ORIGINAL ARTICLE

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, K Y Yuen

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 RNA 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.

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.1 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 use of this technique for the identification of bacterial strains with ambiguous biochemical profiles,2–10 species that are rarely encountered clinically,11–15 and a bacterium that is non-cultivable.16 In addition, we have also reported the discovery of a novel clinical syndrome17–18 and three novel species,19–21 and the characterisation of β-haemolytic Lancefield group G streptococcal bacteria and Granulicatella adiacens and Abiotrophia defectiva bacteraemia.22–23

“We have recently shown that a large number of bacterial strains identified as gemella species by commercially available phenotypic systems are actually Granulicatella adiacens”

Members of the genus gemella consist of catalase negative, facultative anaerobic, Gram positive cocci that phenotypically resemble viridans streptococci. Traditionally, the identification of, and hence the study in the epidemiology of and spectrum of clinical diseases caused by gemella species depend on the phenotypic characterisation of suspected isolates. However, we have recently shown that a large number of bacterial strains identified as gemella species by commercially available phenotypic systems are actually Granulicatella adiacens.23 No data are currently available that have used 16S rRNA gene sequencing as the gold standard. Therefore, in our study, we used 16S rRNA gene sequencing, assisted by traditional phenotypic tests, to describe the epidemiology, clinical diseases, and outcome of gemella bacteraemia. We also examined the usefulness of the Vitek system (bioMerieux Vitek, Hazelwood, Missouri, USA), the API system (bioMerieux Vitek), and the ATB expression system (bioMerieux Vitek), which are commonly used for microbial identification of α-haemolytic streptococci in clinical microbiology laboratories, for the identification of the two gemella isolates.

METHODS AND MATERIALS

Patients and microbiological methods

The patients with gemella bacteraemia in our study were hospitalised at the Queen Mary Hospital in Hong Kong during a six year period (July 1995 to June 2001). All clinical data were collected as described in our previous publication.24 The BACTEC 9240 blood culture system (Becton Dickinson, Sparks, Maryland, USA) was used. To identify potential cases of gemella bacteraemia, in addition to identifying all blood culture isolates by standard conventional biochemical methods,25 the Vitek system (GPI; bioMerieux Vitek) and the API system (20 STREP; bioMerieux Vitek) were used for species identification of all α-haemolytic streptococci other than Streptococcus pneumoniae isolated from blood cultures during the six year period. 16S rRNA gene sequencing was performed on all isolates that were identified by both kits as gemella with > 95% confidence, or by either kit as any bacterial species with

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 \( \alpha \) haemolytic streptococci. Multiple positive blood cultures with the same isolate obtained from the same patient were counted only once.

### Extraction of bacterial DNA for 16S rRNA gene sequencing

Bacterial DNA extraction was modified from our previously published protocol. Briefly, 80 µl of NaOH (0.05M) was added to 20 µl of bacterial cells suspended in distilled water and the mixture was incubated at 60°C for 45 minutes, followed by the addition of 6 µl of Tris/HCl (pH 7.0), to achieve a final pH of 8.0. The resultant mixture was diluted \times 100 and 5 µl of the diluted extract was used for PCR.

### PCR, gel electrophoresis, and 16S rRNA gene sequencing

PCR amplification and DNA sequencing of the 16S rRNA genes were performed according to our previous publications. Briefly, DNase I treated distilled water and the PCR master mix (which contains dNTPs, PCR buffer, and Taq polymerase) were used in all PCR reactions by adding 1 U of DNase I (Pharmacia, Uppsala, Sweden) to 40 µl of distilled water or PCR master mix, incubating the mixture at 25°C for 15 minutes, and subsequently at 95°C for 10 minutes to inactive the DNase I. The bacterial DNA extracts and control water (or PCR master mix) were added to 20 µl of bacterial cells suspended in distilled water or PCR master mix, incubating the mixture at 25°C for 15 minutes, and subsequently at 95°C for 10 minutes to inactivate the DNase I. The resultant mixture was diluted \times 100 and 5 µl of the diluted extract was used for PCR.

### RESULTS

16S rRNA gene sequencing

Among a total of 302 \( \alpha \) 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 (GPI) 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 genes of \( G. morbillorum \) (GenBank accession number L14327), and another (table 1; patient 2) had > 99% nucleotide identity with the 16S rRNA genes of \( G. haemolysans \) (GenBank accession number L14326) (fig 1). Therefore, gemella accounted for 0.7% of bacteraemia caused by \( \alpha \) haemolytic streptococci other than \( S. pneumoniae \).

### Table 1 Characteristics of patients with gemella bacteraemia

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
</tr>
</thead>
<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>Penicillin</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>Penicillin+gentamicin</td>
</tr>
<tr>
<td>Outcome</td>
<td>Remission</td>
</tr>
</tbody>
</table>

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discharged two months from admission. Euryasectomy with insertion of an aortic graft and wasotics were continued for a total of six weeks with residualmultiple subacute infarcts at both occipito–temporal regions,admission. Magnetic resonance imaging of the brain showedHe developed sudden retrograde amnesia 17 days aftertive endocarditis with intravenous penicillin G and netilmicin.

Computer tomography of the abdomen confirmed an infrarenalabdominal aortic aneurysm of 7.5 cm in diameter, without signsleft lower sternal border, and a pulsatile mass in the abdomen.

was admitted because of occasional abdominal and low backpain for one month. He was afebrile. Examination revealed awas admitted because of autologousbone marrow transplantation. On day two postmarrow

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Phenotypic characterisation and identification ofgemella by commercial systems

Gram smears of the two isolates showed Gram positive cocci inpairs. The Vitek System (GPI) identified the G morbillorumand G haemolysans strains as 63% G morbillorum/S agalactiae/S acaliforniae, 22% S pneumoniae, and 68% G morbillorum/ S agalactiae/S acaliforniae, 24% S pneumoniae, respectively. TheAPI 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 Vitek System (ID32 STREP) identified the G morbillorum and G haemolysans strains as “unidentified” and99% G haemolysans, respectively.

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Figure 1  Phylogenetic tree showing the relation of the two gemellaisolates to other species. The tree was inferred from 16S rna

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Gemella bacteraemia by 16S RNA sequencing

**Take home messages**

- Gemella bacteraemia 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 species by the commercial kits was unsatisfactory, especially when the Vitrek 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 gemella bacteraemia during the pre-engraftment period and the oral flora was the source of the bacteraemia in this case.

Species isolated from clinical specimens were highly sensitive to penicillin G and ampicillin. In 1993, a strain of glycopeptide resistant G haemolytics that showed reduced susceptibility to penicillin (minimum inhibitory concentration (MIC) of 0.5 μg/ml) was recovered from the blood culture of a 20 month old boy. In 1996, another strain of G morbillorum that was resistant to penicillin (MIC of > 4 μg/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 μg/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 to penicillin G and ampicillin.

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