

## Aortic Valve Endocarditis Possibly Caused by a *Haematobacter*-Like Species<sup>▽</sup>

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***Haematobacter* is a newly proposed genus for a group of fastidious Gram-negative aerobic bacilli isolated mostly from blood samples from patients with septicemia. The *Haematobacter* genus currently includes two species, *H. massiliensis* and *H. missouriensis*. We report isolation of a novel *Haematobacter*-like species from the blood of a 65-year-old man who suffered from probable aortic valve endocarditis. The possible causative role was suggested by the monomicrobial culture and the absence of another causative agent in a patient with probable endocarditis by Duke criteria. This fastidious organism could not be identified by routine biochemical tests. Sequencing analysis of the 16S rRNA gene (1,425 bp) best matched the known *Haematobacter* species yet was substantially different with a nucleotide similarity of 96.7%. This strain also reduced nitrate to nitrite, unlike known species. This case is likely the first reported case of endocarditis possibly caused by a *Haematobacter*-like organism.**

### CASE REPORT

A 65-year-old man presented to the emergency room in December 2008 with a 3-week history of worsening shortness of breath, orthopnea, subjective fevers, chills, night sweats, dry cough, and sore throat. The shortness of breath occurred initially during exertion, but on the day of presentation, it also occurred at rest. The patient had little prior medical care. He had a 50-pack-per-year history of smoking as well as a history of alcohol abuse.

On physical examination, the patient was in moderate respiratory distress. He was afebrile with a heart rate of 116/min and blood pressure of 149/88 mm Hg. Chest auscultation revealed diffuse wet rales in the lung fields, a III/VI early diastolic murmur heard best at the left upper sternal border with radiation to the apex, and an S3 gallop. Oral thrush, bilateral axillary lymphadenopathy (1 to 2 cm), and bilateral lower extremity pitting edema were also noted.

Laboratory tests revealed a white blood cell count of  $18.9 \times 10^3/\mu\text{l}$  with 85% neutrophils, hemoglobin level of 11.4 g/dl, blood urea nitrogen level of 42 mg/dl, creatinine level of 2.4 mg/dl, total bilirubin level of 2.2 IU/ml (normal range, 0.2 to 1.2 IU/ml), and erythrocyte sedimentation rate of 94 mm/h (normal range, 0 to 20 mm/h). The following laboratory results were also abnormal: lactate dehydrogenase level of 346 U/ml (normal range, 100 to 190 U/ml), creatinine kinase level of 524 U/ml (normal range, 25 to 250 U/ml), creatinine kinase level in muscle and brain of 15.4 ng/ml (normal range, 0 to 5 ng/ml), and troponin I level of 7.70 ng/ml (normal range, 0.01 to 0.08 ng/ml). The levels of transaminases and alkaline phosphatase were within normal limits. A urinalysis conducted 5 days after

urinary catheter placement revealed large amounts of blood and 71 red blood cells per high-power microscopy field. HIV test results were negative.

Admission chest X-ray showed patchy densities in the right upper and lower lung fields as well as the perihilar region of the left lung consistent with pulmonary edema. An electrocardiogram showed sinus tachycardia with premature atrial complexes, a right bundle branch block, and left ventricular hypertrophy. A bedside transthoracic echocardiogram showed moderate to severe aortic insufficiency as well as a lesion on the aortic valve.

Blood cultures using two sets of aerobic and anaerobic bottles were obtained on admission. Antimicrobial therapy with vancomycin and piperacillin-tazobactam was initiated 6 h later, along with a regimen of diuretics, beta-blockers, and statins for his heart failure and hypertension. On hospital day 3, a transesophageal echocardiogram revealed further details of the diseased aortic valve, i.e., a partially flail left coronary cusp along with the presence of two small mobile vegetations, one (0.4 by 0.3 cm) on the tip and another (0.2 by 0.1 cm) at the base. The ejection fraction was slightly impaired (45 to 49%) (normal value, >55%).

Meanwhile, a Gram-negative bacillus, strain BC14248, grew in one of the two aerobic bottles after an incubation of 5 days in the BacT/Alert automated culturing system (bioMérieux Inc., Durham, NC). The patient then met Duke criteria for probable endocarditis (echocardiographic findings of infective endocarditis, positive blood culture not meeting major criteria, fevers, and hematuria) (4). On subculture, the organism grew best on 5% sheep blood agar, but the appearance of colonies (1-mm size) required 48-hour incubation at 37°C with 5% CO<sub>2</sub>. The colonies were light gray, round, raised, glistening, mucoid, and sticky. There was no hemolysis. Similar growth was also seen with nonselective buffered charcoal yeast extract agar. However, the organism grew even more slowly on chocolate

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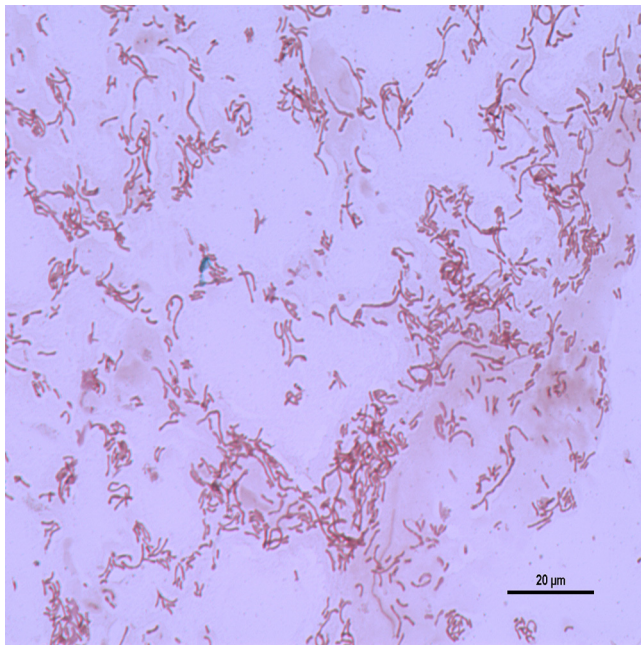


FIG. 1. Gram stain of the organism showing serpentine rod forms.

agar or plain Trypticase soy agar and did not grow at all on MacConkey agar. With Gram staining, the organism was seen as short to long serpentine rods (Fig. 1).

Biochemically, the organism was positive for catalase, oxidase, and urease. It reduced nitrate to nitrite and produced  $H_2S$  by the lead acetate method. However, it did not produce indole or hydrolyze gelatin or esculin. The API 20NE system (bioMérieux) yielded a biocode of 1041044 at 48 h, and the Vitek GNI+ card result was 40504004140, but neither code gave confident identification. The strain was also sent to the Centers for Disease Control and Prevention (CDC) for further work-up, but it did not match with any known organism. The organism was tested for susceptibility to antimicrobial agents by Etest (bioMérieux) using standard Mueller-Hinton agar and a 40-hour reading; it was pansensitive to ceftazidime, cefepime, ciprofloxacin, amoxicillin-clavulanate, piperacillin-tazobactam, amikacin, imipenem, and trimethoprim-sulfamethoxazole with MICs of  $\leq 0.5$   $\mu\text{g}/\text{ml}$  for all these antimicrobial agents. Subsequently, the patient's antibiotic was changed to ceftriaxone for ease of administration.

During the patient's hospitalization, an axial computed tomography of the neck and thorax showed evidence of irregular soft-tissue lesions involving the anterior portion of the right vocal cord; these lesions were suspected to be laryngeal carcinoma. He also had a spiculated nodule in the right upper lobe of the lung. However, the patient refused further work-up. He improved clinically on treatment in that his acute heart failure symptoms, leukocytosis, renal failure, and hematuria resolved. He was discharged from the hospital and went home on ceftriaxone to complete 6 weeks of therapy for this probable native aortic valve endocarditis. After this treatment, he was clinically stable until 3 months later, when he was admitted for endocarditis again. However, this time only *Enterococcus*, not the

original Gram-negative bacillus, was found in multiple blood cultures.

To identify the fastidious Gram-negative rod, we amplified and sequenced its 16S rRNA gene using the MicroSeq 500 system (Applied Biosystems, Inc., Foster City, CA) and two more sets of 16S primers, primers 5'-TGCCAGCAGCCGCGGTAATAC and 5'-CGCTCGTTGCGGGACTTAACC and primers 5'-GCA CAAGCGGTGGAGCATGTG and 5'-AGGAGGTGATCCA ACCGCA (3). Nearly the full length (1,425 bp) of the gene was obtained (GenBank accession no. GU396991), and BLAST searches showed the best match with an uncultured bacterium (6) with 98.9% identity (1,402 of 1,417 bp without gap sites). The second best matches were with *Haematobacter massiliensis* (GenBank accession no. AF452106) for 96.7% identity (1,354/1,400 bp), with *Haematobacter missouriensis* (GenBank accession no. DQ342315) for 96.8% identity (1,342/1,387 bp), and with *Haematobacter* genomospecies (GenBank accession no. DQ342319) for 96.5% (1,339/1,387 bp). Yet, these matches all contained four gap sites. Other close matches were with two *Rhodobacter* species (GenBank accession no. DQ342322 and CP000661) for identities of 96.5% to 95.0%. Therefore, this organism probably represents a novel *Haematobacter*-like species.

**Discussion.** Definitive or probable infective endocarditis (IE) by modified Duke criteria consists of either a pathological cardiac specimen or fulfillment of a range of clinical criteria (4). This patient fulfilled the clinical criteria for probable IE. He satisfied one major criterion by having oscillating mobile vegetations attached to the aortic valve and a new valvular regurgitation. He also met three minor criteria in that he had subjective fevers at home prior to admission, a positive blood culture of an organism not known to cause endocarditis, and hematuria (albeit after urinary catheter placement). The patient's presentation of new congestive heart failure was also impressive, along with IE-associated laboratory abnormalities, including anemia, marked leukocytosis, and an elevated erythrocyte sedimentation rate (5). He showed a right bundle branch block on his admission electrocardiogram as well. The possible causative role of the *Haematobacter*-like species for this patient's endocarditis was suggested by the monomicrobial culture at the time of his initial presentation along with associated signs and symptoms. Although *Enterococcus* grew from his blood cultures 3 months later, this was possibly because his initial endocarditis with the *Haematobacter*-like organism had damaged his heart valves and created a predisposition for endocarditis.

The genus *Haematobacter* was proposed in 2007 by a team at the CDC based on studies of 13 strains isolated in France (1 strain) and the United States (12 strains) (2). The French strain was initially named *Rhodobacter massiliensis* based mainly on the close match of its 16S rRNA gene with a few known *Rhodobacter* species (up to 96%) (1). However, this strain differed from the *Rhodobacter* species by the apparent lack of red pigmentation and several biochemical reactions, and further characterizations of similar CDC strains led to revision to the novel genus *Haematobacter* (2). Organisms within *Haematobacter* are fastidious, nonfermentative, aerobic, and short to long serpentine Gram-negative rods. They are

positive for catalase, oxidase, and urease but negative for nitrate reduction or indole production. Approximately half of the strains of *Haematobacter* also grow on MacConkey agar. Due to the limited number of positive biochemical reactions, definitive identification requires 16S rRNA gene sequencing at present. The genus currently includes two described species, *H. massiliensis* ( $n = 7$ ) and *H. missouriensis* ( $n = 5$ ), and one unnamed genomospecies ( $n = 1$ ), all separated mainly by <70% hybridization of genomic DNA. These organisms have similar biochemical features, and the 16S rRNA gene sequences of *H. massiliensis* and *H. missouriensis* differ by only 2 nucleotides (of 1,389 bp).

Our strain (BC14248) exhibits the general features of *Haematobacter* in its fastidiousness, Gram stain morphology, and limited biochemical reactions. However, this strain is also mucoid and glistening on culture plates, similar to the French strain, whereas other U.S. *Haematobacter* strains have not been reported to have these features. The main difference of our isolate lies in the 16S rRNA gene sequence; the 3.4% divergence including four gap sites would satisfy new species definition in view of the general acceptance that a 2 to 3% divergence connotes a separate bacterial species. Proposal of a new *Haematobacter* species can be considered in the future if other similar strains are isolated.

The MicroSeq 500 system and our additional 16S primers would normally amplify and determine a 1,500-bp segment of the 16S rRNA gene. The 1,425-bp sequence from this *Haematobacter*-like species was shorter than expected. By comparing the sequence with the full-length gene of *Escherichia coli* (1,541 bp; J01859), numerous deletions in the *Haematobacter* gene were noted, which would account for the full length being only 1,467 bp. The available lengths from the *H. massiliensis* and *H. missouriensis* genes were also shorter at 1,414 bp and

1,389 bp respectively (3, 6). The biologic relevance of a shorter 16S rRNA gene remains to be determined.

In summary, our case is likely the first reported case of endocarditis possibly caused by a *Haematobacter*-like organism. This entity should be added to the vocabulary of clinical microbiologists.

**Nucleotide sequence accession number.** The 16S rRNA gene sequence of the *Haematobacter*-like species strain BC14248 has been deposited in GenBank under accession number GU396991.

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