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First Report of Isolation and Characterization of *Aurantimonas altamirensis* from Clinical Samples[▽]

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The genus *Aurantimonas*, proposed in 2003, encompasses four species from environmental sources, including *Aurantimonas altamirensis*, isolated from a cave wall in Spain. Here, we report what we believe are the first cases of the recovery of *A. altamirensis* from human clinical materials.

The genera *Aurantimonas* and *Fulvimarina* are two members of the recently proposed family *Aurantimonadaceae* (8). The genus *Aurantimonas* currently consists of *Aurantimonas coralicida*, *A. altamirensis*, *A. ureilytica*, and *A. frigidaquae*, with the type species, *A. coralicida*, found previously to be the causative agent of the white plague type II on Caribbean scleractinian corals (7). *A. altamirensis* was isolated in the subterranean environment of the Altamira Cave (Cantabria, Spain) from a white microbial growth on the walls of the cave (9). *A. ureilytica* was isolated from an air sample collected from the Suwon region of South Korea (13), and *A. frigidaquae* was recovered from a water-cooling system in the Gwangyang region of South Korea (10).

During 2006 and 2007, strains of *A. altamirensis* were isolated from three different patients in two Canadian provinces. The first isolate was cultured from a contact lens and the lens cleansing solution from a patient with keratitis (case 1). The second isolate was recovered from a corneal culture from a patient who suffered a penetrating eye injury while sharpening a metal tool. Despite treatment with trifluridine, tobramycin-dexamethasone, and fluconazole eye drops, the infection progressed to form a dendritic corneal ulcer with perforation. The culture of corneal scrapings subsequently revealed *Alternaria* sp. and a gram-negative bacillus (case 2). The third isolate was recovered from the sputum of a cystic fibrosis patient who had recently received a 2-week course of tobramycin and piperacillin-tazobactam to treat the exacerbation of the respiratory condition by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Upon discharge, a regimen of ciprofloxacin for 3 weeks and daily inhaled colistin was instituted. Surveillance culture 3 weeks later yielded 2+ growth of *S. aureus* (as assessed using a scoring system in which 0 represents no growth, 1+ represents minimal growth, 2+ represents moderate growth, 3+ represents extensive growth, and 4+ represents maximal growth), as well as one colony of a gram-negative bacillus. The organism was left untreated, and the patient remained well (case 3).

The identification of patient strains at the primary-care level by using commercial identification kits (API 20NE and Vitek [both from bioMérieux, Inc.] and RapID NF [Remel]) gave rise to conflicting results. API 20NE strip identifications included *Ochrobactrum anthropi* and *Sphingomonas paucimobilis*, or the test could not provide adequate identification. The RapID NF strip (Remel) showed 99.9% agreement with the identification of *Shewanella putrefaciens.* The Vitek identification system (bioMérieux, Inc) could not provide adequate identification. Such ambiguities prompted clinicians to forward the isolates to a reference center for further characterization.

The three strains were identified by nearly full 16S rRNA gene sequencing in the Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, and the National Microbiology Laboratory, Winnipeg, Canada (1, 2). The strains were designated NML 070723 (the case 1 isolate), NML 070722 (the case 2 isolate), and NML 060898 (the case 3 isolate). Alignment was done using Clustal X (11), and phylogenetic relationships were inferred using the neighbor-joining method in MEGA 4.0 (12). The strains were found to have >99% identity to one another and 99.4% or greater identity to the *A. altamirensis* type strain (GenBank accession no. DQ372921) (Fig. 1). Pairwise analysis of 16S rRNA gene sequences from recently described species *A. frigidaquae*, which is closely related to, but discernible from, *A. altamirensis* (10), demonstrated 98.5% identity to the clinical isolates as well as the type strain of *A. altamirensis* (Fig. 1).

Conventional phenotypic test methods were carried out at the National Microbiology Library (14), and the results were difficult to relate directly to those used previously to describe environmentally derived *Aurantimonas* spp. (7, 9, 10). The clinical isolates were strict aerobes, had a creamy yellowish pigment, grew well in air or 5% CO_2 at 35°C and in air at 25°C,

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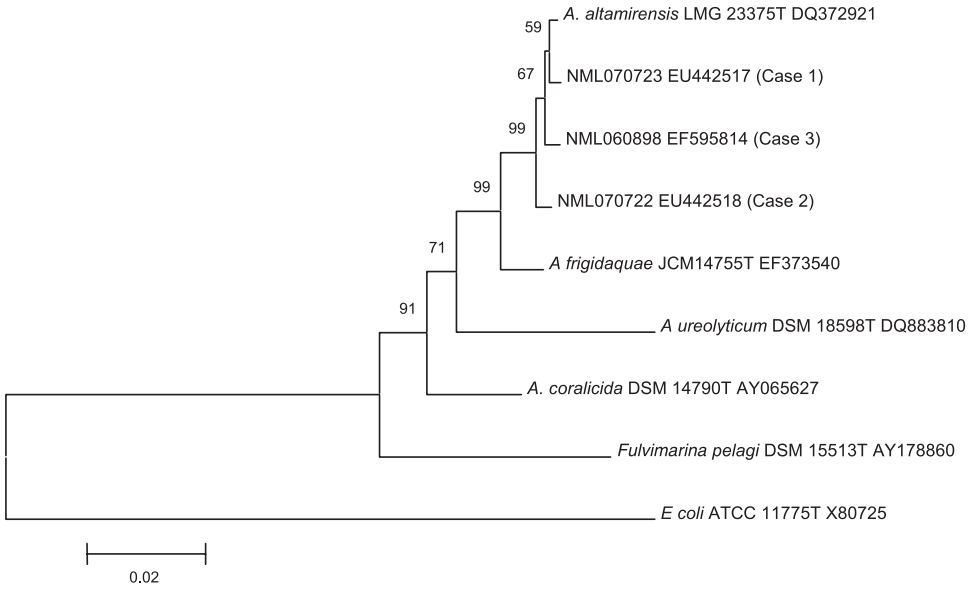

FIG. 1. Phylogenetic relationships inferred using the neighbor-joining method with 16S rRNA gene sequence data for species in the genus *Aurantimonas* and clinically derived isolates. The scale represents percentages of substitutions. *Escherichia coli* was used as a distant outlier, and *Fulvimarina pegali* was used as a family-level outlier.

but showed no growth at 42°C. This observation differed from that for the nonclinical type strain of *A. altamirensis*, which grows best at 28°C (9). All strains were nonmotile, positive for catalase and oxidase, and metabolically oxidative, with triple sugar iron reaction results for the slant and butt being alkaline and neutral or neutral and neutral, respectively (9). All strains hydrolyzed urea but were not reactive with conventional esculin, citrate, cetrimide, indole, and gelatin. The deamination of phenylalanine to phenylpyruvic acid and growth on MacConkey agar were variable. Tests for nitrate reduction were negative except for NML 060898, which reduced nitrate, but not nitrite, as determined by conventional methods. In tests using oxidation-fermentation medium, glucose and xylose were oxidized. NML 070723 also oxidized mannitol, whereas in tests using oxidation-fermentation medium, lactose, sucrose, maltose, and fructose were not oxidized by any strain. The lack of motility, negativity for indole production, and the inability to hydrolyze esculin were consistent with the identification as *A. altamirensis* but not *A. frigidaquae* (10). API CH50 strip (bioMérieux, Inc) reactions differed slightly among the three isolates and were done for comparison with reactions used by Jurado et al. to characterize the type strain of *A. altamirensis* (9). The reaction results are summarized in Table 1.

Cellular fatty acid composition analyses were done as described previously (3) with the MIDI Sherlock system (Newark, DE), except that MIDI software version 4.5 was used. Library generation system software (MIDI) was used to compare profiles of the strains after growth on 5% sheep blood agar for 24 h in 5% CO_2. Cellular fatty acid composition analyses of the three strains showed significant quantities (given as the average total volumes for the three strains, expressed as percentages rounded to the nearest integer) of $C_{16:0}$ (8%), $C_{17:1\omega8c}$ (2%), $C_{17:1\omega6c}$ (2%), $C_{17:0}$ (3%), $C_{18:1\omega7c}$ (71%), $C_{18:0}$ (2%), and $C_{19:0\ cyc\omega8c}$ (6%), with trace to small amounts of $C_{15:0}$, $C_{18:1\omega9c}$, 2-OH $C_{18:1}$, 3-OH $C_{18:0}$, and $C_{20:1\omega7c}$ and summed features 2, 3, and 5. These data were deemed to be highly consistent with those for members of the genus *Aurantimonas* (9, 10, 13).

TABLE 1. API CH50 substrates which were reactive with clinically derived *Aurantimonas* strains and the type strain of *A. altamirensis*[a]

Reaction result	Substrates tested with:			
	NML 070723 (case 1)	NML 070722 (case 2)	NML 060898 (case 3)	*A. altamirensis* LMG 23375T
Positive	Galactose, glucose, D-fucose, L-fucose	Arabinose, arabinose, ribose, D-xylose, galactose, glucose, mannose, melibiose, D-fucose	Erythritol, L-arabinose, ribose, D-xylose, galactose, glucose, melibiose, D-fucose	Erythritol, DL-arabinose, ribose, D-xylose, galactose, glucose mannose, melibiose, gentiobiose, rhamnose, fucose
Negative[b]	Erythritol, D- or L-arabinose, ribose, xylose, mannose, melibiose, gentiobiose, rhamnose	Erythritol, gentiobiose, rhamnose	D-Arabinose, mannose, gentiobiose, rhamnose	

[a] Data are from this study and reference 9.
[b] The result was negative for the indicated strain, but the substrates listed reacted positively with at least one other strain.

TABLE 2. Results of antimicrobial susceptibility testing for *A. altamirensis*[a]

Drug	MIC[b] (μg/ml) (classification) for:		
	Strain NML 070723 (case 1)	Strain NML 070722 (case 2)	Strain NML 060898 (case 3)
Ampicillin	≤4 (S)	≤4 (S)	≤4 (S)
Ampicillin-sulbactam	≤4/2 (S)	≤4/2 (S)	≤4/2 (S)
Aztreonam	≤8 (S)	≤8 (S)	≤8 (S)
Cefazolin	≤4 (S)	≤4 (S)	≤4 (S)
Cefotetan	≤8 (S)	≤8 (S)	≤8 (S)
Cefpodoxime	≤2 (S)	≤2 (S)	≤2 (S)
Cefoxitin	≤4 (S)	≤4 (S)	≤4 (S)
Cefuroxime	≤4 (S)	≤4 (S)	≤4 (S)
Ceftriaxone	≤1 (S)	≤1 (S)	≤1 (S)
Ceftazidime	≤1 (S)	≤1 (S)	≤1 (S)
Cefepime	≤4 (S)	≤4 (S)	≤4 (S)
Ticarcillin-clavulanic acid	≤16/2 (S)	≤16/2 (S)	≤16/2 (S)
Piperacillin	≤16 (S)	≤16 (S)	≤16 (S)
Piperacillin-tazobactam	≤16/4 (S)	≤16/4 (S)	≤16/4 (S)
Imipenem	≤2 (S)	≤2 (S)	≤2 (S)
Meropenem	≤1 (S)	≤1 (S)	≤1 (S)
Amikacin	≤8 (S)	≤8 (S)	≤8 (S)
Gentamicin	≤2 (S)	≤2 (S)	≤2 (S)
Tobramycin	≤4 (S)	≤4 (S)	≤4 (S)
Gatifloxacin	>8 (R)	>8 (R)	4 (I)
Ciprofloxacin	>4 (R)	≤0.5 (S)	1 (S)
Nitrofurantoin	>128 (R)	>128 (R)	>128 (R)
Trimethoprim/sulfamethoxazole	>4/76 (R)	>4/76 (R)	1/19 (S)

[a] Testing was done using broth microdilution (6).
[b] S, susceptible; R, resistant; I, intermediate. When two values are given, they correspond respectively to the components in the drug combination.

Antimicrobial susceptibilities were determined by broth microdilution using Sensititre GN2F panels and cation-adjusted Mueller-Hinton broth (Nova Century Scientific Inc., Burlington, ON) according to the instructions of the panel manufacturer (Trek Diagnostics Inc.) and the CLSI guidelines for *Pseudomonas aeruginosa* and other non-*Enterobacteriaceae* (6). Antibiotic susceptibilities of the three strains are shown in Table 2.

To our knowledge, this is the first description of strains of the genus *Aurantimonas* isolated from clinical specimens and possibly associated with human infection. Phenotypic, chemotaxonomic, and genetic data shown here are unambiguously consistent with those described previously for this genus.

Because the recovery of this organism from humans has not been described previously, the clinical contribution to disease appears to be variable. It is possible that the organism was simply a contaminant derived from environmental and/or water sources (e.g., lens cleansing solution, medicated ocular solutions, or nebulized aerosols and related equipment). Contact lens cases can harbor organisms, such as gram-negative bacilli, that are not the causative organism of keratitis (5). Aerosols and nebulizing equipment can also act as reservoirs for microorganisms (4). In case 1, the simultaneous isolation from two related sources, in the absence of other organisms, from a patient with clinical disease is suggestive of an etiological role. In case 2, the concomitant recovery of *Alternaria* (a known ocular pathogen) from the corneal scrapings obscures the clinical relevance of the isolated *Aurantimonas*. Case 3 supports the possibility that the organism has no or low-level pathogenicity in certain clinical settings. These three cases illustrate the spectrum of clinical significance of *A. altamirensis* and provide guidance to clinical microbiologists on its accurate identification. Further studies are required to elucidate its clinical relevance.

This report also highlights the difficulty of correctly identifying this organism by phenotypically based laboratory methods alone. This agent gave rise to ambiguous identifications depending on which rapid identification method was used, and in such situations, microbiologists and clinicians should consider further characterization by 16S rRNA sequencing.

Nucleotide sequence accession numbers. Sequences obtained for NML 070723 (the case 1 isolate), NML 070722 (the case 2 isolate), and NML 060898 (the case 3 isolate) have been deposited in GenBank under accession no. EU442517, EU442518, and EF595814, respectively.

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