Severe *Achromobacter xylosoxidans* infection and loss of sputum bacterial diversity in an adult patient with cystic fibrosis.

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ABSTRACT

Achromobacter spp. are emerging pathogens in the lungs of patients with cystic fibrosis. We report the case of an adult patient with cystic fibrosis and chronic *A. xylosoxidans* infection who experienced rapid, progressive clinical deterioration. Metagenomic analysis of the sputum revealed that the airway microbiota was almost entirely dominated by *A. xylosoxidans*. We review the impact of this organism on lung function and the airway microbiome in cystic fibrosis, and discuss the potential for cross-infection between patients.

KEYWORDS

Cystic fibrosis, *Achromobacter xylosoxidans*, sputum microbiome, cross-infection

INTRODUCTION

Achromobacter spp. are aerobic Gram-negative bacilli that have historically been considered infrequent opportunistic pathogens in humans. However, these bacteria have been isolated with increasing frequency from the sputum of patients with cystic fibrosis (CF). *Achromobacter xylosoxidans* is the most common species to cause respiratory infection in CF [1,2], although relatively little is known about its clinical impact and optimal management. We review the case of an adult patient with CF and chronic *A. xylosoxidans* infection who experienced rapid, progressive clinical deterioration. The case raises several issues relating to the treatment of *Achromobacter* infection, the impact of this organism on the lung microbiome and the potential for cross-infection between CF patients.

CASE REPORT

A 44 year-old man with CF (genotype F508del/3659delC) was reviewed in February 2014. He was working full time, running daily and living with his wife and two young children. He had recently completed a half marathon. His lung function was stable with an FEV_1 of 2.5 litres (69% predicted). His weight was 75 kg. He was known to have been colonised by a highly antibiotic-resistant *A. xylosoxidans* for many years. He used inhaled tobramycin and colistin on alternate months and typically experienced minor exacerbations requiring oral antibiotics once or twice a year.

In April 2014, the patient developed influenza-like symptoms and minor haemoptysis. He was treated with minocycline with initial improvement. Unfortunately, his symptoms recurred and

despite one month of oral antibiotics and prednisolone, he reported progressive breathlessness and declining exercise tolerance. By June he was no longer able to run, and his FEV_1 had fallen to 1.7 litres (47% predicted). He deteriorated further despite two 14-day courses of intravenous antibiotics at home and was admitted for inpatient management in August 2014. A CT scan of the thorax revealed extensive small airways nodularity and bronchoalveaolar lavage samples grew *Aspergillus fumigatus* and *A. xylosoxidans*.

Despite intensive inpatient physiotherapy, continuous combination intravenous antibiotics and oral intraconazole, his clinical condition and lung function continued to deteriorate (Figure 1). By September 2014 the FEV_1 was 1.1 litres (31% predicted) and the weight 69 kg. The patient developed type II respiratory failure and required supplementary oxygen and nocturnal non-invasive ventilation (NIV). Echocardiography revealed elevated pulmonary artery pressures. Multiple sputum samples were sent for bacterial and mycobacterial culture but were consistently positive for *A. xylosoxidans* only. In October 2014, a single sputum sample was positive for *Mycobacterium intracellulare* and he was commenced on combination anti-mycobacterial therapy with azithromcycin, rifampicin and ethambutol alongside continued broad-spectrum intravenous antibiotics. No other new sputum pathogens were identified during the remainder of his admission. His FEV_1 reached a nadir of 0.5 litres (14% predicted) in November 2014.

Given the rapidity of the patient's deterioration, and the uncertainty regarding the causative organism, we sought to characterise the microbial communities within the airways using metagenomic sputum analysis. An Illumina next-generation sequencing platform and Kraken taxonomic sequence classification system were used to determine that >99.9% of the bacterial

DNA present in a sputum specimen from our patient was attributable to *A. xylosoxidans* (Figure 2), suggesting a profound lack of airway microbial diversity.

As a further consideration, the potential for patient-patient transmission of the patient's *Achromobacter* strain was investigated. *A. xylosoxidans* isolates from the patient and his sibling, who also has cystic fibrosis, were compared by pulsed field gel electrophoresis and found to be identical. Comparisons were also made with *Achromobacter* isolates from other patients attending the same CF centres as the patient and his sibling, but no other evidence of cross-infection was identified.

The patient remained in hospital for six months before eventually becoming sufficiently stable to be discharged home in February 2015. He remained on long-term oxygen therapy and nocturnal NIV. He was subsequently accepted onto the active lung transplant list at the local transplant centre, and underwent lung transplantation surgery in December 2015.

ACHROMOBACTER XYLOSOXIDANS **INFECTION IN CYSTIC FIBROSIS**

Several CF centres have reported an increasing prevalence of *A. xylosoxidans* [3-5]. Chronic infection is commonly reported in around 5-10% of patients, although this figure may be an underestimate given the difficulty of distinguishing *Achromobacter* from other Gram-negative bacilli, including *Pseudomonas spp.* [1].

The explanation for increasing rates of *A. xylosoxidans* infection is unknown, but there is evidence that infection is associated with increasing age and severity of structural lung disease

[5]. There remains uncertainty over the effect of chronic *A. xylosoxidans* infection on lung function. Some groups have reported no effect on the rate of FEV_1 decline [5,6], while others have suggested a significantly more rapid loss of FEV_1 in those patients with chronic *A. xylosoxidans* infection [3,4,7].

In our case, the deterioration of FEV_1 was unusually persistent and unremitting. There are other reports of significant clinical deterioration in patients with *A. xylosoxidans*. Hansen and colleagues, for example, reported that a subset of patients with chronic *A. xylosoxidans* infection demonstrated rapid loss of lung function associated with an increase in precipitating *Achromobacter* antibiodies [3]. Similarly, rapid deterioration has been reported in paediatric patients after acquisition of *A. xylosoxidans* [8], with no other obvious trigger. The potential for such a severe clinical deterioration, albeit within a subset of those with *A. xylosoxidans*, raises the question of whether aggressive eradication strategies are appropriate or effective after initial isolation of *Achromobacter* in the sputum. A recent retrospective analysis of a large CF cohort in Denmark, for example, provides some evidence that inhaled antibiotics commenced after the first *Achromobacter* isolate could delay or prevent chronic infection [9].

CHRONIC *ACHROMOBACTER* INFECTION AND THE RESPIRATORY MICROBIOME

The advent of culture-independent techniques for the study of airway microbiology has contributed to the concept of a respiratory microbiome in CF, whereby the airways harbour diverse communities of bacteria, fungi and viruses. Increasing age and declining lung function are associated with a loss of microbial diversity [10-12]. It has also been reported that chronic infection with Gram-negative organisms including *Achromobacter* is also associated with a loss

of diversity [13]. In the case reported above, the airway microbiota appeared to be almost entirely dominated by *A. xylosoxidans.* Similar findings have previously been reported in a child with CF [14] and also in explanted lungs from an adult undergoing transplantation for severe CF lung disease [15]. However, we are not aware of any other descriptions of such a profound lack of microbial diversity alongside a severe clinical decline.

An important question that arises in this context is whether the frequent use of broad-spectrum antibiotics in patients with CF might disrupt the respiratory microbiome, contributing to the lack of diversity and predisposing to a decline in lung function. There is conflicting data on this question, with at least one study supporting antibiotic use as the primary driver of decreasing microbial diversity in sputum samples from CF patients [12], but another reporting no statistically significant association between antibiotic use and diversity [10]. Our patient received prophylactic inhaled antibiotics (nebulised tobramycin or colistin) at baseline, but had required only relatively infrequent course of systemic antibiotics in recent years. Between July 2014 and January 2015, however, he received almost continuous broad-spectrum antibiotics. His antibiotic regimen generally included agents likely to be active against *Achromobacter*, but it is unknown to what extent the use of antibiotics contributed to the apparent dominance of *A. xylosoxidans* in the airways.

Similarly, the significance of *M. intracellulare* in our patient remains uncertain. This organism was isolated from only one of many sputum specimens sent during the period described and was not present in the initial bronchoscopy specimens. Nevertheless, triple anti-mycobacterial treatment was commenced and was followed by moderate improvement in his lung function. It

is possible that the immune-modulatory properties of the macrolide component of this regimen played a role in arresting the progressive clinical deterioration [16].

PATIENT-TO-PATIENT TRANSMISSION OF *ACHROMOBACTER XYLOSOXIDANS*?

The final issue raised by our case is the potential for transmission of *Achromobacter* between patients with CF, particularly since our patient and his sibling harboured an identical strain of *A. xylosoxidans.* Clusters of identical strains have previously been identified both within CF cohorts [1,3,17,18] and within families [19]. These studies suggest the potential for patient-to-patient transmission, and highlight the need for rigorous infection control measures in patients with chronic infection. Nevertheless, evidence from Denmark suggests that the primary cause of the increasing *Achromobacter* prevalence in their CF population is acquisition from an environmental source, rather than patient-patient transmission [20].

CONCLUSION

Achromobacter infection has previously been linked with a loss of airway microbial diversity and declining lung function in patients with CF. We report a case of an unusually rapid and severe clinical deterioration in a previously stable patient with chronic *A. xylosoxidans* infection, in whom this organism appeared to completely dominate the respiratory microbiota. The case illustrates the considerable challenge posed by highly antibiotic-resistant Gram-negative pathogens, such as *A. xylosoxidans*, in CF. It also highlights many areas of uncertainty in the clinical management of *Achromobacter* infection, including the role of eradication therapy, infection control requirements and the effect of *Achromobacter* on microbial diversity within

the lung. Future research studies should be directed towards addressing these uncertainties with the aim of improving outcomes for patients with CF infected by these organisms.

Words: 1514

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FIGURE LEGENDS

Figure 1. Rapid decline in Forced Expiratory Volume in 1 Second (FEV_1) in a 44 year-old patient with cystic fibrosis and chronic *Achromobacter xylosoxidans* infection.

Figure 2. Classification of DNA within a sputum specimen from the index case. Metagenomic analysis revealed that 27% of the DNA was of human origin and 73% was of bacterial origin, of which >72.9% was attributable to *Achromobacter xylosoxidans*.

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FIGURE 1

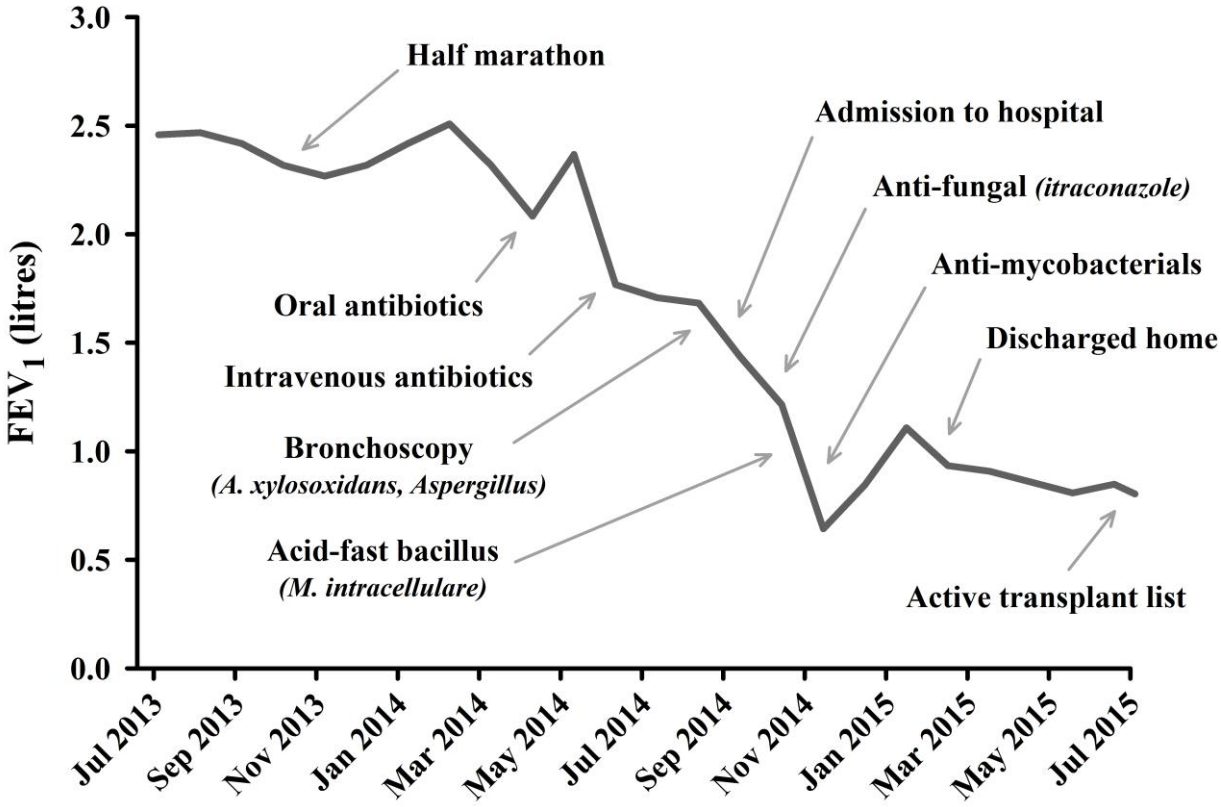

FIGURE 2