Airway Infection with a Novel *Cupriavidus* Species in Persons with Cystic Fibrosis[▽]

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We describe the recovery and identification of a bacterium that represents a new species of the genus *Cupriavidus* from cultures of respiratory tract specimens from two patients with cystic fibrosis (CF). The elucidation of the role of this species in CF lung disease will require an evaluation of a greater number of cases.

Persons with cystic fibrosis (CF) have chronic airway infection with gram-negative bacterial species that are usually not pathogenic for healthy persons. In addition to *Pseudomonas aeruginosa*, which infects the majority of CF patients, several other species, including *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*, as well as a variety of *Ralstonia*, *Cupriavidus*, and *Pandoraea* species, also cause infection in CF (1, 8). However, the precise role these species play in contributing to lung disease in CF is unclear. Obstacles to better elucidating the potential role of these species in CF are their rapidly evolving taxonomy and the difficulty with their proper identification in culture. Here, we describe the recovery and identification of a novel *Cupriavidus* species from respiratory specimens from two CF patients, one living in Germany and the other residing in the United States.

Case 1. The patient was an 11-year-old boy with CF and trisomy 21. His body weight was 20.4 kg, and he was 120 cm tall (5th and 25th percentile, respectively, for boys with trisomy 21). He had undergone operative correction of a patent ductus arteriosus 2 months after birth. Chronic respiratory tract colonization with *P. aeruginosa* had been treated with anti-pseudomonas antibiotics (ceftazidime or meropenem with tobramycin) for a maximum of 2 weeks, twice a year. Analyses of serum antibody titers to *P. aeruginosa* alkaline phosphatase, *P. aeruginosa* elastase, and *P. aeruginosa* exotoxin (Mediagnost) were consistently negative. *P. aeruginosa* was not detected in sputum cultures during the year before he presented with a slight increase in mucus production and dyspnea. At that time, positive serum antibody titers to *P. aeruginosa* alkaline phosphatase (1:1,000), *P. aeruginosa* elastase (1:1,900), and *P. aeruginosa* exotoxin (1:1,600) (positive cutoff for all three titers is 1:500) were detected. Pulmonary function testing could not be performed. Microbiological examination of throat and nasal swabs did not yield *P. aeruginosa*, but *Staphylococcus aureus*, *Haemophilus influenzae*, and a gram-negative rod, designated

M318-3, were recovered. Bronchoalveolar lavage fluid culture yielded the same results. The lavage fluid also contained 4.2×10^4 cells/ml; 58% of the cells were macrophages, 33.3% were neutrophils, and 9% were lymphocytes. PCR assays for *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, mycobacteria, cytomegalovirus, and Epstein-Barr virus were negative. Hematologic testing showed a slight elevation of segmented granulocytes to 64% (normal range, 25% to 60%) and an elevated level of immunoglobulin A at 2.93 g/liter (normal range, 0.3 to 2.3 g/liter).

Strain M318-3 grew on Columbia agar with 5% sheep blood and on MacConkey agar but not on *Pseudomonas* or *Burkholderia cepacia* selective agars at 36°C and 42°C (10). Colonies that were 2 mm in diameter, convex in elevation, smooth, grayish-yellowish in color, and without hemolysis were visible after 1.5 days of cultivation. After 1 day of incubation at room temperature in thioglycolate broth, the bacteria showed a light yellow pigment that disappeared thereafter. The strain was nonfermenting and catalase and oxidase positive and did not hydrolyze urea. It was immotile and showed negative results in the following tests using the API 20NE system (bioMérieux): nitrate and nitrite reduction, indole production, L-arginine dihydrolysis, gelatin and esculin hydrolysis, β -galactosidase, and oxidation-fermentation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, trisodium citrate, and phenylacetate. Positive results were obtained using the following tests: oxidation-fermentation of potassium gluconate, capric acid, adipic acid, and malic acid. The code number obtained with API 20NE was 0000474, which correlated with *Cupriavidus pauculus* at a probability of 47.6%, *Alcaligenes faecalis* at 29.5%, and *Comamonas testosteroni* at 19.9%. The Vitek 2 (bioMérieux) did not provide an identification result. Antimicrobial susceptibility was determined according to CLSI guidelines using Mueller-Hinton broth. The MICs for the antimicrobial agents tested, expressed in μ g/ml, were as follows: ampicillin, 16; ampicillin-sulbactam-clavulanate, 0.047; piperacillin-tazobactam, <0.016; doxycycline, 0.125; cefotaxime, 0.75; ceftazidime, 3.0; imipenem, 0.19; meropenem, 0.25; ciprofloxacin, 0.094; levofloxacin, 0.023; moxifloxacin, 0.016; trimethoprim-sulfamethoxazole, 0.064; and colistin,

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0.047 (Etest). Susceptibility to aztreonam and amikacin was shown using the agar diffusion test.

Because strain M318-3 was not identified by biochemical profiling with commercial systems, it was analyzed by 16S rRNA sequencing. Sequence comparison to publicly available sequences indicated that the organism had the highest similarity to *Cupriavidus*. The patient was treated with amoxicillin (amoxicilline)-clavulanate at a dose of 60 mg per kg per day. Two weeks after beginning the antibiotic treatment, the *Cupriavidus* species was again cultured from a throat swab; however, mucus secretion and dyspnea declined slowly. After treatment, this bacterium has not again been recovered.

Case 2. The patient was a 22-year-old young man who had been born preterm and small for gestational age and who had a protracted neonatal hospitalization, including mechanical ventilation. He was diagnosed with CF during an evaluation of failure to thrive at 5 months of age. His sweat test at that time was unequivocally positive, and he was subsequently found to be homozygous for the delta F508 mutation. He was short of stature throughout his childhood, attaining full height as an adult at 150 cm. He was microcephalic and had a significant developmental delay, with an IQ of 65. He also had a mild form of congenital myotonia characterized by intermittent muscle pain and mild movement abnormalities. After the first year of life, his respiratory course had been remarkably stable. Sputum cultures first became positive for *P. aeruginosa* when he was 15 years of age and remained positive for the next 7 years. During this interval, only one sputum culture was positive for *S. aureus*, one for *Aspergillus fumigatus*, one for *Pseudomonas fluorescens*, and two for *S. maltophilia*. He presented at 22 years of age with symptoms of a mild upper respiratory tract infection. His pulmonary function studies were unchanged from baseline, however, and no antibiotic therapy was prescribed. A culture from a throat swab was positive for mucoid *P. aeruginosa* and a gram-negative rod, designated AU3369. The latter organism grew well aerobically on Mueller-Hinton broth (Becton Dickinson) supplemented with 1.6% (wt/vol) agar and incubated at 32°C for 24 h and showed no growth on *Burkholderia cepacia* selective agar (10). It was nonfermenting, catalase positive, and weakly oxidase positive. It showed negative results for lysine dehydrogenase and *o*-nitrophenyl- β -D-galactopyranoside as well as oxidation-fermentation of sucrose and lactose. Analysis with the RapID NF Plus kit (Remel) did not provide an identification result. DNA was prepared from a culture of this organism, as previously described (18). Two 16S rRNA gene-targeted PCR assays, one specific for all *Burkholderia*, *Ralstonia*, and *Pandoraea* species and one specific for *Ralstonia* species, were performed, as previously described (5, 12), and both showed positive results. Four other 16S rRNA gene-targeted PCR assays specific for *Ralstonia pickettii*, *Ralstonia mannitolilytica*, *Ralstonia insidiosa*, and *Cupriavidus respiraculi*, respectively, were negative (3, 6, 7). Previously published 16S rRNA gene-directed PCR assays specific for other CF-related bacterial species, including *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter* (13, 18, 19), were also negative. Complete 16S rRNA gene PCR amplification, sequencing, and editing were performed, as previously described (18). Edited and assembled sequences were compared to those available in the NCBI GenBank bacterial DNA database. Strain AU3369 was also identified as a *Cupriavidus* species. The patient re-

mained relatively well, not requiring hospitalization for an exacerbation of pulmonary symptoms until the age of 25. The *Cupriavidus* species was never again recovered from culture of respiratory tract specimens.

Individuals with CF are susceptible to life-threatening respiratory infections due to a plethora of opportunistic bacterial species. Although much remains unknown regarding the epidemiology of these species, evidence indicates that they may be acquired by patient-to-patient transmission or through independent events from environmental sources (1). The detection of particularly unusual species, such as the novel *Cupriavidus* species identified in the two patients described herein, strongly suggests acquisition from an as yet undefined environmental source. Both patients had relatively mild lung disease, which seemed to only transiently worsen coincident with the recovery of the novel *Cupriavidus* strain. Of note, at the time that the *Cupriavidus* was recovered from the first patient, serum antibody titers developed to *P. aeruginosa* alkaline phosphatase, elastase, and exotoxin, suggesting the possibility of serologic cross-reactivity between *Pseudomonas* and *Cupriavidus* antigens.

Comparison of the 16S rRNA gene sequences of strains M318-3 and AU3369 revealed that they were 100% identical; the highest similarity levels (between 97% and 99%) were obtained toward 16S rRNA gene sequences of reference and other strains of several *Cupriavidus* species, including *Cupriavidus gilardii*, *Cupriavidus respiraculi*, and several environmental *Cupriavidus* species. At present, *Cupriavidus* species are known mainly as environmental bacteria, including root nodule symbionts and metal resistant soil saprophytes (2, 14–17); nevertheless, a growing number of *Cupriavidus* species, including *C. respiraculi*, *C. gilardii*, *C. pauculus*, *C. metallidurans*, and *C. basilensis* (5, 7), have been isolated from specimens from CF patients. Strains M318-3 and AU3369 were subsequently included in a polyphasic taxonomic study of unusual CF pathogens, which revealed that they represent a novel *Cupriavidus* species. The details of the latter taxonomic study will be published elsewhere (P. Vandamme, E. De Brandt, E. Falsen, and J. J. LiPuma, unpublished data). The recovery of this species highlights the fact that unusual microorganisms may be recovered from the respiratory secretions of CF patients (4). Identification of *Cupriavidus* species is difficult, and misidentification as *Pseudomonas fluorescens* or *B. cepacia* complex is frequent (5, 9, 11). Accurate identification of these species is a necessary prerequisite to further studies aimed at determining the clinical relevance of these species to CF. Therefore, identification of *Cupriavidus* species based on conventional methods should be confirmed with molecular (PCR-based) assays.

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