ORIGINAL ARTICLE

Isolation of *Aggregatibacter aphrophilus* from bronchoalveolar lavage in a paediatric patient presenting with haemoptysis

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Abstract

We report a rare case of non–cystic fibrosis bronchiectasis accompanied by protracted infection with *Aggregatibacter aphrophilus* in a 12-year-old boy with haemoptysis.

Keywords: *Aggregatibacter aphrophilus,* BAL, bronchoscopy, HACEK, non-CF bronchiectasis
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Aggregatibacter aphrophilus, formerly *Haemophilus aphrophilus* and *Haemophilus paraphrophilus,* is a fastidious Gram-negative coccobacillus that belongs to the HACEK group of bacteria (*Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella* and *Kingella*). It is usually a commensal of the oropharyngeal cavity, but it is also an infrequently encountered cause of invasive infections in humans, such as endocarditis, osteoarticular infections and intracranial abscesses [1,2]. Pulmonary involvement is exceedingly rare, with very few cases being reported wherein *A. aphrophilus* was isolated from pleural fluid or lung abscess aspirate [3–6]. We report the isolation of *A. aphrophilus* from bronchoalveolar lavage (BAL) in a paediatric patient with haemoptysis.

A 12-year-old boy was admitted to the 3rd Pediatrics Department of the Attikon University Hospital of Athens with a 4-day history of haemoptysis (six episodes in total). From his medical history, the patient had repeated episodes of bronchitis until the age of 5 years which were occasionally treated with bronchodilators. During the 6 months that preceded admission, he had two episodes of prolonged wet cough that abated after about 2 to 3 weeks without any pharmaceutical intervention. He also reported that occasionally had mild morning cough.

At admission the boy was afebrile, with a heart rate of 76 bpm, blood pressure 116/76 mm Hg and oxygen saturation 99%. Examination of his respiratory system revealed normal breath sounds. The rest of the physical examination was likewise unremarkable. Laboratory investigations demonstrated a white blood cell count of 7.27 × 10^9/L (normal range, 4.5–13.5 × 10^9/L), hemoglobin 13.6 g/dL (normal range, 13–16 g/dL), platelets 362 × 10^9/L (normal range, 150–350 × 10^9/L) and C-reactive protein 3.23 mg/L (normal range, <10 mg/L). Blood biochemistry, coagulation, serum immunoglobulin levels and spirometry were normal. The tuberculin skin test (Mantoux) was negative. Cultures of sputum, nasal and pharyngeal swabs showed normal flora of the upper respiratory tract. A sweat chloride test was performed and yielded normal results (25 mEq/L).

A chest x-ray performed on the first day of haemoptysis in another hospital had shown perihilar markings but had been otherwise unremarkable. Chest computed tomographic scan in our hospital revealed diffuse bilateral bronchial wall thickening and mild bronchiectasis. Flexible bronchoscopy was performed with a 4.0 mm external diameter bronchoscope (Olympus,

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Tokyo, Japan) with the patient under deep sedation. The bronchoscope was inserted through the nasal route, and no suctioning was performed before entering the trachea. BAL was obtained using three aliquots of 0.9% NaCl, 20 mL each, which were instilled into the right middle bronchus and suctioned immediately into a mucus trap. The first aliquot was used for microbiologic analysis and the two others for cytology. Macroscopic findings on bronchoscopy included mucopurulent secretions and red, inflamed mucosa.

Culture of the BAL isolate revealed a Gram-negative, oxidase–weakly positive, catalase-negative coccobacillus, which was best grown on chocolate agar after 48 hours' incubation in 5% CO_2. However, growth on blood agar was very slow and completely absent on MacConkey agar. The isolate was identified as *A. aphrophilus* (biotype 777044) with a probability of 99.99%, by means of the MicroScan HNID panel (Beckman Coulter, Atlanta, GA, USA). There was no growth dependence on either V- or X-factor, which is consistent with the phenotype of the former species *Haemophilus aphrophilus* [2].

Biochemical tests included on the identification panel were as follows: hydrolysis of indoxyl phosphate positive; reduction of nitrate and nitrite positive; acid production from glucose, sucrose, maltose, fructose and lactose positive; hydrolysis of ortho-nitrophenyl-β-D-galactopyranoside positive; production of urease, ornithine decarboxylase and indole negative; hydrolysis of L-propyl-β-naphthylamide and p-nitrophenyl-α-D-glucoside positive; hydrolysis of γ-glutamyl-α-napthylamide and benzoyl-DL-arginine-β-napthylamide negative; acid production from starch negative and production of β-lactamase negative.

Using the agar diffusion method (Kirby-Bauer), with Clinical and Laboratory Standards Institute (CLSI) interpretation for fastidious microorganisms (*Haemophilus influenza*), the isolate was susceptible to all tested antimicrobials (ampicillin, ampicillin/sulbactam, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, meropenem and trimethoprim/sulfamethoxazole). In addition, it was susceptible according to CLSI for Gram-negative bacteria to all antimicrobials tested per clinician request (amikacin, amoxicillin/clavulanic acid, cefepime, cefuroxime, ciprofloxacin, imipenem, lomefloxacin, piperacillin/tazobactam, rifampicin, tetracycline, ticarcillin, ticarcillin/clavulanic acid and tobramycin).

On the basis of the aforementioned investigations, a diagnosis of non–cystic fibrosis bronchiectasis was established, and oral amoxicillin/clavulanic acid was administered as monotherapy for a total of 3 weeks. Although the isolate showed no evidence of β-lactamase production *in vitro,* clavulanic acid was included in the regimen in order to avoid potential *in vivo* neutralization of amoxicillin by β-lactamases produced by normal respiratory flora. The boy was discharged in good clinical condition on the fifth day of hospitalization, during which he remained haemoptysis free and afebrile. By 3-month follow-up, he remained asymptomatic and reported no episodes of wet cough.

This case report highlights an uncommon presentation of *A. aphrophilus,* which in children is more commonly associated with infective endocarditis and intracranial abscesses [1,2,7]. Recent dental procedures and close contact with dogs have been suggested as predisposing factors for such invasive infections [1,2,7]. Our patient, however, had no such history, and thorough cardiologic, ear, nose and throat, and neurologic assessment did not reveal any pathologic findings. Nevertheless, given his medical history of repeated episodes of bronchitis and the recent bouts of prolonged wet cough, along with the bronchoscopic and radiologic findings suggestive of bronchiectasis, we presume that colonization of the tracheobronchial tree took place much earlier. We therefore attribute the patient's haemoptysis to chronic inflammation-induced rupture of bronchial mucosal vessels.

As far as we know, this is the first case referring to the isolation of *A. aphrophilus* from BAL of a symptomatic patient with non–cystic fibrosis bronchiectasis. This finding corroborates the aspect that microbial pathogenicity is indefinable, and every microorganism isolated from naturally sterile clinical specimens should be considered as a putative causative agent warranting further investigation.

Conflict of Interest

None declared.

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