

co-trimoxazole, imipenem, meropenem, ertapenem and vancomycin. *Escherichia coli* ATCC 25922 was used as a quality control strain.

RESULTS

Aerobic hemoculture yielded a pure growth of mucoid, reddish pink colonies (Fig 1) after 1 day of incubation on blood agar at 35°C. The colonies were opaque, circular, smooth, with a butyrous consistency, approximately 1 mm in diameter. The organism grows well on Mueller Hinton agar, but develops larger colonies on blood agar. There was no hemolytic activity. The pink pigment was seen more easily on Mueller Hinton agar. The Gram stain revealed a gram-negative coccobacillus bacterium appearing in pairs or short chains. Anaerobic culture was negative after 5 days of incubation.

For biochemical tests, growth occurred on chocolate agar (equally well comparable to blood agar), Sabouraud dextrose agar (much slower growth than Mueller Hinton agar) within 1-2 days at 25°, 30° or 42°C, but not on MacConkey agar. We found that this organism grew slowly on MacConkey agar; it needs 4 days at 35°C incubation before any growth was seen. The organism was catalase, urease, and citrate utilization positive. It was nonmotile and the triple sugar iron agar's reaction was K/N, with no H₂S. It was negative on the oxidase test, and for utilization of glucose, inositol, lactose, maltose, mannose, mannitol, raffinose, salicin, sorbitol, sucrose, trehalose, xylose, esculin, arginine dihydrolase, ornithine decarboxylase, nitrate reductase, and did not grow in 6.5% sodium chloride broth. It was differentiated from *Methylobacterium* species, another pink-pigmented rod, by the inability of the colony to absorb long-wave UV light and nonvacuolated on Gram stain. Based on cellular morphology and biochemical tests, our



(a)



(b)

Fig 1—*Roseomonas gilardii* subsp *rosea*: mucoid colonies on (a) blood agar (b) Mueller Hinton agar.

organism was identified as *R. gilardii* subsp *rosea*.

From molecular technique by gene sequencing, the results showed the 16 S rDNA of this strain is closely related to or could be a variant of *R. gilardii* 1 or *R. gilardii* 2. The phylogenetic affiliation of the related taxa is shown in Fig 2.

As for the API 20 NE commercial system, the biotype code for this strain was 0201461, which did not match any organism except a 58.6% match with *Methylobacterium mesophilicum*.

The organism was sensitive to ampicillin/

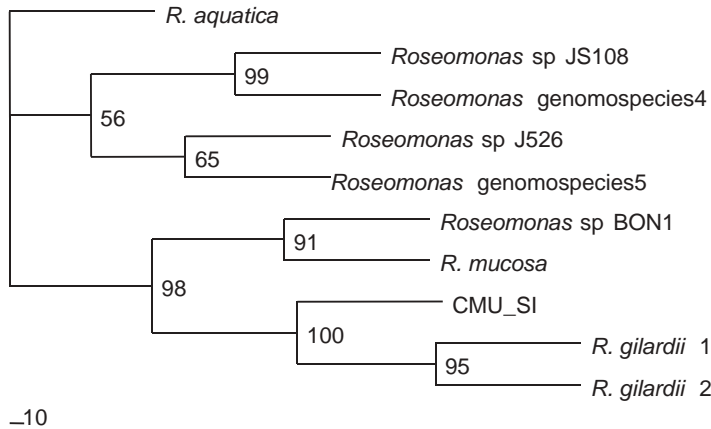


Fig 2—The phylogenetic tree using the first half of the 16S rRNA gene (693 bp) from DNA sequencing of the 16S PCR product from the tested strain against 9 other *Roseomonas* spp from the GenBank. The accession numbers are AM231587, *R. aquatica*; DQ010108, *Roseomonas* sp JS108; AY150048, *Roseomonas* genomospices 4; AY369981, *Roseomonas* sp J526; AF533356, *Roseomonas* genomospices 5; AY219712, *Roseomonas* sp BON1; AF538712, *Roseomonas mucosa*; AY220740, *R. gilardii* strain 1; AY150045, and *R. gilardii* strain 2. CMU_SI was the strain identified in this study. Numbers at the nodes obtained from bootstrap analysis that reflect percentages of particular strains are in the same nodes.

amoxicillin, amoxicillin + clavulanate, cefepime, ceftriaxone, sulbactam/cefoperazone, amikacin, gentamicin, netilmycin, ciprofloxacin, cotrimoxazole, imipenem, meropenem, ertapenem, but resistant to cefotaxime, ceftazidime, ceftazidime, cefuroxime, tazobactam/piperacillin and vancomycin.

DISCUSSION

Roseomonas was the most likely cause of illness in this case because the patient's systemic symptoms were at their height on the day the hemoculture became positive. No other organisms were found in hemoculture. This is consistent with other cases reported in the literature, all of which report that only one of several hemocultures grew out the organism (Korvick *et al*, 1989; Struthers *et al*, 1996). Our finding of the clinical response to its susceptible antibiotic (imipenem) also support a *Roseomonas* infection in this case. We would like to emphasize that *Roseomonas* is a rare human pathogen which may cause se-

rious infection. Since the patient was admitted to a local hospital for two months, the infection could have been nosocomial. According to previous reports, *Roseomonas* cases may be either community-acquired or nosocomial (Shokar *et al*, 2002; Nolan and Waites, 2005).

The natural reservoir for *Roseomonas* infection is unknown (Shokar *et al*, 2002). Although it has been isolated from water supplies (Rihs *et al*, 1993; Sandoe *et al*, 1997), it may also exist as a commensal in humans. However, most strains in case reports have been isolated from potentially significant clinical specimens, such as blood and pus (Gilardi and Aur, 1984; Wallace *et al*, 1990; Rihs *et al*, 1993; Sungkanuparph *et al*, 2000). We investigated the source of infection in this case but none was found. On physical examination, there were no other sites of infection. We did not perform surveillance cultures on the water supply in this case.

In a number of the cases of *R. gilardii* in-

fection reported in the literature, the initial symptoms were indicative of infection and bacteremia. Furthermore, 80% of patients reported had underlying disease, most commonly malignancy, followed by renal disease, inflammatory bowel disease, diabetes, or others (alcohol abuse, osteoarthritis, and circulatory insufficiency) (Shokar *et al*, 2002). However, the patient in our report had no underlying disease except old age.

Since the literature is very limited in regard to identification of *R. gilardii* subsp *rosea* in the clinical microbiology laboratory, this study may prove useful clinically and academically. This organism may be confused with *Rhodococcus* because it had slow growth on MacConkey agar (4 days of incubation), and on Gram stain, it was a gram-negative coccoid to short rod, sometimes chaining, and it occasionally retained some purple stain, leading the clinical microbiology laboratory to misidentify it as gram-positive bacteria (Han *et al*, 2003).

Based on the suggested guidelines for 16S rDNA analysis (Stackerbrandt and Goebel, 1994; Kolbert *et al*, 2004), we can identify our strain as *R. gilardii*. Our phylogenetic tree findings suggest that subspecies differentiation may not be achieved based only on the 16S rDNA sequence. Therefore, we identified the subspecies of our strain as *R. gilardii* subsp *rosea* according to routine methods *eg*, Gram stain and colonial morphology on various agar and extensive biochemical tests (Bibashi *et al*, 2000; Han *et al*, 2003).

The API 20 NE does not contain *Roseomonas* in its database. Therefore, the identification of each species in this genus by using the API 20 NE is not applicable.

Regarding antimicrobial susceptibility testing, according to the CLSI standards, there are no zone diameter interpretive standards for *Roseomonas* (CLSI, 2006). We used the zone diameter interpretive standards for Enterobac-

teriaceae for all drugs tested. According to previous reports, *R. gilardii* subsp *rosea* is usually susceptible to amikacin, ciprofloxacin and frequently susceptible to imipenem, ticarcillin-clavulanate, ampicillin, and ceftriaxone (Han *et al*, 2003). In our study, the results correlated well with these studies, but was partially different from a previous report at Ramathibodi Hospital (Sungkanuparph *et al*, 2000).

In summary, our report contributes to the recognition of *R. gilardii* subsp *rosea* for clinicians and microbiologists in Thailand. It may be useful for identification by clinical microbiologists and helps delineate its role as a human pathogen.

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REFERENCES

- Bibashi E, Sofianou D, Kontopoulou K, Mitsopoulos E, Kokolina E. Peritonitis due to *Roseomonas fauriae* in a patient undergoing continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 2000; 38: 456-7.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Sixteenth Informational Supplement 2006; 25(1), M100-S16.
- Eden PA, Schmidt TM, Blackemore RP, Pace NR. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *Int J Syst Bacteriol* 1991; 41: 324-5.
- Edwards U, Rogall T, Blocker H, Ende M, Bottger EC. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* 1989; 17: 7843-53.
- Euzéby J. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. *Int J Syst Evol*

- Microbiol* 2003; 53: 1701-2.
- Gilardi GL, Aur YC. *Pseudomonas mesophilica* and unnamed taxon, clinical isolates of pink-pigmented oxidative bacteria. *J Clin Microbiol* 1984; 20: 626-9.
- Han YX, Audrey S, Tarrand JJ, Rolston KV, Hesel LO, Levett PN. Bacteriologic characterization of 36 strains of *Roseomonas* species and proposal of *Roseomonas mucosa* sp. nov and *Roseomonas gilardii* subsp *rosea* subsp nov. *Am J Clin Pathol* 2003; 120: 256-64.
- Kolbert CP, Rys PN, Hopkins M, et al. 16 S ribosomal DNA sequence analysis for identification of bacteria in a clinical microbiology laboratory. In: Persing DH, Tenover FC, Vesalovic J, et al. eds. *Molecular microbiology : diagnostic principles and practice*. Washington DC: ASM Press, 2004: 361-90.
- Korvick JA, Rihs JD, Gilardi GL, Yu VL. A pink-pigmented, oxidative, nonmotile bacterium as a cause of opportunistic infections. *Arch Intern Med* 1989; 149: 1449-51.
- Lewis L, Stock F, Williams D, Weir S, Gill V. Infections with *Roseomonas gilardii* and review of characteristics used for biochemical identification and molecular typing. *Am J Clin Pathol* 1997; 108: 210-6.
- Nolan JS, Waites KB. Nosocomial ventriculitis due to *Roseomonas gilardii* complicating subarachnoid hemorrhage. *J Infect* 2005; 50: 244-51.
- Rihs JD, Brenner DJ, Weaver RE, Steigerwalt AG, Hollis DG, Yu VL. *Roseomonas*, a new genus associated with bacteremia and other human infections. *J Clin Microbiol* 1993; 31: 3275-83.
- Sandoe JA, Malnick TH, Loudon KW. A case of peritonitis caused by *Roseomonas gilardii* in a patient undergoing continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 1997; 35: 2150-2.
- Shokar NK, Shokar GS, Islam J, Cass AR. *Roseomonas gilardii* infection: case report and review. *J Clin Microbiol* 2002; 40: 4789-91.
- Stackerbrandt E, Goebel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 1994; 44: 846-9.
- Struthers M, Wong J, Janda JM. An initial appraisal of the clinical significance of *Roseomonas* species associated with human infections. *Clin Infect Dis* 1996; 23: 729-33.
- Sungkanuparph S, Vorachit M, Vibhagool A. *Roseomonas* bacteremia as a cause of febrile neutropenia: a case report. *J Infect Dis Antimicrob Agents* 2000; 17: 119-21.
- Thomson RB Jr, Miller JM. Specimen collection, transport, and processing : bacteriology. In : Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC, eds. *Manual of clinical microbiology*. 8th ed. Washington, DC: ASM Press, 2003: 286-330.
- Wallace PL, Hollis DG, Weaver RE, Moss CW. Biochemical and chemical characterization of pink-pigmented oxidative bacteria. *J Clin Microbiol* 1990; 28: 689-93.
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991; 173: 697-703.