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Identification of *Erysipelothrix rhusiopathiae* by DNA Sequencing in a Culture-Negative Intra-Abdominal Abscess

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Erysipelothrix rhusiopathiae is a Gram-positive bacillus that causes infections primarily in animals. In humans, the bacteria usually cause localized or generalized cutaneous infections. A 75-year-old man with chronic alcoholism presented with abdominal pain. Abdominal computed tomography and laboratory findings suggested an intra-abdominal abscess in the periaortic soft tissue. While no definitive infectious source was identified, *E. rhusiopathiae* was identified by 16S rRNA-based gene sequencing from culture-negative, periaortic necrotic tissue, subsequent to empiric antibiotic treatment. It is suggested that *E. rhusiopathiae* has the potential to cause intra-abdominal abscesses. This case report highlights the usefulness of DNA sequencing to identify pathogens in patients pretreated with antibiotics. **(Ann Clin Microbiol 2014;17:132-135)**

Key Words: Abscess, *Erysipelothrix rhusiopathiae*, Sequencing, 16S rRNA

INTRODUCTION

Erysipelothrix rhusiopathiae is a pleomorphic, Gram-positive, non-sporulating bacillius that has a worldwide distribution. It has been seen primarily as a veterinary pathogen [1,2], normally infecting animals, birds, and fish [2]. In humans, it usually causes occupational diseases in people exposed to infected animals [1,3]. Three well-defined clinical categories of human disease have been described: erysipeloid, generalized cutaneous form, and bacteremia, which is often associated with endocarditis [4]. In addition, new disease manifestations, such as septic arthritis, meningitis, pneumonia, and intra-abdominal abscess, have been reported [5-9].

Herein, we report a case of intra-abdominal abscess and identification of *E. rhusiopathiae* by 16S rRNA-based gene sequencing from culture-negative, periaortic necrotic tissue, suggesting the potential of the bacterium to cause intra-abdominal abscess.

CASE REPORT

A 75-year-old man was admitted to the hospital with 2 weeks of abdominal pain. His past medical history included hypertension, benign prostatic hypertrophy, and chronic alcoholism. There was no history of occupational exposure to live animals; however, he frequently consumed raw fish. There was no fever, and the abdomen was tender with no palpable masses. Blood tests showed an elevated leukocyte count of 11,180/mm^3 with 77% neutrophils, an elevated C-reactive protein (CRP) level of 16.49 mg/dL (reference interval, 0-0.3 mg/dL), and an elevated erythrocyte sedimentation rate of 87 mm/hr (reference interval, 0-27 mm/hr). Computed tomography (CT) showed segmental periaortic soft tissue infiltration and ischemic soft tissue lesions (Fig. 1). Empiric antibiotic treatment with intravenous ceftriaxone and metronidazole was initiated. While no definitive infection source was identified from blood and urine cultures and other microbiological workup for tuberculosis, syphilis, brucellosis, and Q fever, he developed an intermittent fever on hospital day 2. The antibiotic treatment was changed to intravenous ampicillin/sulbactam on hospital day 4, however, the fever continued. On hospital day 10, surgical excision of the periaortic abscess was performed. Intra-operatively, a sample of ab-

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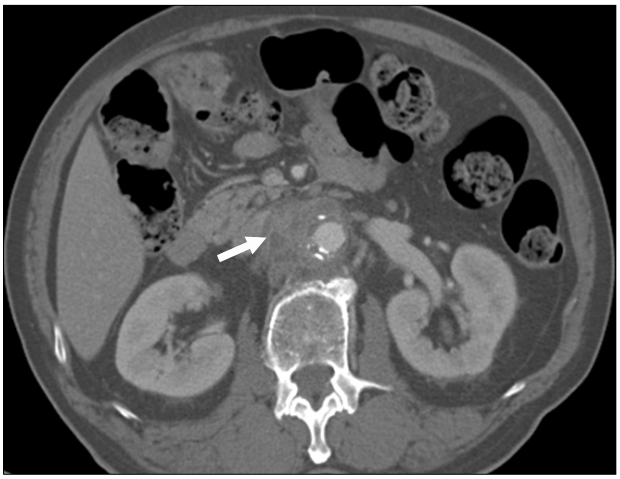

Fig. 1. Abdominal computed tomography scan showing soft tissue infiltration and ischemic lesions around the aorta (arrow).

scess material was obtained and investigated by conventional culture in the blood agar, MacConkey agar, Brucella agar plates, and thioglycollate broth as well as molecular bacteriological diagnostics. Gram staining and aerobic and anaerobic bacterial culture from this specimen yielded no growth after 7 days of incubation.

For broad-range 16S rRNA PCR, complete DNA was extracted from specimen using the MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany) after proteinase K treatment. A semi-nested PCR for bacterial 16S rRNA gene was performed using two pairs of oligonucleotide primers (Table 1). Amplification parameters were as follows: a denaturation step at 95°C for 5 min; then 25 cycles of denaturation at 95°C for 20 sec, annealing at 55°C and 57°C during the first and second rounds, respectively, for 30 sec and extension at 68°C for 45 sec; and extension for 5 min during the final cycle. Sequencing of the amplicon and a database comparison (NCBI BLAST, EzTaxon database, and BIBI database) revealed 99.7% (701/703 bp) identity with an *E. rhusiopathiae*-type strain (GenBank accession number, AB055905.1).

Cultures of drainage fluids subsequently performed on hospital day 11 and 15, but yielded no growth. The patient continued ampicillin/sulbactam treatment and became afebrile after hospital day 14. He was discharged on hospital day 25, the 15th day after surgery, and was switched to oral amoxicillin/clavulanate and moxifloxacin. The inflammatory parameters improved steadily, and CRP decreased to 0.68 mg/dL by the 10th week after surgery and normalized to 0.11 mg/dL by the 18th week after surgery.

Table 1. Primers used in this study for a semi-nested PCR for bacterial 16S rRNA gene

Primer	Sequence (5'-3')	Position*	Length (bp)
First PCR			
1F	AGAGTTTGATCCTGGCTCAG	1-20	20
1R	CCGTCAATTCCTTTGAGTTT	897-916	20
Product		1-916	916
Second PCR			
1F	AGAGTTTGATCCTGGCTCAG	1-20	20
2R	GGCGTGGACTACTAGGGTATCT	787-808	22
Product		1-808	808

*GenBank accession No. AB055905.

DISCUSSION

We have described a case of molecular identification of *E. rhusiopathiae* from culture-negative, periaortic necrotic tissue. There was no involvement of the endocardium, which frequently occurs in invasive *Erysipelothrix* infections, in this case. The invasive form associated with localized infection due to this pathogen is extremely rare. Previous reports of invasive infection in humans have described the following sterile sites: endocardium, bone, joint, and cerebrospinal fluid [5-7]. To the best of our knowledge, only one case of an intra-abdominal abscess due to *E. rhusiopathiae* has been reported [9].

Since the inception of broad-range PCR in the late 1980s, many clinical microbiology laboratories have implemented this technique. Sequence-based typing using broad-range PCR offers two potential benefits: it can often identify pathgens when patents were pretreated with antibiotics, and it can detect culture-resistant, fastidious, damaged, and slow-growing microorganisms [10-12]. In the present case, the pathogen was identified by nucleotide sequence analysis of the 16S rRNA gene from a surgical specimen of the abscess; however, the culture result was negative. Although it might be overlooked due to its slow growth rate and small colony size, antibacterial therapy was started 10 days before specimen collection, which may have caused the negative culture result despite bacterial infection. Since there are no reliable serologic tests for the diagnosis of *Erysipelothrix* infection in humans, the molecular diagnostic assay is important, especially in culture-negative cases. The case reported here demonstrates the diagnostic value of amplification and nucleotide sequence analysis in *Erysipelothrix* infection. Rampini et al. [13] emphasized that amplification and nucleotide sequence analysis from primary sterile body sites should be

performed for patients with a high clinical suspicion of infection and negative culture results. Additionally, they showed that 16S rRNA gene PCR is particularly useful for the identification of bacterial pathogens in patients taking antibiotics. Although direct amplification of rRNA genes from clinical sepcimens can be hobbled by the presence of contaminating DNA in PCR reagents, the use of negative control and double-strand specific DNase pretreatment on PCR reagents can be used to resolve this limitation [10,13].

Most cases of *Erysipelothrix* infection occur after occupational exposure to animals (i.e., butchers, fishermen, fish handlers, veterinarians, housewives, etc.) [1,3,4]. *E. rhusiopathiae* can enter the human body by penetration into the skin or through the gastrointestinal system by ingestion of contaminated food products [3]. Cases of infection that do not have an occupational link have occurred mainly in immunocompromised hosts, and chronic alcohol ingestion is accepted as the most common underlying medical condition in systemic infection [4,6,14]. Although our patient denied occupational exposure or history of contact with animals, he was a chronic alcoholic and frequently consumed raw fish.

Susceptibility data for *E. rhusiopathiae* are limited; however, most strains are highly susceptible to penicillins, cephalosporins, imipenem, clindamycin, and ciprofloxacin, whereas they are resistant to vancomycin, sulfonamides, trimethoprim-sulfamethoxazole, teicoplanin, and aminoglycosides [4,15]. The patient's improvement in this case suggests that intravenous ampicillin/sulbactam and an oral combination of amoxicillin/clavulanate and moxifloxacin were effective.

In conclusion, we identified *E. rhusiopathiae* by 16S rRNA-based gene sequencing from culture-negative necrotic tissue in a patient with an intra-abdominal abscess. Although *E. rhusiopathiae* infection is rare, we should consider it to be one of the possible causes of intra-abdominal abscesses. This case report highlights the usefulness of the 16S rRNA gene sequencing method to identify pathogens in patients pretreated with antibiotics.

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=국문초록=

배양 음성인 복강내 농양에서 16S rRNA 염기서열분석으로 동정된 *Erysipelothrix rhusiopathiae* 1예

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*Erysipelothrix rhusiopathiae*는 사람에서는 주로 피부 감염증을 일으키는 인수 공동 감염 균주인 그람 양성 막대균이다. 만성 알코올 중독인 75세 남자 환자가 복통으로 내원하여 시행한 복부컴퓨터단층촬영에서 복부대동맥 주위에 복강내 농양으로 보이는 연조직의 괴사가 관찰되었다. 경험적 항생제를 투여한 뒤, 수술적으로 제거한 농양 조직으로 시행한 배양 검사는 음성이었으나, 16S rRNA 염기서열분석에서 *E. rhusiopathiae*가 동정되었다. 본 증례는 복강내 농양에서 *E. rhusiopathiae*를 분리한 국내 첫 증례로, 배양 음성인 경우 농양조직에 대한 직접 염기서열 분석을 통하여 원인균을 동정하는 것이 유용할 수 있음을 보여주었다. [Ann Clin Microbiol 2014;17:132-135]

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