

## One Case Each of Recurrent Meningitis and Hemoperitoneum Infection with *Ralstonia mannitolilytica*

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**Two clinical cases of infection with *Ralstonia mannitolilytica* are described: a recurrent meningitis on an implanted intraventricular catheter and an infected hemoperitoneum as a complication of a cholangiocarcinoma. The strains were first misidentified as *Pseudomonas fluorescens* and *Burkholderia cepacia*. Further testing lead to the identification as *Ralstonia pickettii* biovar 3/“*thomasii*,” which was recently shown to represent a separate species, *R. mannitolilytica* (List editor N. Weiss, *Int. J. Syst. Evol. Microbiol.* 51:795–796, 2001), originally described as *R. mannitolilytica* (De Baere et al., *Int. J. Syst. Evol. Microbiol.* 51:547–558, 2001). *R. mannitolilytica* can be distinguished from all described *Ralstonia* species by its acidification of D-arabitol and mannitol and by its lack of nitrate reduction and of alkalization of tartrate. In order to determine the true prevalence of infections with this species, colistin-resistant “*P. fluorescens*” strains and strains growing on *B. cepacia* selective medium deserve further attention.**

### CASE REPORTS

**Case report 1.** In 1997, a Caucasian woman, 38 years old, presented with fever of unknown origin. At the age of 17 she had received a ventriculoatrial draining for hydrocephalus after an intracerebral hematoma. Twenty years later, after a localized epileptic insult, further neurological testing and imaging pointed to a diagnosis of cavernous hemangiomas, for which she was treated surgically. The nonfunctional ventricular drain was partially removed, leaving the intrathoracic part in place. Postoperatively, the patient developed meningitis. Culture remained negative, and the patient was treated with 2 g of ceftriaxone intravenously (i.v.) twice daily during 2 weeks. Two days after antibiotic therapy was stopped, the meningitis recurred. Culture of the liquor yielded a gram-negative bacillus that could grow on *Burkholderia cepacia* selective medium (Mast Diagnostics, Merseyside, United Kingdom) and therefore was first identified as *B. cepacia*. The same organism was cultured from the removed intracerebral catheter segment. Ceftriaxone (2 g i.v. twice per day) was started, and ciprofloxacin (400 mg i.v. thrice per day) was added. Fever subsided and liquor cultures remained negative.

In February 1998, the patient presented in a private hospital with a generalized epileptic insult and high fever (39.5°C) and was treated with amoxicillin and clavulanic acid. The fever subsided, and the single blood culture, positive for “*Pseudomonas fluorescens*,” was considered contaminated. From March 1998 onwards, the patient had repeatedly febrile episodes and lost 10 kg of weight. Blood cultures were not performed. In November 1998, the patient was admitted in the same private hospital for high fever and tonic-clonic insults. Seven blood cultures were found positive for “*P. fluorescens*,”

and the patient was referred to the Ghent University Hospital for removal of the endovascular catheter segment. Cultures of the removed catheter were positive and were identified as *Ralstonia pickettii* biovar 3/“*thomasii*.” Retrospectively, it was shown that this was also the correct identification for the “*P. fluorescens*” isolates that had been obtained from the private hospital. The strain was resistant to ampicillin, gentamicin, temocillin, and aztreonam but was susceptible to cotrimoxazole, piperacilin, cefuroxime, cefotaxime, ceftazidime, imipenem, and quinolones. The patient was treated, according to the susceptibility testing results, with cotrimoxazole and doxycycline. Since then, the patient has been doing well.

**Case report 2.** In December 1997, primary cholangiocarcinoma with extensive hepatic involvement was diagnosed in a 32-year-old woman. Chemotherapy with *cis*-platinum and 5-fluorouracil was started with good clinical response and improvement of the hepatic lesions. Six months later, the patient was seen in the surgery department for partial resection of the liver in order to reduce the tumor mass and to improve the effect of chemotherapy. One week after the resection, computerized axial tomography (CAT) scanning revealed that the Kehr drain was leaking into the abdomen. A hemoperitoneum was diagnosed, and a review of the abdomen revealed bleeding of the right vena subhepatica. Intraoperatively, a specimen was taken from the hematoma for culture. After enrichment in thioglycolate broth, *Enterococcus* sp. and a gram-negative nonfermenting bacillus were isolated. Three days after review, the patient developed fever with peaks up to 39°C and antibiotic therapy with cefuroxime was started. Abdominal drainage fluid culture yielded the same gram-negative nonfermenter. This strain was resistant to ampicillin, gentamicin, colimycin, and temocillin but susceptible to cotrimoxazole, cefuroxime, and quinolones. Despite cefuroxime treatment, fever persisted and metronidazole was added. A CAT scan of the abdomen showed an excessive amount of free abdominal fluid. One week later, small numbers of the nonfermenter and of *Enterococcus*

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TABLE 1. Characteristics useful for differentiating *R. mannitolilytica* from other gram-negative nonfermenters<sup>a</sup>

Phenotypic test used	Test results for:							
	<i>R. mannitolilytica</i>	<i>R. pickettii</i>	<i>R. solanacearum</i>	<i>P. aeruginosa</i>	<i>P. fluorescens</i>	<i>Pseudomonas putida</i>	<i>B. cepacia</i>	<i>B. multivorans</i>
Reduction of nitrate	–	+	+	+	–/+	–	V	+
Reduction of nitrite	–	+	–	+/-	–	–	–	–
Colistin susceptibility	R	R	R	S	S	S	R	R
Desferrioxamine susceptibility	R	S	S	R	R	R	R/S	S
Acidification of sucrose	–	–	+	–	V	–	+/-	–
Acidification of mannitol	+	–	–	V	V	V	+	+
Acidification of D-arabitol	+	–	–	–	ND	ND	ND	ND
Pyrrrolidone peptidase	+	+	–	V	V	–	–	–
Trypsin <sup>b</sup>	–	–	–	+	+	+	–	–
Arginine dihydrolase	–	–	–	+	+	+	–	–
Lysine decarboxylase	–	–	–	–	–	–	+/-	V
Alkalinization of tartrate	–	+	+	ND	ND	ND	ND	ND
Pyoverdin production	–	–	–	+/-	+	+	–	–
Growth on salmonella-shigella agar	–	–	–	+/-	+	+	–	ND

<sup>a</sup> ND, not done; +/-, majority of strains positive; -/+, majority of strains negative; R, resistant; S, susceptible, and V, variable.

<sup>b</sup> Trypsin = benzyl arginine arylamidase.

sp. could still be isolated from the abdominal drainage fluid. Antibiotherapy was switched to piperacillin and tazobactam, and a new abdominal drain was placed. As the patient remained febrile, drug fever was suspected and antibiotics were stopped. A subsequent fluid specimen again grew *Enterococcus* sp. and a gram-negative, nonfermenting bacillus, later identified as *R. mannitolilytica*. The patient's condition improved very slowly, and finally the patient was discharged from the hospital knowing that new intrahepatic lesions were detected on CAT scan.

Strain LMG 19090, obtained from patient 1, was isolated on conventional media and could grow on *Burkholderia cepacia* selective medium, containing 100 mg of ticarcillin/liter and 300 U of polymyxin B/ml. API 20NE (BioMérieux, Marcy l'Etoile, France) testing identified the strain as *P. fluorescens* (profile code 0 054 555). Because of colistin resistance, this strain was studied in more detail (3, 7, 13, 14), which led to an identification as *R. pickettii* biovar 3/"*thomasii*".

The further data gathered by means of polyphasic taxonomy led to the description of this biovar as a separate species, named *R. mannitolilytica*, referring to its characteristic acidification of mannitol, unlike all other described *Ralstonia* species (5). The original spelling of the specific epithet "*mannitolytica*" (5) was corrected to "*mannitolilytica*" (8).

In retrospect, strain LMG 19091 from patient 2, which had been identified previously as *P. fluorescens* (API 20NE profile code 0 045 555), and strain LMG 6866<sup>T</sup>, isolated at St. Thomas' Hospital (London, United Kingdom) during an outbreak and deposited as "*Pseudomonas thomasii*" in 1972 (NCIB 10805) (11, 12), could both be identified as *R. mannitolilytica*.

The G+C content for all three *R. mannitolilytica* strains tested was 66.2 mol%, which is higher than the values for *R. pickettii* (64.0 to 64.1%) (5). The 16S rDNA sequences of the clinical strains (GenBank accession numbers AJ270256 and AJ270257) were identical and clustered at more than 99.5% sequence similarity with the *R. mannitolilytica* type strain LMG

6866<sup>T</sup> (GenBank accession number AJ270258) (5). The 16S rDNA sequences for the *R. pickettii* biovar Va-1 and Va-2 strains clustered at 96% similarity versus *R. mannitolilytica*. DNA hybridization confirmed that the two clinical strains and the type strain belonged to a separate species (5). When tRNA PCR was performed (1, 9), all three strains had a PCR fragment of 108.4 bp (standard deviation, 0.06 bp), in combination with one or two other variably present fragments. The obtained tRNA PCR fingerprints were sufficiently discriminative for us to recognize each strain as being *R. mannitolilytica*.

The two clinical *R. mannitolilytica* strains were motile by a single polar flagellum, while motility was not observed for the culture collection *R. mannitolilytica* type strain LMG 6866<sup>T</sup>. It was observed that freshly isolated strains were very motile and that motility decreased upon prolonged preservation and subculture, which could explain the nonmotility of the type strain. All three strains grew at 30, 37, and 42°C and were viable for less than 6 days on tryptic soy agar (Becton Dickinson, Cockeysville, Md.) at 25°C. Oxidase and catalase were positive. They were resistant to desferrioxamine, O:129, and colistin. No acid was produced from ethylene glycol. Urease, pyrrolidonyl arylamidase (Rosco, Taastrup, Denmark), Tween esterase, and phenylalanine deaminase were positive. Acid was oxidatively produced from glucose, L-arabinose, lactose, maltose, mannitol, D-arabitol, and D-xylose. Alkalinization occurred on minimal mineral agar with acetate, serine, malonate, β-alanine, 4-aminobutyrate, azelate, succinate, fumarate, butyrate, formate, malate, mucate, galacturonate, citrate, histidine, and lactate but not with acetamide, adipate, alginate, allantoin, amygdalin, L-arginine, benzoate, L-ornithine, maleate, and tartrate (5).

In the routine clinical laboratory, *R. mannitolilytica* can be differentiated from *P. fluorescens* and *Pseudomonas aeruginosa* by a negative pyoverdin test, by its inability to grow on salmonella-shigella agar, and by a negative arginine dihydrolase test (Table 1). Growth on *B. cepacia* selective medium pointed to an identification as *B. cepacia*. Differentiation from *B. cepacia*, especially from the genomovar II strains (i.e., *Burkholderia multivorans*), which do not decarboxylate lysine or acidify su-

crose (15), is difficult and is only possible by means of a positive pyrrolidonyl arylamidase test for *R. mannitolilytica*.

Finally, several clear phenotypic differences exist between *R. mannitolilytica* and the other *Ralstonia* species (Table 1). *R. mannitolilytica* can be differentiated from the other described *Ralstonia* species through its assimilation and acidification of mannitol and D-arabitol. *R. mannitolilytica* strains differ from *R. pickettii* and *Ralstonia solanacearum* by their resistance towards desferrioxamine and from *R. pickettii* because of their lack of alkalization of tartrate and of nitrate reductase. Strains of *R. mannitolilytica* were previously reported to be adonitol and ethanol acidification negative, like the *R. pickettii* biovars Va-1 and Va-2, and cellobiose positive, like *R. pickettii* biovar Va-1 (13).

A limited number of cases of hospital outbreaks with "*P. thomasi*" and *R. pickettii* biovar 3/"*thomasi*" isolates have been reported in the literature (2, 6, 10, 11). The first report (12) dealt with bacteremia and bacteriuria in 25 patients due to parenteral fluids prepared with deionized water contaminated with "*P. thomasi*" (11, 12). Pan et al. (10) reported that 23 of 39 *R. pickettii* isolates of an epidemic involving 24 patients that was caused by contaminated saline solution (prepared by the hospital pharmacy) belonged to "*P. thomasi*." A pseudo-outbreak has been described as well (4). Although no serious non-outbreak-related infections have been described thus far, the clinical importance of *R. mannitolilytica* may have been overlooked, possibly due to misidentification as *P. fluorescens*, *B. multivorans*, and/or *R. pickettii*.

We reported two cases of infection with *R. mannitolilytica*, first identified as *P. fluorescens* and/or *B. cepacia*. Colistin-resistant "*P. fluorescens*" isolates and strains growing on *B. cepacia* selective medium should be considered to be possibly *R. mannitolilytica*, a species that was formerly known as *R. pickettii* biovar 3/"*thomasi*" and that can be differentiated from *P. fluorescens* by its colistin resistance and its absence of arginine dihydrolase activity, from *B. cepacia* and *B. multivorans* by its pyrrolidonyl peptidase activity, and from other *Ralstonia* species by the acidification of mannitol. Correct identification of this organism may be of importance, since appropriate treatment was postponed in at least case 1, due to misidentification as *P. fluorescens* and *B. cepacia*, pointing to the presence of a

contaminant and also obscuring the long-term presence of the same bacterial organism.

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