Identification of *Leptotrichia goodfellowii* infective endocarditis by next-generation sequencing of 16S rDNA amplicons

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**Abstract** The oral aerotolerant anaerobe *Leptotrichia goodfellowii* is an unusual cause of endocarditis and is amenable to treatment with β-lactam antibiotics. Because this organism is difficult to identify by conventional methods, molecular detection is a key diagnostic modality. Broad-range 16S rDNA PCR followed by Sanger sequencing constitute the first-line molecular approach, yet poor DNA quality, contaminating DNA, or low template quantity make identification challenging. Here we report a case of culture-negative, aortic and mitral valve endocarditis in a 66-yr-old woman with a history of cardiomyopathy, atrial fibrillation with intracardiac pacer, poor dentition, and recent tooth infection. In this case, 16S rDNA amplicon Sanger sequencing was not sufficient for pathogen identification because of interfering DNA, but deconvolution of the clinical sample using reflexive next-generation amplicon sequencing enabled confident identification of a single pathogenic organism, *L. goodfellowii*. The patient developed a sigmoid colon perforation and died despite additional surgical treatment. Most *Leptotrichia* endocarditis cases have been subacute and have been successfully treated with antibiotics, with or without valve replacement. This case highlights both an unusual etiologic agent of endocarditis, as well as the rational utilization of advanced molecular diagnostics tools for characterizing serious infections.

**INTRODUCTION**

*Leptotrichia goodfellowii* is a fastidious, aerotolerant anaerobe, Gram-negative, filamentous rod- or spindle-shaped bacterium that colonizes the human oropharynx and constitutes one of seven known species in the genus (Eribe et al. 2004). *Leptotrichia* spp have been implicated in a variety of human infections, including animal bite wounds, bloodstream infections, and hepatic abscesses (Eribe and Olsen 2017). Although bacteremia and endocarditis caused by *Leptotrichia* spp are considered uncommon, in a retrospective analysis, three of 39 bacteremia cases (7.7%) caused by anaerobic bacteria among patients with hematologic neoplasms were due to *Leptotrichia spp* (Blairon et al. 2006). *L. goodfellowii* infections have primarily been reported in immunocompetent patients, including all five previously reported cases of endocarditis caused by that organism (Hammann et al. 1993; Caram et al. 2008; Lim et al. 2016; Matias et al. 2016). Similar to other causes of bacterial endocarditis,
important risk factors for Leptotrichia spp endocarditis include valvular pathology, presence of a prosthetic valve and mucosal lesions including periodontitis (Duperval et al. 1984; Hammann et al. 1993; Caram et al. 2008; Eribe and Olsen 2008; Matias et al. 2016).

Most cases of L. goodfellowii endocarditis have been subacute, usually presenting after 2–3 mo of symptoms including fevers and fatigue (Caram et al. 2008; Matias et al. 2016), although a case of acute endocarditis evolving over 1 wk has also been reported (Caram et al. 2008). To our knowledge, few fatal cases of Leptotrichia infection have been described and all previously reported cases of L. goodfellowii endocarditis have been successfully treated with antibiotics, with or without valve replacement (Hammann et al. 1993; Caram et al. 2008; Matias et al. 2016). Antibiotic sensitivities have not been explored in all cases of L. goodfellowii infection, but existing data support low minimum inhibitory concentrations (MICs) to penicillins, most cephalosporins, vancomycin, and clindamycin (Hammann et al. 1993; Caram et al. 2008; Lim et al. 2016).

Even when L. goodfellowii grows in culture, identification in the clinical laboratory can be challenging and may not be successfully accomplished by phenotypic methods or matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Lim et al. 2016). Therefore, molecular identification using broad-range 16S rDNA polymerase chain reaction (PCR) amplification and subsequent Sanger sequencing is a critical diagnostic method for definitive identification for this organism (Caram et al. 2008; Lim et al. 2016; Matias et al. 2016). Molecular methods are important adjunct diagnostic tools for evaluating infective endocarditis (IE) particularly when cultures are negative (Maneg et al. 2016; Liesman et al. 2017), as in the current case. This approach typically requires DNA predominantly from a single species, with minimal interfering templates (Salipante et al. 2013). Because of these limitations, next-generation sequencing (NGS) is emerging as a valuable tool for identifying pathogens in polymicrobial specimens (Cummings et al. 2016). In this case report, we present an important application of NGS: resolution of bacterial 16S rDNA in a monomicrobial infection from a background of contaminating DNA that was nonspecifically amplified from patient tissue. The definitive identification of L. goodfellowii as the etiologic agent in culture-negative endocarditis by this approach highlights the pathogenicity of this organism in immunocompetent patients.

RESULTS

Clinical Presentation

A 66-yr-old woman was hospitalized with complaints of worsening shortness of breath and new chills, night sweats, fevers, loss of appetite, and weight loss. She reported loosening and “infection” of her three remaining left maxillary teeth. Five years earlier she had been diagnosed with hypertrophic cardiomyopathy and atrial fibrillation and had placement of an intracardiac device. For 2 years before admission she had had complaints of exertional dyspnea and fatigue. Prior history was negative for rheumatic fever, diabetes mellitus, or immunodeficiency. She had previously used prescription opioid medications but had not used drugs by injection.

On initial examination she was afebrile and her remaining teeth were in poor repair. There was a III/VI apical holosystolic murmur and a II/VI diastolic murmur best heard at the left sternal border. The abdomen was nontender and there was no organomegaly. Blood cultures obtained on admission were negative after 5 d incubation. Transthoracic echocardiogram showed both moderate aortic regurgitation across a tricuspid valve with a central vegetation and severe mitral regurgitation with a vegetation. Right ventricular systolic pressure was 67 mmHg. Transesophageal echocardiography on hospital day 12 demonstrated mobile vegetations and regurgitation across both the aortic and mitral valves.
A clinical diagnosis of endocarditis was made on admission, and IV antibiotic treatment was provided, initially with vancomycin and then with piperacillin/tazobactam (administered for 18 d). Her remaining teeth were removed on hospital day 3 and the intracardiac device removed on hospital day 12. She developed worsening heart failure and required intubation and pressor support with dobutamine. On hospital day 16 the patient underwent surgery to replace both valves with bioprosthetic devices. Pulmonary artery pressure at the conclusion of surgery was 28/16 mmHg. Valve material was not submitted for pathological examination but was reserved for molecular microbiology testing in the event tissue cultures were negative after 7 d of incubation. Valve tissue showed few white blood cells (WBCs) and no organisms on Gram stains and no growth on bacterial cultures after 7 d. Following cardiac surgery, she became alert and was extubated on hospitalization day 18. As an oral organism was thought to be the most likely cause of her endocarditis, antimicrobial treatment was changed to ampicillin/sulbactam (administered for 5 d).

On hospital day 20 she developed abdominal pain after eating. Imaging demonstrated free intra-abdominal air. Intra-abdominal surgical exam showed peritonitis and perforation at the rectosigmoid junction; sigmoid colon was resected, an end colostomy was created, and the peritoneum was irrigated. Blood cultures prior to surgery showed no growth and surgical cultures were not obtained. Postoperatively she remained hypotensive, and she died on hospitalization day 23. Concurrently, frozen valve tissue was submitted as planned to the Molecular Microbiology Laboratory at the University of Washington Medical Center for broad-range bacterial PCR, where 16S NGS can be ordered as reflexive testing for samples complicated by the presence of multiple templates.

**Genomic Analyses**

Broad-range PCR primers targeting the V1–V2 regions of the 16S rDNA locus amplified an ~370-bp product (278 bp after trimming primer sequences and 5’ and 3’ ends with low-sequence quality) that was faintly visible by capillary electrophoresis (Fig. 1A). The QV+ scores, a measure of total sequenced bases with an acceptable quality value (QV > 20), was

![Figure 1](image-url)
computed and relatively low for both forward and reverse sequences. The forward sequence (QV20+ score: 216) was 98.67% identical to the 16S rRNA gene sequences from three *L. goodfellowii* type strains (NR_025649, NR_113160, AP019822) in the NCBI 16S nucleotide database and 98.67% identity to the 16S rRNA sequence from a single strain of *L. goodfellowii* isolated in our reference laboratory. This percent identity was below the laboratory’s threshold for reporting species-level identification (≈99.6%–99.7% for an ~300-bp 16S rDNA amplicon) (Claridge 2004). Significant similarity to other species was not detected: The next closest species were canine oral *Leptotrichia spp* (87.81%–89.57% identity: JQ294932.1, JQ296172.1). The electropherograms of both forward (Fig. 1B) and reverse sequences (QV20+ score: 67) demonstrated peak over peak, indicating interference from contaminating DNA or additional bacterial templates. Because of these overlapping base calls, the reverse sequence was of insufficient quality to form a contig. Given these findings, 16S rDNA amplicon-based NGS was attempted.

Clinical NGS was performed essentially as previously described (Cummings et al. 2016). A total of 424,512 sequencing reads were analyzed, 91.0% of which (386,306) corresponded to bacterial 16S rRNA gene sequences derived from the patient’s sample (Supplemental Table 1). A single sequence classified as *L. goodfellowii* was identified, with 100% identity to the three type sequences of *L. goodfellowii* listed above (Fig. 2; GenBank accession number MN653151). No other pathogens were identified; the remaining 8.2% of sequence reads largely represented known biotechnology/environmental contaminants and were also recovered from extraction controls processed in parallel (Supplemental Table 1). These findings were consistent with an infection by a single clonal population.

**DISCUSSION**

This case highlights the important role of molecular diagnostic testing when organisms do not grow in culture. Although amplicon-based Sanger sequencing of 16S rDNA or organism-specific PCR assays are usually sufficient to diagnose IE (Liesman et al. 2017), this case was complicated by the presence of interfering DNA templates in the specimen excised from the patient. Therefore, we leveraged clinical NGS to identify the unusual pathogen *L. goodfellowii*. Analysis of more than 400,000 sequence reads generated a high-quality consensus sequence and a clinically reportable identification.

Amplicon-based NGS assays have typically been used to enumerate individual components of polymicrobial infections, often including fastidious bacteria (Salipante et al. 2013; Cummings et al. 2016). Although unbiased metagenomic NGS assays allow broad-range detection of pathogens including viruses (Chiu and Miller 2019), amplicon-based 16S rDNA NGS is well-suited for low-abundance bacterial pathogens (Salipante et al. 2013) and for specimens where there is an overwhelming amount of DNA derived from the human host (Schlaberg et al. 2017), because the amplification step increases analytical sensitivity and specificity.

Despite appropriate antibiotic therapy and valve replacement, the patient deteriorated rapidly and died because of a colonic perforation. This was likely secondary to hypoperfusion because of her underlying cardiomyopathy and progressive heart failure in the setting of a valve infection. Although the patient died from complications before NGS results were available, the results elucidated the etiology of her infection, which was consistent with the empiric antibiotic selection. In the absence of such severe complications, this information would have conferred a favorable prognosis. This scenario supports the early use of molecular techniques to identify pathogenic organisms in IE, which, in some series, has resulted in therapeutic changes in >15% of cases (Marsch et al. 2015).
Figure 2. Midpoint rooted tree generated by NCBI Blast algorithm and Tree Viewer application, v1.17.5, by fast minimum evolution with maximum sequence difference of 0.75 and limited to family Leptotrichiaceae (taxid: 1129771). The clinical sequence ("Query Sequence") is highlighted in yellow and forms a clade with five L. goodfellowii strains, including three with 100% identity (highlighted in green). Scale bars represent a hamming distance of 0.03.
Balancing diagnostic speed, laboratory costs, and clinical need is an important concern when selecting molecular assays, and therefore a rational approach to test selection is advised. Algorithmic approaches have been suggested (Liesman et al. 2017), and in keeping with this philosophy NGS sequencing in this case was performed reflexively. Because Sanger sequencing of a 16S rDNA amplicon is often successful, escalation to NGS-based pathogen identification is reserved for cases with multiple templates and/or significant contaminating DNA that otherwise precludes a clinical result. This stepwise approach prioritizes the most economical tests having the highest probability of success while reserving NGS as a powerful diagnostic technique to be used in unusual clinical scenarios.

METHOD

Clinical Broad-Range PCR and Sanger Sequencing
Broad-range 16S rDNA PCR and Sanger sequencing was performed as a clinical assay as previously described (Lee et al. 2015) targeting the V1–V2 region of the 16S locus, which usually provides sufficient species-specific resolution to render an organism identification (Salipante et al. 2013). Briefly, DNA extraction was confirmed by amplifying and quantifying the human β-globin gene by qPCR, which was present at 0.8 ng/μL. PCR products were visualized by capillary electrophoresis and successful amplifications submitted for Sanger sequencing.

16S Amplicon Next-Generation Sequencing
Clinical NGS, including amplification and library preparation, was performed essentially as previously described (Cummings et al. 2016). Following reamplification of the 16S rDNA product with barcoded primers, paired-end sequencing was performed on an Illumina MiSeq and analyzed with the dada2 pipeline (Callahan et al. 2016).

Clinical Data
Medical records were reviewed and interpreted by the treating infectious diseases physician. Clinical molecular testing was evaluated by the staff and medical directors of the Molecular Microbiology Laboratory at the University of Washington Medical Center following established clinical procedures.

ADDITIONAL INFORMATION

Data Deposition and Access
The 16S rDNA sequence has been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession number MN653151.

Ethics Statement
This case report was prepared in compliance with institutional ethics policies that state that case reports involving a single patient that do not include an investigational device do not require IRB review. The patient died as a result of complications from her infection prior to the organism identification by novel method utilization; thus consent to participate could not be obtained. No next of kin or family members could be identified for this patient in the hospital records or after consulting with the patient’s primary care doctor. We have excluded identifying information.
Acknowledgments
We thank the staff of the California Pacific Medical Center and of the University of Washington Medical Center Molecular Microbiology Laboratory for their work in caring for this patient.

Author Contributions
J.A.L. was a major contributor to writing the manuscript and interpreted sequencing results. K.K., D.S., B.T.C., and S.J.S. analyzed and interpreted the sequencing data and helped prepare the manuscript. J.A.L. and K.K. prepared the figures. D.B. participated in the clinical care of the patient and was a major contributor to writing the manuscript.

Funding
This case report was not supported by research funding.

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Competing Interest Statement
The authors have declared no competing interest.

Received September 18, 2020; accepted in revised form November 23, 2020.
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