Case Report

Tracheobronchitis caused by *Aeromonas veronii* biovar *sobria* after near-drowning

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A 19-year-old man developed an acute tracheobronchitis shortly after having been rescued from a near-drowning in a river where previous investigations had demonstrated the presence of 500 c.f.u. ml⁻¹ of *Aeromonas* sp. in the water. An isolate of *Aeromonas veronii* biovar *sobria* was identified as the causative agent of the tracheobronchitis. The causality was supported by the massive growth of *A. veronii* in bronchial secretion, the presence of a type III secretion system in the bacterial isolate, and the strong haemolytic activity of the strain on blood agar.

Introduction

Infection of the respiratory tract by water-inhabiting micro-organisms after near-drowning is quite common. In fact, only about 10 % of near-drowning victims do not inhale liquids despite the voluntary apnoea at the time of immersion (Ender & Dolan, 1997). The likelihood of infection is considerably increased if the accident occurs in polluted freshwaters.

Several agents of pneumonia have been isolated from near-drowning patients, *Aeromonas* strains being the most regularly reported Gram-negative bacilli (Ender & Dolan, 1997; Mukhopadhyay *et al.*, 2003). *Aeromonas hydrophila* was the most frequent species mentioned in a recent review of approximately 20 cases of *Aeromonas* pneumonia (Kao *et al.*, 2003).

Eighteen *Aeromonas* species have been described so far (Saavedra *et al.*, 2006), but only five (*A. hydrophila*, *Aeromonas caviae*, *Aeromonas veronii*, *Aeromonas jandaei* and *Aeromonas schubertii*) have been identified as human pathogens (Janda & Abbot, 1998). *A. veronii* comprises two biovars, *veronii* and *sobria*, that are two heterogeneous phenotypes of the same species. *A. veronii* biovar *sobria* is one of three species (together with *A. hydrophila* and *A. caviae*) recovered predominantly from clinical materials, and involved in extraintestinal and systemic bacterial infections (Janda & Abbot, 1998). *A. veronii* biovar *veronii*, on the other hand, is rarely reported as a human pathogen (Janda & Abbot, 1998), and little information is available on its occurrence in clinical samples and its ability to cause infections.

Here we report a case of tracheobronchitis caused by an *Aeromonas* isolate that was found to possess determinants of virulence. The strain was identified as *A. veronii* biovar *sobria* by biochemical and molecular analyses.

Case report

A young, apparently healthy, 19-year-old black man with no known anamnesis was rescued from near-drowning after submersion for a few minutes in the waters of the Cassarate river in Lugano (Ticino, Switzerland). The subject had a cardiac arrest, but was successfully resuscitated and transported to the district hospital where he was admitted to the intensive care unit and intubated. On neurological examination, the patient was not reactive to stimuli, but was reactive for clonic and coughing reactions when the orotracheal tube was stimulated. Myoclonic contractions of the arms were sporadically observed. He was given ventilatory support of 5 cmH₂O [PEEP (positive end-expiratory pressure) 10 cmH₂O and PaO₂/FiO₂ ratio of 150].

Bronchoscopy showed an acute tracheobronchitis of moderate severity with the presence of a non-occluding, yellow-reddish bronchial secretion in which an *Aeromonas* sp. was the predominant micro-organism. Antibiotic therapy with cefepime was immediately started. Microbiological analysis showed that the isolated strain was resistant to ampicillin and amoxicillin/clavulanic acid, but sensitive to cephalosporins, gentamicin, tobramycin, tetracycline, ciprofloxacin and trimethoprim/sulfamethoxazole. Thus, the antibiotic therapy was not changed. Chest X-ray revealed bilateral roundish alveolar thickenings and a right basal paracardiac opacity congruent with an infiltration following inhalation. Clinical evaluation showed an improvement in the respiratory indexes and the patient

Abbreviation: TTSS, type III secretion system.

The GenBank/EMBL/DBJ accession number for the *gyrB* sequence of the *Aeromonas veronii* strain is AM490258.

was extubated. Neurological signs indicated a severe post-anoxic encephalopathy leading to the discontinuation of the therapies on day nine. A few hours after discontinuation of therapy the patient died.

The bronchial secretion was plated on classical microbiological media and yielded an almost pure culture of Gram-negative rods. These bacilli were oxidase-positive, resistant to the vibriostatic agent O/129 and strongly haemolytic on blood agar. The API-20 NE (bioMérieux) profile suggested the genus *Aeromonas* and further molecular identification of the strain was carried out using *gyrB* gene sequence analysis (Demarta *et al.*, 2004). Sequence alignment and comparison with other *gyrB* sequences of *Aeromonas* available in the GenBank database was performed using MEGA 3.1 software (Kumar *et al.*, 2004), and the strain was identified as *A. veronii* (GenBank accession no. AM490258). The positive reactions for the Voges–Proskauer and aesculin tests indicated that the strains belonged to the biovar sobria. The presence of a type III secretion system (TTSS), used by many Gram-negative bacteria to deliver effector proteins into host cells (Hueck, 1998), was confirmed by hybridization with a probe consisting of *ascV*, one of the genes encoding a TTSS (Burr *et al.*, 2002).

Discussion

The potential of *Aeromonas* strains to cause respiratory tract infections is often underestimated and largely unknown. *Aeromonas* can be found in the upper part of the respiratory tract, but their presence is generally considered transient (Janda & Abbot, 1998).

Aeromonas spp., however, are known to be among the most important aetiological agents of pneumonia following near-drowning events, with a reported mortality of almost 60% (Ender & Dolan, 1997). As a result, *Aeromonas* must be always suspected as the first, potential cause of a pneumonia when the anamnesis reports contact with water. In our case, the near-drowning accident occurred in the mouth of a river where extensive investigations of the microbiological water quality carried out over a 10 year period had recorded *Aeromonas* concentrations between 250 and 500 c.f.u. ml⁻¹ water (Istituto Cantonale Batteriosierologico, 1995).

The identification of most *Aeromonas* species by conventional biochemical tests is often inaccurate. In the case of the two biovars of *A. veronii*, results of biochemical tests do not always correlate well with genetic identification methods. For example, three *A. veronii* biovar sobria isolates identified by biochemical methods were assigned to *Aeromonas allosaccharophila* by *gyrB* sequence analysis (Saavedra *et al.*, 2006). Graf (1999) demonstrated that the

16S rRNA sequences in the species *A. veronii* biovar sobria are variable. Therefore we decided to perform *gyrB* sequence analysis to confirm species identification. The results of the sequence analysis and biochemical tests allowed the identification of the strain as *A. veronii* biovar sobria.

The role of our *A. veronii* biovar sobria strain as the causative agent of tracheobronchitis was supported by its massive growth in the bronchial secretion, the presence of a TTSS and the strong haemolytic activity on blood agar.

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