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***Bordetella holmesii*–Like Organisms Associated with Septicemia, Endocarditis, and Respiratory Failure**

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We recovered an unusual bacterial strain from blood or sputum of three patients with septicemia, endocarditis, and/or respiratory failure. The three isolates were thin, curved, gram-negative, light brown, pigment-producing bacilli with variable catalase activity. They were asaccharolytic, oxidase-negative, nonmotile, and fastidious. Identification was not possible on the basis of these characteristics alone or in combination with cellular fatty acid profiles. Nucleic acid amplification and sequence analysis of the 16S rRNA gene revealed that all three isolates were identical and most closely related to the emerging pathogen *Bordetella holmesii*, diverging from the published sequence at three nucleotide positions (99.8% similarity). Isolation of a *B. holmesii*–like pathogen from sputum suggests that, in addition to producing septicemia, the organism may inhabit the respiratory tract like other *Bordetella* species.

We describe three patients who developed septicemia, endocarditis, and/or respiratory failure due to a *Bordetella holmesii*–like bacterium that was identified only with use of nucleic acid amplification and sequence analysis of 16S rRNA.

Case Reports

Patient 1

A 15-year-old boy from Austin, Minnesota, presented with fever, headache, and cough. His cough had begun 2 weeks earlier and was productive of green sputum. The cough persisted, and on the morning of admission, the patient awoke with a temperature of 104°F, headache, malaise, anorexia, and neck tenderness. While waiting in the emergency department, he vomited. The patient had had a splenectomy at age 4 years because of hereditary spherocytosis. He had remained healthy until the previous year, during which he had several episodes of fever and cough, often associated with nausea and vomiting. He had a regular history of immunization, including diphtheria-pertussis-tetanus vaccination 10 years earlier. The patient had not traveled outside the United States during the previous 3 months.

On physical examination, the patient was flushed, pleasant, and in no apparent distress. His temperature was 38.4°C (oral);

pulse, 95/minute; respirations, 24/minute; and blood pressure, 112/66 mm Hg. His head, eyes, ears, nose, and throat appeared normal on examination, and the lungs were clear. The blood hemoglobin level was 15.2 g/dL, the platelet count was 407,000/mm³, and the leukocyte count was 37,500/mm³, with 87% neutrophils and 7% monocytes. Urinalysis was normal. Nasopharyngeal swab specimens obtained for tests for group A streptococcus and pertussis antigens and for culture were negative. Urine and throat swab cultures for bacteria were unrevealing. Small, curved gram-negative bacilli with similar colony and microscopic morphology grew in aerobic bottles used for both blood samples collected on admission day. One isolate (X69095) was chosen for further identification.

The patient was given a dose of ampicillin/sulbactam, was hospitalized, and began receiving intravenous fluid support and ceftazidime. Subsequent cultures of blood and urine were negative. Ceftazidime was changed to cefazolin 5 days later on the basis of susceptibility findings. Stabilized and defervesced, the patient was discharged 7 days later with a normal leukocyte count and differential. Oral penicillin V (250 mg b.i.d. indefinitely) was prescribed for prophylaxis.

Patient 2

A 25-year-old man from Little Falls, Minnesota, with a 3-week history of fever was admitted. Five years earlier, in 1992, he had received radiation therapy for stage IIA Hodgkin's disease, and there had been no evidence of recurrence. Over the previous 3 days, he had had a constant fever refractory to acetaminophen. The patient was thirsty and fatigued and had intermittent pain in the thorax and shoulder blade. He had a bicuspid aortic valve that was diagnosed in 1988, and echoradiography in June 1996 showed thickened leaflet edges and mild regurgitation. The patient was allergic to penicillin.

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During the physical examination the young man was pleasant and in no apparent distress. His temperature was 39.3°C (oral); pulse, 110/minute; and blood pressure, 120/70 mm Hg. Head, eye, ear, nose, and throat findings were unremarkable. The lungs were clear. The patient was tachycardic with normal S1 and S2 sounds and no murmur. The blood hemoglobin level was 14.2 g/dL, the platelet count was 210,000/mm³, and the leukocyte count was 17,400/mm³, with 71% segmented and 18% banded neutrophils. A bone marrow biopsy was negative for recurrent lymphoma. Urine and throat swab cultures for bacteria were negative. Blood cultures were performed 1 and 2 days after admission, with one aerobic bottle from each set yielding gram-negative rods (190788).

Transesophageal echocardiography revealed a posterior leaflet vegetation, substantiating the diagnosis of aortic valve endocarditis. Ceftriaxone (2 g iv q.d. for 28 days) was administered, resulting in complete recovery.

Patient 3

A 41-year-old woman from Uniontown, Pennsylvania, was admitted because of respiratory failure due to acute pulmonary edema and concomitant bronchitis. The patient had had a cough productive of purulent sputum for ~4 days before admission. At 5:00 A.M. on the morning of admission, she was awakened by severe dyspnea. Cyanotic and confused, she was transported to the emergency department in a 100% non-rebreather mask and intubated upon arrival. The patient had systolic congestive heart failure with ejection fraction of ~20% following myocardial infarctions 10 and 15 years before admission. She had had a seizure disorder since 1987. Her medical history included chronic obstructive pulmonary disease and tobacco abuse (at least 40 packs/year).

Initial blood gas analysis showed the following values: pH, 7.09; PaCO₂, 86; and PaO₂, 154. The chest roentgenogram revealed cardiomegaly with diffuse interstitial and alveolar infiltrates consistent with pulmonary edema. The blood hemoglobin level was 13 g/dL, the platelet count was 223,000/mm³, and the leukocyte count was 15,700/mm³, with 75% segmental and 4% banded neutrophils. Cardiac enzyme levels were normal. Sputum gram staining showed >25 WBCs per low-power field. Urine and blood cultures were negative. After 3 days, sputum cultures yielded a gram-negative organism (O65172) susceptible to ampicillin, first-generation cephalosporins, and trimethoprim-sulfamethoxazole.

The patient underwent mechanical ventilation for 48 hours. She received renal-dose dopamine, nitrates, furosemide, beclomethasone, and cefuroxime. After defervescence, the chest radiographic findings normalized, and she was discharged after 5 hospital days, at which time she was receiving cefpodoxime.

Results

All three isolates grew on blood and chocolate agar plates after 2 days; however, they did not grow on eosin methylene

blue medium. Microscopic morphology included thin, curved gram-negative bacilli. Light brown pigment generated after 3 days of growth. Biochemical reactions were negative except for variable catalase activity (table 1).

The antibiotic susceptibility profile was determined by an agar dilution method previously described [2]. All three isolates were susceptible to the following antibiotics: amikacin, ampicillin, cefazolin, cefotaxime, ceftazidime, chloramphenicol, gentamicin, mezlocillin, trimethoprim-sulfamethoxazole, imipenem, ciprofloxacin, and piperacillin/tazobactam.

Bacterial cells after 48 hours of growth were saponified, and the liberated fatty acids were methylated and analyzed by capillary gas-liquid chromatography [3] (MIDI, Newark, DE). The system read out “*Bordetella avium*” for isolate O56172 and “no match” for isolates X69095 and 190788. All three isolates yielded large amounts of hexadecanoate and cyloheptadecanoate and relatively small amounts of tetradecanoate and *cis*-9-hexadecanoate. Only trace or small amounts of other fatty acids were detected (table 1).

A primer set (8FPL and 1492RPL, 5'-AGTTTGATCCTGGCTCAG-3' and 5'-GGTTACCTTGCCACGACTT-3') spanning the region of the 16S rRNA gene corresponding to 8–1,510 nucleotide positions of *Escherichia coli* was used to amplify the DNA fragment by PCR [4, 5]. The PCR amplified products were sequenced with use of seven additional internal primers as previously described [4–8]. Phylogenetic analysis using both neighbor-joining and maximum parsimony algorithms was performed as described previously [9]. Sequences determined for the three isolates were 100% identical and most closely related to the emerging pathogen *Bordetella holmesii* [1], diverging from the published sequence at only three nucleotide positions (99.8% similarity).

Phylogenetic analysis revealed that the isolates were closely related to but distinct from the 16S rRNA gene sequence for *B. holmesii*. Neighbor-joining (figure 1) and parsimony analyses gave similar results. Our clinical isolates and *B. holmesii* clustered separately from other *Bordetella* species [1], including *B. pertussis* (99% “bootstrap” confidence level), *B. parapertussis* and *B. bronchiseptica* (86%), and *B. avium* (100%). *Alcaligenes xylosoxidans* [10] and *Kinetoplastibacterium crithidii* [11] clustered distinctly from each other and from the three clinical isolates (92% and 64%, respectively). On the basis of the biochemical reactions, cellular fatty acid profiles, and 16S rRNA gene sequence analyses, these new isolates were identified as *B. holmesii*-like gram-negative bacilli.

Discussion

We identified a *B. holmesii*-like bacillus in three patients, including an asplenic adolescent, a patient with previous Hodgkin's lymphoma, and a patient with chronic obstructive pulmonary disease. *B. holmesii*, originally called “CDC nonoxidizer group 2,” was named in 1995 in honor of Barry Holmes for his substantial contributions to characterization, classification,

Table 1. Biochemical reactions and cellular fatty acid profiles of the three isolates of a *Bordetella holmesii*-like gram-negative bacillus.

Test parameter	Data per isolate (source)			
	X69095 (Blood)	O65172 (Sputum)	190788 (Blood)	<i>B. holmesii</i> * (Blood)
Biochemical reaction[†]				
Nitrate/nitrite reduction	—	—	—	—
Motility	—	—	—	—
Catalase	w+	+	—	+
Oxidase	—	—	—	—
Urease	—	—	—	—
H ₂ S production (tri-sugar ion tube)	—	—	—	—
Decarboxylase production				
Lysine	—	—	—	—
Arginine	—	—	—	—
Ornithine	—	—	—	—
Acid from (oxidative fermenter):				
Dextrose	—	—	—	—
Maltose	—	—	—	—
Xylose	—	—	—	—
Cellular fatty acid profile (%)				
Hexadecanoate (C _{16:0})	56.1	37.4	47.6	41 [‡]
Cyloheptadecanoate (C _{17:0_{cy}})	33.4	35.2	27.6	30 [‡]
Tetradecanoate (C _{14:0}) and <i>cis</i> -9-hexadecanoate (C _{16:1_{w7c}})	10.5	4.4	1.1	7 [‡]
Straight-chain hydroxy acids				
2-OH-C _{12:0}	Trace	3.2	3.1	4 [‡]
2-OH-C _{14:0}	Trace	2.6	3.3	4 [‡]

* Data adapted from [1].

[†] + = positive; w+ = weak positive; — = negative.

[‡] Pooled results from eight strains.

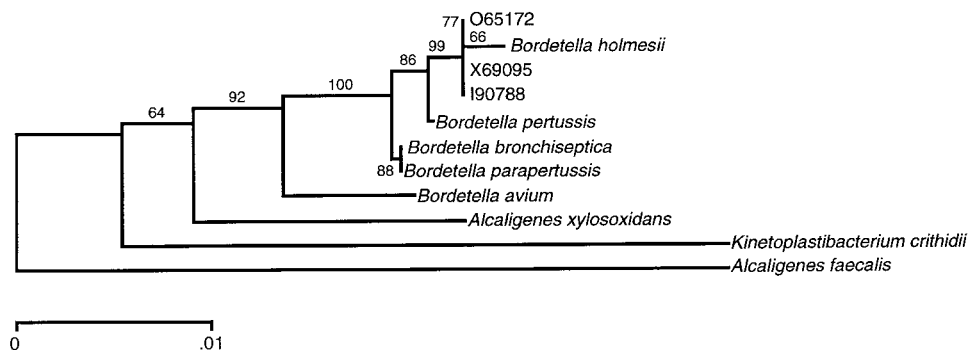
and identification of unusual pathogenic and opportunistic bacteria [1]. Since 1989, >15 isolates have been recovered in blood cultures, mostly of specimens from young adults [1, 12]. Isolation of *B. holmesii*-like pathogens from different clinical specimens from immunocompromised patients with septicemia, endocarditis, and respiratory failure has confirmed their pathogenicity in humans. However, we believe ours is the first description of a pathogenic *B. holmesii* isolated from sputum.

Bordetella species are small, obligately aerobic, gram-negative coccobacilli or short rods, traditionally regarded as respiratory tract parasites. Previously, the genus *Bordetella*

consisted of only four species: *B. pertussis*, *B. parapertussis*, *B. bronchiseptica*, and *B. avium*. They are closely related, showing a DNA homology of 72%–94% [13] and very limited diversity by multilocus enzyme analysis [14]. *Bordetella* species do not survive outside the body for appreciable periods of time. All four original species uniquely localize on cilia of the mammalian respiratory epithelium.

B. pertussis, the causative agent of whooping cough, and *B. parapertussis* are the most important human pathogens among the genus. *B. bronchiseptica* and *B. avium* are respiratory pathogens in various animal hosts; the former occasionally

Figure 1. Neighbor-joining analysis of DNA sequences from several of the species found to have homology with the three clinical isolates, X69095, O65172, and 190788. Phylogenetic analysis was based on 16S rRNA gene sequences. The “bootstrap” replicate percentages appear above each branch. The scale is provided as a measurement of the relative phylogenetic distance.



infects humans as well. Recent genotypic and phenotypic analyses have categorized *Bordetella hinzii* [15, 16], *Bordetella trematum* [17], and the recently reported emerging pathogen *B. holmesii* [1]. *B. hinzii* is a nonpathogenic inhabitant of the respiratory tracts of fowl that occasionally infects humans [18]. Our isolation of *B. holmesii* from sputum indicates that this organism may inhabit the human respiratory tract like other *Bordetella* species.

Biochemical reactions are helpful to differentiate *B. holmesii* from other *Bordetella* species; however, the lack of oxidase activity and of carbohydrate utilization makes it difficult to differentiate this organism from other phenotypically similar bacteria, including asaccharolytic *Acinetobacter* species [19] and CDC nonoxidizer group 1 [20]. Herein, we distinguished *B. holmesii* by sequencing the 16S rRNA gene, in which it is possible to find species-specific regions of 20–30 bases [21]. Identification of fastidious pathogens can be accomplished by sequencing of the 16S rRNA gene [6, 7], which can be amplified directly from bacterial colonies without prior extraction [22]. Our confirmation of *B. holmesii* on the basis of 99.8% sequence homology illustrates the utility of this technique.

The *B. holmesii*-like organisms reported herein are susceptible to most antibiotics intended for treatment of gram-negative bacillus infection. Our laboratory investigations and those of others [12] suggest that use of an intravenous third-generation cephalosporin (ceftriaxone, ceftazidime, or cefotaxime) would likely lead to a favorable clinical outcome, even for patients with invasive infections as described herein.

The normal habitat of *B. holmesii* remains to be determined. Isolation directly from the respiratory tract suggests that this organism may be carried in the oral and/or respiratory tract of humans and/or animals. Underlying immunocompromising conditions such as sickle cell anemia, diabetes, lymphoma, Hodgkin's disease, and prior splenectomies may enhance susceptibility to infection.

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References

- Weyant RS, Hollis DG, Weaver RE, et al. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. *J Clin Microbiol* 1995;33:1–7.
- Washington JA, ed. Laboratory procedures in clinical microbiology. 2nd ed. New York: Springer-Verlag, 1985:293–303.
- Eerola E, Lehtonen OP. Optimal data processing procedure for automatic bacterial identification by gas-liquid chromatography of cellular fatty acids. *J Clin Microbiol* 1988;26:1745–53.
- Eden PA, Schmidt TM, Blakemore RP, Pace NR. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction–amplified 16S rRNA-specific DNA. *Int J Syst Bacteriol* 1991;41:324–5.
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S Ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.
- Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992;327:293–301.
- Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary aniomatosis: an approach to the identification of uncultured pathogens. *N Engl J Med* 1990;323:1573–80.
- Chen K, Neimark H, Rumore P, Steinman CR. Broad range DNA probes for detecting and amplifying eubacterial nucleic acids. *FEMS Microbiol Lett* 1989;48:19–24.
- Kolbert CP, Bruinsma ES, Abdulkarim AS, et al. Characterization of an immunoreactive protein from the agent of human granulocytic ehrlichiosis. *J Clin Microbiol* 1997;35:1172–8.
- Dewhirst FE, Paster BJ, Bright PL. *Chromobacterium*, *Eikenella*, *Kingella*, *Neisseria*, *Simonsiella* and *Vitreoscilla* species comprise a major branch of the beta group *Proteobacteria* by 16S rRNA sequence comparison: transfer of *Eikenella* and *Simonsiella* to the family *Neisseriaceae* (emend.). *Int J Syst Bacteriol* 1989;39:258–66.
- Du Y, McLaughlin G, Chang KP. 16S Ribosomal DNA sequence identities of beta-proteobacterial endosymbionts in three *Crithidia* species. *J Bacteriol* 1994;176:3081–4.
- Lindquist SW, Webber DJ, Mangum ME, Hollis DG, Jordan J. *Bordetella holmesii* sepsis in an asplenic adolescent. *Ped Infect Dis J* 1995;14:813–5.
- Kloos WE, Mohapatra N, Dobrogosz WJ, Ezzell JW, Manclark CR. Deoxyribonucleotide sequence relationships among *Bordetella* species. *Int J Syst Bacteriol* 1981;31:173–6.
- Musser JM, Hewlett EL, Pepler MS, Selander RK. Genetic diversity and relationships in populations of *Bordetella* species. *J Bacteriol* 1996;166:230–7.
- Cookson BT, Vandamme P, Carlson LC, et al. Bacteremia caused by a novel *Bordetella* species, “*B. hinzii*.” *J Clin Microbiol* 1994;32:2569–71.
- Vandamme P, Hommez J, Vancanneyt M, et al. *Bordetella hinzii* sp. nov., isolated from poultry and humans. *Int J Syst Bacteriol* 1995;45:37–45.
- Vandamme P, Heyndrickx M, Vancanneyt M, et al. *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of *Alcaligenes denitrificans* Ruger and Tan 1983. *Int J Syst Bacteriol* 1996;46:849–58.
- Funke G, Hess T, van Gracevenitz A, Vandamme P. Characteristics of *Bordetella hinzii* strains isolated from a cystic fibrosis patient over a 3-year period. *J Clin Microbiol* 1996;34:966–9.
- Gerner-Smidt P, Tjernberg I, Ursing J. Reliability of phenotypic tests for identification of *Acinetobacter* species. *J Clin Microbiol* 1991;29:277–82.
- Hollis DG, Moss CW, Daneshvar MI, Meadows L, Jordan J, Hill B. Characterization of Centers for Disease Control group NO-1, a fastidious, nonoxidative, gram-negative organism associated with dog and cat bites. *J Clin Microbiol* 1993;31:746–8.
- Woese CR. Bacterial evolution [review]. *Microbiol Rev* 1987;51:221–71.
- Ruano G, Kidd KK. Coupled amplification and sequencing of genomic DNA. *Proc Natl Acad Sci USA* 1991;88:2815–9.