

Actinobacillus ureae: an unusual cause of tree-in-bud pattern in a case of pneumonia on lung computed tomographic scan—first clinical case report and review of the literature from India

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Abstract

A 62-year-old man with asthma sought care for intermittent fever, cough with expectoration, breathlessness and orthopnoea with grunting. Computed tomography revealed clusters of centrilobular nodules on both sides with a tree-in-bud appearance and mild diffuse bronchial wall thickening. Sputum sample grew pure colonies of *Actinobacillus ureae* which was confirmed by MALDI-TOF and 16SrRNA gene sequencing. *A. ureae* may be an additional bacteriologic causative agent of the tree-in-bud pattern on computed tomographic scan.

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Keywords: 16sRNA sequencing, *Actinobacillus*, Bronchial asthma, MALDI-TOF, Pneumonia, Tree in bud appearance

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Introduction

A 62-year-old man sought care at the Indraprastha Apollo Hospitals in April 2016 with a 4-day history of intermittent fever, cough with expectoration, breathlessness and orthopnoea with grunting. There was no history of palpitation or chest pain. He had had bronchial asthma for the last 40 years and received oral corticosteroid therapy. The patient regularly used nebulizers/inhalers with Levosalbutamol/Levosalbutamol and ipratropium and budesonide solution. He was recently diagnosed with dilated cardiomyopathy. The patient was not diabetic or alcoholic and was not a smoker. There were no existing comorbidities. White blood count was elevated at $15.3 \times 10^3/\text{mm}^3$ (reference range, $4\text{--}11 \times 10^3/\text{mm}^3$), and erythrocyte sedimentation rate was 22 mm/first hour (reference range, 0–15 mm/first hour). Chest examination revealed basal crepitations. The lung parenchyma

showed clusters of centrilobular nodules on both sides with a tree-in-bud appearance and mild diffuse bronchial wall thickening on 160-slice multidetector computed tomographic (CT) scan (Fig. 1).

A sputum sample was sent to the clinical microbiology laboratory for Gram stain, fungal smear and aerobic and fungal cultures. Sputum showed presence of mucus, and there were more than 25 neutrophils and two to three epithelial cells in approximately thirty $10\times$ fields; the Bartlett score was thus +3. The sample showed Gram-negative vacuolated bacilli and coccobacilli. No Gram-positive cocci were observed. Ziehl-Neelsen staining of sputum sample was negative for acid-fast bacilli. Fungal smear (KOH mount) did not show any fungal elements.

Culture was performed with Columbia blood agar, MacConkey agar and chocolate agar (kept in a CO_2 incubator) and on Sabouraud dextrose agar. Sputum culture showed pure growth of mucoid opaque colonies on Columbia blood agar with greening of the media after incubation at 37°C for 24 hours. On Gram stain, Gram-negative vacuolated bacilli were seen. The isolate was oxidase positive, catalase weak positive and hydrolyzed urea. Indole and bile esculin reactions were negative, and the organism produced acid from glucose, sucrose

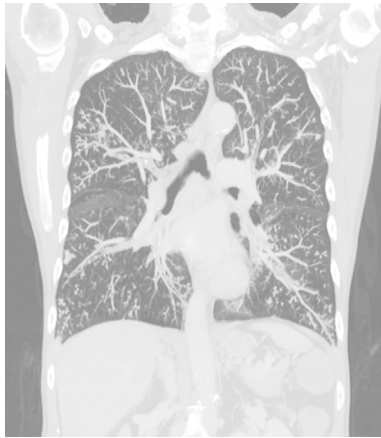


FIG. 1. Coronal MIP image (Maximum Intensity Projection; image reconstruction thickness 10 mm) of high-resolution computed tomographic chest scan (performed on 160-slice MDCT scanner; Toshiba Aquillon) showing multiple clusters of small centrilobular nodules in bilateral lungs, predominantly in lower lobes. Bronchial wall thickening is also evident.

and mannitol. There was no growth on MacConkey agar. No colonies resembling *Haemophilus influenzae* were seen even after 72 hours' incubation on chocolate agar. In addition, no fungal growth was observed until 3 weeks of incubation.

On VITEK 2 Compact, using the ID GN card (bioMérieux, Marcy l'Étoile, France), the organism was identified as *Pasteurella canis* with a contraindication-typical pattern being urease pattern. *Pasteurella* is most likely to be found in clinical specimens of humans associated with pet animals, but the patient had no history of contact with animals. In addition, the isolate was urease positive, while most species of *Pasteurella* are urease negative.

Thus, to resolve the discrepancy, the isolate was tested on VITEK MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS); bioMérieux) for identification. The organism was identified as *Actinobacillus ureae* with a 99.9% confidence value.

To confirm the identity of the isolate, 16S rRNA gene sequencing was performed. A single isolated colony from an overnight culture was used to perform a colony PCR by universal primers 27F and 1492R. The sequences of the primers were 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-CGGTTACCTTGTTACGACTT-3'. Both forward and reverse sequencing was performed.

The PCR product was run on a gel, and an amplicon of ~1400 bp was eluted. The eluted DNA was further purified and sequenced by the Sanger dideoxy sequencing method. The sequence obtained was BLASTed using the National Center for Biotechnology Information (NCBI) nucleotide Basic Local Alignment Search Tool (BLAST). The sequence was submitted to NCBI (accession no. KX355773.1).

The sequence of the present strain *A. ureae* RD (KX355773.1) has the highest alignment score of 2519 bits with the Henriksen strain with 99% query coverage. It was followed by *Actinobacillus ureae* strain CCUG 2139, with a score of 2455 bits with 94% query coverage. We were thus able to differentiate it from other closely related species like *Actinobacillus equuli*. The BLAST results confirmed that the isolate was *Actinobacillus ureae*.

The strain was susceptible to penicillin, ampicillin, cefoxitin, tetracycline, trimethoprim/sulfamethoxazole, amikacin, gentamicin and tobramycin.

A blood sample was also sent for culture at the same time and did not reveal any growth. The patient was treated with amoxicillin with clavulanate for 14 days, and his respiratory symptoms showed marked improvement. He was discharged in a significantly better state, with resolution of fever and respiratory symptoms. White blood cell count was normal after treatment ($9.76 \times 10^3/\text{mm}^3$). He has been undergoing regular follow-up ever since for bronchial asthma. However, a repeat CT scan could not be performed to check for radiologic improvement.

Discussion

The family *Pasteurellaceae* includes bacteria from genera *Pasteurella*, *Actinobacillus* and *Haemophilus*. *A. ureae* is a rarely isolated commensal from human respiratory tract [1]. Predisposing conditions for respiratory colonization include old age, atrophic rhinitis, periodontal disease, bronchitis, bronchiectasis and emphysema, and tumors of the lung and peritonitis have been reported in patients with alcohol-associated cirrhosis, trauma, surgery and HIV infection [2,3]. It has been documented as a rare cause of bacteraemia, endocarditis, meningitis, bone marrow infection, conjunctivitis, otitis media, peritonitis and pneumonia [4,5].

Pasteurella ureae was first described by Henriksen and Jyssem in 1960 as a new variety of *P. haemolytica* from samples taken from cases of sinusitis and chronic bronchitis [6]. On the basis of DNA-DNA hybridization, *Pasteurella ureae* was transferred to the genus *Actinobacillus* [7].

Among *Actinobacillus*, the species *hominis* and *ureae* are regarded as human pathogens [8]. *Actinobacillus hominis* has been isolated from cases of bacteraemia, pleural empyema and bronchopneumonia [9]. *Actinobacillus (Pasteurella) ureae* may be an occasional commensal of the upper respiratory tract. Jones and O'Connor [1] reported 17 cases of this organism isolated exclusively from the respiratory tract associated with cases of chronic chest disease and asymptomatic acute bronchitis. It was thought that this organism was a commensal of the upper respiratory tract, and its pathogenicity was questioned. It has also been isolated from the sputum sample of a patient with

carcinoma of the lung [10,11]. When we reviewed the reports about this organism, we found that most of the cases related to respiratory disease were reported in the 1960s and 1970s, and it was primarily isolated from sputum and respiratory samples. It is evident from later reports that *A. ureae* is the causative organism of wide array of disease conditions such as ocular prosthesis infection [12] and conjunctivitis [13].

A. ureae has also been isolated from more than ten cases of *A. ureae*-caused meningitis in the last two decades [3–5,14]. HIV and history of skull fracture or intracranial surgery were thought to be risk factors [3,4]. It was later reported as the causative agent of meningitis and bacteraemia [5,14]. The organism has also been reported from bone marrow aspirate cultures of a patient with rheumatoid arthritis with low-grade fever for 3 months [15]. It is also a rare causative agent of spontaneous bacterial peritonitis, first reported from the United States. Alcoholism and chronic liver disease, such as cirrhosis and chronic active hepatitis, are predisposing factors for the infection [2,16]. However, in our case the patient had none of the above predisposing conditions.

Old age, male sex and chronic asthma may be the predisposing conditions in the case we report here. These were also the predisposing conditions in the earlier study of 17 patients' respiratory samples [1].

Actinobacillus (Pasteurella) ureae grows easily on blood agar and is cultured under standard laboratory conditions. Colonies with greening of the agar may be at a glance overlooked as α -haemolytic streptococci, which are normal commensal flora. Identification of the organism by conventional or automated systems which do not have this organism in their database may cause erroneous identification. Only awareness of this organism can guide microbiologists to further analyze it for identification and susceptibility testing after Gram staining.

When BLASTed in NCBI, the present isolate *Actinobacillus ureae* strain RD 16S ribosomal RNA gene, partial sequence (KX355773.1), had the following significant hits, with highest scores for *Actinobacillus ureae* strain Henriksen 3520/59 (NR_118761.1) and *Actinobacillus ureae* strain CCUG 2139 (NR_042874.1), followed by *Actinobacillus ureae* partial 16S rRNA gene, strain P161 (AJ438663.1), and *Actinobacillus ureae* partial 16S rRNA gene, strain P524 (AJ438664.1).

Figure 1 shows the relationship between *A. ureae* RD and different *A. ureae* strains.

Radiologic findings correlated with infective aetiology. Documented bacterial causes of tree-in-bud appearance on CT are *Mycobacterium tuberculosis*, *M. avium-intracellulare* complex, *Staphylococcus aureus* and *Haemophilus influenza* [17].

CT scan findings of centrilobular nodules with tree-in-bud appearance and their clinical/pathologic correlation remains unclear. In patients with tree-in-bud appearance, the

overwhelming likelihood is that the underlying cause is related to infection.

The tree-in-bud pattern can also be found in noninfective conditions like congenital disorders, idiopathic disorders (obliterative bronchiolitis, panbronchiolitis), aspiration, inhalation, immunologic disorders and connective tissue disorders, as well as peripheral pulmonary vascular diseases such as neoplastic pulmonary emboli. However, none of these conditions was found to be associated with the presentation in our patient. Our patient had not had tuberculosis in the past; nor did sputum samples or his present history suggest tuberculosis. Indeed, the patient's history was in no way related any of the above-mentioned causes. The sputum sample was cultured under special culture conditions and media required for the growth of *H. influenzae* and *Streptococcus pneumoniae*, but none was isolated.

Keeping this in mind, and also keeping in mind the findings of several studies of *Actinobacillus ureae* in the sputum of patients with chronic respiratory distress, we speculate that this rare species might be the causative organism even though we could not analyse a second sputum sample and it appears that our patient had an endogenous infection in view of his chronic asthma. Continuous corticosteroid therapy may have predisposed to this commensal organism to cause infection.

In conclusion, we report what is to our knowledge the first case of *A. ureae* from India. Identification of such rare microorganisms should be attempted. A high index of suspicion and awareness among microbiologists about the relevance of *Actinobacillus ureae* in such clinical presentations and the need for confirmation by MALDI-TOF MS or 16S rRNA gene sequencing is highlighted by our study. Any one suffices for confirmation, as there was a 100% correlation on identification by the two methods, although MALDI-TOF MS has the advantage, as it is cheaper, quicker and easier.

Identification by 16srRNA gene sequencing is suggested, as it not only confirms the result by other automated and biochemical systems but also provides evolutionary data. The isolate in this case is 99% identical to *A. ureae* Henriksen 3520/59 (NR_118761.1).

A. ureae may be an additional bacteriologic causative agent of the tree-in-bud pattern on CT to be kept in mind.

Conflict of Interest

None declared.

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