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...
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/sulfoperazone, 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 25º, 30º or 42ºC, but not on MacConkey agar. We found that this organism grew slowly on MacConkey agar; it needs 4 days at 35ºC 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 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/
amoxicillin, amoxicillin + clavulanate, cefepime, ceftriaxone, sulbactam/cefoperazone, amikacin, gentamicin, netilmicin, ciprofloxacin, co-trimoxazole, imipenem, meropenem, ertapenem, but resistant to cefotaxime, cefoxitin, 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 serious 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; Sungkanuparp 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-
Infection 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 \textit{R. gilardii} subsp rosea in the clinical microbiology laboratory, this study may prove useful clinically and academically. This organism may be confused with \textit{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 \textit{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 \textit{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 \textit{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 \textit{Roseomonas} (CLSI, 2006). We used the zone diameter interpretive standards for Enterobacteriaceae for all drugs tested. According to previous reports, \textit{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 (Sungkanuparp et al., 2000).

In summary, our report contributes to the recognition of \textit{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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