Case of Catheter Sepsis with Ralstonia gilardii in a Child with Acute Lymphoblastic Leukemia

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Received 29 May 2001/Returned for modification 20 September 2001/Accepted 27 September 2001

Acute lymphoblastic leukemia was diagnosed in a 7-year-old girl. Two months after insertion of a central venous catheter, she developed fever and complained of headache and abdominal pain. Physical examination revealed no focus of infection. A gram-negative nonfermenting bacillus was recurrently cultured from blood. Extensive biochemical testing and 16S ribosomal DNA sequencing led to the identification of Ralstonia gilardii.

CASE REPORT

Acute lymphoblastic leukemia (ALL) was diagnosed in a 7-year-old girl in May 2000, and treatment was initiated according to the EORTC-CLCG-58951 protocol for children with very-low-risk ALL. The girl achieved hematologic remission after induction chemotherapy. A central venous catheter was inserted in June 2000. The girl tolerated the treatment uneventfully until September 2000, when, during a course of chemotherapy (high-dose methotrexate), she developed spiking fever as high as 40°C. She complained of headache and abdominal pain and vomited twice. Physical examination revealed no focus of infection. The leucocyte count was 5,800/ml, with an absolute neutrophil count of 4,760/ml and elevated C-reactive protein (87 mg/dl). The chemotherapy was stopped, and the girl was treated with intravenous (i.v.) ampicillin (100 mg/kg of body weight/day). One day later blood cultures grew gram-negative bacilli, and (i.v.) netromycin (7.5 mg/kg/day) treatment was added. The spiking fever disappeared and the girl’s health improved.

A gram-negative nonfermenting bacillus was isolated and found to be resistant to ampicillin, pipercillin, aztreonam, gentamicin, and tobramycin and susceptible to cefuroxime, ceftriaxone, ceftazidime, imipenem, co-trimoxazole, ofloxacin, and amikacin. The girl was treated as an outpatient with i.v. ceftriaxone (100 mg/kg/day) once daily for 4 more days. Thirty-six hours after the ceftriaxone treatment was stopped, she again developed spiking fever, and i.v. ceftriaxone (100 mg/kg/day) was restarted in combination with i.v. amikacin (15 mg/kg/day). Again, a gram-negative nonfermenting bacillus was cultured from blood. The girl showed an allergic reaction to ceftriaxone with rash and pruritus, and the ceftriaxone was replaced with i.v. ciprofloxacin (20 mg/kg/day). The spiking fever disappeared again, and amikacin and ciprofloxacin i.v. treatment was given for 7 more days. Blood cultures remained negative, and the central venous catheter was not removed. Three months later the intensive chemotherapy was completed and the girl was doing well. The catheter was removed on January 8 2001. No nonfermenting gram-negative bacilli were cultured from the tip.

Discussion. The gram-negative bacillus was isolated in pure culture from all eight FAN aerobic blood cultures and from one of the eight BacT/Alert anaerobic cultures (Organon Teknika, Turnhout, Belgium), collected over a period of 10 days. Initial identification based on the AP120NE system (bio-Mérieux, Marcy l’Etoile, France) yielded code 1000474, leading to an identification as Alcaligenes faecalis, Comamonas acidivorans or Comamonas testosteroni, or Pseudomonas alcaligenes or Pseudomonas pseudoalcaligenes. However, more elaborate biochemical testing led to the identification of the organism as Ralstonia gilardii. The strain in the present case report (designated GUH 00 09 2123); another clinical isolate of R. gilardii (UCL NF 926), isolated from a cerebrospinal fluid in 1977, without further information on clinical relevance; and three reference strains (LMG 5886T, LMG 15537, and LMG 3400) were tested extensively. The bacteria were motile with peritrichous flagella, grew at 42°C, and were viable for more than 15 days on tryptic soy agar (TSA) at room temperature. Positive reactions were observed for catalase; oxidase; alkaline phosphatase (tablets; Rosco, Taastrup, Denmark); Simmons citrate; and alkalization of acetate, allantoin, lactate, and mucate on Simmons base agar. The strains were negative or very weakly positive for pyrrolidonyl arylamidase (Rosco) and gave a delayed positive result for alkalization of maleate on Simmons base agar. All five strains were susceptible to colistin and resistant to desferrioxamine. Negative reactions were observed for Tween 80 hydrolysis (read after 5 days); urease; phenylalanine deaminase; nitrite reduction; esculin and gelatin hydrolysis; arginine dihydrolase; ornithine decarboxylase; lysine decarboxylase; hydrogen sulfide (H₂S) and indole production; acidification of glucose, saccharose, maltose, manni-

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tol, arabitol, l-arabinose, inositol, lactose, and D-xylose; and alkalinization on Simmons base agar of galacturonate.

Variable reactions were observed for alkalinization of malonate, oxalate, and tartrate on Simmons base agar. Nitrate reduction tested by conventional methods was positive only for strains NF933 and LMG 3400, and it was additionally positive for strain LMG 15537, when tested with the API20NE system.

Reactions on ID32GN (BioMérieux) were positive for utilization of itaconate (n = 5), suberate (n = 5), acetate (n = 5), lactate (n = 5), l-alanine (n = 4), propionate (n = 1), caprate (n = 5), valerate (n = 3), citrate (n = 1), histidine (n = 3), 3-hydroxybutyrate (n = 5), 3-hydroxybenzoate (n = 5), and proline (n = 5). Reactions in the API20NE system (bioMérieux) were positive for gluconate (n = 5), caprate (n = 5), and malate (n = 5) and were variable for adipate (n = 3) and citrate (n = 1). This biochemical profile was consistent with an identification as R. gilardii (2, 3). It should be mentioned that all strains were found to have multiperitrichous flagella instead of a single polar flagellum as described previously (2). Table 1 summarizes the phenotypic characteristics used to differentiate R. gilardii from other oxidase-positive, motile, asaccharolytic, nonfermenting gram-negative rods.

Sequencing of 1,466 bp of the 16S rRNA gene was carried out as described previously (6) for the case report strain. The sequence obtained contained six ambiguities, which could not be resolved upon repeated sequencing and which are probably caused by the presence of multiple 16S rRNA operons with slightly differing sequences. Comparison to all known sequences of the GenBank by using the Blast program (http://www.ncbi.nlm.nih.gov/blast) resulted in a 98% similarity with two Ralstonia species strains (AF239160 and AY005039), and a Ralstonia pacauna strain (AF067657). The only R. gilardii sequence present (LMG 5886T [AF076645]) was only fourth in choice. This relatively low similarity could be largely explained by the fact that the sequence of the R. gilardii type strain (AF076645) contained 18 ambiguities. After detailed visual analysis of the sequences, only seven true mismatches were left, which raised the similarity to the highest observed, confirming the biochemical identification.

Using primers aimed at the amplification of tRNA intergenic spacer regions (1, 4, 5), no amplification signal could be obtained, as is the case for most Ralstonia species (unpublished results). Therefore, tRNA-PCR appears not to be useful for the identification of most Ralstonia species.

R. gilardii may be of more clinical importance than is currently assumed but may have been largely overlooked due to identification problems and due to previously poor taxonomy. Indeed, the original publication describing this species (2) mentions several clinical strains that had been isolated from cerebrospinal fluid (n = 2), bone marrow (n = 1) and a furuncle (n = 1), without reference to published reports. A strain isolated from cerebrospinal fluid back in 1977 was present in our collection, and a new case of R. gilardii sepsis is reported here.

In summary, a gram-negative motile, nonfermenting, asaccharolytic bacillus with a positive oxidase and alkaline phosphatase reaction that is susceptible to colistin and alkaline phosphatase reaction that is susceptible to colistin can be suspected to be R. gilardii and should warrant further identification, especially among the nonsaccharolytic nonfermenters.

Nucleotide sequence accession number. The sequence obtained for the present strain has been assigned GenBank accession no. AJ306571.

REFERENCES


