JOURNAL OF CLINICAL MICROBIOLOGY, June 2002, p. 2300–2301 Vol. 40, No. 6
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.6.2300–2301.2002
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CASE REPORTS

Pneumonia Due to *Bordetella bronchiseptica* in a Cystic Fibrosis Patient: 16S rRNA Sequencing for Diagnosis Confirmation

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Received 3 December 2001/Returned for modification 21 January 2002/Accepted 12 February 2002

Bordetella bronchiseptica **was identified as an unusual etiologic agent of pulmonary recurrent exacerbations and pneumonia in a cystic fibrosis (CF) patient by utilizing a 16S rRNA molecular kit in our hospital's clinical laboratory. This method appears to be a useful approach for identifying new emerging CF pathogens when discrepancies exist between phenotypical tests.**

CASE REPORT

A 27-year-old female patient was diagnosed with cystic fibrosis (CF) at the age of 4 years after persistent pulmonary disease. The diagnosis was confirmed by sweat test and genotype assay showing a homozygous ΔF_{508} mutation of the CF transmembrane conductor regulation gene. Bronchial colonization with *Staphylococcus aureus* had been noticed since childhood and diffuse bronchiectasis had been confirmed by computed tomography scan. She was never colonized by *Pseudomonas aeruginosa.* In April 1999, the patient was admitted to the respiratory care unit for a pulmonary exacerbation with a fever of 38°C, dyspnea, increased sputum, and a significant spirometric deterioration (forced expiratory volume in 1 s [FEV_1] = 1.2 liters, versus 1.4 liters in October 1998; 15% decrease from baseline). In the mucopurulent sputum both *S. aureus* (10^8 CFU/ml) and an unusual gram-negative bacillus (10^8 CFU/ml) were isolated and quantified (1). After the failure of oxacillin treatment, an intravenous antimicrobial treatment (ticarcillin, 250 mg/kg of body weight/day, plus tobramycin, 10 mg/kg/day) was administered for 15 days and the patient clinically recovered. During the follow-up between May 1999 to October 2001, she presented with two episodes of pneumonia in the left lower lobe and two exacerbations. On each occasion, a gram-negative bacillus and *S. aureus* were isolated in the sputum. In October 2001, her FEV_1 under stable condition had significantly decreased to 0.95 liter. This patient lived in town and did not have any pets at home.

In each exacerbation and in the two episodes of pneumonia, sputum was cultured for aerobic organisms. Cultures on bromocresol purple, blood agar, and chocolate agar plates yielded a bacillus culture mixed with *S. aureus*. Gram staining from the bacillus culture showed small gram-negative coccobacilli. This rod was catalase and oxidase positive, motile, non-spore-forming, and strictly aerobic. It was able to reduce nitrate to nitrite, and it did not produce indole. Phenotypical identification performed with the API 20NE system (bioMérieux, Marcy l'Etoile, France) with an inoculum of 0.5 McFarland standard confirmed *Bordetella bronchiseptica* (code no. 1200066; p = 99%; T = 0.84), whereas the identification performed with the API 32GN system gave *Pseudomonas alcaligenes* (code no. 00170063022; p = 95%; T = 0.95). The discrepancy between these two phenotypical identifications and the fact that the rod did not rapidly hydrolyze urease prompted us to use the Microseq 500 16S ribosomal DNA bacterial sequencing kit (Applied Biosystems, Foster City, Calif.). A 496-bp portion of the amplified DNA was sequenced with an automated DNA sequencer (ABI Prism 377; Applied Biosystems). These 496 bp were compared with National Center for Biotechnology Information GenBank entries by using the BLAST algorithm, giving 100% homology with *B. bronchiseptica* (accession no. X 57026) and *B. pertussis* (accession no. AF 142327). *P. alcaligenes* was not indicated in the BLAST algorithm results. The molecular identification of *B. bronchiseptica* was the most reliable one, corresponding to the API 20NE phenotypical identification, since the bacteria grow on simple nutritive medium (bromocresol purple). *B. pertussis* needs enriched media such as Bordet-Gengou or Regan-Lowe medium to grow and does not grow on simple medium. At last, the in vitro susceptibility tests using a disk diffusion technique on Mueller-Hinton agar according to the Antibiogram Committee of the French Microbiology Society (15) showed that this bacterium was susceptible to ticarcillin, piperacillin, ceftazidime, imipenem, gentamicin, tobramycin, trimethoprim-sulfamethoxazole, and doxycycline and resistant to fluoroquinolones.

B. bronchiseptica is generally a common commensal or pathogen in the respiratory tract of several mammalian species (18). Since 1975, fewer than 50 cases of respiratory infections have been published. They were mainly found in immunocompromised patients and were often associated with animals (2, 6, 8, 11, 13). Among the new emerging pathogens in CF (e.g., *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, and *Alcaligenes xylosoxidans*), *B. bronchiseptica* has not been men-

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tioned in recent reviews (3, 4). This is why our first identification of *B. bronchiseptica* by the API ID20 NE system had to be confirmed by another phenotypical method. The API ID 32GN system giving *P. alcaligenes* was perhaps most plausible in the context of CF. Moreover, the case described by Peltroche-Llacsahuanga et al. (12), reporting a misidentification of *A. xylosoxidans* as *B. bronchiseptica* by an API ID20 NE system, was a good reason to check our identification with another method (7). The genotypical approach utilizing a 16S rRNA molecular sequencing method allowed us to confirm rapidly and with good agreement the identification of *B. bronchiseptica*. When an unusual microorganism is identified in CF patients, the main question is whether it plays a role in pulmonary deterioration. Such uncertainties remain for pathogens such as *S. maltophilia* (3). A follow-up over 2 years for our patient showed a significant deterioration of pulmonary function. Such a decline appears unusual for patients with long-term isolated *S. aureus* colonization. In addition, oxacillin treatment during initial exacerbations did not provide a clinical response. The role of *B. bronchiseptica* in lung disease remains unclear, but the ability of this bacterium to inhibit leukocyte function and to adhere to respiratory epithelial cells explains the seriousness of the infection and the persistence in the lower respiratory tract (8, 9, 16). Moreover, immunocompetent patients have been described as infected by *B. bronchiseptica* (14, 16, 17). In our patient, the two pneumonias developed while she was pregnant. Finally, a strict questioning of the patient revealed that several months prior to the first exacerbation of bronchitis, which occurred 3 years ago, she practiced occasional horse riding, which could explain the colonization of the respiratory tract of the patient by *B. bronchiseptica* (5, 10). *B. bronchiseptica* could potentiate the obstructive lung disease by altering the ciliary kinesia and thus reducing mucociliary clearance and local defenses.

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