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Gemella bacteraemia characterised by 16S ribosomal RNA gene sequencing

P C Y Woo, S K P Lau, A M Y Fung, S K Chiu, R W H Yung and K Y Yuen

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Since the discovery of the polymerase chain reaction (PCR) and DNA sequencing, comparisons of the gene sequences of bacterial species have shown that the 16S rRNA gene is highly conserved within a species and among species of the same genus, and hence can be used as the new standard for speciation of bacteria. Using this new standard, phylogenetic trees, based on base differences between species, are constructed, and bacteria are classified and reclassified into new genera. Recently, we have reported the discovery of a novel clinical syndrome and three novel species, Abiotrophia adiacens, Granulicatella adiacens and Abiotrophia defectiva. In addition, we have also reported the discovery of a novel clinical syndrome and three novel species. The characterisation of β haemolytic Lancefield group G streptococci other than α haemolytic streptococci other than S pneumoniae isolated from blood cultures during a six year period were identified by conventional biochemical methods, the Vitek system, and the API system. 16S rRNA gene sequencing was performed on all isolates identified by both kits as gemella with ≥ 95% confidence or by either kit as any bacterial species with < 95% confidence. The ATB expression system was used to identify the two isolates that were defined as gemella species by 16S rRNA gene sequencing. Of the 302 α haemolytic streptococci other than S pneumoniae isolated, one was identified as Gemella morbillorum, and another as Gemella haemolysans by 16S rRNA gene sequencing. The patient with monomicrobial G morbillorum bacteraemia was a 66 year old man with community acquired infective endocarditis with septic thromboemboli. The patient with G haemolysans bacteraemia was a 41 year old woman with hospital acquired polymicrobial bacteraemia during the neutropenic period of an autologous bone marrow transplant for non-Hodgkin’s lymphoma, the first case of its kind in the English literature. The API and ATB expression systems only identified the second strain as G haemolysans at 94% and 99% confidence, respectively, whereas the Vitek system identified none of the two strains correctly at > 70% confidence. Gemella bacteraemia is uncommon. 16S rRNA gene sequencing is the method of choice for identification of gemella and gemella-like isolates.

Aims: To define epidemiology, clinical disease, and outcome of gemella bacteraemia by 16S RNA gene sequencing. To examine the usefulness of the Vitek, API, and ATB systems in identifying two gemella species.

Methods: All α haemolytic streptococci other than Streptococcus pneumoniae isolated from blood cultures during a six year period were identified by conventional biochemical methods, the Vitek system, and the API system. 16S rRNA gene sequencing was performed on all isolates identified by both kits as gemella with ≥ 95% confidence or by either kit as any bacterial species with < 95% confidence. The ATB expression system was used to identify the two isolates that were defined as gemella species by 16S rRNA gene sequencing.

Results: Of the 302 α haemolytic streptococci other than S pneumoniae isolated, one was identified as Gemella morbillorum, and another as Gemella haemolysans by 16S rRNA gene sequencing. The patient with monomicrobial G morbillorum bacteraemia was a 66 year old man with community acquired infective endocarditis with septic thromboemboli. The patient with G haemolysans bacteraemia was a 41 year old woman with hospital acquired polymicrobial bacteraemia during the neutropenic period of an autologous bone marrow transplant for non-Hodgkin’s lymphoma, the first case of its kind in the English literature. The API and ATB expression systems only identified the second strain as G haemolysans at 94% and 99% confidence, respectively, whereas the Vitek system identified none of the two strains correctly at > 70% confidence.

Conclusions: Gemella bacteraemia is uncommon. 16S rRNA gene sequencing is the method of choice for identification of gemella and gemella-like isolates.

Abbreviations: MIC, minimum inhibitory concentration; PCR, polymerase chain reaction.
< 95% confidence. To examine the usefulness of the ATB expression system (ID32 STREP; bioMerieux Vitek) for the identification of gemella species, this system was also used for identification of the two isolates that were finally defined as gemella species by 16S rRNA gene sequencing. Antimicrobial susceptibility was undertaken by the E test for penicillin and the Kirby Bauer disk diffusion method for the other antibiotics using Muller Hinton agar supplemented with 5% horse blood, and the results were interpreted according to the NCCLS criteria for α haemolytic streptococci. Multiple positive blood cultures with the same isolate obtained from the same patient were counted only once.

**Table 1** Characteristics of patients with gemella bacteraemia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
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<tbody>
<tr>
<td>Year of isolation</td>
<td>1997</td>
</tr>
<tr>
<td>Age/sex</td>
<td>66/M</td>
</tr>
<tr>
<td>Underlying disease</td>
<td>Abdominal aortic aneurysm</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Infective endocarditis with septic thromboemboli</td>
</tr>
<tr>
<td>Community/hospital acquired</td>
<td>Community</td>
</tr>
<tr>
<td>Number of positive blood cultures</td>
<td>3</td>
</tr>
<tr>
<td>Monomicrobial/polymicrobial bacteriaemia</td>
<td>Monomicrobial</td>
</tr>
<tr>
<td>Positive cultures from other specimens</td>
<td>None</td>
</tr>
<tr>
<td>Identification by 16S rRNA sequencing</td>
<td>Gemella morbillorum</td>
</tr>
<tr>
<td>Antibiotic susceptibility</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Treatment</td>
<td>Remission</td>
</tr>
<tr>
<td>Outcome</td>
<td>Remission</td>
</tr>
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</table>

Among a total of 302 α haemolytic streptococci other than *S pneumoniae* isolated from blood cultures of patients admitted to Queen Mary Hospital during the six year period (July 1995 to June 2001), none was identified by both the Vitek system (API) and the API system (20 STREP) as any gemella species with ≥ 95% confidence. A total of 74 were identified by either kit as any species with < 95% confidence. PCR of the 16S rRNA genes of these isolates showed bands at approximately 1410 bp. Sequencing of the 16S rRNA genes revealed that one isolate (table 1; patient 1) had > 99% nucleotide identity with the 16S rRNA gene of *G morbillorum* (GenBank accession number L14327), and another (table 1; patient 2) had > 99% nucleotide identity with the 16S rRNA genes of *G haemolytans* (GenBank accession number L14326) (fig 1). Therefore, gemella accounted for 0.7% of bacteraemia caused by α haemolytic streptococci other than *S pneumoniae*.

**RESULTS**

**16S rRNA gene sequencing**

Among a total of 302 α haemolytic streptococci other than *S pneumoniae* isolated from blood cultures of patients admitted to Queen Mary Hospital during the six year period (July 1995 to June 2001), none was identified by both the Vitek system (API) and the API system (20 STREP) as any gemella species with ≥ 95% confidence. A total of 74 were identified by either kit as any species with < 95% confidence. PCR of the 16S rRNA genes of these isolates showed bands at approximately 1410 bp. Sequencing of the 16S rRNA genes revealed that one isolate (table 1; patient 1) had > 99% nucleotide identity with the 16S rRNA gene of *G morbillorum* (GenBank accession number L14327), and another (table 1; patient 2) had > 99% nucleotide identity with the 16S rRNA genes of *G haemolytans* (GenBank accession number L14326) (fig 1). Therefore, gemella accounted for 0.7% of bacteraemia caused by α haemolytic streptococci other than *S pneumoniae*.
Phenotypic characterisation and identification of gemella by commercial systems

Gram smears of the two isolates showed Gram positive cocci in pairs. The Vitek System (GPI) identified the *G. morbillorum* and *G. haemolysans* strains as 63% *G. morbillorum*/S. agalactiae/S. acidominimus, 22% *S. pneumoniae*, and 68% *G. morbillorum*/S. agalactiae/S. acidominimus, 24% *S. pneumoniae*, respectively. The API system (20 STREP) identified the *G. morbillorum* and *G. haemolysans* strains as 60% *Leuconostoc* sp., 28% *Streptococcus mitis*, and 94% *G. haemolysans*, 6% *G. morbillorum*, respectively. The ATB expression system (ID32 STREP) identified the *G. morbillorum* and *G. haemolysans* strains as “unidentified” and 99% *G. haemolysans*, respectively.

Patient characteristics

Table 1 summarises the characteristics of the two patients with gemella bacteraemia. Briefly, the first patient, a 66 year old man, was admitted because of occasional abdominal and low back pain for one month. He was afebrile. Examination revealed a collapsing pulse, an early diastolic murmur of grade 3/6 at the left lower sternal border, and a pulsatile mass in the abdomen. Computer tomography of the abdomen confirmed an infrarenal abdominal aortic aneurysm of 7.5 cm in diameter, without signs of rupture. Transthoracic echocardiogram showed severe aortic regurgitation and vegetations on both the aortic and mitral valves. Three sets of blood cultures all recovered the same strain of *G. morbillorum*. He was treated for community acquired infective endocarditis with intravenous penicillin G and netilmicin. He developed sudden retrograde amnesia 17 days after admission. Magnetic resonance imaging of the brain showed multiple subacute infarcts at both occipito–temporal regions, compatible with septic thromboembolism. Intravenous antibiotics were continued for a total of six weeks with residual memory loss. He subsequently underwent an elective endoanurectomy with insertion of an aortic graft and was discharged two months from admission.

The second patient, a 41 year old woman with non-Hodgkin’s lymphoma, was admitted because of autologous bone marrow transplantation. On day two postmarrow infusion, she developed neutropenic fever. Examination did not reveal a heart murmur or other focus of infection. Blood cultures were performed and recovered *G. haemolysans*, *S. mitis*, and *Escherichia coli*. She responded to empirical intravenous imipenem and subsequent blood cultures were negative. Her bone marrow engrafted on day 15.

DISCUSSION

The identification of infrequently encountered bacterial species in clinical microbiology laboratories has always been a problem. Because the number of reference strains used for building 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. In our study, we described our experience in using 16S rRNA gene sequencing to characterise two strains of a rarely encountered bacterial genus, gemella, recovered from blood cultures of our patients in the past six years.

The genus *gemella* consists of six species. Before 1998, there were only two known gemella species, *G. haemolysans* and *G. morbillorum*. *G. haemolysans* was originally classified as *Neisseria haemolysans*; and was subsequently reclassified as *G. haemolysans* in 1960; whereas *G. morbillorum* was originally classified as *Diplococcus morbillorum*, subsequently as *Peptostreptococcus morbillorum* and *Streptococcus morbillorum*, and was finally reclassified as *G. morbillorum* in 1988. In the past five years, four additional *gemella* species have been discovered. *Gemella bergeriae* and *G. sanguinis* were recovered from human clinical specimens, whereas *G. palcatans* was recovered from the oral cavity of a dog, and *G. canicatula* from an abscess of a rabbit.

The identification of “gemella-like” species by the commercial kits was unsatisfactory, especially when the Vitek system (GPI) was used. In the present series, the Vitek system (GPI) was not able to identify a single isolate successfully; whereas the API system (20 STREP) and the ATB expression system (ID32 STREP) were only able to identify one of the two gemella species correctly. This poor accuracy in identifying gemella species by commercial kits was also demonstrated in a recent study, in which none of the three gemella species was successfully identified by the rapid phenotypic identification systems. Interestingly, among the nine isolates of *Granulicatella adiacens* and *A. definitiva* that we reported recently, the Vitek system (GPI) incorrectly identified seven of them as gemella, and the API system (20 STREP) and the ATB expression system (ID32 STREP) incorrectly identified one of them as gemella. Therefore, we think that 16S rRNA gene sequencing should be used as the method of choice for identifying gemella and “gemella-like” species.

Species of gemella are important causes of infective endocarditis. Among the reported cases of gemella infections, infective endocarditis is the most common diagnosis. Over 25 cases of gemella endocarditis have been reported in the literature, with the incidence of *G. morbillorum* and *G. haemolysans* endocarditis being approximately the same. In our present report, one of the two patients had gemella endocarditis. However, further studies using 16S rRNA gene sequencing need to be carried out to ascertain the true incidence of gemella endocarditis, because gemella species are frequently misidentified as species of streptococcus, leuconostoc, and abiotrophia, and granunicatella and abiotrophia species are frequently misidentified as species of gemella.

“...we think that 16S rRNA gene sequencing should be used as the method of choice for identifying gemella and gemella-like species”

We report the first case of gemella bacteraemia during the pre-engraftment period in a bone marrow transplant recipient. *V. alginans* streptococci are commonly isolated from the blood cultures of bone marrow transplant recipients during the pre-engraftment period. As members of the oral flora, it is not unexpected that gemella species would also cross the mucosal barrier and cause bacteraemia in those patients with mucositis. The concomitant isolation of *S. mitis* and *E. coli* was in line with the hypothesis that the oral flora was the source of the bacteraemia in case 2 (in immunocompromised patients, part of the normal oral flora is replaced by enterobacteriaceae and non-fermentative Gram negative bacilli).

The emergence of penicillin and macrolide resistance in gemella species is of great concern. In the past, all gemella
species isolated from clinical specimens were highly sensitive to penicillin G and ampicillin. In 1993, a strain of glycopeptide-resistant \textit{G haemolytans} that showed reduced susceptibility to penicillin (minimum inhibitory concentration (MIC) of 0.5 \textmu g/ml) was recovered from the blood culture of a 20 month old boy. In 1996, another strain of \textit{G morbillorum} that was resistant to penicillin (MIC of \geq 4 \textmu g/ml) was recovered from the blood culture of an 11 year old girl with nasopharyngeal Burkitt's lymphoma. In our present series, one of the two gemella strains showed reduced susceptibility to penicillin (MIC of 0.5 \textmu g/ml). Furthermore, it was also resistant to erythromycin, clarithromycin, and azithromycin. Because amoxicillin or macrolides are used as prophylaxis for infective endocarditis, the rising incidence of penicillin and macrolide resistance may mean the failure of these agents in the prophylaxis of infective endocarditis in a considerable proportion of these patients undergoing dental or other invasive procedures.

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BIBLIOGRAPHY


Take home messages

- \textit{Gemella} bacteremia is rare and 16S RNA gene sequencing should be the method of choice for identification of gemella and gemella-like isolates.
- The identification of ‘gemella-like’ species by the commercial kits was unsatisfactory, especially when the \textit{Vitek} system (GPI) was used.
- Species of gemella are important causes of infective endocarditis and one of the two patients reported here had gemella endocarditis.
- The other patient was a bone marrow transplant recipient who had \textit{gemella} bacteremia during the pre-engraftment period and the oral flora was the source of the bacteremia in this case.

REFERENCES


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