Corynebacterium pseudotuberculosis
Pneumonia in a Veterinary Student Infected During Laboratory Work

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We present a case of Corynebacterium pseudotuberculosis pneumonia in a veterinary student, with molecular genetic evidence of acquisition during laboratory work, an observation relevant for laboratory personnel working with C pseudotuberculosis isolates. The patient was clinically cured with 14 months trimethoprim/sulfamethoxazole and rifampicin combination treatment.

Keywords. Corynebacterium; pneumonia; zoonosis.

CASE REPORT

A 23-year-old, previously healthy female veterinary student presented to hospital in July 2007 with a 4-week history of mild airway symptoms with initial globus sensation and dysphagia developing to cough with intermittent purulent expectorate, some night sweats, but no fever. Computed tomography (CT) of the thorax revealed a 60 mm consolidated structure in the posterior upper lobe with enlarged lymph nodes in the right hilum and mediastinum (Figure 1A and B). Laboratory analysis revealed moderate increase in inflammation markers with sedimentation rate 30 mm/hour, C-reactive protein (CRP) 33 mg/L, leukocytes 13.2 × 109 cells/L with slight neutrophilia; 8.0 × 109 cells/L, moderate eosinophilia; 2.4 × 109 cells/L and thrombocytosis; 480 × 109 cells/L. She initially received 2 courses of macrolide treatment and 1 course with penicillin V without clinical response. Infectious serology samples were negative (Pertussis, Mycoplasma, Chlamydia, Francisella, Puumula, Toxoplasma, and human immunodeficiency virus) as well as markers of immune-mediated disease (antinuclear antibodies and antineutrophil cytoplasmic antibodies). Angiotensin-converting enzyme was also in the normal range. Two bronchoscopy procedures with 2 bronchoalveolar lavages (BAL) and 1 blind transbronchial biopsy were performed in September and October 2007. Microbiological cultivation of BAL showed normal airway flora at both occasions, and in the lung biopsy Streptococcus salivarius was found; however, these findings were not considered clinically relevant. Neither atypical bacteria (Mycoplasma, Chlamydia or Legionella), yeast or airways virus were found. Mycobacterium tuberculosis PCR was positive in the first BAL sample. However, this sample was transported in an inadequate medium (Copan viral transport medium) and in the second BAL, transported in isotonic salt water, the Mycobacterium tuberculosis PCR was negative, furthermore there were negative Mycobacterium cultures on both occasions. Thus, we believe that the first Mycobacterium tuberculosis PCR was false positive.

In December 2007, a CT-guided transthoracic fine-needle biopsies from the infiltrate in the right upper lung lobe was performed. The biopsies were immediately transported in isotonic salt water to the microbiological department for analysis. Corynebacterium spp was identified from cultures by means of Gram stain, catalase reaction, and Api Coryne (bioMérieux sa, Marcy l’Etoile, France). Api Coryne gave low discrimination (84%) for Corynebacterium renale group. Corynebacterium ulcerans, but not Corynebacterium pseudotuberculosis, is included in the Api Coryne identification database. Histological examination revealed necrotic material with signs of granulomatous inflammation; the pathologist observed rod-shaped bacteria. DNA extracted from the lung biopsy was amplified and sequenced (ABI Prism 3730 DNA analyzer; Applied Biosystems, Foster City, CA) using 2 primers within the 16S rRNA gene (5′-AGAGTTTGATCCTGGCTCAG and 5′-GTATTACCGTGCTGCTG). The obtained sequence was compared with sequences in the National Center for Biotechnology Information database using BLAST (version 2.2.17) and revealed, as the only species, a 100% shared identity with C pseudotuberculosis. Susceptibility testing was done according to the instructions from bioMérieux sa, and the minimum inhibitory concentrations (MICs) were read after 48 hours of incubation at 37°C. Break points for susceptibility testing were not standardized for this bacterium in 2008, but the isolate was considered susceptible to
the antimicrobial agents tested (penicillin, trimethoprim/sulfa-
methoxazole, clindamycin, erythromycin, rifampicin, and tetracycline) due to the low MIC levels (Table 1). When this bacterium, which rarely causes human disease, was identified, contagion during veterinary studies was suspected. The patient had not been exposed to ungulates during the previous weeks but recalled that she had classes in veterinary microbiology, including work with *C. pseudotuberculosis* cultures, approximately 3 weeks before debut of symptoms. The patient had not been exposed to ungulates during the previous weeks but recalled that she had classes in veterinary microbiology, including work with *C. pseudotuberculosis* cultures, approximately 3 weeks before debut of symptoms. The patient received treatment with 200 mg of doxycycline daily for the first 2 weeks followed by 100 mg daily for the next 2 weeks. Clinically, the patient was in good shape, but she had persistent productive cough and still had some night sweats. A CT scan in February 2008 showed no major differences from July 2007, but the sedimentation rate, CRP, and leukocyte levels were normalized. A supplementary 18-fluorine positron emission tomography (PET)-CT was performed in April 2008, displaying marked metabolic activity in the lung lesion as well as in a lymph node conglomerate in the hilum and mediastinum (Figure 2). Consequently, a second CT-guided fine-needle lung biopsy was done in June 2008. Histological findings were unchanged from the examination 6 months earlier. *Corynebacterium pseudotuberculosis* was again detected both by cultivation and by 16S rDNA PCR, sequencing directly from the biopsied material. Compared with previous analysis, the isolate displayed no significant changes in MIC levels (Table 1). Polymerase chain reaction and cultivation for *M*

![Figure 1](http://ofid.oxfordjournals.org/)

**Figure 1.**  (A and B; 2007) Contrast-enhanced chest computed tomography showing a 60 mm consolidation in the posterior upper right lobe in July 2007 (A) (courtesy of Centrum Radiology Institute, Oslo). There were concomitant enlarged lymph nodes in the right hilum and in the mediastinum in November 2007 (B) (courtesy of Department of Radiology, Drammen Hospital). (C and D; 2013) Nonenhanced chest computed tomography showing the lung lesion reduced to 20 mm (C). Except for one 18 mm calcified lymph node, the right hilum and mediastinum were normalized (D) (courtesy of Department of Radiology, Drammen Hospital).

<table>
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<tr>
<th>MIC (mg/L)</th>
<th>Penicillin</th>
<th>Trimethoprim-Sulfamethoxazole</th>
<th>Clindamycin</th>
<th>Rifampicin</th>
<th>Tetracycllin</th>
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<td>Isolate 2007</td>
<td>0.064</td>
<td>0.125</td>
<td>0.125</td>
<td>0.002</td>
<td>0.064</td>
</tr>
<tr>
<td>Isolate 2008</td>
<td>0.094</td>
<td>0.125</td>
<td>0.064</td>
<td>0.002</td>
<td>0.032</td>
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tuberculosis complex were still negative. In June 2008, she was treated with the combination of trimethoprim/sulfamethoxazole and rifampicin. After 6–7 weeks, the cough and night sweats gradually disappeared. The treatment was continued for the following months, and a new CT scan in May 2009 showed minimal changes from 1 year earlier, prompting continued treatment. Thus, 14 months treatment of trimethoprim/sulfamethoxazole and rifampicin was given in total. During the following 4 years, the patient has had some intermittent stinging chest pain but no relapse of cough or night sweats. The inflammation markers have been in the normal range during this period. A CT scan in October 2013, 4 years after treatment was completed, showed that the lung lesion was reduced to 20 mm with some calcification of a single lymph node, otherwise the hilum and mediastinum were normalized (Figure 1C and D). Since 2007, the patient has regularly performed lung function tests, always with normal results. She is now working full time as a veterinarian.

Genomic Analysis

Genomic analysis was performed to elucidate the possibility of contagion during veterinary studies. The isolate used during the laboratory course originated from goat. The strain isolated from the patient (48252, CP008922), the course strain (CS_10, CP008923), the original goat strain (Ft_2193/67, CP008924), and an equine C pseudotuberculosis reference strain (CCUG 27541, JPJB00000000) have all been sequenced and published [1]. Efficient Database Framework for Comparative Genome Analyses using BLAST Score Ratios (EDGAR) was used to compare the genomes and construct a phylogenetic tree of the species C pseudotuberculosis, including these genomes (https://edgar.computational.bio.uni-giessen.de/) [2]. The newick tree files from EDGAR was visualized in TreeView, version 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) [3]. The phylogram shows that the biovar equi strains all cluster together (C pseudotuberculosis Cp162, 31, 1-06-A, 316, 258 and CIP 5297 [4–9]), whereas the biovar ovis strains [10–14] are divided into 2 main clusters (Figure 3). The patient strain (48252) and the course strain (CS_10) are highly similar and cluster together with the original goat strain (Ft_2193/67). The reference strain CCUG 27541 cluster together with the other equine strains.

The phylogenetic tree presented includes 1 human strain, C pseudotuberculosis FRC41, in addition to our patient strain. This strain, isolated from the inguinal lymph node of a 12-year-old girl with necrotizing lymphadenitis, clusters in the same main biovar ovis group as our patient strain, course strain, original goat strain, and 2 strains isolated from caseous lymphadenitis in sheep and goat [12, 13, 15]. The course strain of C pseudotuberculosis was originally isolated from Norwegian goat herd from Hemsedal, a mountain valley in Southern Norway, in May 1967. The animals demonstrated low appetite with chronic loss of weight.

High similarity between the patient and course strain was further supported by a comparison of gene content. Most genes were common between the strains with only 12 genes unique to the patient strain and 15 genes unique to course strain (most of these genes code for hypothetical proteins except a putative antisigma factor and a conserved membrane protein ML1361 unique to the course strain). The high level of shared genomic identity between the patient and course strain strongly supports the hypothesis that the infection was acquired during laboratory work.

Search for virulence genes and genomic islands potentially involved in virulence was conducted at the Rapid Annotation using Subsystem Technology (RAST) server, version 4.0 (http://rast.nmpdr.org/) [16] and with GIPSy: Genomic Island Prediction Software (http://www.bioinformatics.org/groups/?group_id=1180) using Corynebacterium glutamicum strain ATCC 13032 (NC 003450) as the non-pathogenic reference strain. As expected, the most important known virulence factor for C pseudotuberculosis,
phospholipase D, was among the potential virulence genes detected [17]. Several genes involved in iron acquisition were also detected, most notably the \textit{fag} operon [18]. Thus, mutations in the \textit{fag} operon, located in close proximity to the phospholipase D gene, has been shown to reduce in vivo virulence of \textit{C. pseudotuberculosis} [18]. Furthermore, putative manganese ABC transporters, as well as a manganese-dependent transcription regulator, were among the potential virulence genes identified. No potential virulence genes unique to the patient strain, compared with the course strain, were detected.

Another potent virulence factor, the diphtheria toxin gene (\textit{tox}), has previously been detected in \textit{C. pseudotuberculosis} strains 31, 992, and 993 (all isolated from buffalo in Egypt), likely as a result of horizontal gene transfer [19–21]. However, this toxin was not detected in either of our strains.

**DISCUSSION**

\textit{Corynebacterium pseudotuberculosis} is a facultative anaerobic pleomorphic Gram-positive rod first identified and described as a cause of renal abscess in sheep in 1891 [22]. The microbe is a well known pathogen in veterinary medicine infecting ungulates, predominantly sheep and goats, although horses, cattle, and deer may also be infected [23]. Caseous lymphadenitis is the dominant clinical manifestation among affected animals, but visceral organs including lungs may also be affected [24].

In humans, the first case of infection was described in 1966 [25], and until now approximately 30 human cases have been described, especially among occupationally exposed sheep farmers in Australia [26]. The mode of transmission is usually by direct skin and wound contact, but drinking of contaminated raw goat milk and raw goat cheese may also represent transmission routes [26, 27]. As in ungulates, most human cases present as necrotizing granulomatous lymphadenitis [26]. There is 1 previous report on human \textit{C. pseudotuberculosis} pneumonia, also in a veterinary student, published 35 years ago [28]. This patient had unspecific symptoms with fatigue, chills, and dry cough with a marked initial eosinophilia of 31% in blood and a left lower lobe infiltrate. The patient was given erythromycin for 2 weeks with clinical, biochemical, and radiological response. The source of infection was unclear, but notable also this student had been working with veterinary microbiology 4 weeks before the development of pneumonia and laboratory transmission was suspected.
In this study, we present the second human case of pneumonia caused by \textit{C. pseudotuberculosis} in a veterinary student. Biochemical identification of \textit{C. pseudotuberculosis} and its differentiation from other corynebacteria such as \textit{C. ulcerans} is difficult, but excellent identification was obtained by both PCR and sequencing. Furthermore, the obtained isolates were later retested and identified as \textit{C. pseudotuberculosis} by matrix-assisted laser-desorption/ionization time-of-flight mass spectroscopy (Daltonics, Bremen, Germany). Thus, the identification of \textit{C. pseudotuberculosis} as a cause of chronic pneumonia in our patient is well documented.

The patient history including laboratory work with \textit{C. pseudotuberculosis} 3 weeks before debut of symptoms and the very high level of shared genomic identity between the course strain and the patient strain strongly indicate laboratory transmission. To our knowledge, this is the first documented laboratory transmitted infection by this bacterium. Epidemiological studies among animals have displayed high levels of antibodies towards \textit{C. pseudotuberculosis} [29], but we do not know whether such tests have been validated for human purposes. If such tests were available, screening of veterinaries, students, laboratory workers, and sheep farmers would be of interest to examine the degree of exposition in such human settings.

A very strong catalase reaction is generated when \textit{C. pseudotuberculosis} is exposed to hydrogen peroxide, routinely performed in veterinary laboratory courses including the course that our patient had attended. Performing catalase reactions on \textit{C. pseudotuberculosis} cultures produce spread of aerosols, containing viable bacteria, several centimeters above agar plates, a process both we (unpublished own data, described in Supplementary data) and others have observed [30]. Dry aerosol particles with \textit{C. pseudotuberculosis} may possibly be transported for relatively long distances in a laboratory together with air currents. Thus, we believe that transmission of \textit{C. pseudotuberculosis} to the lungs in our patient was potentially due to inhalation of bacteria aerosols during laboratory work. Consequently, our observation may have implications for how laboratory work with \textit{C. pseudotuberculosis} is organized to prevent new human cases.

The virulence of \textit{C. pseudotuberculosis} is partly due to their ability to produce phospholipase D, hydrolyzing sphingomyelin in mammalian cell membranes such as in endothelial cells, with potential establishment and spread of the infection in the host [26]. Other important virulence factors are proteins involved in iron acquisition, in particular the \textit{fag} operon, responsible for extracellular iron acquisition and survival in hostile environments. The genomic analyses demonstrate that both phospholipase D and \textit{fag} operon as well as other potential virulence genes indeed were detected in the sequenced pathogenic patient strain.

In vitro, \textit{C. pseudotuberculosis} is, like \textit{M. tuberculosis}, able to survive and grow intracellularly in macrophages, thus escaping immune responses and causing necrotizing granulomatous inflammation [31]. However, to our knowledge, the exact mechanism for this property is unknown.

In vitro, \textit{C. pseudotuberculosis} is usually considered susceptible to a broad range of antibiotics including penicillin, as we found in our isolate. However, in almost all human cases previously reported, clinical response is not achieved by drug therapy alone, and additional surgical excision of the affected tissue has been necessary for clinical cure [26]. In this \textit{C. pseudotuberculosis} pneumonia, we observed treatment failure with 4 weeks of tetracycline treatment, but we noted gradual clinical and radiological response with long-term trimethoprim/sulfamethoxazole and rifampicin treatment, without need for surgical removal of the infected tissue. The rationale for using trimethoprim/sulfamethoxazole and rifampicin is the high concentration of these drugs in lung tissue and the beneficial intracellular antimicrobial effect [32]. Hence, long-term treatment with an antibiotic possessing intracellular effect may be necessary because \textit{C. pseudotuberculosis} survive inside macrophages.

It is interesting to note that only low grade and temporarily displayed elevated systemic inflammation markers were observed early in the disease progress in spite of chronic infection. Thus, it was the low-grade, enduring symptoms in combination with radiological changes, in particular observed by PET-CT, that indicated persistent infection, which was subsequently confirmed by repeated microbiological sampling.

**CONCLUSIONS**

In conclusion, we hereby present the first documented laboratory-transmitted infection by \textit{C. pseudotuberculosis}. Moreover, we believe that transmission was due to inhalation of bacteria containing aerosols possibly when catalase reactions were performed during a laboratory course. Hence, our observation may have implications for laboratory personnel working with \textit{C. pseudotuberculosis} to prevent new cases. Finally, a clinical and radiological treatment response was achieved with long-term trimethoprim/sulfamethoxazole and rifampicin combination treatment, without need for surgical removal of the infected tissue.

**Supplementary Material**

Supplementary material is available online at Open Forum Infectious Diseases (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.
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