Case Report

Isolation of *Aurantimonas altamirensis* from pleural effusions

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Received 10 February 2010
Accepted 15 June 2010

Here we report two cases of isolation of *Aurantimonas altamirensis* from pleural fluid and blood. The strains were identified by 16S rRNA gene sequencing. *A. altamirensis* appears to be a rare pathogen involved in unusual infectious processes, and must be isolated and studied at the molecular level for correct clinical diagnosis.

Case report

This paper reports two cases of isolation of *Aurantimonas altamirensis* from two human clinical samples. The first bacterial isolate was cultured from pleural fluid (Case 1) while the second isolate was recovered from both cultured blood and pleural fluid (Case 2). Both patients were diagnosed with pleural effusion.

The first case involved a 69-year-old male patient with numerous prior hospitalizations involving chronic ischaemic heart disease and grade III gastric adenocarcinoma (with total gastrectomy 1 year prior to hospitalization). On admission, the patient presented dysphagia, dyspnoea and chest pain. Significant findings on physical examination included dyspnoea [arterial oxygen saturation <95 %], and severe chest pain exacerbated by deep inspiration, decreased breath sounds and dullness on percussion over the right lung. The following tests were performed: blood workup [haemoglobin, 11 g dl⁻¹; platelets, 258 000 µl⁻¹; total white blood cell count, 4 × 10⁹ l⁻¹ with a differential count of 56.2 % neutrophils]; C-reactive protein (25.2 mg l⁻¹); creatinine (1.1 mg dl⁻¹); and urea (132 mg dl⁻¹). Results indicated normal coagulation profile, blood glucose, total cholesterol, high-density lipoprotein and triglycerides. Radiological evaluation of the thorax revealed slight bilateral pleural effusion.

Diagnostic thoracentesis was performed and 1.2 l pleural liquid with a serohaematic appearance was extracted. The clinical materials were submitted for biochemical, micro-

biological and cytological analyses. The biochemistry laboratory reported lactate dehydrogenase, 479 UI l⁻¹ (quotient lactate dehydrogenase in pleural liquid/serum >0.6, denoting an exudative nature); the exudate presented a predominance of polymorphonuclear cells, 5.1 g proteins dl⁻¹, 88 mg glucose dl⁻¹, 30.3 UI adenosine deaminase l⁻¹ and pH of 7.9. The cytological examination did not disclose atypical cells; however, the microbiological study revealed the presence of a Gram-negative coccobacillus.

In the second case, an 81-year-old man presented with a fever of 39 °C, chest pain and coughing for 7 days prior to hospitalization. Significant findings on physical examination included dyspnoea (arterial oxygen saturation <92 %), and severe chest pain exacerbated by deep inspiration, decreased breath sounds and dullness on percussion over the left lung. The laboratory analysis revealed: haemoglobin, 12 g dl⁻¹; platelets, 317 000 µl⁻¹; total white blood cell count, 11.4 × 10⁹ l⁻¹ with a differential count of 91.7 % neutrophils; and C-reactive protein, 600 mg l⁻¹. A posteroanterior chest radiograph revealed a consolidation in the left lung representing a passive atelectasis with a moderate amount of left pleural effusion.

A thoracentesis obtained serohaematic fluid, exudative by lactate dehydrogenase and protein levels (651 UI l⁻¹ and 4.4 g dl⁻¹, respectively), with a normal pH (7.4), predominantly polymorphonuclear cells (90 %), an adenosine deaminase level of 29.6 UI l⁻¹ and 135 mg glucose dl⁻¹. Atypical cells were not observed in the cytology analysis but again the microbiological analysis revealed a Gram-negative coccobacillus.

After thoracentesis, the patients were hospitalized and levofloxacin (500 mg l⁻¹) was administered intravenously.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequences of isolates PGE8817 and JGG6850/JGG68502 are FN658985 and FN658986, respectively.

This antibiotic was selected according to the clinical protocol followed in our hospital for this type of pathology.

Microbiology

In both cases, two sets of blood cultures were drawn [Becton Dickinson (BD) Bactec 9240 Blood Culture System]. In Case 1, both sets of aerobic blood culture bottles yielded a Gram-negative coccobacillus on the fourth day of hospitalization. In Case 2, the samples in the aerobic blood culture bottles had not grown by the seventh day of hospitalization.

In the pleural fluid of both patients, yellow, mucoid, convex and smooth colonies measuring about 1 mm in diameter were observed after 5 days of aerobic incubation. The incubation of pure culture was carried out on sheep blood agar (5%), chocolate agar and MacConkey agar (BD).

In both patients, the organism was initially identified using the BD Phoenix Automated Microbiology System and MicroScan Walk-Away (Dade Behring). Conventional biochemical tests showed that the isolates were catalase- and oxidase-positive and gave a negative result in the indole test, while the automated methods identified the strains as *Wautersia pauculus* (now called *Cupriavidus pauculus*) and 'CDC group EO-2' (now called *Paracoccus yeei*). The isolates showed susceptibility to levofloxacin (5 mg), ampicillin (10 mg), chloramphenicol (30 mg), rifampicin (5 mg), tetracycline (30 mg), norfloxacin (10 mg), novobiocin (30 mg), streptomycin (10 mg), carbenicillin (100 mg) and erythromycin (15 mg) (BBL Sensi-Disc Antimicrobial Susceptibility Test Discs; BD). Susceptibility was assessed by the methods described by the CLSI (2006, 2008). However, there is no standard methodology for testing this species for susceptibility. The patients were administered levofloxacin (500 mg) and clinical and radiological improvements were observed in both cases.

The BD Phoenix and MicroScan Walk-Away identification systems could not provide adequate identification; therefore, the isolates were forwarded to another centre for further characterization.

The three isolates, two from Case 1 and one from Case 2, were identified as *Aurantimonas altamirensis* by 16S rRNA gene sequencing at the Instituto Valenciano de Microbiología, Valencia (Betera), Spain, and the Instituto de Recursos Naturales y Agrobiología, CSIC, Sevilla, Spain.

The isolates from Case 1 were designated JGG6850 and JGG68502 (GenBank accession no. FN658986) and the isolate from Case 2 was designated PGE8817 (GenBank accession no. FN658985).

The methods used in this study were described previously by Jurado *et al.* (2006). Phenotypic characteristics of strains

PGE8817, JGG6850/JGG68502 and *A. altamirensis* are shown in Table 1.

Bacterial DNA was extracted following the method described by Marmur (1961). The 16S rRNA gene was amplified by PCR using the conserved primers 27F (5'-AGA GTT TGA TCC TGG CTC AG) and 1522R (5'-AAG GAGGTG ATC CAG CCG CA). PCR thermal conditions were as follows: 95 °C for 1 min; 35 cycles of 95 °C for 15 s, 55 °C for 15 s, 72 °C for 2 min; and a final extension cycle at 72 °C for 10 min. Forward and reverse strands of the amplified DNA fragment were sequenced in an ABI 3700 sequencer (Applied Biosystems). A similarity search was performed using the BLAST algorithm (Altschul *et al.*, 1990) at the NCBI database (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/>). For phylogenetic analysis, nucleotide

Table 1. Phenotypic characteristics of strains PGE8817 (GenBank accession no. FN658985), JGG6850/JGG68502 (GenBank accession no. FN658986) and *A. altamirensis*

Strains: 1, *A. altamirensis* CECT 7138^T; 2, PGE8817; 3, JGG6850/JGG68502. –, Negative; +, positive; (+) weakly positive.

Characteristic	1	2	3
Nitrate reduction	–	–	–
Catalase reaction	+	+	+
Oxidase test	+	+	+
Acid produced from:			
D-Arabinose	(+)	(+)	(+)
L-Arabinose	+	+	+
Erythritol	+	+	+
L-Fucose	(+)	(+)	(+)
D-Galactose	+	+	+
Gentiobiose	–	–	–
D-Mannose	+	+	+
Melibiose	(+)	(+)	(+)
L-Rhamnose	+	+	+
D-Ribose	+	+	+
D-Xylose	+	+	+
L-Xylose	–	–	–
Assimilation of:			
Arabinose	+	+	+
Glucose	+	+	+
Maltose	+	+	+
Mannitol	+	+	+
Mannose	+	+	+
N-Acetyl-D-glucosamine	+	+	+
Potassium gluconate	+	+	+
Enzymic activity			
Alkaline phosphatase	+	+	+
Esterase	+	+	+
Esterase lipase	+	+	+
Leucine arylamidase	+	+	+
Trypsin	+	+	+
Acid phosphatase	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	+
Urease	+	+	+

sequences were aligned using the program CLUSTAL W (Larkin *et al.*, 2007) with the corresponding sequences of representative *Aurantimonas* species from the GenBank (16S rRNA) database.

Phylogenetic trees were reconstructed using the software package MEGA3 (Kumar *et al.*, 2004). The overall topologies of neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods were similar (data not shown). The robustness of the trees was assessed by bootstrap resampling (1000 replicates). Representative *Aurantimonas* strains EU544515, EU442517, EU442518, EU697964 and EF595814 (Lagrange, 2009; Mendes *et al.*, 2009; Luong *et al.*, 2008) were found to have 99.4% or greater similarity to the *A. altamirensis* type strain (GenBank accession no. DQ372921). The results of our phylogenetic analysis showed that these strains fell within the evolutionary radiation encompassed by the genus *Aurantimonas*. In the phylogenetic tree based on the 16S rRNA gene sequence (Fig. 1), both strains from our cases formed a clade with the type strain of *A. altamirensis*, which is supported by a bootstrap value of 100% in the neighbour-joining analysis.

Discussion

Both Case 1 and Case 2 were identified as pleural effusion caused by *A. altamirensis*. This bacterium belongs to the 'Aurantimonadaceae', a small family of marine bacteria; the

first described species was *Aurantimonas coralicida*, the causative agent of white plague type II on Caribbean corals (Denner *et al.*, 2003). *A. altamirensis* was first isolated in a subterranean environment, the Altamira Cave (Cantabria, Spain), from white colonies thriving on the walls of the cave (Jurado *et al.*, 2006). Furthermore, *A. altamirensis* was isolated from human clinical samples from four different patients in Canada and the USA between 2006 and 2008. The bacterium was isolated from different clinical samples, such as: a contact lens and the lens cleansing solution from a patient with keratitis; culture of corneal scrapings; the sputum of a cystic fibrosis patient (Luong *et al.*, 2008); and a bloodstream infection (Mendes *et al.*, 2009).

The clinical relevance of *A. altamirensis* remains uncertain. *A. altamirensis* has not previously been isolated from pleural effusions, and only one prior report concerning the respiratory system (cystic fibrosis) has been published (Luong *et al.*, 2008). The bacterium is considered a contaminant found in environmental and/or water sources (Jurado *et al.*, 2006). The clinical symptoms of this disease appear to be variable, due to the diversity of the reported pathologies. Moreover, neither the previous reports nor ours give evidence of any important epidemiological factors or indicate the possible transmission route of this bacterium. However, interestingly, recent studies have revealed that strains closely related to this bacterium have been isolated from rice plants as plant endophytes and can be found inside and outside the rice leaves (Mano & Morisaki, 2008). The patients in this case study live at a

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships between the isolates obtained from the patients and an extended set of reference sequences belonging to the genus *Aurantimonas*. The tree was constructed by using the neighbour-joining method and was based on a comparison of 1339 nucleotides. The tree was rooted with *Fulvimarina pelagi* HTCC 2615^T as the outgroup. Bootstrap values are expressed as percentages of 1000 replications. Bar, 0.005 substitutions per nucleotide position. '*Aurantimonas littoralis*' (AY178863) and '*Aurantimonas manganoxydans*' (U53824) have been described by Anderson *et al.* (2009) but the names have not been validly published to date. GenBank database accession numbers are given in parentheses.

distance of some 10 km from each other and at 30 km from a wetland area with extensive rice production (cities of Valencia and Albufera), and although direct aetiology cannot be traced, this coincidence is suggestive.

Immune deficiency associated with AIDS, chemotherapy for cancer, immune-mediated diseases, transplantation, as well as ageing of the population, has contributed to the emergence of infectious diseases in the immunocompromised population. The difficulty of diagnosing patients with unusual infectious processes, like those caused by *A. altamirensis*, and the limitations of automated identification systems emphasize the importance of the clinical diagnosis of rare micro-organisms using molecular biological techniques. Furthermore, it points to the need for an epidemiological study of this bacterium, considered a rare pathogen, within the context of a diversity of infectious diseases in which it may be involved.

Bacteria from the genus *Aurantimonas* can be dispersed through water (Kim *et al.*, 2008; Jurado *et al.*, 2006) and air (Weon *et al.*, 2007). Isolation of *A. altamirensis* strains from environments and patients separated by a considerable geographical distance (Spain, Canada, the USA, Japan and New Caledonia) indicate that this bacterium may be of fairly common occurrence. The present study would indicate that other cases using automated identification methods may have misidentified *A. altamirensis* and, therefore, other *A. altamirensis*-related cases may exist.

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