

Case Report

카르노박테리아 균혈증: 증례 보고와 문헌 고찰

정인화, 안규대, 김남희, 김경희, 신상동, 한진영, 우광숙

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Isolation of *Carnobacterium divergens* from Blood Culture in Korea : A Case Report and Literature Review

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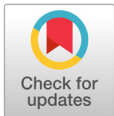
ABSTRACT

Carnobacterium is a genus of gram-positive bacilli belonging to the family *Lactobacillaceae*. Generally, *Carnobacterium* species are considered nonpathogenic to humans and are mostly found in the natural environment, food, and food packaging. Furthermore, some *Carnobacterium* species play a bioprotective role in the food industry. Isolation of *Carnobacterium* from human blood or other sites, such as skin or abscess, has rarely been reported—there are only four published case reports worldwide, and none of them is from Korea. In all the reported cases, the patients reported contact with an aqueous environment or were administered nutrition via a parenteral route. Herein, we report the detection of *Carnobacterium divergens* bacteremia in an immunocompromised patient by using mass spectrometry in Korea.

Keywords: Blood cultures, *Carnobacterium divergens*, MALDI-ToF/MS

INTRODUCTION

Carnobacterium species are heterofermentative lactic acid bacteria usually isolated from the environment or food such as fish, meat, and dairy products [1]. They share resemblance to lactobacilli in several aspects such as morphology and absence of a cytochrome system [2]. *Lactobacilli* are generally known to live in human intestines and female vagina but *Carnobacteria* are not known members of the human gastrointestinal microbial community. The first mention of such unusual lactobacilli was made in 1950, and referred this strain to isolates from poultry meat [2]. Currently 11 species are known in the *Carnobacterium* genus [3], but among them, only *Carnobacterium divergens* and *Carnobacterium maltaromaticum* are frequently isolated in food or fish. These two species are known to inhibit growth of *Listeria monocytogenes* in fish and meat products by production of antimicrobial peptides. Especially, *C. maltaromaticum* (or *C. piscicola*) is known to show antilisterial activity in-vitro and in cooked ham [4]. Up to date, there are only four reports of isolation of such pathogen in human world-wide. No reports in Korea have been published



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until now. Herein, we report a case of *C. divergens* bacteremia in an adult central nervous system (CNS) lymphoma patient who was receiving chemotherapy in Dong-A University Hospital.

CASE REPORT

A 65-year old Chinese male patient diagnosed with CNS lymphoma on July 2019 was admitted to our hospital due to mild fever of 37.8°C and severe oral mucositis for 3 to 4 days. Laboratory findings showed pancytopenia with moderate neutropenia (white blood cell 2,240 /uL, segmented neutrophils 41.0%, hemoglobin 9.9 g/dL, platelet 70,000 /uL) and increased CRP (2.91 mg/dL). No other physical findings were observed. Due to his primary CNS lymphoma, he had had osteoplastic craniotomy with tumor removal on July 29 2019. After surgery, treatment was started with R-MPV (rituximab, methotrexate, procarbazine and vincristine) induction chemotherapy from August 10 for five days. On November 8, he was treated with etoposide for peripheral blood stem cell transplantation. Due to oral mucositis, total parenteral nutrition was applied to his central venous catheter on the 6 day of his admission. For antimicrobial therapy and treatment of fever, two pairs of blood cultures were drawn on the day of admission, 5, 6 and 8 day of admission. They were inoculated into two aerobic and two anaerobic blood culture bottles and incubated in BacT/ALERT 3D blood culture instrument (bioMérieux, Marcy-L'Étoile, France). On the 8 day of admission, after 48 hours of incubation at 37°C, one of the anaerobic bottles showed positive results. White to gray colonies with 1-2 mm diameter were grown on blood agar plates (Fig. 1). On biochemical studies, the colonies were catalase and oxidase negative, and alpha-hemolytic. Microscopic examination revealed either Gram-positive rods or cocci (Fig. 2). *Carnobacterium divergens* was identified by using MALDI-ToF/MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; bioMérieux, Marcy-l'Étoile, France) with 99.9% validity. The sample for MALDI-ToF was taken from the colony, not directly from blood samples. For confirmation, 16S rRNA gene sequencing using the MiSeq Microbial Identification System (Macrogen, Seoul, Korea) was performed.

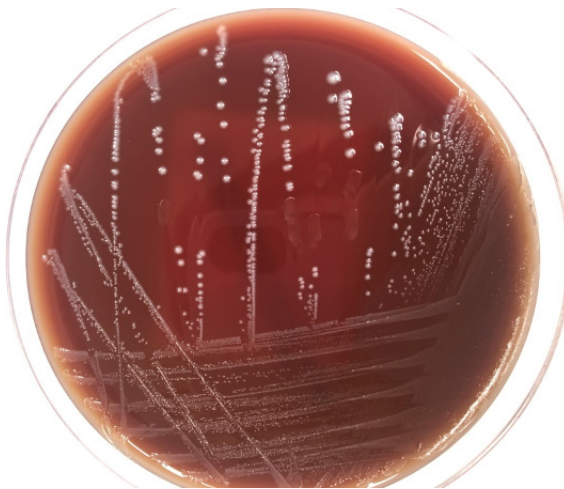


Figure 1. *Carnobacterium divergens* on the surface of sheep blood agar after a two day cultivation at 37°C

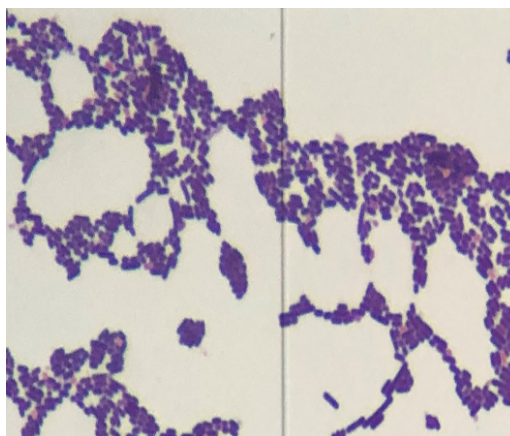


Figure 2. Gram staining of *Carnobacterium divergens* observed on microscopy (×1,000)

The sequencing confirmed *C. divergens* with homology of 99%. Antimicrobial susceptibility test was performed using VITEK 2 system (bioMérieux, Marcy-L'Etoile, France). The isolate was resistant to oxacillin (minimum inhibitory concentration (MIC) of 4 mg/L) but susceptible to vancomycin (MIC of 0.5 mg/L) and linezolid (MIC of 2 mg/L). Antimicrobial therapy with piperacillin/tazobactam (Tabaxin) was empirically started on the date of his admission to cover both Gram-positive and negative bacteria, but after isolation of *C. divergens*, vancomycin was added due to resistance to oxacillin. Two days later, subsequent peripheral and central venous catheter blood cultures showed no growing bacteria. As his fever subsided and oral mucositis was improved, the patient was discharged.

DISCUSSION

Unlike the previously published cases of *Carnobacterium* species (Table 1), this case was identified by MALDI-ToF mass spectrometry, which is now applied widely in clinical microbial laboratories. In comparison to classical methods of bacterial identification such as API or VITEK, mass spectrometry uses

Table 1. Literature review of *Carnobacterium* bacteremia

Authors (year)	Sex/Age	Diagnosis	Suspected route of infection	Specimen	Method	Identification
XU <i>et al.</i> (1997) [5]	F/13	Finger necrosis	Contamination from pool water	Finger gangrene	API 20A System 16S rRNA sequencing	<i>Carnobacterium</i> species
Chmelar <i>et al.</i> (2002) [6]	M/35	Traumatic amputation of hand	Water sawmill worker, parenteral nutrition	Post-surgical abscess	API 50 16S rRNA sequencing	<i>C. piscicola</i>
Hoenigl <i>et al.</i> (2010)	M/43	Meningitis, sepsis	Contact with seafood	Peripheral blood	N/S 16S rRNA sequencing	<i>Carnobacterium</i> species
Smati <i>et al.</i> (2015)	F/57	Diabetic ketoacidosis, necrotizing esophagitis	Parenteral nutrition	Peripheral blood	API Coryne 16S rRNA sequencing	<i>C. divergens</i>

Abbreviation: N/S, not specified.

proteomic approach that allows rapid and accurate identification of bacteria as well as yeast and fungi [3]. In fact, the conventional bacterial detection method, VITEK 2 (bioMérieux), identified the same colony as *Enterococcus gallinarum* with VITEK 2 GP card (code 2420916203). This implies that the conventional method cannot accurately identify such Gram-positive rod. The most recent case introduced a 57-year old female patient from France who was diagnosed with acute necrotizing esophagitis [7]. She began parenteral nutrition after surgery, and her four sets of blood culture bottles showed positive results. Unclear results were showed on API Coryne and API Listeria systems (bioMérieux) but the physicians presumed the isolate was *L. monocytogenes*. Following 16S rRNA sequencing confirmed the pathogen was *C. divergens*. The second case from Austria presented a 43-year male patient with history of contact with seafood [8]. He was diagnosed with meningitis, and bacterial growth was detected in one of his culture bottles. They also suspected the isolate to be *L. monocytogenes*. However, gene sequencing discovered that the pathogen was *C. divergens*. The specimens of the other two cases of *Carnobacteria* were necrotic tissue or pus [3,9]. They both had history of either contact with aqueous environment, feeding with parenteral nutrition or both.

It is well known that the mass spectrometry has higher diagnostic sensitivity and specificity than the conventional microbial identification methods [8] but there are three other factors that also support the causative relationship of the patients' bacteremia and such pathogen in our case report. First, the patients' fever subsided after adding vancomycin which covers methicillin/oxacillin resistant Gram-positive bacteria. Subsequent culture from central catheter showed negative results. Second, although the patient history of contact with seafood or other special environment was not definitely confirmed, he was on parenteral nutrition, like the other two published reports. Third, his previous three sets of blood cultures showed no growth, but only after he started nutrition on feeding tube, bacterial growth was observed. However, since the bacteria were present in only one of the four bottles, the possibility of contamination cannot be completely ruled out. We assume the origin of infection was bacterial contamination of the parenteral nutrition bag or colonization of the feeding tube. To confirm the pathogen, culture of parenteral nutrition solution could have been implemented.

Similar to lactobacilli, which is used as biological preservatives in food, *Carnobacteria* can also rarely cause bacteremia [9,10]. In summary, we report a case of *C. divergens* bacteremia in an immunocompromised patient identified by MALDI-ToF MS and review of the literature. As more Gram-positive bacteria and other atypical bacteria, which were previously neglected or undetected, are detected with such relatively new diagnostic tool, more attention should be paid.

요약

카르노박테리아는 락토바실러스에 포함되는 그람 양성 간균이다. 카르노박테리아는 일반적으로 인간에게는 병원성으로 간주되지 않고 대부분 자연 환경과 식품 또는 식품 포장에서 발견된다. 그 중 일부 종은 식품 산업에서 다른 균에 감염되지 않게 생물보호적 역할이 있음이 알려져 있기도 하다. 여태까지 발표된 문헌에 따르면, 흔하지 않게 인간의 혈액이나 피부 및 농양에서 분리되기도 한다. 전세계적으로 카르노박테리아의 분리 및 동정 사례는 4건에 불과하지만 한국에는 아직 보고된 바가 없다. 카르노박테리아 균혈증으로 보고된 모든 사례들은 환자가 수성 환경

과 접촉한 과거력이 있거나 입원 기간 동안 비경구 영양식을 했다는 과거력이 있다. 우리는 질량 분석기를 통해 면역결핍환자에서 확인된 *Carnobacteria divergens* 균혈증 증례를 보고한다.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Xu J, Yang H, Lai X, Fu X, Wu J, Huang L, et. al. Etiological study for a case of multi-bacterial synergistic gangrene. *Chinese Sci Bull* 1997;42:511-7.
2. Chemelar D, Matusek A, Korger J, Durnova E, Steffen M, Chmelarova E. Isolation of *Carnobacterium piscicola* from human pus – case report. *Folia Microbiol* 2002;47:455-7.
3. Leisner JJ, Laursen BG, Prevost H, Drider D, Dalgaard P. *Carnobacterium*: positive and negative effects in the environment and in foods. *FEMS Microbiol Rev* 2007;31:592-613.
4. Hoenigl M, Grisold AJ, Valentin T, Leitner E, Zarfel G, Renner H, et. al. Isolation of *Carnobacterium* sp. from a human blood culture. *J Med Microbiol* 2010;59:493-5.
5. Carbonnelle E, Mesquita C, Bille E, Day N, Dauphin B, Veretti JL, et. al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011;44:104-9.
6. Smati M, Palacios C, Cohen Y, Mechai F, Tankovic J, Fleche-Mateos A, et. al. *Carnobacterium divergens* bacteremia in woman. *Emerg Infect Dis* 2015;21:1081
7. Danielski GM, Imazaki PH, Andrade Cavalari CM, Daube G, Clinquart A, Freitas de Macedo RE. *Carnobacterium maltomaticum* as bioprotective culture *in vitro* and in cooked ham. *Meat Sci* 2020;162:108035.
8. Schillinger U and Holzapfel WH. The genus *Carnobacterium*. In: Wood BJB, Holzapfel WH, eds. *The genera of lactic acid bacteria*. Vol 2, Boston; Springer, 1995:307-26.
9. Scarpellini M, Mora D, Colombo S, Franzetti L. Development of genus/species-specific PCR analysis for identification of *Carnobacterium* strains. *Curr Microbiol* 2002;45:24-9.
10. Barakat RK, Griffiths MW, Harris LJ. Isolation and characterization of *Carnobacterium*, *Lactococcus*, and *Enterococcus* spp. from cooked, modified atmosphere packaged, refrigerated, poultry meat. *Int J Food Microbiol* 2000;62:83-94.