A First Report of *Rothia aeria* Endocarditis Complicated by Cerebral Hemorrhage

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Abstract

We herein report the first case of infective endocarditis attributable to *Rothia aeria*, which had a fatal outcome after cerebral hemorrhagic infarction and was not susceptible to vancomycin. If Gram-positive bacillary or filamentous bacteria that form white, coarse, dry colonies are detected, keeping the possibility of *Rothia* species in mind is advisable because members of this species can cause severe infections.

Key words: *Rothia aeria*, infective endocarditis, *Nocardia*, acid-fast stain

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Introduction

Rothia species belong to the *Micrococcus* family. *Rothia dentocariosa*, the *Rothia* species most commonly isolated from humans, has been reported to be a causative agent of infective endocarditis and other serious infections (1). This case report describes the first case of infective endocarditis caused by *R. aeria* with subsequent hemorrhagic cerebral infarction.

Case Report

A 40-year-old Japanese man with a history of smoking visited a neighborhood physician complaining primarily of fever, headache and arthralgia. The physician prescribed a two-week course of oral levofloxacin, and the patient's condition began to improve. However, after complaining of fever again one month after the onset of symptoms, the patient consulted our institution and was hospitalized three days after the initial consultation.

The patient had a history of allergic conjunctivitis; however, he had no prior hospitalizations or history of any dental treatments, including treatment for dental caries. He had never been diagnosed with valvulopathy or cardiac murmurs. His body temperature was 37.3°C. A mild diastolic

murmur was audible on auscultation. He did not present with dental diseases, petechiae on the skin or mucosa, Osler's nodes, Janeway lesions or splenomegaly. The patient's white blood cell count was 16,930/μL, and test results for human immunodeficiency virus infection and diabetes mellitus were negative (Table 1). Other tests to detect viral or immunodeficiency diseases were not conducted. Three days prior to and two days after hospitalization, blood samples for culture were collected in BACTEC Plus Aerobic/F and Plus Anaerobic/F bottles (BD Diagnostic Systems, Sparks, MD, USA), which were incubated in an automated blood culture system (Bactec 9240; BD Diagnostics Systems). Transthoracic echocardiography revealed an ejection fraction of 78%. Cystic structures were present on the anterior mitral valve leaflet (Fig. 1A), and transesophageal echocardiography showed a vegetation measuring 20×9 mm on the same leaflet (Fig. 1B). The three cusps of the aortic valve demonstrated mild hypertrophy and severe aortic regurgitation (AR). A diagnosis of infective endocarditis was suspected based on the presence of vegetation on echocardiography. Combination therapy with 1 g of ceftriaxone and 30 mg of gentamicin administered every 12 hours was initiated according to the Guidelines for the Prevention and Treatment of Infective Endocarditis released by the Japanese Circulation Society in 2008 (http://www.j-circ.or.jp/guideline/pdf/JCS2008_miyatake_h.pdf, in Japanese).

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Table 1. Laboratory Findings

	values		values
White blood cells (/μL)	16,930	AST (IU/L)	30
neutrophils (%)	81.2	ALT (IU/L)	64
lymphocytes (%)	12.5	LDH (IU/L)	257
monocytes (%)	5.0	ALP (IU/L)	344
eosinophils (%)	0.9	γ-GTP (IU/L)	122
basophils (%)	0.4	Creatinine (mg/dL)	0.73
Red blood cells (×10 ⁶ /μL)	537	BUN (mg/dL)	8
Hemoglobin (g/dL)	15.4	Glucose (mg/dL)	105
Pletlets (×10 ⁴ /μL)	27.1	HbA1c (%JDS)	5.4
CRP (mg/dL)	3.05	HIV-EIA	(-)
		RF (IU/mL)	<5
		ANA-FA (titer)	<40
		IgG (mg/dL)	1,155

CRP: C-reaction protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase, γGTP: γ-glutamyltranspeptidase, BUN: blood urea nitrogen, HIV-EIA: human deficiency virus-enzyme immunoassay, RF: rheumatoid factor, ANA-FA: antinuclear antibody-fluorescent antibody method, IgG: Immunoglobulin G

The patient developed a headache on the second day of hospitalization. Magnetic resonance imaging showed faint high-intensity areas in the dorsal aspects of the left thalamus (near the hippocampus) and the left occipital lobe (Fig. 2A). Based on a diagnosis of acute cerebral infarction with ischemia of the left posterior cerebral artery region, a treatment regimen of intravenous infusion with edaravone and glycerin was administered. Magnetic resonance angiography showed no evidence of cerebral aneurysms. The patient's headache intensified and he vomited on the third day of hospitalization. Computed tomography indicated the presence of hemorrhagic infarction or intraventricular hemorrhage as well as acute hydrocephalus (Fig. 2B). Emergency bilateral ventricular drainage was therefore performed.

Bacteria were detected in the first blood culture set on the fifth day of incubation and were streaked onto a blood agar plate, after which white, coarse, dry colonies appeared on the second day (Fig. 3A). Microscopic observation revealed the presence of Gram-positive rods (Fig. 3B). The bacterium was presumptively identified as a *Nocardia* species based on the morphology and the appearance of colonies without using the automated system of bacterial identification. A genetic analysis was performed as described below to confirm the identification. Since the same organism was detected on a second examination conducted on the eighth day of hospitalization, a definite diagnosis of infective endocarditis was made, and the antibiotic therapy was changed to imipenem based upon a previous report (2). Bacterial elimination from the bloodstream was not assessed. The patient developed hypotension, most likely induced by a brainstem disorder, and died on the fifteenth day of hospitalization. Consent for an autopsy could not be obtained.

The 16S rRNA gene of the isolated bacterium was analyzed for genetic identification. A 1,517-bp DNA fragment was sequenced and compared to published sequences retrieved from the GenBank database (National Center for

Biotechnology Information, accession number EU293888). The query sequence was found to have 99.7% (1,406/1,407 bp) identity with the sequence of the 16S rRNA gene of *Rothia aeria*. The next closest match was to *R. dentocariosa* (98.0% identity). Twenty-two days after the patient was hospitalized, we identified the isolate as *R. aeria*, as described in the guidelines recommended by Janda and Abbott (3). Drug susceptibility was characterized using Etest strips (BioMérieux, Marcy l'Etoile, France) and a disk diffusion test (BD Diagnosis Systems) (Table 2). Although no Clinical and Laboratory Standards Institute (CLSI) protocols exist for *R. aeria*, we assessed the organism's drug susceptibility utilizing the 2009 CLSI criteria (M100-S19) for *Staphylococcus*, as described in previous reports (4-7). The bacterium was susceptible to most antibacterial agents, except for clindamycin and vancomycin.

Discussion

Rothia species are Gram-positive bacteria belonging to the *Micrococcus* family. *Rothia dentocariosa*, the *Rothia* species most commonly isolated from humans, was first detected by Onishi in a patient's mouth in 1949 (8) and has been reported to be a causative bacterium of infective endocarditis and other serious infections (1). *R. aeria* was isolated from an air sampler in Russia's Mir space station (9). Since *R. aeria* was originally classified as *R. dentocariosa* genomovar II before the report of Li et al. (9), *R. aeria* might have been involved in cases of *R. dentocariosa* infection. *R. aeria*, like *R. dentocariosa*, has been detected in the mouths of healthy individuals (10, 11). Although some microorganisms in the oral flora are known to cause infective endocarditis, the present case appears to be the first case of infective endocarditis caused by *R. aeria* with subsequent hemorrhagic cerebral infarction. The few cases of *R. aeria* infection reported to date include cases of multifocal nodular pneumonia (4), bronchitis (5), sepsis (6) and septic arthritis (7). The reported cases of *R. aeria* infection have been reported to be associated with rheumatoid arthritis (5, 7) and neurosarcoidosis (4) as underlying diseases, in addition to steroids (4, 7), immunosuppressants (4, 7) and anti-tumor necrosis factor therapy (5).

R. aeria is an aerobic, Gram-positive, coccoidal, coccobacillary or filamentous bacterium that forms white, coarse, dry colonies. *Rothia* species can be mistaken for bacteria such as *Dermabacter hominis*, *Actinomyces viscosus*, *Propionibacterium avidum*, *Corynebacterium matruchotii* and *Nocardia* species because many laboratories are unfamiliar with these organisms, which may be difficult to culture due to their variable aerotolerance (12-14). The colonies of *Nocardia* typically have a powdery appearance (15) similar to those of *R. aeria*. However, *Nocardia* grows slightly slower than *R. aeria*. In the present case, the isolated bacterium was misidentified as a *Nocardia* species based on the morphology and the appearance of the colonies without the use of modified acid-fast staining such as Kinyoun or Grocott.

Figure 1. Echocardiography. (A) A cystic structure (arrow, 11×16 mm) on the anterior mitral leaflet was found on transthoracic echocardiography; however, no vegetation was observed. (B) Vegetation (arrow, 20×9 mm) on the anterior mitral leaflet was found on transesophageal echocardiography.

Figure 2. Neurological images. (A) Diffusion-weighted imaging indicated a cerebral infarction on the second day of hospitalization. (B) Computed tomography indicated a hemorrhagic infarction on the third day of hospitalization.

Figure 3. Microbiological examinations. (A) *Rothia aeria* colonies on blood agar. (B) A Gram stain of *Rothia aeria* under high power magnification (1,000×).

Table 2. Antibiotic Susceptibility of *Rothia aeria* in the Present Case

Antibiotic	Susceptibility	MIC ($\mu\text{g/mL}$)	Inhibition zone diameter (mm)
Benzylpenicillin	S	0.002	
Ampicillin	S	0.016	
Ceftriaxone	S	0.016	
Imipenem	S	0.064	
Clindamycin	I	2	
Minocycline	S	1	
Vancomycin	I	3	
Linezolid	S	0.38	
Piperacillin	S		72
Amoxicillin-clavulanic acid	S		53
Cefazolin	S		53
Cefdinir	S		43
Erythromycin	S		50
Gentamicin	S		20
Trimethoprim-sulfamethoxazole	S		26
Levofloxacin	S		30

The drug susceptibility of *Rothia aeria* was characterized using Etest strips and disk diffusion and was assessed according to the 2009 CLSI (M100-S19) *Staphylococcus* criteria. MIC: minimum inhibitory concentration, S: susceptible, I: intermediate

These acid-fast stains are additional methods available to detect acid-fast and partially acid-fast bacteria (e.g., *Mycobacterium* and some members of the genus *Actinomyces*, except for *Rothia*) and are typically used to help diagnose *Nocardia* infections in hospital laboratories (15, 16). Since the presence of *Nocardia* species was strongly suspected in this case, only genetic identification was performed to confirm the diagnosis. In this case, the bacterium was not a *Nocardia* species. However, identifying *Nocardia* species using acid-fast staining only carries a risk for misidentification because some *Nocardia* species are negative on acid-fast stains (17). A genetic examination is helpful for making an accurate diagnosis.

R. aeria is susceptible to many antibiotics (4-7); however, it reportedly demonstrates only intermediate susceptibility to clindamycin and vancomycin (7). Resistance to clindamycin (4, 6), aztreonam (4) and ofloxacin (5) determined using Etest and disk diffusion tests has also been reported. The *R. aeria* strain that we detected was not susceptible to clindamycin or vancomycin, neither of which had been given to the patient. In addition, *R. dentocariosa* is reportedly not resistant to clindamycin or vancomycin (14); however, Salamon et al. reported that the minimum inhibitory concentration (MIC) of vancomycin for a particular strain of *R. dentocariosa* determined using an Etest is 3 $\mu\text{g/mL}$ (18). If vancomycin, which is usually used in empirical therapy for infective endocarditis caused by Gram-positive organisms, had been prescribed to the patient during hospitalization, the efficacy would have been poor due to the organism's lack of susceptibility to this drug. We empirically used ceftriaxone, which is listed in the Japanese guidelines for the treatment of Gram-positive infective endocarditis. We also chose imipenem for target therapy against the *Nocardia* species presumed to be present, based on a previous report (2). Un-

fortunately, the effectiveness of ceftriaxone and imipenem could not be assessed based on the physical findings and laboratory tests because the patient developed a severe cerebral infarction and bacterial elimination in the bloodstream was not assessed. It is known that appropriate antibiotic therapy may decrease the risk of neurological complications. In the ICE-PCS study, the rate of stroke after one week of antimicrobial therapy was only 3.1% (19). In this case, if the use of an antimicrobial agent susceptible to *R. aeria* had been continued before hospitalization, the stroke would not have occurred. The patient may thus have benefitted from surgical therapy in light of the large focus of infection and the possibility for acute AR if he had not developed hemorrhagic infarction immediately after diagnosis.

Central nervous system complications are seen in as many as 20% to 40% of infective endocarditis cases and are often the result of infarction due to vegetation (20). Affected patients are at an increased risk of cerebral infarction if the vegetation diameter is at least 10 mm or if vegetation is present on the anterior mitral valve leaflet (21, 22). The present patient had both of these risk factors. Affected patients are also prone to developing hemorrhagic cerebral complications within 48 hours of hospitalization (23), as did the present patient, who suffered hemorrhagic infarction soon after admission.

To our knowledge, this is the first report of infective endocarditis followed by hemorrhagic cerebral infarction caused by *R. aeria* that is not susceptible to vancomycin. With any serious infection such as infective endocarditis, identification of the causative agent is critical, as identification influences the choice of therapy, which in turn influences the outcome. When Gram-positive bacillary or filamentous bacteria are detected on bacteriological examinations, acid-fast staining should be examined, even if a *Nocardia* species is strongly suspected. When the stain is negative, the presence of a *Rothia* species should be suspected in addition to other Gram-positive bacteria. Genetic identification should therefore be made down to the species level because members of this species can cause severe infections, and some species are not susceptible to specific antibacterial agents, including vancomycin.

The authors state that they have no Conflict of Interest (COI).

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