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Discrepant Satellitism for Identification of *Granulicatella adiacens* Isolates

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Granulicatella adiacens is a catalase-negative, oxidase-negative, facultatively anaerobic, Gram-positive coccus [1]. This bacterium is considered nutritionally variant streptococci (NVS) because of its requirement for pyridoxal, L-cysteine, or additional nutrients, such as sulfhydryl compounds, for growth [2]. *G. adiacens* can grow well on culture medium containing these required nutrients. *G. adiacens* colonies can grow as satellite colonies around other bacteria, such as *Staphylococcus aureus* [3]. However, most clinical microbiologists have overlooked the fact that *G. adiacens* isolates can show discrepant satellite testing results based on the different kinds of nutrients contained in blood agar plates (BAPs) produced by different manufacturers. Here, to the best of our knowledge, we firstly report a discrepant satellite testing result of *G. adiacens* isolates on BAPs produced by different manufacturers.

A 62-yr-old man was admitted to our hospital after 2 days of fever. He had no history of cardiac disease. There was no vegetation on his cardiac valves on the basis of echocardiography results.

Blood samples were collected from peripheral veins at two other sites and inoculated into broth media in aerobic and anaerobic blood culture bottles. The BacT/Alert 3D system (BioMérieux Inc, Durham, NC, USA) was used to incubate and monitor bacterial growth. After blood culture tests were performed, the patient received empirical antibiotic treatment with 2 g intravenous cefotaxime every 8 hr.

After 3 days of incubation, two sets of aerobic blood culture bottles appeared positive on the basis of visual inspection. Gram staining of the positive smear preparations revealed Gram-positive pleomorphic cocci in pairs and short chains. The cultured blood specimens were inoculated onto BAP, MacConkey agar (MAC), and chocolate agar plates (ASAN Pharmaceutical, Hwaseong, Korea). After 72 hr, pin-point sized, grayish-white colored colonies grew on the BAP and chocolate agar. No colony growth was observed on MAC, and catalase and oxidase tests were negative.

We performed a satellite test to identify the organism because the Gram staining and biochemical testing results suggested NVS. A streak of *S. aureus* ATCC 25923 was made across the BAP surface for satellite testing. These microorganisms exhibited no satellite effect on BAPs. We used the same method to perform a satellite test using another BAP (MICROMEDIA Corp., Busan, Korea), and found that the microorganism exhibited a satellite effect (Fig. 1). The isolate was identified as *G. adiacens* with 98.7% probability using the VITEK 2 Gram-Positive Identification Card (BioMérieux, Marcy-l'Etoile, France).

We performed 16S ribosomal RNA (rRNA) gene sequencing analysis to confirm the identity of the isolate. PCR was used to amplify the first 500 bp of the 5′ end of the 16S rRNA gene using the MicroSeq 500 16S rDNA Bacterial Identification PCR kit

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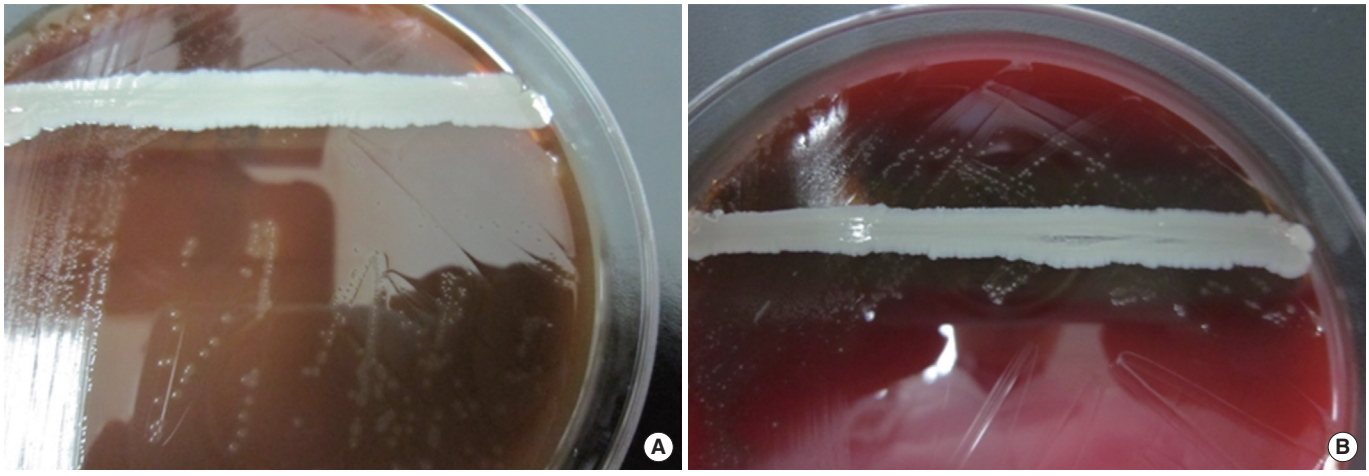

Fig. 1. Growth of *Granulicatella adiacens* colonies after 3 days of incubation. (A) No satellite effect on blood agar plate (BAP) manufactured by ASAN. (B) Satellite effect on BAP manufactured by MICROMEDIA.

Table 1. Three cases of *Granulicatella adiacens* infection

Case No.	Sex	Age (yr)	Symptom	Source	Diagnosis	Outcome	Satellitism	
							ASAN	MICROMEDIA
1	M	62	Fever	Blood	Sepsis	Recovered	No	Yes
2	M	21	Fever, Cough	Blood	Pneumonia	Recovered	No	Yes
3	M	49	Fever, Abdominal distension	Peritoneal fluid	Peritonitis	Recovered	No	Yes

Abbreviation: M, male.

(Applied Biosystems, Foster City, CA, USA). Cycle sequencing reactions were performed with a MicroSeq 500 16S rDNA Bacterial Identification Sequencing kit (Applied Biosystems). The purified sequencing products were analyzed on a 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

The resulting sequence was compared with sequences in GenBank (http://www.ncbi.nlm.nih.gov). A BLAST search revealed that the 16S rRNA gene sequence of the isolate was 100% homologous with *G. adiacens* strain CCUG 60768 (GenBank accession number, FR822389.1). This strain showed percent identity with >0.8% separation from other species that were identified in the search [4]. Therefore, the 16S rRNA gene sequencing analysis confirmed that the microorganism was *G. adiacens*.

Antimicrobial susceptibility testing was performed by using microdilution methods with 0.001% pyridoxal hydrochloride and cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood [5]. The isolate was susceptible to clindamycin, rifampin, and vancomycin, and it was resistant to penicillin, cefotaxime, ceftriaxone, and meropenem. Because the isolate was resistant to cefotaxime, the patient received antibiotic therapy with intravenous injection of 1 g vancomycin every 12 hr. After the third day of vancomycin therapy, the patient's fever subsided, and subsequent blood cultures were negative for *G. adiacens* and any other microorganisms.

We reviewed the medical records of all patients who had visited our hospital, and found two other patients with *G. adiacens* identified from clinical specimens. One patient was a 21-yr-old man who had a persistent cough and fever for 7 days. The condition of the patient was diagnosed with pneumonia. An isolate from blood culture tests was identified as *G. adiacens*. We inoculated this isolate onto two kinds of BAPs produced by ASAN and MICROMEDIA manufacturers, and satellite testing was performed as described above. This isolate showed no satellitism on the BAP manufactured by ASAN, but was positive for satellitism on the BAP from MICROMEDIA. The second patient was a 49-yr-old woman who had abdominal distension and fever. Her condition was diagnosed with peritonitis and advanced gastric cancer. An isolate from the peritoneal fluid was identified as *G. adiacens*. The isolate also showed no satellitism on the BAP manufactured by ASAN, but did show satellites on the BAP from MICROMEDIA (Table 1).

Granulicatella spp. should be suspected when Gram positive

pleomorphic cocci are revealed in pairs and short chains that fail to grow in subculture. Once a suspected *Granulicatella* isolate is cultured, its identity should be confirmed by establishing its requirement for pyridoxal. Organisms can be cultured on media supplemented with filter-sterilized 0.01% pyridoxal hydrochloride at a final concentration of 0.001%. Pyridoxal disks may also be used in the satellite test. When pyridoxal-supplemented blood agar is not available, *Granulicatella* spp. can also be identified by the demonstration of satellitism around a *S. aureus* streak on BAP [6]. Because *S. aureus* provides nutrients, such as pyridoxal, to *Granulicatella* spp. by hemolyzing erythrocytes on BAP, *Granulicatella* species can grow well around *S. aureus*.

Chocolate agar and brucella agar media contain pyridoxal hydrochloride; thus, *Granulicatella* can grow well independently on these media. *Granulicatella* may not grow on conventional BAPs without pyridoxal hydrochloride or L-cysteine. However, *Granulicatella* may grow on blood agar independently, if the isolation medium containing pyridoxal hydrochloride or L-cysteine offers enough enrichment for growth [7].

In our three cases, we performed satellite tests of the *G. adiacens* isolates. These isolates exhibited a satellite effect on BAPs manufactured by MICROMEDIA but not on BAPs obtained from ASAN. We found that the ASAN BAPs contained thioketone, which is one of the required nutrients for *G. adiacens* growth. This result indicates that this isolate may grow well independently and show no satellite effect on BAPs containing thioketone without L-cysteine or pyridoxal hydrochloride. Thus, we suggest that clinical microbiologists should be aware of all nutrients contained in media used to culture *Granulicatella* spp. We also recognize that streaking *S. aureus* for satellite testing may not be needed to inoculate BAPs containing nutrients required for *G. adiacens* growth.

G. adiacens isolates can be identified by biochemical testing with or without molecular confirmation. However, biochemical testing has been reported to lead to incorrect identification of this organism. *G. adiacens* can be misidentified as other species of *Granulicatella*, *Abiotrophia*, or *Gemella* [8]. It can be difficult for clinical microbiologists to separate these genera by only using standard biochemical tests. When biochemical tests show discrepant or conflicting results, molecular methods, such as 16S rRNA gene sequencing analysis, can be used to confirm the identity of the microorganism.

In conclusion, we showed that streaking *S. aureus* for satellite testing may not be necessary when BAPs contain the required nutrients for *G. adiacens* growth. Furthermore, we demonstrated that16S rRNA gene sequencing analysis can be used to confirm the identity of *G. adiacens* when biochemical tests do not provide clear results.

Author's Disclosures of Potential Conflicts of Interest

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

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