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Case report

Ralstonia mannitolilytica in cystic fibrosis: A new predictor of worse outcomes

I. Coman [a], L. Bilodeau [b], A. Lavoie [a], M. Carricart [a], F. Tremblay [a], J.E. Zlosnik [d], Y. Berthiaume [a, c, *]

[a] Centre hospitalier de l'Université de Montréal (CHUM Hôtel-Dieu), Respiratory Service, 3840 St Urbain St, Montreal, QC, H2W 1T8, Canada
[b] Institut universitaire de cardiologie et de pneumologie de Québec (IUCPQ), 2725 Chemin Ste-Foy, Quebec, QC, G1V 4G5, Canada
[c] Institut de recherches cliniques de Montréal (IRCM), 110 Pine Avenue Ouest, Montreal, QC, H2W 1R7, Canada
[d] University of British Columbia, 2329 West Mall, Vancouver, BC, V6T 1Z4, Canada

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ABSTRACT

Background: Patients with Cystic Fibrosis are subject to repeated respiratory tract infections, with recent increasing isolation of unusual pathogens. Ralstonia species have lately been isolated at our institution, an organism historically frequently misidentified as Burkholderia or Pseudomonas. The prevalence of Ralstonia spp. in cystic fibrosis populations has yet to be determined, along with its clinical implications.
Case presentations: Seven patients out of the 301 followed at our cystic fibrosis clinic have had Ralstonia strains identified in their respiratory tract. Most strains identified were multi-drug resistant. After aquisition of Ralstonia spp., the patients' clinical course was characterized by more frequent and more severe respiratory infections along with prolonged hospitalizations, greater decline of lung function, and greater mortality. The mortality rate in this group of patients was 86%. No other factor that could explain such a dramatic evolution was identified upon review of patient data. Some of the strains involved were recognized as clones on Pulse Field Electrophoresis Gel, raising the question of person-to-person transmission.
Conclusion: New pathogens are identified with the evolution of the microbiota in cystic fibrosis respiratory tracts. In our cohort of patients, acquisition of Ralstonia spp. was associated with dramatic outcomes in terms of disease acceleration and raised mortality rates. It is of critical importance to continue to better define the prevalence and clinical impact of Ralstonia in cystic fibrosis populations.

1. Background

Cystic fibrosis (CF) patients are vulnerable to a variety of respiratory tract infections, resulting in chronic endobronchial inflammation eventually leading to respiratory failure and death. The organisms most frequently isolated are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophila*, *Achromobacter xylosoxidans* and *Burkholderia* species [1].

There have been recent changes in the epidemiology of bacterial organisms found in the respiratory tract of CF patients. A number of unusual pathogens are increasingly isolated, such as *Ralstonia*, *Cupriavidus* and *Pandoraea* species [2–5]. These are usually organisms of low virulence and can be found in the environment and the commensal flora but in CF patients, they can cause severe infections.

Over the past few years, *Ralstonia* species have been identified in the respiratory tract of CF patients at our institution. Since its recognition in 1995, the *Ralstonia* genus was split into *Ralstonia* (5 species) and *Cupriavidus* (11 species) [6,7]. *Ralstonia picketti*, previously known as *Burkholderia picketti* and *R. mannitolilytica*, formerly known as *R. picketti biovar 3* or *P. thomasii*, have been associated with nosocomial outbreaks [8,9]. *R. respiraculi*, *R. gilardii and R. taiwanensis* are now part of the *Cupriavidus* species [10].

The frequency of identification of these organisms in CF patients has not been studied, in part because of strain misidentification. *Ralstonia* strains are able to grow on *B. cepacia* specific agars and are often mistakenly identified as *Burkholderia cepacia complex (BCC)* or *Pseudomonas* species [11,12]. One group (Coenye et al.) identified 38 patients infected with *Ralstonia* species that were only identified as

* Corresponding author. IRCM, 110 Pine Avenue Ouest, Montreal, QC, H2W 1R7, Canada.
E-mail address: yves.berthiaume@ircm.qc.ca (Y. Berthiaume).

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such after repeated comparative PCR-based assays [13].

While the prevalence of *Ralstonia spp.* in the CF population is presumed to be low, it has not yet been defined. Here we describe the clinical course of 7 patients from our center, in whom *Ralstonia mannitolilytica (RM)* strains were identified.

2. The cases

Of the 301 patients followed at our CF clinic since 1984, 7 patients (2,33%) had *RM* isolated from their respiratory tract cultures. At the time of identification of the strains, the mean age of these 7 patients was of 28.6 years (range 19–39 years). Their chronic therapy included inhaled tobramycin or inhaled colistimethate as well as oral azithromycin. One patient was on chronic oral prednisone but none of them were treated with ibuprofen. During the year preceding their first *RM* culture, the patients were hospitalized 2.6 times on average (median 2, range 1–3 hospitalizations), with a mean duration of hospitalization of 46.4 days (range 16–53 days). Their mean FEV1 was 45% predicted (range 24%–54%, N/A for 1 patient).

R. mannitolilytica was identified in the sputum of all seven patients, four of which had *RM* cultured in specimens other than sputum; blood cultures [2], sinus culture [1] and mediastinal lymph node biopsy [1]. Most patients had at least one multidrug resistant *RM* strain from the first identification of the organism; 4 patients had one strain that was resistant to all antibiotics on the antibiogram and 5 patients had strains resistant to all but Piperacillin-Tazobactam. Whereas other bacteria and fungus were identified in their sputum cultures (Table 1), no atypical mycobacteria were identified.

The hospitalizations during which the *RM* strains were initially detected were characterized by higher temperatures and higher WBC than previous infectious episodes; six patients had fever ≥39.2 °C (≥102 F) and their average WBC was of 18.3 (range 8.5–33). As for the subsequent infectious episodes, the fever and leucocytosis were inconsistently elevated. A total of 60 hospitalizations for intravenous antibiotic treatments occurred during the patients' follow-up after detection of the *RM* strains (3.7 hospitalizations per patient-year), with a mean duration of hospitalization of 30.9 days (range 3–124).

The patients' FEV1 also rapidly deteriorated during follow-up; 4 patients had predicted FEV1 decline rates were of 3%, 4%, 9% and 11% per year, hence averaging a predicted FEV1 decline rate of 4.44% per patient-year. 3 patients did not have sufficient FEV1 data to be included this analysis.

Despite numerous prolonged courses of intravenous antibiotic combinations for recurrent respiratory infections, most patients progressively deteriorated. Multiple combinations of intravenous antibiotics were used to treat these recurrent infections. These combinations included aminoglycoside (tobramycin or amikacin), penicillins (ticarcillin or piperacillin), carbapenem (meropenem), cephamycin (ceftazidime or cefepime), monobactam (aztreonam), colistimethate or tigecycline. During follow-up, six of the seven patients died, most of which (5/6) were due to progressive hypoxemic respiratory failure due to severe pneumonia following admission to the Intensive Care Unit. Two patients also developed severe septic shock. As for the last patient, she has required an average of 5 hospitalizations per year for recurrent respiratory infections since acquiring a *RM* strain.

Analysis of the *R. mannitolylitica* by Pulse Field Gel Electrophoresis showed that 5 samples from 3 separate patients (Patients 1, 3 and 5, Table 1) appeared to be the same clone by Tenovar's criteria, as there were less than 3 band changes in the clones (data not shown). After reviewing social contacts and clinic visits of these patients, we could not find any encounters suggesting person-to-person transmission. Unfortunately, at the time of writing this case report, the other four samples are not available to determine if they are the same clone.

3. Discussion

Although the presence of *Ralstonia mannitolylitica* has been previously reported in CF, this is to our knowledge, the first report of an accelerated evolution of CF disease in patients infected with *R. mannitolylitica*. In our series of 7 patients with *RM*, the FEV1 decline rates were steeper (3%–11) than expected [14], as well as mortality rates (86%). We could not identify any other clinical conditions that could explain the rapid progression of their CF disease. Interestingly, the only patient that has survived, since the isolation of RM in her secretions, has a diversified flora in her sputum culture. Recently, it has been shown that low bacterial diversity in CF secretions is associated with more severe lung disease

Table 1
Characteristics of CF patients chronically infected with *Ralstonia mannitolilytica* and outcomes after first identification of *RM* strains in their respiratory tracts.

Patient no	1	2	3	4	5	6	7
CF mutation	ΔF508/ΔF508	ΔF508/621+1G→T	621+1G→T/ G542X	ΔF508/ΔF508	ΔF508/A455E	ΔF508/I148T	ΔF508/ΔF508
Gender	F	F	F	F	F	M	M
Date of 1st isolation (age)	Nov 7, 2008 (27)	Sept 4, 2004 (29)	Mar 5, 2009 (28)	Aug 25, 2008 [19]	Sept 14, 2011 (39)	Nov 26, 2008 [21]	Jul 16, 2008 (37)
RM strains isolated	2 RM strains[a]	3 RM strains	1 RM strain[a]	1 RM strain	1 RM strain[a]	1 strain[a]	2 strains[a]
Other organisms	*B. cepacia* *P. aeruginosa* MS *S. aureus* *S. maltophilia* *A. xylosoxidans* *A. fumigatus*	*R. picketti* MR *S. aureus*	*P. aeruginosa* MR *S. aureus* *A. xylosoxidans*	*P. aeruginosa* MR *S.aureus* *A. fumigatus*	*P. aeruginosa*	*P. aeruginosa* MR *S. aureus* MS *S. aureus*	*P. aeruginosa* MR *S. aureus*
IV antibiotic courses	21	11	16	3	1	6	2
Cumulative hospital days	354	457	525	84	14	362	56
Cause of death	N/A	Multilobar pneumonia	Hypoxemic CRA	RM septic shock, lung abscess	RM septic shock	Multilobar pneumonia	Pneumonia
Date of death	N/A	Mar 12, 2009	Jan 12, 2013	Dec 3, 2008	Sept 26, 2011	Feb 13, 2011	Sept 13, 2008
Survival with RM (days)	ongoing	1638	1409	100	12	809	59

[a] Based on the biochemical, morphological and antibiotic resistance observed in the clinical microbiological laboratory; RM: *Ralstonia manninolitytica*; *B. cepacia: Burkholderia cepacia*; *P. aeruginosa: Pseudomonas aeruginosa*; MS *S. aureus*: Multi sensitive *Staphylococcus aureus*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *A. xylosoxidans*: *Achromobacter xylosoxidans*; *A. fumigatus*: *Aspergillus fumigatus*; *R. picketti*: *Ralstonia picketti*; MR *S. aureus*: Methicillin resistant *Staphylococcus aureus*; CRA: Cardiorespiratory arrest.

[15]. So it is unclear if the lack of diversified flora might have contributed to the evolution of their disease. We have also identified MRSA in 5 of the patients. Isolation of MRSA in the respiratory tracts of CF patients is also associated with worse outcomes, namely FEV1 [16,17] and survival, with reported FEV1 decline rates 43% more rapid in patients colonized with MRSA when compared with patients without MRSA (a difference of −0.62% predicted/year; 95% CI, −0.70 to −0.54) and mortality hazard ratios of 1.27 (adjusted; 95% CI, 1.11 to 1.45) [18]. However, the presence of MRSA is not associated with disease evolution we observed which was similar if not identical to the clinical evolution described with the 'cepacia syndrome' [19]. Indeed, all our patients that had a fatal outcome had, in the course of their disease evolution, high fever, elevated WBC, severe pneumonia and in two of them severe septic shock. Furthermore, two of them had positive blood culture to RM and not MRSA. We were also able to observe the presence of RM in the lymph node of one of our patients, a condition also reported previously in a CF patient who died of 'cepacia syndrome' [20]. In order to ascertain that we did not misidentify or miss the presence of *B. cepacia* complex in these patients, 5 samples from 3 patients were further analyzed in a separate lab (the Canadian *Burkholderia cepacia* complex research and referral repository) and confirmed to be *R. mannitolylitica* and negative for *B. cepacia* complex. All specimens produced a brown pigment and Pulse Field Electrophoresis Gel also confirmed that the specimen had identical banding indicating they were clonal. So the clinical evolution of our patients suggests that, as for *B. cepacia* complex infection, infection with RM can lead to an acute deterioration of CF disease and increased mortality of the patients secondary severe bronchopneumonia and sepsis.

It is worrisome to note that at least some of the strains identified in the secretion of our patients were clones. We were unable to identify social contexts or potential contact during clinic visit that could explain person-to-person transmission. None of the previous identification of RM in CF secretions has suggested the potential of RM to be transmissible from patients to patients. However, since pathogens found in the respiratory droplets of infected patients may survive on dry surfaces and inanimate objects for months [21], there may have been indirect person-to-person transmission even if they were never in direct contact at the clinic or on the wards [22]. Hence, this raises the question of whether these patients should be isolated from one another, as has been recommended in patients colonized with resistant strains of *Pseudomonas aeruginosa (PA)*, *B. cepacia complex (BCC)*, and *Methicillin-resistant Staphylococcus aureus (MRSA)*. Since the drastic evolution observed in six of our patients, we have adopted the same segregation policy for RM colonized patients as the one we have for patients colonized with *B. cepacia complex*.

4. Conclusion

Even though chronic infection with *Ralstonia spp*. is uncommon, our review of the evolution of these patients at our center suggests acceleration of the course of cystic fibrosis after acquisition of RM. Our cohort of patients reminds us of the constant evolution of the microbiota in CF respiratory tracts and of the importance of recognizing potential new aggressive pathogens when patients follow a rather unexpected unfavorable course. This study and the resultant bad outcomes suggest that it is now important to better define the prevalence of *Ralstonia spp* in the CF population and to characterize its impact on disease evolution.

Declaration of conflict of interest statements

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