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# *Globicatella sanguinis* Bacteremia in a Korean Patient

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*Globicatella sanguinis* is an unusual pathogen causing bacteremia, meningitis, and urinary tract infection, and can be misidentified as *Streptococcus pneumoniae* or viridans streptococci due to its colonial morphology. A 76-year-old female patient with hypertension and degenerative arthritis was admitted to the hospital complaining of knee joint pain. Blood culture revealed the presence of Gram-positive cocci, and the isolated organism was equally identified as *S. pneumoniae* using the MicroScan identification system (Beckman Coulter, USA) and Vitek 2 identification system (bioMérieux, USA). However, the isolate showed optochin resistance based on the optochin disk susceptibility test. The organism was finally confirmed to be *G. sanguinis* based on 16S rRNA sequencing and hydrogen sulfide production testing. Accurate identification of *G. sanguinis* isolated from aseptic body fluids including blood is important for appropriate antibiotic selection based on accurate application of interpretative criteria of antimicrobial susceptibility test. **(Ann Clin Microbiol 2018;21:40-44)**

**Key Words:** Bacteremia, *Globicatella sanguinis,* 16S rRNA sequencing

## INTRODUCTION

*Globicatella sanguinis* was described in 1992 as a new genus and species when several isolates with phenotypically resemblance to *Streptococcus uberis* were characterized by Collins et al. [1]. *Globicatella* strains showed a negative leucine aminopeptidase (LAP) reaction and growth in the presence of 6.5% NaCl, totally opposite phenotypes of viridans streptococci [2,3]. Subsequently, a new species of the genus, *Globicatella sulfidifaciens*, was described, when several animal isolates from Belgium with resemblance to *G. sanguinis* were studied [4]. Although there was 99.2% similarity in 16S rRNA of *G. sulfidifaciens* to those of *G. sanguinis*, *G. sulfidifaciens* were classified as a new species based on differences in their whole cell protein patterns and biochemical profiles [4]. Until now, only 43 *G. sanguinis* isolates from clinical specimens and 14 case reports about bacteremia, meningitis or urinary tract infection have been reported [5]. To best of our knowledge, this is the second report of *G. sanguinis* bacteremia case identified by 16S rRNA gene sequencing and biochemical test in Korea [6].

## CASE REPORT

A 76-year-old woman visited to the orthopedic surgery department complaining of left knee joint pain. The patient had 2-year history of hypertension and 10-year history of rheumatoid arthritis, but did not take the medication regularly. The pain of left knee joint began to develop 10 years ago and the pain has become worse. The cause of the pain was due to the fluid retention in the joints, and the patient underwent continuous joint aspiration. Her knee pain did not improve with several procedures, so she was admitted to the hospital for the definite diagnosis. The patient was diagnosed with degenerative arthritis and admitted on October 17, 2017. On the day after admission, total left knee replacement was performed with elective surgery. On the 4$^{th}$ day after admission, deep wound aspirations were inoculated but there were no microorganisms. Until the 14$^{th}$ day of hospitalization, surgical site pain persisted and C-reactive protein and erythrocyte sedimentation rate were elevated to 7.70 mg/dL and 96 mm/h, respectively. Surgical site infection was diagnosed by reoperation. Flomoxef, an antibiotic used to pre-

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vent surgical site infection, was discontinued and vancomycin and levofloxacin were used instead. On the 15th day of hospitalization, two pairs of aerobic and anaerobic blood cultures were incubated in the BACTEC FX (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). Blood cultures showed positive signals in both aerobic bottles at 47 and 64 hours, respectively. Gram stain revealed Gram-positive cocci, and specimens were inoculated into 5% sheep blood agar plate (KOMED Life Science Co., Seongnam, Korea) and MacConkey agar plate, respectively. Viridans colored $\alpha$-hemolytic colony was observed in the sheep blood agar plate. Because the isolate was suspected as *Streptococcus pneumoniae*, optochin disk (5 $\mu$g-ethylhydrocupreine hydrochloride, Becton Dickinson Microbiology System, Cockeysville, MD, USA) susceptibility test was done, and microbial identification was performed by using Gram Positive ID Type 3 panels (PID3, Beckman Coulter, Brea, CA, USA). Antimicrobial susceptibility was determined using MicroScan MICroSTREP Plus Type 1 panel (Beckman Coulter) containing lysed horse-blood supplemented cation-adjusted Mueller-Hinton broth designed specifically for minimal inhibitory concentration (MIC) breakpoints for *Streptococcus* species viridans group [7]. The isolate was susceptible to all tested antimicrobials (penicillin MIC 0.03 $\mu$g/mL, ampicillin 0.06, cefepime 0.25, cefotaxime 0.25, ceftriaxone 0.25, aztreonam 0.25, meropenem 0.06, vancomycin 0.25, erythromycin 0.06, clindamycin 0.06, chloramphenicol 1, levofloxacin 0.25). Optochin disk susceptibility test of the isolate was interpreted as resistant because inhibition zone diameter including optochin disk was 6 mm, but the isolate was susceptible to optochin in PID3 (Beckman Coulter) and additionally tested VITEK 2 Gram-positive ID card (bioMérieux, Durham, NC, USA) (Table 1). The isolate was identified as *S. pneumoniae* by PID3 (Beckman Coulter, probability 94.79%) and VITEK 2 (bioMérieux, probability 93.0%). The optochin disk susceptibility test was performed one more time, but the result showed optochin resistance. The 16S rRNA sequence analysis was performed to confirm the species identification, after PCR amplification of a region of the 16S rRNA by using primers 27F (AGAGTTTGA

**Table 1.** Phenotypic characteristics of our isolate identified by commercial kits and *Globicatella* species previously reported

| Characteristics | Our isolate* | *G. sanguinis* | | | *G. sulfidaciens* |
|---|---|---|---|---|---|
| | | Previous study by | | | Previous study by Vandamme et al. [4] |
| | | Facklam [3]† | Shewmaker et al. [2]‡ | Collins et al. [1] | |
| Leucine aminopeptidase | ND | − | − | ND | ND |
| Pyrrolidonylarylamidase | ND | V | V | + | − |
| Growth in 6.5% NaCl | − | + | + | ND | + |
| Bile-esculin reaction | − | + | ND | ND | ND |
| Esculin hydrolysis | ND | + | + | ND | + |
| Voges-Proskauer reaction | − | − | ND | ND | − |
| $H_2S$ production | − | ND | ND | − | + |
| $\beta$-galactosidase | + | ND | ND | + | − |
| $\beta$-glucuronidase | − | ND | ND | − | + |
| Hippurate hydrolysis | ND | + | + | + | − |
| Optochin resistance | − | ND | ND | ND | ND |
| Acidification | | | | | |
| Arginine | − | − | ND | ND | ND |
| Mannitol | − | + | + | + | − |
| Sorbitol | − | V | V | ND | − |
| Arabinose | − | ND | V | ND | − |
| Lactose | − | ND | + | ND | − |
| Maltose | + | ND | + | ND | + |
| Sucrose | − | ND | + | ND | + |
| Inulin | − | ND | ND | − | + |
| Ribose | − | ND | ND | + | − |

*+, positive; −, negative; †+, positive reactions ≥92%; −, positive reactions ≤8%; v, variable reactions positive in 9 to 91% of strains; ‡+, positive reactions ≥85%; −, positive reactions ≤15%; v, variable reactions positive in 16 to 84% of strains.
Abbreviation: ND, not done.

TCMTGGCTCAG) and 801R (GGCGTGGACTTCCAGGGTA TCT) [8]. Sequencing was conducted using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 3730 genetic analyzer (Applied Biosystems). All sequences were analyzed by using the basic local alignment search tool (BLAST, a genome database of the National Center for Biotechnology Information) and ribosomal database project (RDP). The 16S rRNA gene sequences from our isolate showed 99% similarity to *G. sulfidifaciens* and *G. sanguinis*, and the 16S rRNA gene sequences showed the highest similarity (99.74%) with *G. sanguinis* based on the analysis that uses the EzBioCloud database (www.ezbiocloud.net) (Fig. 1). For differential identification to *Globicatella* species level, the isolate was inoculated on triple sugar iron (TSI) agar and incubated at 35°C ambient air for 2 days. Since *G. sulfidifaciens* produce hydrogen sulfide when grown in TSI agar, the medium turns black when this bacterium grows [4]. As the isolate did not produce hydrogen sulfide, we finally confirmed it as *G. sanguinis*. No microorganisms were isolated on wound cultures at the 16$^{th}$ day after admission. Also vancomycin and levofloxacin treatment was discontinued because no more isolates were found in two additional blood cultures on 21$^{st}$ day and 23$^{rd}$ day after admission.

## DISCUSSION

*G. sanguinis* could be misidentified with aerococci, streptococci, and enterococci due to their phenotypical resemblance [2]. The major differentiating characteristic between *Globicatella* and the aerococci is the cellular arrangement of the cells in the Gram stain. *Globicatella* forms chains while the aerococci form tetrads and clusters. The colonial morphology of *Globicatella* strains most closely resembles the viridans streptococci [2]. However, these strains are readily distinguished with a negative LAP and growth in the presence of 6.5% NaCl. The viridans streptococci are pyrridonylarylamidase (PYR) negative and LAP positive and do not grow in the presence of 6.5% NaCl. The enterococci are PYR and LAP positive and grow at 10°C. None of the *Globicatella* isolates grew at 10°C or gave positive LAP reactions [2]. As *Globicatella* is rarely encountered in clinical microbiology laboratories, most laboratory personnel are not familiar with their phenotypic characteristics and identification. Moreover, *Globicatella* shows various biochemical reactions depending on strain [9] and *Globicatella* may not be included on the database of the commercial identification system [5]. As a result, the bacterium may be overlooked when isolated, reported as unidentified *Streptococcus*-like organisms, or misidentified as another species. Miller et al. [10] reported two cases of *G. sanguinis* infection and they used matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Identification scores were 2.26 and 2.22 respectively, which were high confidence identification. If our identification procedure contained MALDI-TOF MS, the blood culture report was finished early.

Our isolate was initially identified as *S. pneumoniae* by two commercial identification systems in spite of *G. sanguinis* and *G. sulfidifaciens* were included in VITEK 2 (bioMérieux) database. Lau et al. [9] reported that two isolates of *Globicatella* were optochin susceptible. However, Jain et al. [11] reported optochin resistance *G. sanguinis*. These reports suggested that *G. sanguinis* showed variable characteristics about optochin resistance. But in our case, there was discrepancy between opto-

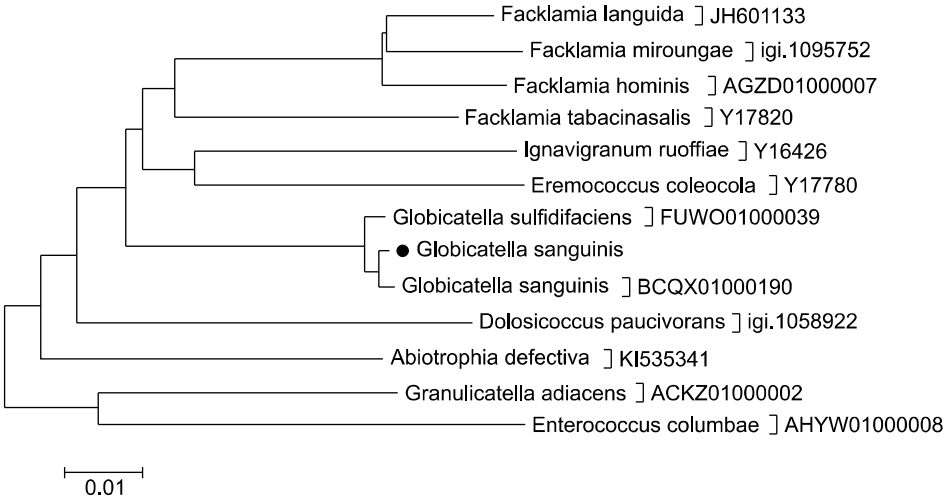


**Fig. 1.** Neighbor joining phylogenetic tree showing the relationship of our case to *Globicatella* species. The black dotted *G. sanguinis* is our isolate. Species name and accession code are given as cited in the EzBioCloud database.

chin disk susceptibility test and identification of commercial kit. There were no reports that the antibiotics susceptibility test of *Globicatella* were influenced by contents of blood agar or lysed horse blood media. More researches need to clear about uncertainty on antibiotics susceptibility of *Globicatella*.

Since there is no Clinical and Laboratory Standards Institute (CLSI) approval interpretation guideline for *Globicatella*, it is recommended that the laboratory should report MICs only [10]. However, in the case reports of several *Globicatella* infections, results about antibiotic susceptibility were judged by the criteria of viridans group *Streptococcus* spp. [2,10,12,13]. By the Shewmaker et al. [2]'s report in antibiotic susceptibility of *G. sanguinis*, resistance rates to cefuroxime, cefotaxime, and meropenem were high as 74%, 48%, and 37%, respectively, in contrast to 100% susceptibilities to penicillin and amoxicillin. While using $\beta$-lactam-based antibiotic and macrolide as empirical regimen which was related to lowering mortality of bacteremic pneumococcal pneumonia, *G. sanguinis* had high resistance to both antibiotics [2,14]. Fluoroquinolone, which was recommended to treat pneumococcal infection, could be an option because *G. sanguinis* was susceptible to levofloxacin [2,15]. As our *G. sanguinis* was fortunately susceptible to cerotaxime, ceftriaxone, meropenem, erythromycin, and levofloxacin, the patient was rapidly recovered from bloodstream infection. While it has been still hard to choose antibiotics, the correct identification of *Globicatella* was the first important step of the treatment.

While reporting of *G. sanguinis* infection was rare, the bacteria were related to severe infection such as meningitis, endocarditis, and osteomyelitis. It is difficult to explain that most cases of *G. sanguinis* infection, including this case, were revealed from aged female patients [5,9,10,12,13]. *G. sanguinis* would infect stockbreeding animals and farmer but actual pathway of infection was unknown [9,12]. In usual, *G. sanguinis* had high MIC level against 3$^{rd}$ generation cephalosporin, which was used popularly as empirical antibiotics, but low MIC level against penicillin [2,12]. Therefore, rapid and correct identification are necessary to treat *G. sanguinis* infection.

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=국문초록=

# 한국인에서 발생한 *Globicatella sanguinis* 균혈증 1예

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**안광진, 황규열, 윤갑준, 어 영**

*Globicatella sanguinis*는 드물게 균혈증, 뇌막염, 요로감염을 일으킬 수 있는 병원성 균종으로서 집락 형태가 유사한 *Streptococcus pneumoniae* 또는 비리단스 사슬알균으로 잘못 동정될 수 있다. 76세 여자 환자가 무릎관절 통증으로 입원하였으며 선행질환으로 고혈압과 퇴행관절염이 있었다. 혈액배양에서 그람양성알균이 관찰되었으며 MicroScan 동정시스템(Beckman Coulter, USA)과 Vitek 2 동정시스템(bioMérieux, USA)에서 동일하게 *Streptococcus pneumoniae*로 동정되었으며, optochin 디스크감수성검사에서는 내성이었다. 원인 균종은 16S rRNA 염기순서분석과 황화수소생성검사에 의해 최종적으로 *G. sanguinis*로 확인되었다. 혈액을 포함한 무균체액에서의 *G. sanguinis*의 정확한 동정은 정확한 항균제감수성검사의 판정 기준 적용과 이에 따른 적절한 항균제 선택에 중요하다. [Ann Clin Microbiol 2018;21:40-44]

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