Native Valve Endocarditis Due to a Novel Strain of *Legionella*[▽]

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Legionellae are Gram-negative bacteria which are capable of causing disease, most commonly in the form of pneumonia. We describe a case of native valve endocarditis caused by a *Legionella* strain which by genotypic (16S rRNA and *mip* gene sequencing) and phenotypic analyses is unlike previously described strains of *Legionella*.

Of the 54 validly named *Legionella* species, 21 have been isolated from human infections (14, 24). *Legionella pneumophila* represents 90% of the cases, with *L. longbeachae* and *L. bozemanii* being the next most common, causing 4% and 2% of cases, respectively (25). The primary site for *Legionella* infection is the lung, but in rare cases, *Legionella* is found in extrapulmonary sites, either as a consequence of dissemination from the lung or as isolated primary infections (13). Here, we describe an unusual case of *Legionella* endocarditis in a native heart valve. Genotypic and phenotypic analyses indicate that the bacterial isolate is unlike previously described strains of *Legionella*.

A 68-year-old woman with Goodpasture's syndrome who underwent living related kidney transplantation in 2004 presented to the hospital in August 2009 complaining of chest pain and dyspnea on exertion. Her history was notable for cytomegalovirus viremia in 2004 and an episode of organ rejection in 2008 requiring methylprednisolone and thymoglobulin therapy. Her maintenance immune suppression was mycophenolic acid and tacrolimus. At presentation, she reported a 1-day history of chest pain associated with nausea, diaphoresis, and anxiety. She was afebrile, with a heart rate of 100 bpm, blood pressure 113/42 mm Hg, and pulse oximetry of 96% on 2 liters of oxygen. A grade II holosystolic murmur was present. Her leukocyte count was 13,500/mm³ (normal range, 4.1 to 10.9/mm³), creatinine level was 3.5 mg/dl (normal range, 0.4 to 1.0 mg/dl), potassium level was 5.7 meq/liter (normal range, 3.4 to 5.1 meq/liter), and troponin level was 0.2 ng/ml (normal range, ≤0.1 ng/ml). A chest radiograph demonstrated an infiltrate in the right lower lobe, which was confirmed by computed tomography (CT) scan of the chest.

Due to a history of penicillin allergy, vancomycin and ciprofloxacin were initiated for suspected health care-associated pneumonia (she had been hospitalized 3 weeks earlier for "gastroenteritis"). *L. pneumophila* urine antigen and urine pneumococcal antigen were undetectable, and blood cul-

tures revealed no growth. Her troponin level continued to rise and peaked at 2.18 ng/ml. A transesophageal echocardiogram showed a 1.09-cm freely mobile mass on the left coronary cusp of the aortic valve, consistent with vegetation.

Vancomycin and ciprofloxacin were continued, and gentamicin was added for empirical coverage of culture-negative endocarditis. Her clinical status deteriorated, and she underwent emergency aortic valve replacement with a bovine bioprosthesis on the 6th day of hospitalization. Intraoperative findings included a trileaflet aortic valve with a large vegetation on the left coronary cusp. No aortic annulus abscess was found. Gram stain of the resected aortic valve revealed lightly staining Gram-negative bacilli; however, routine tissue culture showed no growth. Broad-range 16S rRNA PCR and sequencing were performed on the resected aortic valve tissue (17, 22). The 16S rRNA genes were PCR amplified with broad-range bacterial primers, using HotStar *Taq* master mix (Qiagen, Inc., Valencia, CA) (3). Bidirectional sequencing was performed using the BigDye terminator kit (v 3.0) on the ABI 3130xl gene analyzer (Applied Biosciences, Foster City, CA). The DNA sequences were aligned in DNASTar SeqMan Pro v7.2 software (DNASTar, Inc., Madison, WI) and compared to reference sequences in GenBank (3). The 16S rRNA sequencing identified the organism as a *Legionella* species (see below). The organism subsequently grew after plating on buffered charcoal-yeast extract (BCYE) agar.

The patient's antibiotics were changed to moxifloxacin, and she completed a 6-week course of therapy. Her clinical condition improved initially, but she subsequently restarted dialysis due to worsening renal failure. Serial transthoracic echocardiograms were unchanged until April 2010, when she was readmitted to the hospital with volume overload. A transthoracic echocardiogram showed evidence of a perivalvular abscess with disruption of the valve. Blood cultures remained sterile. She was restarted on therapy with vancomycin, azithromycin, and rifampin. Despite treatment, her clinical status deteriorated, and she became hypotensive, requiring vasopressors to maintain her blood pressure. A repeat valve replacement and debridement of infected tissue were recommended. However, the patient refused repeat surgery and expired 2 weeks following admission. A request for an autopsy was declined.

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Extrapulmonary manifestations of *Legionella* infections include myocarditis, pericarditis, and endocarditis. There have been 16 cases of *Legionella* pericarditis or myocarditis, attributed mostly to *L. pneumophila* and *L. dumoffii*, and 15 cases of *Legionella* endocarditis linked to *L. pneumophila*, *L. micdadei*, and *L. dumoffii* (2, 5, 6, 10, 11, 13, 15, 18, 20). All but one of the endocarditis cases occurred in patients who had received aortic homografts or prosthetic valve replacements, and in most cases, the suspected route of infection was via direct inoculation with contaminated surgical equipment. The single case of native valve endocarditis reported in the literature was due to *L. pneumophila* (18). In contrast, 16S rRNA PCR and sequencing of the aortic valve tissue and culture isolate in our case revealed that the *Legionella* strain, designated H63, shared the highest similarity with *L. brunensis* ATCC 43878^T (98.4%), an environmental strain originally isolated from a cooling tower in Czechoslovakia and never implicated in disease (23). Among the next closest species were *L. jordanis* ATCC 33623^T (96.5%), *L. nautarum* ATCC 49506^T (96.5%), and *L. jamestowniensis* ATCC 35298^T (96.0%).

While bacterial strains displaying less than 97% similarity in 16S rRNA sequence are to be considered distinct species, it has recently been suggested, based on retrospective analysis of 16S rRNA data and DNA-DNA hybridization values, that the cutoff value be raised to 98.7% (19). Indeed, *Legionella* species can share up to 99.4% 16S rRNA similarity but still be defined as distinct based on DNA-DNA hybridization values below 70% (7, 12). The *mip* gene has been proposed as an additional sequencing target because of its wide distribution among legionellae and increased resolution over the 16S rRNA gene (16). In congruence with the 16S rRNA analysis, *mip* of H63 shared its highest degree of similarity with *mip* in *L. brunensis* ATCC 43878^T (85.6%), followed by *L. hackeliae* ATCC 35250^T (85.3%), *L. jamestowniensis* ATCC 35298^T (84.1%), and *L. feeleii* ATCC 35072^T (84.3%). The 14.5% difference between H63 and *L. brunensis* is within the range of variation that is typical of *Legionella* species (3 to 31%) (16). In sum, genomic data suggest that H63 represents either a novel strain of *L. brunensis* or a new *Legionella* species.

H63 grew well on BCYE agar and displayed L-cysteine auxotrophy (21). It produced a brown pigment upon growth in buffered yeast extract (BYE) broth, did not autofluoresce under UV light, and was negative for nitrate reduction, glucose fermentation, and urease (1, 21). Regarding the published chemotaxonomic traits that vary among *Legionella* species, H63 produced catalase, β -lactamase, and gelatinase. As related to a final variable trait, H63 hydrolyzed hippurate, unlike *L. brunensis*, the nearest neighbor based on 16S rRNA sequence (8, 9). Cellular fatty acids of H63 were determined with the Microbial Identification System (MIDI, Newark, DE) after 72 h of incubation at 35°C on BCYE agar (4). The main fatty acids of H63 were C_{15:0} anteiso (29%), C_{16:1w6c/w7c} (22%), and C_{16:0} (21%). Compared to entries in the Sherlock CLIN database, H63 had a fatty acid composition most similar to *L. steigerwaltii* and *L. jamestowniensis*, with similarity indices of 0.397 and 0.357, respectively. H63 was further distinguished from *L. brunensis* by the fact the main fatty acid of that type strain is C_{15:0} at 39%, followed by C_{17:0} anteiso at 24% and C_{16:0} at 12% (23). Taken together, the results from standard

phenotypic analysis support the view that H63 represents a novel strain of *Legionella*.

Strain H63 displays the typical genetic, physiological, and biochemical characteristics of the *Legionellaceae* family. Based upon 16S rRNA and *mip* sequencing, the closest *Legionella* species is *L. brunensis*; however, there are notable differences between the strains in terms of hippurate hydrolysis and fatty acid makeup. While it has yet to be determined if H63 is a novel strain of *L. brunensis* or represents a new species of *Legionella*, this is only the second reported case of native valve endocarditis due to a *Legionella* species and a reminder that *Legionella* should be considered in the differential diagnosis of culture-negative endocarditis.

Nucleotide sequence accession number. The 16S rRNA and *mip* sequences have been deposited in GenBank under accession no. JF831047 and JF831048, respectively.

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REFERENCES

- Baine, W., J. Rasheed, J. Feeley, G. Gorman, and L. Casida. 1978. Effect of supplemental tyrosine on pigment production in cultures of the Legionnaires' disease bacterium. *Curr. Microbiol.* 1:93–94.
- Burke, P. T., R. Shah, R. Thabalingam, and S. Saba. 2009. Suspected *Legionella*-induced perimyocarditis in an adult in the absence of pneumonia: a rare clinical entity. *Tex. Heart Inst. J.* 36:601–603.
- Claridge, J. E., III. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev.* 17:840–862.
- Diogo, A., A. Verissimo, M. F. Nobre, and M. S. da Costa. 1999. Usefulness of fatty acid composition for differentiation of *Legionella* species. *J. Clin. Microbiol.* 37:2248–2254.
- Friedland, L., et al. 1984. Ocular and pericardial involvement in Legionnaires' disease. *Am. J. Med.* 77:1105–1107.
- Hagl, C., et al. 2002. Replacing the ascending aorta and aortic valve for acute prosthetic valve endocarditis: is using prosthetic material contraindicated? *Ann. Thorac. Surg.* 74:S1781–S1785.
- Hookey, J. V., N. A. Saunders, N. K. Fry, R. J. Birtles, and T. G. Harrison. 1996. Phylogeny of *Legionellaceae* based on small-subunit ribosomal DNA sequences and proposal of *Legionella lytica* comb. nov. for *Legionella*-like amoebal pathogens. *Int. J. Syst. Bacteriol.* 46:526–531.
- Hwang, M.-N., and G. M. Ederer. 1975. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci. *J. Clin. Microbiol.* 1:114–115.
- Kuroki, H., et al. 2007. *Legionella impletisoli* sp. nov. and *Legionella yabuuchiae* sp. nov., isolated from soils contaminated with industrial wastes in Japan. *Syst. Appl. Microbiol.* 30:273–279.
- Larive, S., et al. 1998. Acute pericarditis of unusual etiology. *Rev. Pneumol. Clin.* 54:152–155.
- Littrup, P., J. K. Madsen, and K. Lind. 1987. Aortic valve endocarditis associated with *Legionella* infection after *Mycoplasma pneumoniae*. *Br. Heart J.* 58:293–295.
- Lo Presti, F., et al. 1999. *Legionella taurinensis* sp. nov., a new species antigenically similar to *Legionella spiritensis*. *Int. J. Syst. Bacteriol.* 49:397–403.
- Lowry, P. W., and L. S. Tompkins. 1993. Nosocomial legionellosis: a review of pulmonary and extrapulmonary syndromes. *Am. J. Infect. Control* 21:21–27.
- Muder, R. R., and L. Y. Victor. 2002. Infection due to *Legionella* species other than *L. pneumophila*. *Clin. Infect. Dis.* 35:990–998.
- Patel, M. C., et al. 2005. *L. micdadei* PVE successfully treated with levofloxacin/valve replacement: case report and review of the literature. *J. Infect.* 51:265–268.
- Ratcliff, R. M., J. A. Lanser, P. A. Manning, and M. W. Heuzenroeder. 1998. Sequence-based classification scheme for the genus *Legionella* targeting the *mip* gene. *J. Clin. Microbiol.* 36:1560–1567.
- Relman, D. A., T. M. Schmidt, R. P. MacDermott, and S. Falkow. 1992. Identification of the uncultured bacillus of Whipple's disease. *N. Engl. J. Med.* 327:293–301.
- Samuel, V., A. A. Bajwa, and J. D. Cury. 2 June 2011, posting date. First case of *Legionella pneumophila* native valve endocarditis. *Int. J. Infect. Dis.* [Epub ahead of print.]
- Stackebrandt, E. J. 2006. Taxonomic parameters revisited: tarnished gold standards. *Microbiol. Today* 8:6–9.

20. **Torres Tendero, D., J. Gutierrez Fernandez, A. Diez Ruiz, J. M. Bermudez Garcia, and J. Rico Irlas.** 1995. Pleuropericarditis as the only manifestation of *Legionella pneumophila* infection. *Arch. Bronconeumol.* **31**:249–251.
21. **Weaver, R. E., and J. C. Feeley.** 1979. Cultural and biochemical characterization of the Legionnaires' disease bacterium, p. 20–25. *In* G. L. Jones and G. A. Hebert (ed.), *Legionnaires': the disease, the bacterium and methodology*. Centers for Disease Control, Atlanta, GA.
22. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**:697–703.
23. **Wilkinson, H. W., et al.** 1988. *Legionella moravica* sp. nov. and *Legionella brunensis* sp. nov. isolated from cooling-tower water. *Ann. Inst. Pasteur Microbiol.* **139**:393–402.
24. **Yang, G., et al.** 18 March 2011, posting date. *Legionella nagasakiensis* sp. nov., isolated from water samples in Japan and Australia and from a patient with pneumonia in the United States. *Int. J. Syst. Evol. Microbiol.* [Epub ahead of print.]
25. **Yu, V. L., et al.** 2002. Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. *J. Infect. Dis.* **186**:127–128.