Detection of the *Streptococcus milleri* group in sputum samples and investigation of its clinical significance in respiratory diseases

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**Abstract**

**Objectives:** This study was conducted to investigate the detection rate of the *Streptococcus milleri* group (SMG) in sputum cultures and clarify its relevance to the clinical presentation of respiratory diseases.

**Methods:** Data from sputum specimens that were submitted for routine clinical bacterial testing since 1997 at our hospital and classified using the Miller and Jones sputum classification system were analyzed based on SMG bacteria detection. SMG streptococci identification, and antimicrobial susceptibility testing. The relationships between SMG detection and respiratory disease and between SMG detection and bacterial volume were also analyzed in 23 patients with respiratory diseases.

**Results:** SMG bacteria were most often detected in sputum samples that were macroscopically purulent (Miller and Jones classification P3) with a high number of leukocytes (Geckler classification G5). Some isolates also exhibited resistance to clindamycin and other antibiotics. SMG bacteria were detected in patients with pneumonia, pyothorax, and other respiratory diseases. Notably, an SMG-positive culture was observed in four patients with bronchial asthma.

**Conclusions:** To our knowledge, this is the first report on the detection of SMG bacteria in patients with bronchial asthma. Considering the complications of asthma and chronic obstructive pulmonary disease overlap syndrome, SMG bacteria could contribute to exacerbation of the symptoms of these diseases. Given these findings, SMG bacteria, a well-established component of normal flora of the human oral cavity, may also serve as pathogens, especially in respiratory diseases.

**Keywords:** *Streptococcus milleri* group, SMG, Viridans streptococci, Oral flora, Respiratory disease

**Introduction**

The *Streptococcus milleri* group (SMG) includes three species, *Streptococcus constellatus* (SC), *Streptococcus anginosus* (SA), and *Streptococcus intermedius* (SI). They are microaerophilic and typically found in the mucous membranes of the human oral cavity, pharynx, digestive tract, and vagina.¹,² These bacteria were first isolated from dental abscesses by Guthof³ in 1956 and named "*Streptococcus milleri*" in honor of the microbiologist W.D. Miller. SMG bacteria cause various infectious diseases in human and are a primary pathogen of abscess formation. In recent years, they have been considered a pathogen of pneumonia, pyothorax, and other infectious diseases of the respiratory system. SMG bacteria are found in sputum, pleural fluid, ascites, bile, vaginal discharge, blood, and other purulent material. However, because these three species cannot be distinguished based on hemolysis or biochemical properties,³ they are often difficult to identify accurately. A kit that utilizes multiple enzymes to achieve highly accurate identification of SMG species as reported by Whiley et al.⁴ has recently become commercially available, thus enabling the clinical identification of these three species. As there are some SMG species that cannot be grown aerobically, carbon dioxide (CO₂) gas culture or anaerobic culture is recommended. After 24–48 h of incubation on blood agar, it is possible to observe SMG colonies that are somewhat smaller (0.4–0.6 mm in size) than those of other streptococcal species and have a distinctive, caramellike smell.⁵

This study was conducted to determine the detection rate of SMG bacteria in sputum cultures at a university hospital in Japan and clarify the association between SMG bacteria and clinical presentations of respiratory illnesses.

**Methods**

**Detection of SMG**

We reviewed 3,278 cases at our hospital (dating back to 1997) for which sputum specimens were cultured for routine clinical bacterial testing and classified macroscopically as M1 to P3 according to the Miller and Jones sputum classification system.⁶ A portion of these specimens were Gram stained to create smear samples and classified as G1 to G6 according to the Geckler classification system.⁷ Specimens were cultured on blood/chocolate agar plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for 24–48 h at 35°C in 5% CO₂ as a routine test. In addition, anaerobic bacterial tests were carried out for some sputum specimens submitted on anaerobic porter when

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anaerobic bacterial testing was requested. Colonies of β-hemolytic streptococci, which grew to approximately 0.5 mm in size, were then placed in a purified culture using sheep blood agar plates (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) and cultured under the same conditions described above. Using the colonies from this purified culture, antimicrobial susceptibility testing (Table 1) and identification using the Streptogram kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and cultured under the same conditions described above. Using the colonies from this purified culture, antimicrobial susceptibility testing (Table 1) and identification using the Streptogram kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were performed. Sputum specimens in which SMG was detected were subjected to additional study to determine the following:

1. SMG detection rate in sputum specimens
2. SMG detection rate according to month
3. SMG detection according to patient sex
4. SMG detection by sputum type according to both the Miller and Jones and Geckler classification systems
5. Minimum inhibitory concentration (MIC) according to antimicrobial susceptibility testing.

Item 5 was performed at the first instance of SMG detection during the testing period.

**Investigation of the clinical significance of SMG in respiratory diseases**

SMG bacteria were detected in the sputum of 23 patients treated on either an outpatient or inpatient basis at the Department of Respiratory Medicine of our hospital. These patients were investigated to determine the following information:

1. SMG species detected from sputum specimens
2. Pathogenic bacteria detected in addition to SMG bacteria
3. SMG bacteria detection according to detailed respiratory disease
4. Comparison of the bacterial volumes of SMG bacteria and normal flora (NF).

As SMG bacteria also constitute NF in sputum, it is difficult to judge the presence or absence of pathogenicity. Thus, we set the balance between NF and SMG bacteria as one criterion. In 19 of 23 cases investigated above, there were fewer SMG bacteria than NF. In the remaining four cases, the bacterial volume of SMG was equal to that of NF or no NF was detected. The following items were investigated in these four cases:

1. Disease
2. Peripheral blood leukocyte count (μL)
3. C-reactive protein (CRP) in peripheral blood (mg/dL)
4. Name of the SMG species detected from sputum

5. Sputum type according to both the Miller and Jones and Geckler classification systems
6. Pathogenic bacteria detected in addition to SMG
7. MIC according to antimicrobial susceptibility testing.

This research was approved by the Ethics Committee of Fujita Health University (No. HM16-321). Patients were able to opt out of the study at any point after providing consent via the study website.

**Statistical analysis**

One-way analysis of variance was used to evaluate statistical differences. P values less than 0.05 were considered significant.

**Results**

**Detection of SMG**

1. The SMG detection rate from sputum was 2.59% (85 of 3,278 specimens). Among these 85 specimens, all from unique patients, SC was detected in 58 specimens, SA in 26 specimens, and SI in 1 specimen.

2. The SMG detection rate was lowest during April–July and highest during February–March. Annual trends were nearly the same, with little difference from year to year. The graph shows all cases since 1997 (Figure 1).

3. SMG bacteria were found in 45 men and 40 women.

4. According to the Miller and Jones classification system, SMG bacteria were primarily detected in P3 samples (27 specimens, Figure 2). According to the Geckler classification system using smear specimens, SMG bacteria were primarily detected in G5 samples (26 specimens, Figure 3).

5. Most SMG isolates were sensitive to antimicrobials. Two SC isolates and two SA isolates were resistant to minocycline.

**Investigation of the clinical significance of SMG bacteria in respiratory diseases**

Of the 23 cases investigated:

1. There were 17 cases of SC, 5 cases of SA, and 1 case of SI.
2. Other species found in SMG infections included *Candida* spp. (n=4), *Staphylococcus aureus* (n=2), *Klebsiella pneumoniae* (n=2), *Haemophilus influenzae* (n=2), *Pseudomonas aeruginosa* (n=2), *Streptococcus pneumoniae*.

### Table 1. Antibiotics used in antimicrobial susceptibility testing

<table>
<thead>
<tr>
<th>Antibiotic used in antimicrobial susceptibility testing</th>
<th>Classification</th>
<th>MIC interpretive criteria (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCG (Penicillin)</td>
<td>β-lactam (Penicillin)</td>
<td>≤0.12</td>
</tr>
<tr>
<td>ABPC (Ampicillin)</td>
<td>β-lactam (β-lactamase inhibitor combination)</td>
<td>≤2</td>
</tr>
<tr>
<td>CRZ (Cefazolin)</td>
<td>—</td>
<td>≤0.5</td>
</tr>
<tr>
<td>CCL (Cefaclor)</td>
<td>—</td>
<td>≤0.5</td>
</tr>
<tr>
<td>CTM (Cefotiam)</td>
<td>—</td>
<td>≤0.5</td>
</tr>
<tr>
<td>IPM (Imipenem)</td>
<td>β-lactam (β-lactamase inhibitor combination)</td>
<td>≤0.12</td>
</tr>
<tr>
<td>C/AMP (Clavulanic acid/amoxicillin)</td>
<td>β-lactam-β-lactamase inhibitor combination</td>
<td>≤2</td>
</tr>
<tr>
<td>MINO (Minocycline)</td>
<td>β-lactam (Penicillin)</td>
<td>≤0.25</td>
</tr>
<tr>
<td>EM (Erythromycin)</td>
<td>—</td>
<td>≤0.25</td>
</tr>
<tr>
<td>CLDM (Clindamycin)</td>
<td>—</td>
<td>≤0.25</td>
</tr>
<tr>
<td>LVFX (Levofloxacin)</td>
<td>—</td>
<td>≤2</td>
</tr>
<tr>
<td>ST (Sulfamethoxazole-trimethoprim)</td>
<td>Tetrahydrofolate synthesis pathway inhibitor combination</td>
<td>≤9.5</td>
</tr>
</tbody>
</table>
(n=1), *Streptococcus pyogenes* (n=1), an unidentified *Streptococcus* spp. (n=1), *Serratia marcescens* (n=1), an unidentified Gram-negative bacillus (n=1), and an unidentified *Mycoplasma* spp. (n=1) (includes multiple instances).

3. SMG detection according to respiratory disease is shown in Figure 4.

4. When comparing the volumes of SMG bacteria and NF detected in sputum, there were 3 cases in which the volumes were equal (SMG = NF), 19 cases in which there were fewer SMG than NF (SMG < NF), and 1 case in which no NF was detected. Details of the three cases (Cases 1–3) in which SMG = NF and the one case (Case 4) in which no NF was detected are shown in Table 2.

5. Regarding antibiotic susceptibility testing of SMG isolates, bacteria detected in Cases 1, 2, and 4 were susceptible to all antibiotics tested. EM and CLDM resistance was only observed in the SMG isolate from Case 3.

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**Discussion**

As SMG bacteria constitute NF of the oral cavity, they may be considered nonpathogenic bacteria in the sputum culture test. However, SMG bacteria may be detected in various pulmonary diseases. Therefore, in this study, we investigated the relationship between SMG detection and clinical findings in our hospital. When comparing sputum samples, we found many SMG-positive specimens were purulent when observed macroscopically and had large numbers of leucocytes in smear tests. A good-quality, highly purulent sample is considered essential for obtaining accurate test results when estimating phlogogenic bacteria. As antibiotic susceptibility testing determined that most SMG species are susceptible to β-lactam antibiotics, penicillin-based medicines are considered to be as effective as they are for conventional viridans streptococci. Although CLDM has been reported to be particularly effective against SMG bacteria, there have been some instances in which...

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**Figure 1** SMG detection rate (%) according to month

The months are shown along the horizontal axis, and the ratio of the number of sputum tests in which SMG was detected to the total number of sputum tests is shown as a percentage along the vertical axis. The SMG detection rate was lowest during April–July and highest during February–March. The graph shows all cases since 1997. SMG: *Streptococcus milleri* group

**Figure 2** Sputum type of SMG-positive specimens according to the Miller and Jones classification system

Each spoke on this radar chart represents a sputum type in the Miller and Jones classification system. The solid line indicates all SMG-positive specimens. SMG bacteria were most frequently detected in G5 sputum (26 samples). SMG: *Streptococcus milleri* group

**Figure 3** Sputum type of SMG-positive specimens according to the Geckler classification system

Each spoke on this radar chart represents a sputum type in the Geckler classification system. The solid line indicates all SMG-positive specimens. SMG bacteria were most frequently detected in G5 sputum (26 samples). SMG: *Streptococcus milleri* group

**Figure 4** Number of cases with SMG detection according to respiratory disease

SMG bacteria were most often detected in patients with pneumonia and bronchitis but were also seen in many common respiratory diseases. SMG: *Streptococcus milleri* group
of this study, we did not find any pathological mixed infection of \textit{S. pneumoniae} SMG bacteria must be considered a potential causative factor. To Japanese society and other factors have resulted in a trend respiratory tract and trachea.

allergens, contraction of the muscles of the respiratory system or the body attempts to clear the respiratory tract and bronchi of detected in four cases of bronchial asthma, a condition which had other common respiratory diseases. Notably, SMG bacteria were before and after collection, thus anaerobes are unlikely to survive. SC or SA exhibited resistance.

The following species were detected in the sputum of patients with respiratory diseases with mixed infections that included SMG: \textit{S. aureus}, \textit{K. pneumoniae}, \textit{H. influenzae}, \textit{P. aeruginosa}, \textit{S. pneumoniae}, \textit{S. marcescens}, and \textit{Mycoplasma} spp. All are potentially phlogogenic bacteria capable of causing bacterial pneumonia. While SMG bacteria are highly likely to coexist with other phlogogenic bacteria in the respiratory tract, bronchi, and lungs, it is difficult to identify pathogenic bacteria without a high-quality sputum specimen, as mentioned above.

Shinzato et al. reported that mixed infection of SMG bacteria and anaerobic bacteria may be a risk factor for increased disease severity during prolonged inflammation. However, in the course of this study, we did not find any pathological mixed infection of both SMG and anaerobic bacteria. Sputum is exposed to oxygen before and after collection, thus anaerobes are unlikely to survive.

The detection of SMG bacteria in cases of pneumonia, bronchitis, pyothorax, and other infectious respiratory diseases was concordant with previously published reports. The present study also found instances of SMG bacteria in patients with asthma, lung cancer, chronic obstructive pulmonary disease (COPD), tuberculosis, obstructive sleep apnea syndrome, and other common respiratory diseases. Notably, SMG bacteria were detected in four cases of bronchial asthma, a condition which had not been previously reported. Symptoms of asthma include excessive secretion of phlegm as the body attempts to clear the respiratory tract and bronchi of allergens, contraction of the muscles of the respiratory system or restricted air movement during episodes, and contraction or thickening of the walls of the respiratory tract and bronchi due to remodeling, all of which can induce retention of phlegm in the respiratory tract and trachea. When SMG bacteria are found in the sputum of an asthma patient, it causes concern for a potentially higher risk of inflammation or infection from other phlogogenic bacteria due to retention of pus comprising the remains of neutrophil granulocytes that phagocytose SMG in the respiratory tract and bronchi. Three of the four cases (Cases 1–3) in which SMG bacteria were found in patients with bronchial asthma during this study involved adults hospitalized because of asthma attacks. Although Japan has recently seen a decline in both hospitalizations and deaths due to asthma, the acute aging of Japanese society and other factors have resulted in a trend toward an increased number of adults with asthma. Our hospital treats a significant number of adults and children with asthma. Given the importance of inflammation due to neutrophil granulocytes in COPD, as well as COPD overlap syndrome SMG bacteria must be considered a potential causative factor. To our knowledge, there have been no reports of SMG bacteria detected in patients with asthma. If sputum is observed at the time of an asthma attack, other diseases associated with asthma must also be considered. The condition of the lungs of asthma patients during an attack and non-seizure changes drastically compared with patients with other respiratory infections. During an asthma attack, the airways and trachea become extremely narrow. Pus is more likely to accumulate in the respiratory tract and trachea, thus, aging of the immune system of asthmatic patients will increase their risk of developing multiple infectious diseases.

Although NF was not detected, both \textit{Candida} spp. and Gram-negative bacilli were found in addition to SI in the last of these four cases (Case 4). Case 4 involved a patient with pyothorax; antibiotic treatment had already been initiated prior to the sputum test, which is thought to be the reason that no NF was detected. In contrast, the fact that SI was found in pus, despite not being resistant to antibiotic treatment, can be considered an indication that the treatment had not had the expected effect.

Of the cases in this study in which SMG bacteria were detected, bacterial volumes of SMG and NF were equal or no NF was detected. When processing specimens derived from the respiratory system and from which SMG bacteria are detected, it is generally considered prudent to treat SMG bacteria as potentially pathogenic bacteria rather than as NF. However, because SMG bacteria are a part of the NF, it is difficult to make an exact determination of their phlogogenic potential from an ordinary sputum test. Tests used in North America and Europe to avoid contamination by NF, such as transtracheal aspiration tests or endotracheal aspiration using fiberoptic bronchoscopy, are considered desirable but also present concerns of their own as they are invasive treatments. Thus, sputum collection and testing will remain a subject of future research.

### Conflict of Interest

The authors have no conflicts of interest directly relevant to the content of this article.

### References

4. Whitey RA, Fraser H, Hardie JM, Beighton D. Phenotypic


