First Isolation of Streptococcus gallolyticus subsp. pasteurianus from a Korean Patient with Severe Septic Shock

Seri Jeong¹, Ji Yeon Park¹, Sang Hoon Han², Yangsoon Lee^{1,3}, Dongeun Yong¹, Kyungwon Lee¹, Yunsop Chong¹

¹Department of Laboratory Medicine and Research Institute of Bacterial Resistance, ²Department of Internal Medicine, Yonsei University College of Medicine, Seoul, ³Department of Laboratory Medicine, National Health Insurance Corporation Ilsan Hospital, Goyang, Korea

A 60-year-old man presented with a 1-day history of fever, vomiting, and diarrhea. He was diagnosed with severe septic shock on the basis of a body temperature of 38.9° C, heart rate of 92/min, respiratory rate of 25/min, WBC count of 22,970/ μ L, C-reactive protein (CRP) level of 136 mg/L, blood urea nitrogen (BUN) of 34.0 mg/dL, and creatinine of 2.98 mg/dL. On blood culture, Gram-positive cocci were detected in all 6 bottles. Small grayish non-hemolytic colonies were found on blood agar plates after incubation at 37°C for 2 days. The isolates were negative for catalase and L-pyrrolidonyl- β -naphthylamide hydrolysis, and positive for bile-esculin and leucine aminopeptidase activity. The strain was identified as *Strep*-

INTRODUCTION

The non-enterococcal group D streptococci include *Strepto-coccus bovis*, which is an indigenous resident in the gastro-intestinal tract of both humans and animals [1]. *S. bovis* causes human infections such as bacteremia, sepsis and endocarditis [2]. Among *S. bovis* species, *Streptococcus gallolyticus* subsp. *pasteurianus* was reported as a microbial cause of meningitis and septic shock [3-5]. However, case of *S. gallolyticus* subsp. *pasteurianus* septic shock has not been reported in Korea yet. This report describes a septic shock case caused by *S. gallolyticus* subsp. *pasteurianus*, a rare causative agent of severe infection.

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tococcus gallolyticus subsp. *pasteurianus* using Vitek 2 GP II systems. We performed 16S rRNA gene sequencing and detected 100% identity with *S. gallolyticus* subsp. *pasteurianus* strain CIP 107122^T (1,345/1,345-bp). The patient recovered after receiving ampicillin-sulbactam. This is the first report of phenotypic and genetic identification of *S. gallolyticus* subsp. *pasteurianus* causing severe septic shock in a Korean patient. (Korean J Clin Microbiol 2011;14:144-147)

Key Words: Streptococcus gallolyticus subsp. pasteurianus, Septic shock, 16S rRNA gene sequencing

CASE REPORT

A 60-yr-old man was admitted to Severance hospital on May 24, 2010, with 1-day history of fever, vomiting and diarrhea after eating a bowel of jjamppong, Chinese-style noodles with vegetables and seafood. He had periodontal surgeries two months ago, and smoked cigarettes (30-pack years). His parents died of gastric cancer and stoke. He had not recently travelled. He complained about lower abdominal pain. On admission, his temperature was 36.0°C because he has already taken an antipyretic. Heart rate was 84 beats/min and blood pressure was 65/45 mmHg. After 6 hours, body temperature was 38.9°C, with pulse rate of 92 beats/min, respiratory rate of 25 breaths/min, and oxygen saturation of 79.7%. A laboratory investigation at the time of admission revealed a peripheral white blood cell (WBC) count of 22,970/ μ L (98.2% neutrophils), a hemoglobin level of 15.1 g/dL, and a platelet count of $216,000/\mu$ L. C-reactive protein (CRP) was elevated to 136 mg/L. Initial routine blood chemistry showed increased blood urea nitrogen (BUN) of 34.0 mg/dL, creatinine of 2.98 mg/dL, aspartate amino-

Correspondence: Dongeun Yong, Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea. (Tel) 82-2-2228-2442, (Fax) 82-2-313-0956, (E-mail) deyong@yuhs.ac

transferase (AST) of 65 IU/L, and alanine transaminase (ALT) of 52 IU/L. The patient was diagnosed as severe septic shock and acute renal failure based on the physical examination and laboratory results indicated above.

Three sets of blood samples were drawn for culture study on admission. After 5 days of incubation in the BacT/Alert 3D blood culture system (bioMérieux, Durham, NC, USA), all the aerobic and anaerobic culture bottles yielded gram-positive cocci in chains and small-sized (0.5-1 mm) gravish non-hemolytic colonies on blood agar plate were obtained after 2 days of subculture in CO₂ incubator. The initial stool and urine culture showed not remarkable results. The isolate of blood was negative for catalase and pyrrolidonylarylaminidase, while bile-esculin and leucine aminopepetidase positive. They failed to grow in heart infusion broth containing 6.5% NaCl. The species identification was achieved by the Vitek 2 GP identification system (bioMérieux, Marcy l'Etoile, France). GP I card suggested S. bovis, but GP II card identified the isolate as S. gallolyticus subsp. pasteurianus. The biochemical profiles of this strain are compared with those of the S. gallolyticus subspecies pasteurianus and S. gallolyticus subspecies gallolyticus Table 1. Additionally, the starch acidification test was performed which are not reported by the Vitek 2 GP identification system. All of biochemical characteristics except for β -glucuronidase supported that the identification of the isolate of this case was S. gallolvticus subsp. pasteurianus. We performed 16S rRNA gene sequencing for more accurate identification. The forward 8F (5'-A GA GTT TGA TCC TGG CTC AG-3') and reverse 1541R (5'- AAG GAG GTG ATC CAG CCG CA-3') primers were used to amplify and sequence the 16S rRNA gene fragments. The isolates showed 100% (1,345/1,345 bp) similarity with the type strain of *S. gallolyticus* subsp. *pasteurianus* CIP 107122^T (GenBank nucleotide accession numbers DQ232528). Antimicrobial susceptibility testing was performed by E-test for penicillin and disk diffusion methods for other antibiotics according to the Clinical and Laboratory Standards Institute guidelines [6]. The initial isolates from the blood cultures were susceptible to penicillin G (minimal inhibitory concentration; 0.06 μ g/mL), clindamycin, cefepime, cefotaxime, levofloxacin, meropenem, vancomycin, and linezolid, while resistant to erythromycin and tetracycline. The results of antimicrobial susceptibility tests showed the similar patterns of S. pasteurianus reported by Beck et al. [7]. Antibiotic therapy with meropenem and vancomycin was started. These drugs were held on the 7th hospital day due to the aggravated renal function and drug eruption. The subsequent blood cultures on the 3rd hospital day were negative. Intravenous ampicillin-sulbactam (Whanin Pharm Co. Ltd, Seoul, Korea) was applied from the 3rd hospital day until his discharge (on the 49th hospital day) due to accompanied L4-5 spondylodiscitis. Additionally, after infusion of normal saline, hydroxyethyl starch and norepinephrine to maintain a mean arterial pressure to be over 65 mmHg, his vital signs and laboratory results were improved on the 3rd hospital day. Laboratory tests showed decreased WBC count from $22,970/\mu$ L to $5,760/\mu$ L and CRP from 136 mg/L to 24 mg/L. On the 10th hospital day, the BUN and creatinine levels normalized to 14.3 mg/dL and

Table 1. Biochemical characteristics of Streptococcus gallolyticus subsp. pasteurianus clinical isolate

Characteristics	Isolates of this case	S. gallolyticus subsp. pasteurianus*	S. gallolyticus subsp. gallolyticus*
Catalase	_	_	_
PYR hydrolysis	—	—	_
Leucine aminopeptidase	+	+	+
Bile-esculin test	+	+	+
β -Glucuronidase	—	+	—
α -Galactosidase	+	v	+
β -Galactosidase	+	+	_
Acid production from			
Starch	†	—	+
Inulin	—	—	+
Lactose	+	+	+
Mannitol	_	—	+
Raffinose	+	v	+
Trehalose	+	+	+

*The biochemical characteristics of the subspecies of *S. gallolyticus* were obtained from a report by Schlegel et al. [9]. [†]The test of starch acidification performed manually. For the rest, Vitek2 GP I and GP II system were used.

Abbreviations: +, ≥80% of strains positive; -, ≤20% of strains positive; v, 21-79% activity compared to the positive control reaction.

0.71 mg/dL, respectively.

Echocardiography and colonoscopy were performed to find the septic foci and entry of *S. gallolyticus* subsp. *pasteurianus*. Echocardiography showed no visible vegetation and normal global left ventricle systolic function (59% of ejection fraction). Colonoscopic examination detected the patient's underlying hemorrhoid and diverticulum. He was discharged from the hospital after receiving conservative care.

DISCUSSION

The S. bovis group includes Streptococcus equines, Streptococcus gallolyticus, Streptococcus infantarius, and Streptoccus alactolyticus, which are encountered in blood cultures of patients with bacteremia, sepsis, and endocarditis. The blood culture growing S. bovis group has a clinical significance due to the association of S. gallolyticus subsp. gallolyticus with gastrointestinal cancer and of S. gallolyticus subsp. pasteurianus with meningitis [2]. These clinical implications require accurate species identification. The S. bovis group was previously classified into three groups according to their biochemical characteristics: biotype I (mannitol fermentation positive), biotype II/1 (mannitol negative and beta-glucuronidase negative), and biotype II/2 (mannitol negative and beta-glucuronidase positive) [8]. Recently, extensive taxonomic changes have occurred in this group, and the new classification designated S. bovis biotypes I, II/1, and II/2 as S. gallolyticus subsp. gallolyticus, S. infantarius subsp. coli, and S. gallolyticus subsp. pasteurianus, respectively, on the basis of genetic characteristics. S. gallolyticus subsp. pasteurianus, also named Streptococcus pasteurianus, was proposed to replace S. bovis II/2 [9]. Although genetic methods showed remarkable achievements in reappraisal of S. bovis, biochemical characteristics still take considerable possessions of identification. We could identify the infecting organism as S. gallolyticus subsp. pasteurianus with high confidence at the subspecies level using the biochemical characteristics and the 16s rRNA gene sequencing.

The patient's condition required urgent, aggressive management on his admission. He received fluid resuscitation through rapid intravenous administration of crystalloid fluid and vasopressor support. After that, echocardiography was performed considering the association of infective endocarditis with *S. bovis* infection [10]. Colonoscopic examination was also carried out because the investigation of the lower gastro-intestinal tract to identify a portal of entry in cases of systemic *S. bovis* infection is important [11]. Colonic carcinoma has been reported in about 30% of patients with *S. bovis* endocarditis or bacteremia [12,13]. Investigation of the gastrointestinal tract has revealed a range of large bowel pathology such as inflammatory colitis, polyps and diverticulosis in the 19 cases of *S. bovis* meningitis [14]. Other colonic benign diseases have also been described in patients with *S. bovis* infection [12,13]. In our case, the patient's underlying hemorrhoid and diverticulum were revealed using the colonoscopic examination. It seems to be a hemorrhoid provided a direct pathway for hematogenous dissemination of the organism.

Several cases of *S. gallolyticus* subsp. *pasteurianus* infections such as meningitis and septic shock were reported in the United States, France, and Japan [3-5]. In Korea, Kim et al. [15] reported an infective endocarditis patient caused by *S. gallolyticus* subsp. *gallolyticus* underlying with colon cancer. However, this is the first report of *S. gallolyticus* subsp. *pasteurianus* severe septic shock in Korea and it was confirmed by phenotypic and genetic methods. This report is arousing the impressive virulence potential of *S. gallolyticus* subsp. *pasteurianus*.

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=국문초록=

Streptococcus gallolyticus subsp. pasteurianus에 의한 한국인에서의 첫 패혈쇼크 1예

¹연세대학교 의과대학 진단검사의학교실, 세균내성연구소, ²연세대학교 의과대학 내과학교실, ³국민건강보험공단 일산병원 진단검사의학교실 정세리¹, 박지연¹, 한상훈², 이양순^{1,3}, 용동은¹, 이경원¹, 정윤섭¹

60세의 남자 환자가 1일 전부터의 발열과 구토, 설사가 있었다. 당시 체온 38.9°C, 심박수 92/min, 호흡수 25/min, 백혈구 수 22,970/μL, C반응성 단백질 136 mg/L, 혈중 요소 질소 34.0 mg/dL, 크레아티닌 2.98 mg/dL을 보여 심각한 패혈쇼크로 진단되었다. 혈액 배양병 6병 모두에서 그람 양성 알균이 증식되었다. 혈액 한천 배지에서 37°C, 2일 동안 배양한 결과, 작은 크기의 비용혈성 회색 집락을 관찰할 수 있었다. 분리주는 catalase와 L-pyrrolidonyl-β-naphthylamide hydrolysis에 음성이었고 bile-esculin test와 leucine aminopeptidase activity에 양성이었다. Vitek 2 GP II systems으로 균주 동정 결과 *Streptococcus gallolyticus* subsp. *pasteurianus*으로 나타났고 16S rRNA 유전자 염기서열 분석에서도 *S. gallolyticus* subsp. *pasteurianus* strain CIP 107122^T (1,345/1,345-bp)의 염기 서열과 100% 일치하였다. 환자는 ampicilin-sulbactam 투여 후 회복 되었다. 본 증례는 국내에서 발생한 *S. gallolyticus* subsp. *pasteurianus*에 의한 첫 패혈쇼크 보고이다. [대한임상미생물학회 지 2011;14:144-147]

교신저자 : 용동은, 120-752, 서울시 서대문구 연세로 50 연세대학교 의과대학 진단검사의학교실, 세균내성연구소 Tel: 02-2228-2442, Fax: 02-313-0956 E-mail: deyong@yuhs.ac