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Achromobacter xylosoxidans infection in an adult cystic fibrosis unit in Madrid



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ABSTRACT

Background: *Achromobacter xylosoxidans* is an emerging pathogen in cystic fibrosis (CF). Although the rate of colonization by this microorganism is variable, prevalence is increasing in CF units.

Methods: A microbiological/clinical study was conducted on of adult CF patients harboring *A. xylosoxidans*. Identification and susceptibility testing were performed using MicroScan (Siemens). Decline in lung function was assessed using the variable, annual percentage loss of FEV1 (forced expiratory volume in 1 s).

Results: *A. xylosoxidans* was isolated in 18 (19.8%) of 91 patients over a 14-year period. Mean age was 26.6 years (18–39 years). Nine patients (9.8%) were chronically colonized. Piperacillin/tazobactam and imipenem were the most active antibiotics. Mean annual decline in lung function in chronically colonized patients was 2.49%.

Conclusions: *A. xylosoxidans* is a major pathogen in CF. A decreased lung function was observed among patients who were chronically colonized by *A. xylosoxidans*. Antibiotic therapy should be started early in order to prevent chronic colonization by this microorganism.

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Infección por *Achromobacter xylosoxidans* en una unidad adulta de fibrosis quística en Madrid

RESUMEN

Introducción: *Achromobacter xylosoxidans* es un patógeno emergente en fibrosis quística (FQ). Aunque la tasa de colonización por este microorganismo es variable, la prevalencia está aumentando en las unidades de FQ.

Métodos: Llevamos a cabo un estudio clínico-microbiológico de los pacientes adultos con FQ portadores de *A. xylosoxidans*. La identificación y sensibilidad fueron realizadas usando MicroScan (Siemens). La pérdida de función pulmonar fue evaluada por la variable porcentaje anual de pérdida de FEV1.

Resultados: *A. xylosoxidans* fue aislado en 18 (19.8%) de 91 pacientes, en un periodo de 14 años. La edad media fue 26.6 años (18–39 años). Nueve pacientes (9.8%) presentaban colonización crónica. Piperacilina/tazobactam e imipenem fueron los antibióticos más activos. La media anual de pérdida de función pulmonar en los pacientes colonizados de forma crónica fue 2.6%.

Conclusiones: *A. xylosoxidans* es un importante patógeno en FQ. Observamos pérdida de la función pulmonar en los pacientes colonizados de forma crónica por *A. xylosoxidans*. El tratamiento antibiótico debe iniciarse lo más rápido posible para prevenir la colonización crónica por este microorganismo.

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Palabras clave:

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Introduction

Cystic fibrosis (CF) is the most frequent fatal genetic disorder in Caucasians. The main cause of morbidity and mortality in patients with CF is chronic lung infection by *Pseudomonas aeruginosa*.¹ Other respiratory pathogens that can cause lung infection in CF patients include *Staphylococcus aureus* and *Haemophilus influenzae* in infants and children and *Burkholderia cepacia* complex, *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*, and non-tuberculous mycobacteria in adults.

A. xylosoxidans is an aerobic, non-fermenting, motile, Gram-negative rod that was previously known as *Alcaligenes xylosoxidans*. Since this entity is frequently misidentified as *P. aeruginosa*, its prevalence is likely to be underestimated in CF patients with lung colonization/infection.^{2–4} *A. xylosoxidans* is widely distributed in the environment^{5,6} and has been isolated from a wide variety of clinical samples such as blood, vascular catheters, cerebrospinal fluid, sputum, and wounds.^{7,8}

According to the U.S. Cystic Fibrosis Foundation National Patient Registry, the prevalence of patients harboring *A. xylosoxidans* has increased over the last few years from 0.5% in 1995 to 6.2% in 2011.⁹

The higher prevalence of *A. xylosoxidans* in the lungs of CF patients could be the result of an increase in patient life expectancy.^{10–12} In addition, the microbiological techniques used to identify *A. xylosoxidans* and other emerging Gram-negative pathogens in the lungs of CF patients have improved.^{4,11}

The clinical significance of *A. xylosoxidans* in the sputum of CF patients and the role of the microorganism in declining lung function in this group remain unclear.¹³ There are few published data on the clinical impact of *A. xylosoxidans* infection. Colonization has been associated with exacerbation of pulmonary symptoms,¹⁴ and strains are often highly resistant to many of the antibiotics commonly used to treat lung infection in CF patients (e.g., β -lactams, aminoglycosides, quinolones, carbapenems, and colistin).

The goal of this study was to assess the isolates and susceptibility of *A. xylosoxidans* and to analyze the clinical progress of CF patients with *A. xylosoxidans*.

Materials and methods

Patients

We performed a retrospective microbiological and clinical study of patients with CF harboring *A. xylosoxidans* treated in our adult CF Unit (Hospital La Princesa, Madrid, Spain) from January 1999 to December 2013. In total, 91 patients received care in the unit, which works in collaboration with the pediatric unit of Hospital Universitario Niño Jesús (Madrid, Spain). Patients are referred to the unit at 18 years of age. Most patients attend the center every 2 months.

We recorded the following variables: age, sex, weight, mutations in the *CFTR* gene, presence of diabetes mellitus, pancreatic insufficiency, and lung function parameters such as forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FVC%, and FEV₁%. Respiratory function tests were performed on a Datospir 120® Silbelmed® spirometer. We define the decline in lung function as follows: annual percentage loss of FEV₁% = [initialFEV₁% – actualFEV₁%]/initialFEV₁% × 100]/follow-up in years]. FEV₁ is reported as the percentage of the theoretical value.

Lower respiratory tract secretions for sputum microbiology testing were obtained by coughing. Co-colonization with other microorganisms, allergic bronchopulmonary aspergillosis (ABPA), and the number of annual exacerbations.

Patients were considered chronically colonized according to the criteria of Pereira et al.,¹⁵ namely at least 3 positive cultures

obtained in 1 year, with a minimum 1-month interval between them, for at least 2 years.

Processing of sputum samples

Sputum samples were pretreated (vol/vol) with N-acetylcysteine to decrease viscosity and then mixed vigorously to obtain a homogenous sample. As many pathogens are present in the respiratory tract of CF patients; selective media are required to identify specific pathogens. We inoculated the following plates: blood agar, bacitracin chocolate agar, mannitol-salt agar, MacConkey agar, Sabouraud agar with chloramphenicol, and *B. cepacia* selective agar (bioMérieux, Marcy l'Etoile, France). The incubation time was 3 to 5 days at 35 °C. Bacitracin chocolate agar was incubated in CO₂ increased atmosphere. We used a quantitative 3 mcl loop to measure bacterial counts.

Isolation and identification

All bacteriological analyses were performed in our microbiology laboratory. Lactose-negative colonies isolated on McConkey agar were re-isolated on blood agar and subsequently tested for oxidase activity.

Isolates were identified as *A. xylosoxidans* by conventional methods including MicroScan WalkAway (Siemens Healthcare Diagnostics Inc., West Sacramento, California, USA), API 20NE (bioMérieux, Marcy l'Etoile, France) and matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper, Bruker). MALDI-TOF MS was used only during the last year of the study. Six patients have at least one strain in which the identification of the organism is confirmed by sequencing at the Microbiology Reference Center (Majadahonda). In the rest of the patients it was not possible to perform the confirmation by sequencing.

These procedures were performed according to the manufacturer's recommendations.¹⁶

Susceptibility testing

Susceptibility testing was performed using broth microdilution (MicroScan WalkAway, Siemens Healthcare Diagnostics Inc., West Sacramento, California, USA).

As there are currently no specific standardized sensitivities for *A. xylosoxidans*, we used minimal inhibitory concentration (MIC) interpretive criteria for "Other Non-Enterobacteriaceae" from the Clinical Laboratory Standards Institute (CLSI).¹⁷

We studied the following antibiotics: piperacillin/tazobactam, ceftazidime, cotrimoxazole, minocycline, imipenem, and meropenem.

Results

A. xylosoxidans was isolated in 18 (19.8%) of 91 adult CF patients during the study period. The mean age was 26.6 years (range, 18–39 years). As for mutations in the *CFTR* gene, 61.1% of the patients had F508del/other, 22.2% had F508del/F508del, and 16.7% had other/other. Table 1 shows the clinical characteristics of CF patients harboring *A. xylosoxidans*. According to the criteria defined by Pereira et al.,¹⁵ 9 patients (9.8%) were chronically colonized with *A. xylosoxidans* and the mean colonization period was 3.9 (± 0.8) years. The most frequent concomitant species were *S. aureus*, *Haemophilus* spp., and *P. aeruginosa*. Most chronically infected patients (8/9) were concomitantly colonized by *S. aureus*. Two strains were methicillin-resistant.

Table 1
Clinical characteristics of cystic fibrosis patients harboring *Achromobacter xylosoxidans*.

Characteristic (s) (n = 18)	Colonization result for characteristic	
	Chronic (n = 9)	Intermittent (n = 9)
Mean $\pm$ SD age (years)	27.4 $\pm$ 5	25.8 $\pm$ 7.1
No. female (%)	5/9 (55.6%)	4/9 (44.4%)
Mean $\pm$ SD weight (kg)	57.8 $\pm$ 6.6	60.5 $\pm$ 9.8
No. (%) with CF transmembrane regulator mutation type		
F508del/F508del	2/9 (22.2%)	2/9 (22.2%)
F508del/other	6/9 (66.7%)	5/9 (55.6%)
Other/other	1/9 (11.1%)	2/9 (22.2%)
No. (%) with		
Diabetes mellitus	1/9 (11.1%)	1/9 (11.1%)
Pancreatic insufficiency	9/9 (100%)	8/9 (88.9%)
ABPA	3/9 (33.3%)	4/9 (44.4%)
Mean $\pm$ SD pulmonary function (%) (year 2013)		
FEV ₁	53.94 $\pm$ 16.03	68.2 $\pm$ 10.59
FVC	69.69 $\pm$ 12.53	62.9 $\pm$ 22.28
Decline mean of lung function $\pm$ SD (%)	–2.49 $\pm$ 3.29	–1.27 $\pm$ 5.04
Annual loss of FEV ₁ (%)		
Mean of annual exacerbations	0.92 $\pm$ 0.63	1.59 $\pm$ 1.73
No. (%) coinfecting with other microorganism(s)		
<i>S. aureus</i>	8/9 (88.9%)	8/9 (88.9%)
<i>Haemophilus</i> spp.	2/9 (22.2%)	3/9 (33.3%)
<i>P. aeruginosa</i>	6/9 (66.7%)	4/9 (44.4%)
<i>B. cepacia</i> complex	1/9 (11.1%)	1/9 (11.1%)
<i>Enterobacteriaceae</i>	1/9 (11.1%)	2/9 (22.2%)
<i>Scedosporium</i> spp.	0/9	1/9 (11.1%)

ABPA, allergic bronchopulmonary aspergillosis; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

FEV₁% and FVC% were calculated with data collected throughout 2013 in all patients except for 3 lung recipients and 1 patient who died. For these four cases, data were collected during the last year of the patients' visit.

Decline in lung function was assessed with the variable *annual percentage loss of FEV₁*, as described above. The mean annual decline in lung function in chronically colonized patients was 2.49%.

As for susceptibility of the last isolate of *A. xylosoxidans* from each patient, 9 (56.3%) isolates were resistant to ceftazidime, 7 (46.7%) to cotrimoxazole, 6 (37.5%) to meropenem, 2 to minocycline (13.3%), and 1 (6.3%) to piperacillin/tazobactam. All the isolates revealed the innate resistance to aztreonam, amikacin, tobramycin, gentamicin, and ciprofloxacin.

Discussion

Few studies have analyzed the clinical progress of patients with CF harboring *A. xylosoxidans*. This microorganism is an opportunistic pathogen whose prevalence is increasing. Of the 91 patients included in the study, 18 had at least 1 positive culture since their first visit (19.8%). The prevalence of *A. xylosoxidans* varies, although a slight and gradual increase has been widely observed.^{18–20}

For instance, Ridderberg et al.²⁰ reported an increase from 6% in 2005 to 10% in 2009, Amoureux et al.¹³ found 13.9% in 2010 and Lambiase et al.²¹ a 17.6% in 2011.

According to the criteria of Pereira et al.,¹⁵ the chronic colonization rate was 9.8% in our unit. Our chronic colonization rate is slightly higher than that reported in other studies.

Diagnosis of *A. xylosoxidans* infection by conventional methods is difficult since the microorganism can be misidentified as *P. aeruginosa*.^{2,3,22} New identification techniques such as MALDI-TOF MS enable accurate identification.^{23,24} Therefore, the increased prevalence observed could be a consequence of improved detection techniques. The rise in prevalence could also be explained

by the increased in life expectancy of CF patients and the increase in antibiotic pressure.

The impact of *A. xylosoxidans* on lung disease is unclear, but the recent study by Jakobsen et al.²⁵ showed significantly enhanced inflammatory parameters in combination with progressed lung disease in CF patients with chronic *A. xylosoxidans* lung infection. In their case-control study, De Baets et al.⁴ reported lower FVC and lower FEV₁ values in patients harboring *A. xylosoxidans*. However, other studies failed to demonstrate the negative influence of *A. xylosoxidans* on lung function in CF patients chronically colonized with this organism.^{26,27}

Using the criteria of the study of Taccetti et al.²⁸ reporting that patients not infected by *P. aeruginosa* have an annual percentage loss of FEV₁ of 1.65% and in chronically infected patients the decline in FEV₁ was 4.74%, we found that patients who were chronically colonized by *A. xylosoxidans* have an annual 2.49% loss of FEV₁ while in patients colonized intermittently, the annual decline in FEV₁ was 1.27%. Ours is the first study to show a decline in lung function associated with chronic colonization by *A. xylosoxidans* in CF patients. It would be interesting to determine the annual percentage loss of FEV₁ in larger numbers of patients chronically colonized by *A. xylosoxidans*.

Antibiotic resistance considerably affects the virulence of *A. xylosoxidans*. While treatment is usually guided by antimicrobial susceptibility testing, eradication of *A. xylosoxidans* (and thus chronic infection) using antibiotic therapy is problematic, probably because of the presence of *A. xylosoxidans* biofilm.^{25,29} Even though treatment of *A. xylosoxidans* infection is hampered by resistance, it should include inhaled antibiotics as this modality seems to be the most successful for eradication of the first isolate.³⁰

Our study is limited by its sample size and the lack of a non-infected control group. Another limitation to be considered is that some patients have other factors that may contribute lung function decline. However, the present study adds interesting data due to the fact that few studies on colonization by *A. xylosoxidans* in CF patients have been published.

Further larger-scale studies are needed to demonstrate the source and route of acquisition of *A. xylosoxidans* and to correlate the outcome of CF in patients infected by the organism.²⁷

Conclusions

A. xylosoxidans is a major pathogen in CF with increasing prevalence. Ours is the first study to report a decline in lung function associated with chronic colonization by *A. xylosoxidans* in CF patients and further studies, assessing the importance of this bacteria in these patients are needed, including a greater number of patients and, if possible, only colonized by *A. xylosoxidans*. It is important to start antibiotic therapy early in order to prevent chronic colonization by this microorganism. Piperacillin-tazobactam and imipenem were the most active agents. Cotrimoxazole, ceftazidime and meropenem had a high rate of resistance (46.7%, 56.3% and 37.5% respectively).

Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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