Empyema due to \textit{Gemella morbillorum} Is Diagnosed by 16S Ribosomal RNA Gene Sequencing and a Phylogenetic Tree Analysis: A Case Report and Literature Review

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\section*{Abstract}
We report a case of empyema due to \textit{Gemella morbillorum}. In this case, an analysis of the aspirate from the pleural effusion revealed empyema and evidence of a Gram-positive coccal bacteria. A biochemical identification system labelled the bacteria as ‘unclassified’, although we initially suspected the bacterium to belong to the \textit{Streptococcus} species. 16S ribosomal RNA (16S rRNA) gene sequencing and a phylogenetic tree analysis of the isolated strain confirmed the presence of \textit{Gemella morbillorum}. To ascertain the true incidence of \textit{Gemella} species in empyema, 16S rRNA gene sequencing should be used when the standard conventional biochemical methods fail to identify the organism or it identifies it with a low degree of reliability.

\textbf{Key words:} \textit{Gemella morbillorum}, empyema, 16S ribosomal RNA gene sequencing

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\section*{Introduction}
\textit{Gemella morbillorum}, formerly known as \textit{Streptococcus morbillorum}, is a catalase-negative, facultative anaerobic, and gram-positive coccus that is present in the normal flora of the oral cavity, upper respiratory tract, gastrointestinal tract, and genitourinary system. Isolates of \textit{G. morbillorum} have been found in cases of soft-tissue abscesses, meningitis, endocarditis, septic shock, and arthritis (1). Although viridans streptococci with similar microbiological properties have been found to be involved in the pathogenesis of pleural empyema and lung abscess, the presence of \textit{G. morbillorum} in this setting has seldom been reported (2). In this case of empyema that was caused by \textit{G. morbillorum}, a diagnosis was made by DNA amplification and sequence analyses of the 16S ribosomal RNA (16S rRNA) and phylogenetic tree analysis.

\section*{Case Report}
The patient was a 77-year-old man who was an ex-smoker and a social drinker. He had been diagnosed with essential thrombocythaemia for which he received hydroxy carbamide and aspirin for eight years. He presented symptoms of sudden fever, dyspnoea, left-sided pleuritic pain, and shivering. He was then admitted to our hospital and a chest computed tomography (CT) scan revealed a left pleural effusion without pulmonary lesions (Fig. 1). His vital signs upon admission were as follows: temperature, 38.6°C; blood pressure, 106/62 mmHg; pulse, 104 beats/min with regular rhythm; and oxygen saturation on room air, 88% on pulse oximetry sensor. Decreased breath sounds in the left lung were detected on auscultation, and heart sounds indicated a regular rhythm without a heart murmur. His oral condition was poor, and he had some decayed teeth. The patient’s C-reactive protein (CRP) level was elevated at 8.4 mg/dL. No blast cells were detected in the peripheral blood. The patient’s HbA1c (NGSP: National Glycohemoglobin
Blood cultures were also negative. We believed that a clinically significant bacteria, fungi, and acid-fast bacilli. One heterogeneous form (Fig. 2). Sputum cultures were negative for the isolated strain showed a Gram-positive diplococci with a heterogeneous form. A gram stain of this specimen revealed a predominance of neutrophils with Gram-positive cocci. This sample was cultured aerobically using Brucella HK agar. After 48 hours of incubation, α-haemolytic, catalase-negative colonies were obtained. A gram stain of this isolate as Gemella morbillorum (ATCC 27824; Sequence accession no. L14327). Based on this result, we identified the organisms G. haemolysans and G. morbillorum with degrees of reliability of 85.7% and 14.1%, respectively, indicating that the strain could not be identified precisely. Routine antimicrobial susceptibility testing with a disc diffusion on Mueller-Hinton agar that contained 5% sheep’s blood showed a good susceptibility of the strain to various drugs, including ABPC/SBT. Subsequently, we asked the Division of Anaerobe Research, Life Science Research Center of Gifu University to perform an additional analysis of this isolated strain using molecular biology techniques. We performed molecular identification by polymerase chain reaction (PCR) amplification and sequence analysis of the 16S rRNA gene using DNA extracted from the isolated strain. The universal primers 8UA (5'-AGAGTTTGATCMTGGCTCAG-3') and 1485B (5'-ACGGG CGGTGTGTRC-3') were used for amplification and sequencing. We performed a sequence analysis using the basic local alignment search tool (BLAST) search and CLUSTAL X (neighbor-joining method) phylogenetic tools. The phylogenetic tree was drawn by TreeView and shown in Fig. 3. The sequence of the 16S rRNA gene showed 98.8 % concordance (1,434 bp over the entire 1,451 bp fragment) with a type strain of G. morbillorum (ATCC 27824; Sequence accession no. L14327). Based on this result, we identified the isolate as G. morbillorum. After insertion of the chest tube, he was maintained on antibiotics (ABPC/SBT), and irrigation of the thoracic cavity with saline solution was started twice a day during hospitalization. The patient’s condition improved gradually after local and systemic therapy was started twice a day during hospitalization. The patient’s condition improved gradually after local and systemic Standardization Program) level was 5.6%. Anti-HIV-1 antibodies were negative. An arterial blood gas analysis on room air revealed hypoxemia with a partial pressure of oxygen in arterial blood (PaO₂) of 53.4 Torr. Contrast-enhanced whole-body CT showed no other lesions, and transthoracic echocardiography showed no evidence of infective endocarditis. An analysis of the pleural aspirate prior to antibiotic therapy showed a pH of 7.2, an elevated lactate dehydrogenase level of 3,063 IU/L, and decreased glucose of 39 mg/dL. A gram stain of this specimen revealed a predominance of neutrophils with Gram-positive cocci. This sample was cultured aerobically using chocolate and sheep’s blood agar/ bromothymol blue (BTB) lactose agar, and anaerobically cultured using Brucella HK agar. After 48 hours of incubation, α-haemolytic, catalase-negative colonies were obtained on the sheep’s blood agar medium. A gram stain of this isolated strain showed a Gram-positive diplococci with a heterogeneous form (Fig. 2). Sputum cultures were negative for clinically significant bacteria, fungi, and acid-fast bacilli. Blood cultures were also negative. We believed that a Streptococcus species was the most likely pathogen responsible for the empyema because the catalase test for the strain in our patient was negative, and Streptococcus species is the most common aetiologic agent responsible for empyema (3). We inserted a chest tube for natural drainage and treated the patient with an antibiotic therapy of ampicillin/sulbactam (ABPC/SBT) (9.0 g/day). Thereafter, an additional biochemical investigation of this isolated strain was performed with the API rapid ID 32 Strep system (bioMérieux sa, Marcy l’Etoile, France), which identified the organisms G. haemolysans and G. morbillorum with degrees of reliability of 85.7% and 14.1%, respectively, indicating that the strain could not be identified precisely. Routine antimicrobial susceptibility testing with a disc diffusion on Mueller-Hinton agar that contained 5% sheep’s blood showed a good susceptibility of the strain to various drugs, including ABPC/SBT. 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therapy, and his CRP level reverted to normal values. The chest tube was removed on the 24th hospital day, and antibiotic therapy with ABPC/SBT was continued for 30 days. He was discharged from our hospital on the 34th hospital day.

**Discussion**

Microorganisms of the *Gemella* species are opportunistic pathogens that are Gram-positive, with a coccic morphology, and are facultative anaerobic. These pathogens have the ability to cause serious local and systemic infections, mainly in immunocompromised patients. The species *G. haemolysans* and *G. morbillorum* are the most important pathogens. *G. haemolysans* can cause endocarditis and meningitis, whereas *G. morbillorum* causes endocarditis, especially of native valves, septic arthritis, and meningitis (4). Respiratory tract infections caused by *Gemella* species have rarely been reported, but they can cause lung abscesses, necrotizing pneumonia, and pleural empyema. However, there are a few publications referring to pleural involvement, and these describe isolated cases by conventional biochemical identification (5).

In the literature, there are 14 cases of empyema due to *G. morbillorum*. Ten patients (71.4%) were men and 4 (28.6%) were women, with a median age of 66 (range: 26-80) years, which indicates that middle-aged men are predominantly affected. All patients had predisposing factors such as poor oral hygiene, smoking, chronic cardiovascular or respiratory disease, drug abuse, alcohol abuse or malignancies, indicating that these patients were frequently associated with conditions that favoured aspiration (4-10). Our patient’s oral condition was poor and he had some decayed teeth. These conditions might result in silent aspiration. Moreover, he had a haematological disorder, which might be an additional risk factor. Prognosis of *G. morbillorum*-associated pleuropulmonary infections is generally good with early and adequate therapy, and species isolated from clinical specimens in the past were highly susceptible to penicillin (6, 9).

The identification of infrequently encountered bacterial species in clinical microbiology laboratories has always been a problem. Since the number of reference strains used to build up databases in commercial kits is usually small for rare bacteria, it is not uncommon to encounter clinical isolates of these rare bacterial species with ambiguous biochemical profiles. Furthermore, even if they are ‘successfully’ identified by the commercial kits, the low prevalence rate would imply a low positive predictive value. Using 16S rRNA gene sequencing, Woo et al. reported that *Gemella* species accounted for 0.7% of bacteraemias caused by *α*-haemolytic streptococci, with the exception of *Streptococcus pneumonia*. Because commercially available phenotypic systems frequently misidentified the organism, they recommended identification of *Gemella* and *Gemella*-like isolates by 16S rRNA gene analysis (11). In fact, biochemical identification with the API Rapid ID 32 Strep system labelled the strain in our patient as ‘unclassified’, although we initially suspected a *Streptococcus* species as the most likely pathogen of empyema because of the negative catalase test for this strain. This abnormality has also been reported for other organisms and may be responsible for the frequent misidentification of *Gemella*, and possibly, the fact that so few cases of *Gemella* infection are reported. On the other hand, Kawanami et al. reported that some cases showed discrepancies between the results from the cultivation method and the clone library method in the analysis of bacterial pleurisy. These inconsistencies suggest that the cultivation method alone may not fully express precise bacterial information of the infected foci. They concluded that the clone library analysis using the 16S rRNA gene of pleural fluid showed a higher incidence of anaerobic bacteria in infectious pleurisy than previously expected, and also provided additional bacterial information of cultivation method (12).

Therefore, clinicians should consider the possibility of a mixed infection in the pleuropulmonary infections. Although the pathogen that was detected in our case was *G. morbillorum*, the possibility of a mixed infection that would include anaerobic bacteria cannot be discounted. We believe that a biochemical and molecular approach is important in order to detect the precise pathogens in the pleuropulmonary infections.

We report the first case, to the best of our knowledge, of empyema due to *G. morbillorum* that was diagnosed by 16S rRNA gene sequencing and a phylogenetic tree analysis. Further studies using 16S rRNA gene sequencing need to be conducted to ascertain the true incidence of empyema that is caused by *Gemella* species because this species might be frequently misidentified as a species of *Streptococcus*.

The authors state that they have no Conflict of Interest (COI).

**References**


