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Case report

Achromobacter xylosoxidans infection in cystic fibrosis siblings with different outcomes: Case reports

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ABSTRACT

Introduction: The clinical relevance of *Achromobacter xylosoxidans* infection in cystic fibrosis (CF) remains controversial. This emerging agent in CF has been associated with increased lung inflammation, more frequent exacerbations and more severe lung disease. We describe a pair of CF siblings chronically colonized by the same multilocus genotype of *A. xylosoxidans* with different clinical courses, and assess whether this species may have developed any virulence traits and antimicrobial resistance that could have contributed to their singular outcomes.

Case presentation: Two siblings were positive for the F508del and Y1092X mutations, and were chronically colonized by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The female patient had a more severe CF phenotype and faster clinical deterioration than her brother. Her pulmonary function and computed tomography scan lesions were worse than those of her brother, and both parameters progressively declined. She died at 14 years of age, when he was 18. All isolates of *A. xylosoxidans* were biofilm producers. *Achromobacter xylosoxidans* showed less swarming motility in the female patient.

Conclusions: Biofilm production and diminution of motility allow persistence. Only swarming motility differed between the isolates recovered from the two siblings, but this finding is not sufficient to explain the different clinical outcomes despite their similar genotypes. Modifier genes, unknown environmental factors and female gender can partially explain differences between these siblings. We were unable to correlate any microbiological findings with their clinical courses, and more translational studies are necessary to decrease the gap of knowledge between laboratory and clinical data to promote better clinical interventions.

1. Introduction

Achromobacter spp. are emergent pathogens in cystic fibrosis (CF) patients. There are 16 species and 2 subspecies already described [1]. Recently there is a proposal to include 4 new species: *Achromobacter agilis* sp. *nov., nom. rev., Achromobacter pestifer* sp. *nov., nom. rev., Achromobacter kerstersii* sp. *nov. and Achromobacter deleyi* sp. *nov.* [2]. The most prevalent species in CF using discriminative molecular tools is *Achromobacter xylosoxidans*, which has been associated with increased lung inflammation [3], more

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frequent CF exacerbations, and more severe lung disease [4,5]. However, evidence of the clinical relevance of these species remains controversial [6]. *Achromobacter ruhlandii, Achromobacter insuavis, Achromobacter dolens* and a few of other *Achromobacter* species may also chronically colonize CF patients, but most are sporadic [7]. The adaptation of *Achromobacter* species to the human host in chronic infection remains uncharacterized, and studies are needed to clarify the pathogenesis of this agent in CF lung disease [8].

We describe 2 cases of CF siblings chronically colonized with *A. xylosoxidans* for more than 10 years who had different clinical courses and assess whether this species may have developed any coping virulence traits and antimicrobial resistance that could have contributed to their singular outcomes. To measure differences in their clinical courses, we reviewed clinical data, computed tomography (CT) scans and lung function. To investigate the possible role of *A. xylosoxidans* in the etiopathology of lung disease, we investigated the presence of well-known virulence traits favouring bacterial colonization of the host mucosa, such as biofilm formation, bacterial motility and antibiotic resistance.

2. Case reports

Two siblings were diagnosed with CF in the same year, when the younger, a female patient, was 15 months old and her brother was 5 years and 10 months old. Both patients always lived with their parents in the same home where they were born. Both were positive for the F508del and Y1092X severe mutations, had exogenous pancreatic insufficiency and elevated sweat chloride concentrations and were chronically colonized by *A. xylosoxidans, Pseudomonas aeruginosa* (both mucoid and non-mucoid from the first colonization) and methicillin-susceptible *Staphylococcus aureus* for more than 10 years [9]. However, they had different clinical courses.

Follow-up at the CF reference centre began when the girl was 15 months old, immediately after diagnosis. She had a past history of meconium ileus (MI), gastroesophageal reflux, failure to thrive, one hospitalization for oedema, hypoproteinaemia and anaemia and an episode of distal intestinal obstruction syndrome. Her parents also related daily productive cough in the previous 2 months, recurrent vomitus and 4 evacuations/day, with greasy malodorous stools. On physical examination, she weighed 6950 g, with a length of 72 cm and body mass index (BMI) of 13.4 kg/m^2 (all less than the 3rd percentile for age and z score −2.01, −2.73 and −2.11 respectively), 44 breaths per minute, subcostal retractions, pulmonary rhonchi, 98% oxygen saturation and hepatomegaly. Oropharyngeal swab culture was positive for *P. aeruginosa* and *S. aureus.* Attempts to eradicate *P. aeruginosa* failed. After 1 year of *P. aeruginosa* and *S. aureus* chronic colonization, at 2 years and 3 months of age, she had the first positive culture for *A. xylosoxidans,* which also evolved to chronic infection. Regarding sporadic colonization, the girl had a single positive sputum culture for *Haemophilus* and one for *Acinetobacter*. In the first 2 years of follow-up in the CF care centre, she was hospitalized 2 times yearly because of CF exacerbation. The period between 3 and 10 years of age was almost free of bad events, except for persistent difficulty in weight gain, with one hospitalization because of CF exacerbation. Thereafter, her clinical condition steadily worsened. In the next 4 years, she was hospitalized 8 times for pulmonary exacerbations, of which 5 occurred during the last year, with progressive deterioration of lung disease and evolution to respiratory failure. She was referred to a lung transplant centre, but died before transplant at 14 years and 4 months of age.

Her brother, 4 years and 7 months older, was diagnosed with CF after her diagnosis. He was born at term without complications and had a past history of frequent vomiting and poor weight gain since his first months of life. He was hospitalized for dehydration at 3 months of age and for enterorrhagia by Meckel's diverticulum at 6 months of age. At 2 years of age, recurrent upper respiratory tract infections started. Despite a voracious appetite, his difficulty in weight gain worsened. In the first consultation at the CF reference centre, at 5 years and 10 months of age, his parents reported that he had frequent coughing. On physical examination, he exhibited pallor, with a weight of 15.7 kg (2nd percentile for age), height of 109 cm (11th percentile for age), BMI of 13.3 kg/m^2 (3rd percentile for age), and z scores of −1.22, −1.94 and −1.81, respectively. From the first consultation, *P. aeruginosa* and *S. aureus* respiratory chronic colonization were detected and, from the following month, also *A. xylosoxidans.* Attempts to eradicate *P. aeruginosa* also failed. Regarding sporadic colonization, the boy had two positive cultures for *Haemophilus*. Until 18 years of age, he had 3 exacerbations of CF treated with hospitalization for 14 days each during the 7th and 8th years and another hospitalization for viral encephalitis at age 11. In the last year, at 17 years and 6 months old, liver disease was detected, and at 18 years of age he was admitted twice: once for vasculitis and another for exacerbation of CF. However, their mother claimed that she could not understand the greater debilitation of her daughter's health compared with her son's, despite the daughter's better compliance with the treatment regimen.

The comparisons of lung function (forced expiratory volume in 1 s - FEV_1), BMI and Bhalla CT score in two siblings were done at the same age and are presented in Fig. 1. Although both siblings exhibited some improvement in their absolute BMI during follow-up at the reference centre, the female's values were significantly lower than the male's (Mann-Whitney test, $p < 0.0001$; interquartile Range (IQR) - male: 2.5 kg/m^2, female: 1.1 kg/m^2).

To measure lung structural damage, 2 radiologists who were blinded to any other information reviewed the CT scans separately. They assigned modified Bhalla scores [10,11] for each CT scan and reassigned the scores 1 month later. The median of the final Bhalla scores was obtained for each available CT scan. The female had lower Bhalla scores than her brother: 18.7 (1 year and 6 months), 9 (7 year and 2 months), 6.7 (10 years and 11 months) and 6.5 (13 years and 6 months). His scores were 16.5 (5 years and 10 months), 12.2 (9 years and 7 months) and 10.2 (14 years).

Their bacteriological backgrounds were studied between 3.3 and 6.5 years of age for the female and 7.7 and 9.9 years of age for her brother. Species identification was performed by conventional methods, and genotype analysis was performed by multi-locus sequence typing (MLST) of 7 housekeeping genes (*nusA, rpoB, eno, gltB, lepA, nuoL, nrdA*), as previously described [12,13]. Allelic profiles and ST were analysed according to the PubMLST Website (http://pubmlst.org/achromobacter/). All isolates were *A. xylosoxidans* and belonged to the same sequence type (ST 201). This is a new ST previously identified in Brazilian CF patients by our group and added to the PubMLST Website [13].

The determination of minimal inhibitory concentrations (MIC) of antibiotics [ceftazidime (CAZ), ciprofloxacin (CIP), imipenem (IMP) and thimethoprim-sulfamethoxazole (TMP-SXT)] was performed using E-test strips (AB Biodisk, Solna, Sweden). The breakpoints used were those recommended by CLSI for non-*Enterobacteriaceae.* The isolates were considered resistant when MIC ≥32 μg/mL for CAZ; MIC ≥4 μg/mL for CIP; MIC ≥16 μg/mL for IMP and MIC ≥4/76 μg/mL for TMP-SXT [14]. The majority of isolates (58.3%) were susceptible to all antibiotics tested (95%CI: 36.5–80.1%). All isolates were susceptible to IMP and CAZ (Fig. 2).

The swimming and swarming motility abilities were determined as described by Rashid et al. [15]. The results are presented as the median diameters obtained in 3 independent assays performed in triplicate (Fig. 2). There were no differences in swimming phenotype between the isolates (unpaired *t*-test, $p = 0.07$; 95%CI - male: 38.1–58.2 mm, female: 27.2–45.4 mm), but the swarming