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Achromobacter xylosoxidans: Characterization of Strains in Brazilian Cystic Fibrosis Patients[▽]

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We investigated the possibility of cross-infection among cystic fibrosis patients in two Brazilian reference centers. *Achromobacter xylosoxidans* isolates ($n = 122$) were recovered over a 5-year period from 39 patients. Isolates were genetically heterogeneous, but one genotype was present in 56% of the patients, suggesting that cross-infection may have occurred.

Achromobacter xylosoxidans has been isolated from respiratory samples from cystic fibrosis (CF) patients, but its propensity to chronically colonize and cross-infect them is still unclear. Widespread clones of *A. xylosoxidans* have been described previously (8, 11, 19), so we addressed this issue in two CF reference centers in Brazil. We aimed to evaluate the occurrence of chronic *A. xylosoxidans* colonization and the genetic relatedness of strains isolated from the same patients and to establish the possibility of cross-infection among CF patients.

This study is a retrospective analysis of 179 CF patients receiving routine care, from January 2003 to January 2008, at Instituto Fernandes Figueira (IFF-Fiocruz) ($n = 130$) and Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro (HUPE-UERJ) ($n = 49$). IFF-Fiocruz and HUPE-UERJ are two reference centers for pediatric and adult patients, respectively, in Rio de Janeiro, Brazil. The study was approved by the Committee on Ethical Practice of the Universidade do Estado do Rio de Janeiro (approval no. 2574-CEP-CAAE: 0037.0.228.000-10).

Respiratory samples were cultured in accordance with bacteriological protocols for CF samples (4). Isolates identified as *Achromobacter* spp. by the Vitek 2 Compact system using Gram-negative (GN) cards (reference no. 21341; bioMérieux) were submitted for further identification using a panel of phenotypic tests as previously described (1, 10). To identify each isolate, DNA was extracted by a boiling lysis method, and the whole 16S rRNA gene was PCR amplified, sequenced, and used for BLAST searches against the GenBank database (6). Phenotypic tests and DNA sequencing identified 122 (93.8%) isolates as *A. xylosoxidans*. Eight isolates could be identified only at the genus level and were excluded from the study.

Over the study period, 39 patients (21.8%) had at least one positive culture for *A. xylosoxidans* (22 females and 17 males). Among these, there were 31 pediatric patients (mean age, 9.8 years; range, 2 to 17 years) and 8 adult patients (mean age, 23.8 years; range, 18 to 37 years). *A. xylosoxidans* colonization among pediatric and adult patients was 23.9% (31/130) and 16.3% (8/49), respectively, indicating a high prevalence of pediatric patient colonization compared to that described in most international reports (2, 5). As, on average, CF patient survival is lower in Brazil than in other countries, perhaps Brazilian pediatric patients have more severe lung disease, resembling the clinical status of adult patients in other countries. As a consequence, pulmonary colonization by emergent pathogens might be present earlier. Another possibility is that improved diagnostic tools allow the characterization of rare CF pathogens (3, 15). In total, 122 *A. xylosoxidans* isolates were recovered from 39 patients and positive cultures per patient ranged from 1 to 20. Five patients (12.8%) were chronically colonized by *A. xylosoxidans*. Patients were considered chronically colonized when at least three positive cultures in 1 year were obtained, with a minimum 1-month interval between them for at least 2 years. In 32 cultures, the only isolated microorganism was *A. xylosoxidans*, while in 90 cultures it was associated with other CF pathogens, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia*, and *Burkholderia cepacia* complex. Antibiotics were administered to treat these latter groups of bacteria but not to specifically target *A. xylosoxidans*. Usually, *A. xylosoxidans* appeared after repeated and prolonged treatment for *P. aeruginosa* lung infection (5). Unlike others (7, 15), our population did not show persistent associations with other pulmonary pathogens in patients chronically colonized with *A. xylosoxidans*. This is possibly linked to patient characteristics, including mean age and years of chronic colonization.

A. xylosoxidans isolates were compared by pulsed-field gel

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TABLE 1. Distribution of clonal groups among 122 *A. xylosoxidans* isolates obtained from 39 CF Brazilian patients

Patient[a]	No. of isolates with indicated clonal group[b]																					
	A	C[1]	D	E[2]	F[1]	G[2]	H[1]	I	J	K	L	M	N	O[1]	P	Q[2]	S	U	V	W	X	Z
1							1															
2						1																
3						1																
4															1							
5						1																
6		3																				
7													2									
8						1																
9				1																		
10	3																					
11														1								
12						1																
13*					1	1		1								15						
14							3												1			
15			1																			
16*						4										15						
17						1																
18						1																
19				1								1										
20		1																				
21						1								1								
22						3			2													
23*				1	10	1										1	1					
24																				1		
25						1																
26						2																
27																		1				
28																1						
29						1										1					1	1
30						1																
31						1																
32						1																
33						1					1											
34						1																
35										5												
36						1																
37						1																
38*				7																		
39*				7																		
Total	3	4	1	17	11	28	4	1	2	5	1	1	2	2	1	33	1	1	1	1	1	1

[a] *, chronically infected patients.
[b] Superscript 1 after clonal group indicates *A. xylosoxidans* clones shared between two patients; superscript 2 after clonal group indicates *A. xylosoxidans* clones shared by more than two patients.

electrophoresis (PFGE) analysis of SpeI-digested genomic DNA (9). Patterns were analyzed by GelComparII software (Applied Maths, Sint-Martens-Latem, Belgium), with Dice coefficient analysis. The unweighted pair-group method using average linkages was applied with the bandwidth tolerance set at 1.5%. Isolates clustering together with an 85% level of similarity were considered to belong to the same PFGE type. Among the 122 isolates, 22 different profiles were found, indicating a considerable genetic heterogeneity. Most patients (15/39) carried individual genotypes, and sometimes, the persistence of those clones was associated with the establishment of chronicity. Nevertheless, some patients (7/39) shared the same clone. Chronically colonized patients carried just one clone at a particular period and occasionally exhibited other clones, often in a single appearance and before the establishment of chronicity (Table 1). Currently, studies minimize the possibility of *A. xylosoxidans* transmission by interhuman contacts, with a common genotype observed among pairs of brothers or pairs of friends who were frequently hospitalized at the same time (13, 14). In our study, patients 13 and 16 and patients 38 and 39 (two pairs of cohabiting siblings who were assisted in the same reference center) exhibited shared clones (Table 1).

Epidemiological studies have reported the presence of predominant *A. xylosoxidans* clones in chronically colonized CF patients, suggesting transmission between patients (7, 15, 16, 19). Kanellopoulou et al. (7) indicated that five CF patients were colonized by genetically related *A. xylosoxidans* strains. Lambiase et al. (8) showed that more than half of the *A. xylosoxidans* isolates could be grouped into seven different clusters, suggesting patient-to-patient transmission. Turton et al.

(18) compared isolates from different patients from the same center as well as other centers, revealing that 3 of 6 in one center shared the same cluster. In our patients, 56.4% (22/39) presented only the G genotype between March and October 2005 (Table 1). This is a substantially higher prevalence than that reported by others for their dominant clones (10, 12, 18). Reports of cross transmission with *A. xylosoxidans* in CF, in general, involve few adult patients. We identified a single clone from a large number of patients, mostly children, which was present in both analyzed centers. Patients who also presented with *P. aeruginosa* and/or *B. cepacia* complex infection had more severe disease, characterized by lower body mass index (BMI), lower forced expiratory volume in 1 s (FEV1), and more respiratory exacerbations and hospitalizations. Even though only patients 13 and 16 were siblings, and patients who were hospitalized at similar dates were admitted to separate wards, our results suggest a cross-infection, since all patients were assisted at the same time at a CF clinic. In addition, we could not completely rule out the occurrence of social contacts outside the hospital or a possible contamination from common environmental sources. Based on these considerations, we believe that this was an isolated episode that is not of sufficient strength to justify the revision of infection control measures because the segregation policy is carefully emphasized both in hospitals and in social contact. Furthermore, the G genotype did not persist, even though we still detect *A. xylosoxidans* in our patients and our control measures have proven to be efficient for the control of other bacteria found in CF patients. Additionally, there are no current control measures specifically aimed at *A. xylosoxidans* (17).

To our knowledge, this is the first case of an *A. xylosoxidans* cross-infection in a large number of CF patients. There is still little consensus as to whether *A. xylosoxidans* cross-infection occurs to a significant extent and whether measures such as segregated cohort clinics should be considered within an infection control strategy. Additional studies with more discriminative molecular tools are necessary to elucidate this issue.

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