### $\Box$ CASE REPORT $\Box$

## Culture-negative Brain Abscess with *Streptococcus intermedius* Infection with Diagnosis Established by Direct Nucleotide Sequence Analysis of the 16S Ribosomal RNA Gene

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### Abstract

A 70-year-old woman developed a headache for a month followed by right upper limb weakness. CT scan and MRI showed multiple ring-enhancing lesions. An intracerebral aspiration of an abscess was performed, but culture results were negative. The nucleotide sequence analysis of the 16S rRNA gene from the specimens identified *Streptococcus intermedius*. Given this result, *S. intermedius* was cultured by enrichment culture, and its sensitivities to antibiotics were determined. The patient exhibited complete remission. Thus, 16S rRNA gene analysis was highly useful not only for pathogen identification with negative culture results but also for the appropriate selection of antibiotics.

Key words: brain abscess, Streptococcus intermedius, Streptococcus anginosus group, 16S rRNA

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### Introduction

Brain abscess is a focal, intracerebral infection that begins as a localized area of cerebritis and develops into a collection of pus surrounded by a well-vascularized capsule (1). *Streptococcus intermedius* is a member of the *Streptococcus anginosus* group (SAG). Several retrospective reviews and case reports have mentioned *S. intermedius* as a significant pathogen among the SAG species in the development of brain abscess (2-5). However, no studies have compared *S. intermedius* brain abscess with abscess caused by other SAG members or other bacteria.

In a previously published series, 20-28% of all intracerebral abscesses reported have been shown to produce negative culture results, presumably owing to concurrent antibacterial therapy (6, 7). We herein report the case of a 70-yearold woman with brain abscess caused by S. intermedius and review the literature describing cases of brain abscess caused by S. intermedius. In the present case, the pathogen was identified by the nucleotide sequence analysis of the 16S ribosomal RNA (rRNA) gene from a specimen obtained by the surgical aspiration of the abscess, the culture of which initially tested negative. The 16S rRNA gene in bacteria consists of conserved sequences interspersed with variable sequences that include 9 hypervariable regions. These hypervariable regions range from approximately 50 to 100 bases in length and are useful for universal microbial identification (8). Here, after the pathogen was identified, S. intermedius was cultured using a medium enriched with dehydrated thioglycollate, which enabled us to determine the sensitivities of the pathogen to various antibiotics. The present case

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Figure 1. Brain CT and MR images of present case. (A, B, C) Brain CT and MRI (A: axial contrast-enhanced CT scan, B: axial T2-weighted MR imaging, C: diffusion-weighted imaging), which were performed 1 week after admission, demonstrate multiple ring-enhancing lesions and surrounding edema; diffusion-weighted imaging shows a high-intensity signal within these lesions, typical of abscess. (D, E, F) In the brain CT and MRI (D: axial contrast-enhanced CT scan, E: axial T2weighted MR imaging, F: diffusion-weighted imaging), which were performed 8 weeks after admission, multiple ring-enhancing lesions and surrounding edema became hardly noticeable compared with those performed 1 week after admission.

emphasizes the efficacy and usefulness of the 16S rRNA gene analysis of clinical specimens from patients with negative culture results, for whom bacterial infection is suggested by clinical observations.

### **Case Report**

A previously healthy 70-year-old woman presented to our ER with a few weeks' history of frontal headache and weakness of her right upper limb. She denied experiencing any neck stiffness, nausea, or vomiting. She also denied any preceding trauma or fever. On hospital admission, she could hardly speak and struggled to get words out; her right upper limb was paralyzed. These symptoms disappeared soon after admission. She had an implant replacement about a month before admission.

Her body temperature was  $37.6^{\circ}$ C, her pulse rate 88 beats per minute, her BP 114/62 mmHg, and her SpO<sub>2</sub> 98%. Her lungs were clear, and her heart showed normal rhythm without any murmurs. Her white blood cell count was  $13.5 \times 10^{\circ}$ / L with 78.3% neutrophilic leukocytes, and her C-reactive protein (CRP) level was slightly elevated at 0.92 mg/dL. Her erythrocyte sedimentation rate was elevated at 86 mm/h. Liver function tests revealed a slightly elevated alanine aminotransferase (ALT) level of 48 (normal <35). CSF analysis revealed the following results: WBC, 49/µL (60% neutrophils); glucose level, 79 mg/dL; and protein level, 59 mg/dL. Gram staining of CSF was negative. CSF and four blood cultures tested negative for bacteria or fungi. CT scan and brain MRI showed multiple ring-enhancing lesions consistent with brain abscess (Fig. 1A-C). Ear, nose, and throatrelated infections were excluded clinically. Endocarditis as the origin of bacterial infection and right-to-left shunt were ruled out by transesophageal echocardiography (TEE). Meropenem (2 g every 8 hours) and vancomycin (1 g every 12 hours) treatment was initiated; however, after the antibacterial therapy was started, her blood biochemistry showed an elevated ALT level of up to 249 U/L, an asparatate aminotransferase (AST) level of 211 U/L, and a  $\gamma$ -glutamyl transpeptidase (y-GTP) level of 310 U/L. Her liver dysfunction and drug eruption required a reduction in meropenem dose (1 g every 6 hours), and the development of drug eruptions required the discontinuance of vancomycin. Eight days after admission, head CT demonstrated an increase in the number and growth of abscesses. On day 15 of admission, the patient began to present disorientation and apraxia. Although her consciousness level was clear, she could not answer questions requiring dates or places correctly. She showed inability to use chopsticks or to put on a shirt. She also showed inability to properly imitate hand gestures. Given the deteriorating clinical course, the patient underwent a surgical aspiration of an abscess for pathogen deter-

# Table 1. Results of Direct Sequencing and Blast-N Analyses of the Products from Bacterial 16S rRNA Sequence-specific PCR

			number of "N"		identity		
primer pair	sequence (5'-3')	(bp)	(with multiple peaks)	accession No.	tophit name	("Ns" are not included)	
338F/140 7R	GTGACGGTACTTAACCAGAAAGGGACGGCTAACTACGTGCCANCAGCCGCGGNAATACGTAGGNNNN NAGCGTTGTCCGGATTTATTGNNAGTNAGNGAGCNCAGGCGGTAGATAAGTCTGAAGTTAAAGGC AGTGGCCAAACCATTGTAGGCTTTGGAAACTGTTTAACTTGAGTGCAGAAGGGGAGAGTGGAATTCCA TGTGTAGCGTGAAAGCGTAGATATATGGAGGAACACGGGTGCAGAAGCGGCTCTNTGGTCTGTA ACGATGGCTGCAGGCTCGAAAGCGTGGGGGAGCGAACACGGATAGATA	736	19	HM59627 5	Streptococcus intermedius strain F0413	717/717 (100%)	
27F/1407 RR	eq:thm:triggalaxedalaxedalaxedalaxegalaxedalaxedalaxedalaxedalaxedalaxedalaxedala	1061	48	HM59627 5	Streptococcus intermedius strain F0413	1013/1013 (100%)	
27F/1492 R	ACTACACCGTATTCTGTGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTG	1382	I	HM59627 5	Streptococcus intermedius strain F0413	1381/1381 (100%)	

mination, but gram staining and culture results from this specimen were negative. The PCR amplification of the 16S rRNA gene was performed on surgically obtained specimens using the 27F and 1429R universal primers (9), which yielded a single DNA band about 1.4 kb in length. The nucleotide sequence analysis of the PCR products revealed 100% identity to the 16S rRNA sequence of S. intermedius strain F0413 (accession no. HM596275) (Table 1). On the basis of this information, the antibiotic regimen was changed to ceftriaxone (2 g every 12 hours). After the pathogen was identified by the nucleotide sequence analysis of the 16S rRNA gene, culture employing a medium enriched with dehydrated thioglycollate was performed and colonies of S. intermedius were obtained, which revealed sensitivities to meropenem, ceftriaxone and ampicillin. Ten days after the antibiotic regimen was changed to ceftriaxone, she presented with eosinophilia and fever. Therefore, the antibiotic regimen was again changed to ampicillin (2 g every 6 hours). Eight weeks after admission, the patient's clinical condition and radiological findings substantially improved (Fig. 1D-F), and 12 weeks after admission the antibiotic regimen was changed to oral levofloxacin (500 mg per day) and the patient was discharged home. At the time of discharge, she was fully recovered without any disorientation or apraxia.

### Discussion

When antibacterial therapy is started before specimen collection, culture from specimens often tests negative despite bacterial infection. The case reported here demonstrates the diagnostic value of the amplification and nucleotide sequence analysis of bacterial genes in specimens obtained from patients with culture-negative infections who have a likelihood of acute bacterial infection. In this case, *S. intermedius* was detected by the bacterial 16S rRNA gene amplification and nucleotide sequence analysis of the specimen aspirated from a brain abscess. Once the pathogen was identified, *S. intermedius* was successfully cultured using a culture medium supplemented with dehydrated thioglycollate, which enabled the antibiotic sensitivity test of the strain.

Pathogen identification in bacterial infection is always essential for establishing the correct diagnosis and appropriate selection of treatment. In clinical practice, however, we frequently experience negative cultures. The sequencing of bacterial 16S rRNA genes has been reported to be useful for pathogen discovery and identification since the 1990s (10). As demonstrated in the present case, 16S rRNA gene amplification and nucleotide sequence analysis can serve as useful methods for establishing a definitive etiology of intracerebral abscess with negative culture results, enabling the selection of antibiotics appropriate for more targeted treatment. In the present case, we had difficulty in antibiotic selection because of the patient's allergic reaction to drugs (vancomycin and ceftriaxone). The result of the antibiotic susceptibility test, which was obtained after the pathogen was identified, was helpful, as we could change ceftriaxone to ampicillin with confidence. Although 16S rRNA gene analysis is not used as a routine laboratory test, this molecular technique should be used as an adjunct method to culture for the diagnosis of brain abscess when the clinical presentation and laboratory findings suggest a bacterial etiology, particularly for patients who have received antibacterial therapy at the time of sample collection, and when the culture results are negative.

*S. intermedius* is a member of the SAG, together with *S. anginosus* and *S. constellatus*, which are the most common pathogens associated with bacterial intracerebral abscess (11, 12). Carpenter et al studied 49 patients with brain abscess, and documented the results of their microbiological investigation. The above SAG species were the most commonly isolated, followed by anaerobes (2). However, no studies have compared *S. intermedius* brain abscess with abscess caused by other members of the SAG or other bacteria.

Brain abscess is almost always secondary to the focus of suppuration elsewhere in the body, and may develop either by spread from a contiguous focus of infection after neurosurgery or head trauma or by hematogenous spread from a distant focus (13). The cause of brain abscess, however, remains obscure in 10 to 37% of patients (14). Headache, fever, and focal neurological deficit are called the classic triad of brain abscess. Headache is both the initial and most common presenting symptom. Additionally, nausea and vomiting occur with headache (15). Most patients have one or more symptoms suggestive of an intracranial cause of their disease (2). The majority of abscesses are solitary and occur in the frontal lobe. Surgery is required in most patients, and the duration of antibiotic treatment ranges from 4 to 8 weeks (16, 17). The SAG has recently been recognized as a common cause of brain abscesses, which were collectively identified with other streptococci as the viridians group streptococci in prior studies (4, 13).

Infections caused by *S. intermedius* are not common in previously healthy individuals. An underlying condition, such as diabetes, cirrhosis, or cancer, is associated with such infections in the majority of patients (18, 19). The present patient had neither chronic illnesses nor evidence of immunosuppression. However, afterwards, papillary carcinoma was detected in our patient's thyroid grand. Our patient presented multiple abscesses that indicated hematogenous spread. She had implant replacement one month before ad-

mission, but presented no arteriovenous fistula or right-to-left shunt.

The literature review in Table 2 shows the clinical characteristics of the patients with brain abscess caused by S. intermedius. Nineteen patients were included in this study, including 13 male patients (68%) and 6 female patients (32%), of whom 3 children were <15 years of age. The mean age of the patients was 34.2 years (range, 6-70 years). Headache was the most common clinical manifestation observed in 11 of the 19 patients. Fever was observed in 8 patients and motor weakness was observed in 4 patients. Convulsion was observed in 3 patients, aphasia was observed in 3 patients, and alterations in consciousness were observed 2 patients. Apraxia, disorientation, dysarthria, and vertigo were observed in 1 patient. CT and MRI detected a single brain abscess in 10 patients (53%) and multiple brain abscesses in 9 patients (47%). A solitary abscess was localized to the frontal lobe in 4 patients and to the parietal lobe, occipital lobe, and cerebellum in 2 patients each. Underlying conditions were noted for 13 patients. Direct microscopic examination of pus revealed S. intermedius in 11 patients and gram-positive cocci in 4 patients. Blood culture was performed in 11 patients, and only 2 patients showed positivity for S. intermedius. In 6 patients (including the present case), direct nucleotide sequence analysis of 16S rRNA was performed, which detected S. intermedius in all of them. Penicillin antibiotics were used in 10 patients (53%), thirdgeneration cephalosporin in 5 patients (26%), carbapenem in 4 patients (21%), vancomycin in 2 patients (11%), chloramphenicol in 2 patients (11%), and metronidazole, aminoglycoside, cephamycin, rifampin, and amphotericin B were used in one patient each. Seven patients (37%) underwent craniotomy, 6 patients (32%) underwent stereotactic drainage, 4 patients (21%) underwent aspiration, and 1 patient underwent lobectomy. Three (16%) of the 19 patients died: one of diffuse cerebritis, one of pneumonia caused by S. intermedius, and one of catheter infection.

It is well recognized that the microbiological identification of the causative organism is crucial in the treatment of cerebral abscess (1). Some reports indicate that the administration of empirical antibiotics before microbiological diagnosis is achieved is associated with the failure to subsequently grow an organism from surgical specimens, making the appropriate choice of antibiotics difficult (3). Microbiological diagnosis can be performed by the aspiration or excision of specimens. In the early cerebritis stage, however, because abscess walls are poorly formed and friable, excision would pose greater risks of injuries to the surrounding cortex than aspiration (20). Aspiration can be carried out at any stage of abscess evolution even when excision is inappropriate in the cerebritis stage (21). Specimen collection for identifying the pathogen of brain abscess should therefore be performed before antibacterial therapy. However, in the majority of cases, antibacterial therapy is started even before specimen collection, as in the present case. In such cases, 16S rRNA gene amplification and sequencing could be a

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Dationt	Detiant Area		Symptom			Underlying	Other		Proin				Modified	
no.	Age, gender	Headache	Fever	Neurological sign	Location	condition	abscesses	Blood	abcess	16S rRNA	Antibiotics	Surgery	Rankin Scale	Year
1	70 F	+	+	aphasia weakness of lt. upper limb apraxia disorientation	multifocal	none	none	negative	negative	S. intermidius	carbapenem vancomycin ceftriaxone ampicillin	aspiration	1	This case
2	61 M	-	-	convulsion	multifocal	none	lung	negative	negative	S. intermidius	done /1y	lobectomy	0	2010
3	22 M	-	+	aphasia rt. hemiplegia	multifocal	tongue piercing	none	-	S. intermidius	-	penicillin chloramphenicol	aspiration	6 (catheter infection)	2009
4	16 F	-	-	-	lt. frontal lobe	chronic sinusitis	sinus	-	S.viridans group	S. intermidius	ceftriaxone /8w	craniotomy	0	2008
5	6 M	+	-	syncope	multifocal	Ebstein anomaly	sinus	negative	gram-positive cocci	S. intermidius	carbapenem /13w	aspiration	2	2008
6	21 M	+	-	dysarthria	multifocal	chronic sinusitis	sinus	histoplasma titer	gram-positive cocci	S. intermidius	ceftriaxone vancomycin amphotericin B /6w	craniotomy	0	2008
7	16 M	+	+	-	multifocal	none	lung	negative	gram-positive cocci	S. intermidius S.constellatus	ceftriaxone rifampin	craniotomy	2	2008
8	63 F	-	-	vertigo	cerebellum	PFO	none	-	S. intermidius	-	done	craniotomy	0	2006
9	43 F	+	-		cerebellum	PFO	none	-	S. intermidius	-	done /8w	craniotomy	0	2006
10	39 M				multifocal	oral infection	oral cavity liver	S. intermidius	-	-	penicillin G metronidazole	stereotactic drainage	0	2006
11	14 F	+	-		lt. frontal lobe	sinusitis	sinus	-	S. intermidius	-	piperacillin/ tazobactam	stereotactic drainage	0	2006
12	25 F	-	+	aphasia rt. hemiplegia	lt.parietal lobe	cyanotic congenital heart disease	none		S. intermidius Actinomyces spp. Capnocytophaga spp.	-	penicillin /10w	craniotomy	0	2002
13	55 M	+	+	confusion	multifocal	alcoholic cirrhosis	lung	(lung biopsy S. intermidius)	gram-positive cocci	-	ceftriaxone ampicillin	none	6 (pneumonia)	2000
14	20 M	+	+	rt. DTR increase	lt.parietal lobe	chronic granulomatous disease	none	-	S. intermidius	-	carbapenem	aspiration	0	1999
15	13 M				rt.frontal lobe	choronic sinusitis	sinus	negative	S. intermidius	-	piperacillin cefinetazole /4w	stereotactic drainage	0	1999
16	52 M				rt.frontal lobe	none	none	negative	S. intermidius	-	carbapenem /5w	stereotactic drainage	0	1999
17	69 M	+	+	aphasia	lt.occipital lobe	arteriosclerotic disease	liver	S. intermidius	S. intermidius	-	penicillinG chloramphenicol	craniotomy	6	1978
18	27 M	+	-	convulsion	lt.occipital lobe	none	none	negative	S. intermidius	-	penicillin /7w	stereotactic drainage	1	1978
19	18 M	+	+	convulsion lt. facial weakness	multifocal	none	none	negative	S. intermidius	-	oxacillin gentamicin/8w	stereotactic drainage	0	1978
	PFO: natent foramen ovale											nen ovale		

sensitive method of identifying pathogens.

This case report highlights the clinical presentation of brain abscess caused by *S. intermedius* infection and the usefulness of the 16S rRNA gene sequencing method to identify the primary causative organism of such abscess. Additional studies will be necessary to more precisely determine the prevalence and range of clinical presentations caused by *S. intermedius* infection.

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

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