

## Molecular Characterization of *Trichomonas tenax* Causing Pulmonary Infection

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### CASE REPORT

A 58-year-old man presented with retrosternal pain 30 months after a surgical operation for reflux esophagitis in relation to Barrett's esophagus. Gastroscopy revealed an ulcer of the inferior part of the esophagus, which on biopsy proved to be an adenocarcinoma. Additional surgery was performed through the abdomen and through the thorax via the sixth intercostal space. Resection of the lower part of the esophagus and high anastomosis were performed because of nondilatable peptic stenosis. Two chest tubes were put in place: one draining the right pleura and the other in contact with the anastomosis. On the second postoperative day, a sepsis-like syndrome including bronchospasm developed with increasing ventilation requirements. A chest radiograph revealed no abnormalities, and a perfusion computed tomography (CT) scan did not show pulmonary thromboembolism. Amoxicillin-clavulanic acid and gentamicin were given for 8 days despite various bacteriological analyses that were all sterile, including 10 blood samples, 3 pleural fluid samples, 1 bronchoalveolar lavage, 1 bronchial aspiration, and 1 urine sample. The patient improved partially, and the pleural tube was removed. On the 13th postoperative day, the patient was still ventilator dependent. Fever recurred, and biological investigations showed a leukocyte count of 11,700/mm<sup>3</sup> and a C-reactive protein level of 128 mg/liter. A chest CT scan revealed a small collection of 1 cm in diameter in contact with the posterior part of the anastomosis and a new infiltrate of the left lung parenchyma located in the apical segment of the lower lobe. Analysis of the fluids from both bronchoalveolar lavage and chest tube showed a large number of neutrophils and numerous flagellated, motile organisms with the typical appearance of trichomonads, visible on wet preparations and Giemsa staining. Culture of bronchoalveolar fluid yielded *Neisseria lactamica*, while culture of tube fluid yielded alpha- and beta-hemolytic streptococci and *Haemophilus parainfluenzae*. Treatment with metronidazole, piperacillin-tazobactam, and gentamicin was started. Trichomonads were not found in the drained fluid, and a chest CT scan showed the resolution of the collection and no change in pulmonary parenchyma 3 and 5 days after the initiation of metronidazole therapy, respectively. The CT scan failed to show passage of contrast material from the esophageal suture line into the

mediastinum. Antibiotics, including metronidazole, were continued for 10 days, and the tube was removed later. However, the patient had remained ventilator dependent since the operation. Ischemic brain damage of unknown origin was revealed by cerebral magnetic resonance imaging, and the patient remained in a deep coma for 1 week and then died.

In order to identify the species of *Trichomonas*, we amplified directly from the bronchoalveolar fluid and sequenced the 5.8S rRNA gene and the internal transcribed spacer flanking regions (ITS1 and ITS2) with the forward and reverse primers TRICHO-F and TRICHO-R (5'-CGGTAGGTGAACCTGC CGTT-3' and 5'-TGCTTCAGTTCAGCGGGTCT-3', respectively) as described previously (6). DNA extraction from clinical specimens and PCR were performed in a protected molecular biology area. Negative (bronchoalveolar fluids) and positive (vaginal specimen with *Trichomonas vaginalis*) controls were included in the series. Sequence analysis of both strands was carried out on a 3700 DNA analyzer (Applied Biosystems, Courtaboeuf, France). The sequence of the amplified 368-bp fragment was compared with the National Center for Biotechnology Information database sequences and exhibited 100% homology to *Trichomonas tenax* (accession no. U86615). The sequence had only 96% identity to *Trichomonas gallinae* (16 nucleotide differences in the 368-bp fragment), 93% identity to *T. vaginalis* (25 nucleotide differences in the 368-bp fragment), and 85% identity to *Pentatrichomonas hominis* (51 differences in the 339-bp fragment).

*T. tenax* is a commensal of the human oral cavity, found particularly in patients with poor oral hygiene and advanced periodontal disease. Its prevalence in the mouth ranges from 4 to 53% (4). Transmission is through saliva, droplet spray, and kissing or use of contaminated dishes and drinking water (4, 11, 15). Bronchopulmonary infections caused by *T. tenax* have been reported mainly in patients with underlying cancers or other lung diseases (4, 6, 9, 11, 13, 15–17). The organism is believed to enter the respiratory tract by aspiration from the oropharynx. The parasite is known to feed on bacteria and therefore is not able to cause pulmonary disease by itself (9). In the present case, the coinfection with trichomonads and bacteria from the oropharyngeal flora supports the hypothesis that the source of trichomonads was most likely the oral cavity. In addition, this association suggests that the cause of the pulmonary infection may not be the sole parasite and the relative contribution of both trichomonads and bacteria to the pulmo-

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nary disease is unknown. However, as in the other reported pulmonary trichomoniasis cases, the search for trichomonads in the mouth remained unsuccessful (13, 17, 18). The number of trichomonads found in oral washing is rather low, and detection by conventional methods such as wet-mount preparations or staining may not be sensitive enough. In addition, staining is not useful for species identification, and culture techniques are not of routine use (15). Recently, molecular diagnostic tools have been developed for both the detection and the identification of *Trichomonas* species (3, 10). Amplification of the 5.8S rRNA gene by PCR followed by sequencing has become a reliable means for more rapid and specific detection and identification of trichomonads. The 5.8S rRNA sequences present the advantages of being present in multiple copies in the genome and of being conserved in certain regions and variable in others, even between very closely related species (2, 7). In a recent case report describing an empyema due to *P. hominis*, identification to the species level was not possible without DNA sequencing (6). In most other reports of trichomonal pleuropulmonary infection, species characterization has not been performed, and since *T. tenax* is the most common cause, it was considered likely to be the responsible pathogen (9, 18). Pulmonary infections caused by other trichomonads such as *P. hominis*, a parasite of the gastrointestinal tract, or *T. vaginalis*, a pathogen of the genitourinary system, are very unusual (4, 6). However as clinical resistance and in vitro resistance to metronidazole have been regularly reported for *T. vaginalis*, species characterization by molecular methods is of clinical importance (1, 5, 8, 12, 14).

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