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A Case of *Streptococcus gallolyticus* subsp. *gallolyticus* Infective Endocarditis with Colon Cancer: Identification by 16S Ribosomal DNA Sequencing

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Although the association between *Streptococcus bovis* endocarditis and colon carcinoma is well known, very few cases of *S. bovis* infection associated with underlying malignancies have been reported in Korea. The *S. bovis* group has been recently reclassified and renamed as *Streptococcus gallolyticus* and *Streptococcus infantarius* subspecies under a new nomenclature system. We report a case of infective endocarditis with colon cancer caused by *S. gallolyticus* subsp. *gallolyticus* (previously named *S. bovis* biotype I). A 59-yr-old woman presented with a 1-month history of fever. Initial blood cultures were positive for gram-positive cocci, and echocardiography showed vegetation on mitral and aortic valves. Antibiotic treatment for infective endocarditis was started. The infecting strain was a catalase-negative and bile-esculin-positive alpha-hemolytic *Streptococcus* susceptible to penicillin and vancomycin. The strain was identified as *S. gallolyticus* subsp. *gallolyticus* with the use of the Vitek 2 GPI and API 20 Strep systems (bioMérieux, USA). The 16S rDNA sequences of the blood culture isolates showed 100% homology with those of *S. gallolyticus* subsp. *gallolyticus* reported in GenBank. The identification of the infecting organism, and the subsequent communication among clinical microbiologists and physicians about the changed nomenclature, led to the detection of colon cancer. The patient recovered after treatment with antibiotics, valve surgery, and operation for colon cancer. This is the first report of biochemical and genetic identification of *S. gallolyticus* subsp. *gallolyticus* causing infective endocarditis associated with underlying colon cancer in a Korean patient. *(Korean J Lab Med 2010;30:160-5)*

Key Words : *Streptococcus gallolyticus, Endocarditis, Colon cancer, rDNA sequencing*

INTRODUCTION

Streptococcus bovis is a member of group D streptococci-common inhabitants of the intestine and causative agents of endocarditis. A well-recognized association has been established between *S. bovis* endocarditis and colon carcinoma, which led to great interest in the identification of this organism [1]. The *S. bovis* group is divided into 3 biotypes according to their biochemical characteristics: biotype I (mannitol fermentation positive), biotype II/1 (mannitol negative and β-glucuronidase negative), and biotype II/2 (mannitol negative and β-glucuronidase positive) [2]. Each biotype has somewhat different pathogenicity. *S. bovis* biotype I has been documented to be the biotype more likely associated with both endocarditis and malignant or premalignant colonic lesions [3]. The taxonomy of the *S. bovis* group has been evolving in the last few decades, and a new nomenclature was adopted on the basis of genetic distances and phylogenetic analyses. The new classification lists *S. bovis* biotypes I, II/1, and II/2 as *Streptococcus gallolyticus* subsp. *gallolyticus*, *Streptococcus infantarius*

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subsp. *coli*, and *S. gallolyticus* subsp. *pasteurianus*, respectively [4]. We report a case of infective endocarditis with colon cancer caused by *S. gallolyticus* subsp. *gallolyticus*. Identification of the causative organism was performed by phenotypic characterization and 16S rDNA sequence analysis.

CASE REPORT

A 59-yr-old woman was admitted to Seoul National University Hospital, with a 1-month history of recurrent episodes of fever and chills. She also had arthralgia of both knees and 3 episodes of fecal incontinence for 1 month. She was a carrier of the hepatitis C virus and had a history of stage 4 gastric marginal zone lymphoma, which had been previously treated with chemotherapy over 2 yr ago. She had no evidence of residual lymphoma after treatment. On physical examination, fever was present (38.9℃); lung fields were clear; heart sounds were regular without murmur; and abdomen was soft, non-distended, and with no palpable mass. Digital rectal examination revealed no melena, hematochezia, or mass. Specimens for laboratory tests and blood cultures were collected, and empirical antibiotic therapy with intravenous ciprofloxacin (400 mg every 12 hr) was started. Initial laboratory studies showed the following results: hemoglobin (11.1 g/dL), white blood cells [12.01×10^9/L; neutrophils (79%), lymphocytes (14%), and monocytes (4%)], platelets (242×10^9/L), and C-reactive protein (5.79 mg/dL). Results of the liver function tests and creatinine, glucose, and troponin tests were within the normal range. Stool occult blood test was not performed. The patient's chest radiograph was unremarkable. She had persistent fever increasing up to 39.1℃ despite antibiotic therapy, and initial blood cultures were positive for gram-positive cocci in pairs and short chains. Twenty-four hours later, ciprofloxacin was discontinued and vancomycin (1,000 mg every 12 hr) was started after collecting additional blood cultures. An echocardiography was performed, which showed vegetations on the mitral and aortic valves. The initial isolate from the first blood cultures was identified as *S. gallolyticus* subsp. *gallolyticus*, which was susceptible to penicillin. The second blood cultures were also positive for similar alpha-hemolytic streptococci. Vancomycin was discontinued and antibiotics were replaced by intravenous penicillin G (3,000,000 U every 4 hr) and gentamicin (63 mg every 8 hr). The fever subsided, and the subsequent blood cultures were negative for streptococci. The isolate from the second blood cultures was initially identified as *Streptococcus mutans*; however, the results of retest and further investigation confirmed that it was the same strain as the isolate from the first blood cultures. The results of blood cultures were discussed among clinical microbiologists and physicians, including the corrected results of the second blood cultures, the information about the causative organism (i.e., *S. gallolyticus* subsp. *gallolyticus*, which was named as *S. bovis* biotype I in the previous nomenclature system), and the risk of colon cancer. The abdomen computerized tomography (CT) image showed newly detected segmental wall thickening of the sigmoid colon. Colonoscopy revealed an ulceroinfiltrative mass, which was confirmed as adenocarcinoma. The patient underwent operation for valve replacement and anterior resection for colon cancer on the 18th and 50th day of hospitalization, respectively. She successfully recovered and was discharged after completing the antibiotic therapy and postoperative care.

Both the first and the second blood cultures showed gram-positive cocci in pairs and short chains after 24 hr of incubation in broths at 37℃ (Bactec, Becton Dickinson, Baltimore, MD, USA). Subsequent subculture on 5% sheep blood and chocolate agars incubated at 35℃ showed small alpha-hemolytic colonies. The isolates were negative for catalase and pyrrolidonylarylaminidase, showed no zone of inhibition around the optochin disk, failed to grow in heart infusion broth containing 6.5% NaCl, and bile-esculin positive after overnight incubation. A tentative identification of viridans group *Streptococcus* was made. The Vitek2 GP identification system (bioMérieux, Durham, NC, USA) was used to identify the isolate from the first blood culture as *S. gallolyticus* subsp. *gallolyticus*; the isolate from the second culture was identified as *S. mutans*. The tests were repeated twice for the subculture of the second isolate, and the results were as follows: *S. gallolyticus* subsp. *gallolyti-*

cus (excellent identification, probability 98.11%), *S. gallolyticus* subsp. *gallolyticus* (low discrimination, 50.52%), and *S. mutans* (49.48%). Both of the repeated tests showed identical biochemical profiles, except that the test with an excellent confidence level showed a positive result in the pullulan acidification test. The API 20 Strep system (bioMérieux, Durham, NC, USA) identified the isolate from the first blood culture as *S. bovis* biotype I (*S. gallolyticus* subsp. *gallolyticus*) (profile 5000453, 78.1%) and *S. bovis* biotype II (13.5%), while the isolate from the second culture was identified as *S. bovis* biotype I (*S. gallolyticus* subsp. *gallolyticus*) (profile 5000553, 99.1%). The first isolate showed negative reactions with leucine arylamidase and mannitol, which are atypical for *S. gallolyticus* subsp. *gallolyticus*. The subculture of the first isolate was tested repeatedly and identified by the API 20 Strep system as *S. gallolyticus* subsp. *gallolyticus*, with 99.1% probability. The biochemical profiles of this strain are compared with those of the *S. gallolyticus* and *S. infantarius* subspecies in Table 1. Antimicrobial susceptibility testing was performed by E test for penicillin and disk diffusion methods for other antibiotics. Isolates from the first and second blood cultures showed identical results: susceptible to penicillin (MIC= 0.032 μg/mL), vancomycin, and chloramphenicol and resistant to erythromycin and clindamycin.

We performed 16S rDNA gene sequencing for the isolates from the first and second blood cultures. The forward (27F: 5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse (1492R: 5′-TACGGYTACCTTGTTACGACTT-3′) primers were used to amplify 16S rDNA gene fragments, representing approximately 98% of the complete 16S rDNA gene. The following primers were used for sequencing of the fragments obtained: 27F, 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′). Both isolates showed identical 16S rDNA gene sequences and showed 100% (1,480/1,480 bp) homology with those of *S. gallolyticus* subsp. *gallolyticus* (EU163500, GenBank). Isolates from the first and second blood cultures showed 99.7% (1,476/1,480 bp) and 98.6% (1,459/1,480 bp) homology with *S. gallolyticus* subsp. *pasteurianus* (EU163502, GenBank) and *S. infantarius* subsp. *coli* (EU163503, GenBank), respectively [5]. The sequences were analyzed by using MicroSeq ID 16S rDNA 500 library v2.1 and analysis software v2.0 (Applied Biosystems, Foster City, CA, USA), and the isolate was

Table 1. Characteristics of the blood culture isolates of this case in comparison with the subspecies of *Streptococcus gallolyticus* and *Streptococcus infantarius*

Characteristics	Isolates of this case		*S. gallolyticus* and *S. infantarius* subspecies*				
	Vitek 2 GP	API 20 Strep	*S. gallolyticus* subsp. *gallolyticus*	*S. gallolyticus* subsp. *pasteurianus*	*S. gallolyticus* subsp. *macedonicus*	*S. infantarius* subsp. *infantarius*	*S. infantarius* subsp. *coli*
Hydrolysis of:							
Esculin	NT	+	+	+	−	v	+
Production of:							
β-Glucosidase	NT	+	+	+	−	v	+
β-Glucuronidase	−	−	−	+	−	−	−
α-Galactosidase	+	−	+	v	v	+	+
β-Galactosidase	−	−	−	+	v	−	−
Acidification of:							
Starch	NT	+	+	−	+	+	v
Glycogen	NT	+	+	−	−	+	−
Inulin	NT	−	+	−	−	−	−
Lactose	+	+	+	+	+	+	+
Mannitol	+	+	+	−	−	−	−
Methyl-β-D-glucopyranoside	+	NT	+	+	−	−	+
Raffinose	+	+	+	v	−	+	−
Trehalose	+	+	+	+	−	−	−

*The biochemical profiles of the subspecies of *S. gallolyticus* and *S. infantarius* were obtained from a report by Schlegel et al. [4].
Abbreviations: NT, not tested; +, ≥80% of strains positive; −, ≤20% of strains positive; v, 21-79% activity compared to the positive control reaction.

identified as *S. bovis*.

DISCUSSION

The relationship between streptococcal endocarditis and colon cancer was first described in 1951 [6], and *S. bovis* was recognized as the pathogen specifically related to colon cancer in 1977 [1]. Although many other bacterial strains have been associated with gastrointestinal tumors, the association between *S. bovis* and colon cancer remains the strongest and best documented one. Several prospective studies have suggested that *S. bovis* infection could be seen as a first sign of colon cancer [7, 8]. Therefore, clinicians should not readily rule out colon cancer or other malignancies when they encounter patients with *S. bovis* infection. In this case, colon cancer can be detected by colonoscopy upon clinical suspicion of *S. bovis* infection, even without clinical findings suggesting colon cancer. The pathogenesis of the association between *S. bovis* infection and colon neoplasms has been widely investigated; however, the pathogenesis is still not clear. One hypothesis is that the *S. bovis* group is a normal inhabitant of the gastrointestinal tract and can readily enter the bloodstream upon mucosal disruption or vascular changes [9].

Since the early 1980s, genetic and biochemical diversities among *S. bovis* bacteria have been recognized. These diversities led to devising schemes to distinguish strains by biotype. *S. bovis* bacteria are said to be biotype I (or typical) if they ferment mannitol and produce glucan, and biotype II (or variant) if they cannot ferment mannitol or produce glucan. The *S. bovis* biotype II strains are further divided into types II/1 and II/2 according to the ability of the latter group to produce β-galactosidase and β-glucuronidase and ferment trehalose but not glycogen [10]. In the 1990s, 4 new species were described: *Streptococcus gallolyticus*, *Streptococcus macedonicus, Streptococcus waius*, and *Streptococcus infantarius* [11]. *S. gallolyticus*, originally isolated from koalas, is phenotypically similar to *S. bovis* biotype I [12], and reexamination of strains previously identified as *S. bovis* by either phenotypic or genetic methods revealed that many of the human strains associated with endocarditis and animal (goat, pigeon, and cow) strains are identical to *S. gallolyticus* [13, 14]. Meanwhile, molecular technologies, such as DNA-DNA hybridization or the amplification of selected targets, have been developed to complement and improve the identification of streptococci at the species level. Farrow et al. demonstrated that the *S. bovis*/*Streptococcus equinus* complex comprised 6 DNA groups [15]. Members of DNA group 2 have been reclassified, according to their genetic and biochemical characteristics, and renamed as *S. gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, or *S. gallolyticus* subsp. *macedonicus* [4]. Biochemical characteristics, such as gallate hydrolysis activity, are useful for the identification at the subspecies level. A recent study showed that the published phenotypic characteristics of *S. gallolyticus* and *S. infantarius* were equivocal and did not allow unambiguous phenotypic differentiation [5]. Beck et al. also showed that although the 16S rDNA gene sequences are very similar between subspecies, full-length 16S rDNA gene sequence analysis clearly assigned the subspecies in most cases because of the extremely low base pair variability within one species or subspecies. They showed that only 3 of 29 *S. gallolyticus* subsp. *gallolyticus* strains, 1 of 12 *S. gallolyticus* subsp. *pasteurianus* strains, and 1 of 17 *S. infantariuss* subsp. *coli* had single point mutations, whereas other strains showed identical 16s rDNA sequences within each subspecies [5]. Our isolates did not ferment inulin but fermented mannitol and raffinose. They also produced acid from starch, glycogen, methyl-β-D-glucopyranoside, and trehalose, but did not produce β-glucuronidase and β-galactosidase (Table 1). On the basis of the biochemical characteristics and the 16S rDNA sequencing data, we could identify the infecting organism as *S. gallolyticus* subsp. *gallolyticus* with high confidence at the subspecies level.

Cases of *S. bovis* infection associated with underlying malignancies have been rarely reported in Korea. There are 2 previous reported cases of *S. bovis* endocarditis and associated colon cancer in Korea [16, 17]; however, they did not provide information about the microbiological characteristics or *S. bovis* biotypes. In a more recent single-center study, the association of *S. bovis* biotypes with the

type of clinical infection and data on antimicrobial susceptibility was studied. In this study, none of the 13 patients with *S. bovis* bacteremia had gastrointestinal malignancies [18]. More studies on the prevalence, underlying diseases, and microbiological characteristics of *S. gallolyticus* subspecies in Korea are needed.

This case shows that accurate identification of *S. gallolyticus* subsp. *gallolyticus* endocarditis makes the detection of underlying cancer possible. Much attention should be given when identifying the causative organisms of streptococcal endocarditis, and investigation for underlying gastrointestinal malignancies should be performed when *S. gallolyticus* is identified. In addition, some clinicians may not be familiar with the new nomenclature system of *S. bovis* bacteria. This lack of awareness can lead to underdiagnosis of serious underlying conditions, including colon cancer; thus, it is important to provide clinicians with sufficient information about the nomenclature change when reporting study results. Moreover, although the use of the name *S. bovis* biotype I or II/2 instead of *S. gallolyticus* is common in clinical microbiology despite the clearly established new nomenclature, it is also important to update and use the proper taxonomic species in reporting, communication, or clinical studies to prevent further confusion in the future.

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