Fatal Infection Caused by *Cupriavidus gilardii* in a Child with Aplastic Anemia

Matthew Karafin,1 Mark Romagnoli,1 Doran L. Fink,2 Tracy Howard,1 Rachel Rau,3 Aaron M. Milstone,2 and Karen C. Carroll1*

Department of Pathology, Division of Medical Microbiology,1 Department of Pediatrics, Division of Pediatric Infectious Diseases,2 and Department of Oncology, Division of Pediatric Oncology,3 the Johns Hopkins University School of Medicine and the Johns Hopkins Hospital, Baltimore, Maryland

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*Cupriavidus gilardii* is a Gram-negative bacterium that has rarely been associated with human infections. We report a fatal case of sepsis caused by *C. gilardii* in a previously healthy 12-year-old female.

CASE REPORT

While vacationing in Europe, a previously healthy 12-year-old female was diagnosed with severe idiopathic aplastic anemia. One week after presentation, the patient was transferred to a hospital in the United States near her home. Laboratory evaluation at the time of admission was significant for a total white blood cell count of 2,590/μl with a differential of 98.8% lymphocytes, 0.4% monocytes, and 0.8% neutrophils, hemoglobin of 7 g/dl, and platelets of 72,000/μl. On hospital day (HD) 1, following placement of a double-lumen Hickman catheter, the patient became febrile to 39.7°C and was started on piperacillin-tazobactam. She remained febrile through HD 12, prompting empirical addition of amikacin and then liposomal amphotericin B to her antibiotic regimen. A contrast computed tomography (CT) scan of the chest, abdomen, and pelvis showed diffuse perirectal thickening, prompting initiation of metronidazole for improved anaerobic coverage. She subsequently defebrilized and remained afebrile from HD 15 to 24, though all antimicrobials were continued due to profound neutropenia and persistent perirectal pain.

A repeat CT scan on HD 25 revealed progression of moderate to marked perirectal inflammation associated with the appearance of new fluid collection not amenable to surgical drainage. On HD 27, a blood culture from the previous day became positive for vancomycin-resistant enterococcus (VRE), and her central line was removed after initiation of linezolid. By HD 30, persistent fevers and perirectal pain resulted in discontinuation of piperacillin-tazobactam in favor of meropenem, and from HD 32 to 38, the patient clinically improved. During this time, amikacin and liposomal amphotericin B were discontinued, and central venous access was reestablished with a peripherally inserted catheter (PICC line).

Low-grade fever (38.0 to 38.3°C) and abdominal pain recurred on HD 40 and persisted through HD 53, when the patient developed high spiking fevers. A repeat CT scan revealed worsening of perirectal inflammation and progression of bowel wall inflammation to involve the cecum, most of the ascending colon, and a portion of the transverse colon. Stool testing for *Clostridium difficile* antigen was negative. However, a stool surveillance culture was positive for *Cupriavidus gilardii*, resistant to piperacillin-tazobactam, aztreonam, imipenem, and meropenem, and susceptible to cefepime, trimethoprim-sulfamethoxazole, and ciprofloxacin. Consequently, meropenem was discontinued in favor of cefepime, and amikacin was added. Fever subsided, and linezolid was discontinued. On HD 60, the patient became febrile again, and blood cultures were positive for VRE and *C. gilardii*, now susceptible only to trimethoprim-sulfamethoxazole and ciprofloxacin.

Ciprofloxacin and linezolid were added, and after removal of the PICC line, the patient began receiving daily granulocyte infusions. However, peripheral blood cultures remained positive for *C. gilardii*, and the patient experienced hypotensive episodes associated with both granulocyte and ciprofloxacin infusions. She was transferred to the pediatric intensive care unit on HD 62, where she developed evidence of systemic inflammatory response syndrome with significant pulmonary edema and hypoxia. She was maintained on vasopressor support and mechanical ventilation but ultimately succumbed to multiorgan failure secondary to sepsis on HD 70. Unlike all previous isolates, the final *Cupriavidus gilardii* isolate recovered from the blood on the day of death was resistant to ciprofloxacin and susceptible only to trimethoprim-sulfamethoxazole.

Seventeen isolates of *C. gilardii* were recovered from the throat (3 isolates), stool (1 isolate), and blood (13 isolates) of this patient over a 40-day period. A computer-generated dendrogram of the cellular fatty acid profiles from 5 of the representative recovered isolates (2 throat isolates, 2 blood isolates, and 1 stool isolate) revealed that the isolates were closely related (Fig. 1). Pulsed-field gel electrophoresis (PFGE) (Bio-Rad Gen Path, Hercules, CA) was performed on all isolates. The bacterial DNA was digested with Smal, and gels were analyzed with the Molecular Analyst Fingerprinting Plus software program (Bio-Rad, Hercules, CA). Isolates were considered genetically related if their PFGE patterns differed by 3 or fewer bands. PFGE verified the dendrogram results. All five...
isolates differed by only two bands and were considered closely related (data not shown).

A single isolate, recovered from the blood 32 days after the initial recovery of C. gilardii from a throat culture and 7 days prior to the death of the patient, was selected for detailed characterization.

The type isolate was identified by both phenotypic and genotypic methods and is described as follows. After approximately 24 h at 35°C, on Trypticase soy agar (TSA) medium with 5% sheep blood (Becton-Dickinson, Sparks, MD), colonies appeared as small (<1 mm in diameter), gray, translucent, round, entire, nonhemolytic, and convex with a glistening appearance. On Gram staining, the organism was shown to be a poorly staining, medium-sized Gram negative rod. On MacConkey agar, after 48 h of incubation, colonies were non-lactose fermenting and approximately 1 to 2 mm in size. Following 5 days of incubation, the isolate was negative for lysine decarboxylase, arginine dihydrolase, urease production, nitrate reduction, nitrogen gas production, hydrolysis of o-nitrophenyl-β-D-galactopyranoside (ONPG), acid production in oxidative-fermentative (OF) carbohydrate media (glucose, xylose, maltose, sucrose, and lactose), indole production, hydrolysis of esculin, acetamide, and Tween, gelatin liquefaction (2 weeks' incubation), and assimilation of N-acetyl-glucosamine (API 20 NE strip; bioMerieux, Durham, NC). The isolate was positive for catalase, oxidase, and alkaline phosphatase activity (Key Scientific Products, Round Rock, TX), motility, and citrate utilization. In addition, the isolate demonstrated resistance (no zone of inhibition) to a 1,000-μg disc of colistin (8).

Further characterization was performed using 16S rRNA gene sequencing and by analysis of cellular fatty acid (CFA) content using gas liquid chromatography (GLC). CFA content was determined using the Sherlock microbial identification system, version 6.1, library version 5.0 (MIDI Inc., Newark, DE) and with an in-house database. The MIDI database does not include C. gilardii and identified the isolates as either Burkholderia cepacia or Ralstonia pickettii. Major fatty acids produced included 18:1 w7c (cis-9-hexadecenoic acid, 33%), 16:0 (hexadecanoic acid, 25%), and summed feature 3 (16:1 w7c/16:1 w6c, cis-9-hexadecenoic acid, 24%). In addition, the MIDI Sherlock software creates a similarity index (SI) value from 0.001 to 1.000, where 1.000 is a perfect match with profiles stored in the library. A SI value of 0.849 was determined for this isolate when data were compared to an in-house database of previously analyzed clinical isolates and type strains of C. gilardii. 16S rRNA gene sequencing was performed on the first 500 bp of isolates 1 to 5 and on the full gene of the blood isolate chosen for complete characterization as per guidelines outlined by Applied BioSystems, Inc. (ABI, Foster City, CA). The consensus sequence generated was submitted to the MicroSeq (ABI), GenBank (National Center for Biotechnology Information [NCBI]) and Ribosomal Data base Project (RDP) (Michigan State University) gene libraries for identification. While MicroSeq did not contain entries for C. gilardii in its database, both GenBank (score, 99%) and RDP (score, 0.992) listed C. gilardii as top choices for species-level identification. All of the above procedures were performed according to the manufacturer’s instructions.

Susceptibility testing was performed using the Phoenix automated identification system (BD Diagnostics, Sparks, MD), which identified the isolate as either Delftia acidovorans or Alcaligenes faecalis, even though Ralstonia gilardii is listed in the Phoenix database. Results on the 5 representative isolates showed that all of the isolates were resistant to amikacin, ampicillin, aztreonam, cefoxitin, cefazolin, ertapenem, gentamicin, meropenem, ticarillin-clavulanic acid, and tobramycin but were susceptible to moxifloxacin, tetracycline, and trimethoprim-sulfamethoxazole. Variability was demonstrated against cefepime, ciprofloxacin, imipenem, and piperacillin-tazobactam.

_Cupriavidus gilardii_, named after a prominent American microbiologist, G. L. Gilardii, is an aerobic, Gram-negative, peritrichously flagellated (motile), glucose-nonfermenting bacillus. The taxonomic history for this species continues to be rather complex, and consequently the species has been known by various names, including *Ralstonia gilardii*, *Wautersia gilardii*, and *Cupriavidus gilardii* (1, 4, 6). This species was first identified in 1999 by Coenye et al. (1). While similar to *Alcaligenes faecalis*, it was found to be distinct enough to be separated into its own diagnostic entity, *Ralstonia gilardii* (1). Later, in 2001, De Baere et al. (4) revealed that the *Ralstonia* genus could be divided into two distinct groups based on phenotype and genotype. Specifically, sequence analysis of these two *Ralstonia* groups differed by >4% using 16S rRNA gene analysis (8). Moreover, the *Ralstonia eutrophus* lineage, of which *C. gilardii* was a member, consisted of 9 species that were all Gram-negative rods, motile by a peritrichous flagellum, aerobic, viable on TSA for more than 9 days, forming smooth colonies on blood agar, positive for oxidase and catalase, nonusers of glucose (it was neither acidified nor assimilated), incapable of reducing nitrate or nitrite, and susceptible to colistin. The second *Ralstonia* group, the *R. pickettii* lineage, was distinct from the *eutropha* group in that *R. pickettii* had polar flagella, were resistant to colistin, were viable on TSA for less than 6 days, and produced acid from carbohydrates (8). Based on these differences, the organism described by Coenye et al. was
renamed *Wautersia gilardii* (8). That same year, however, the entire genus was entirely reclassified to the genus *Cupriavidus* due to the fact that *Wautersia eutropha* was genetically identical to a previously identified organism, *Cupriavidus necator* (6). Consequently, *Wautersia gilardii* was given its current name, *Cupriavidus gilardii*.

*C. gilardii* has been isolated from a number of ecological niches, including plants and soils contaminated with heavy metals (3). While this organism has not been known for its pathogenicity, it has been isolated from multiple human tissues and has rarely been identified as the cause of opportunistic human infection. The organism has been isolated from cerebrospinal fluid (CSF), a furuncle, bone marrow, wounds, and the respiratory tract (1, 3, 7). Furthermore, *C. gilardii* has been identified in respiratory secretions of cystic fibrosis patients (2, 3). The only reported clinical infection caused by *C. gilardii* was described by Wauters et al. in 2001. In this case, the organism was the cause of catheter-related sepsis in a 7-year-old female patient with acute lymphoblastic leukemia (9). A bloodstream isolate from the patient was found to be resistant to ampicillin, piperacillin, aztreonam, gentamicin, and tobramycin and susceptible to cefuroxime, ceftriaxone, ceftazidime, imipenem, cotrimoxazole, ofloxacin, and amikacin. The child was treated with intravenous ampicillin, netromycin, ceftriaxone, ciprofloxacine, and amikacin at different points in time based in part on the blood culture results and in part on the patient’s response to therapy. After treatment, her sepsis resolved completely (9).

The true pathogenicity of *C. gilardii* is unknown, and the frequency with which it has caused human disease has been masked by the difficulty in accurate species identification. To our knowledge, the presented case represents the first fatal infection caused by this organism. Intriguingly, our case of *C. gilardii* infection is quite similar to the case presented by Wauters et al. (9). In both situations, the patients were young and immunocompromised. Additionally, the organism in each case was resistant to many antibiotics, resulting in difficulty with treatment. The main difference between the cases is that the patient in our hospital did not survive the septic reaction induced by the bloodstream infection. The hypotension that our patient experienced with antibiotic administration suggests the presence of lipopolysaccharide (LPS) or other cytokine-stimulating factor. It seems that the organism was able to gain access to the bloodstream due to the patient’s underlying immunosuppression and inability to clear the initial intestinal focus of infection. Further complicating the picture, the organism in our case continued to acquire new antibiotic resistances over time. Once the patient became bacteremic, the combination of pan-resistance and the patient’s septic response likely resulted in the fatal outcome.

In summary, *Cupriavidus gilardii* is a poorly understood Gram-negative bacterium that has been identified as a rare colonizer of human tissues. Difficulty remains in correctly classifying the organism both by biochemical and genetic means. Further changes in the classification of this organism may occur as more is learned about this organism’s genetics and phenotypic behavior. The present case represents the first identified fatal infection caused by *Cupriavidus gilardii*. Based on the known clinical history, this organism may represent an emerging pathogen in immunocompromised patients due to its innate antimicrobial resistance and its ability to acquire new resistances as it colonizes its human host.

REFERENCES