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<td>Author(s)</td>
<td>Lau, SKP; Ng, KHL; Woo, PCY; Yip, KT; Fung, AMY; Woo, GKS; Chan, KM; Que, TI; Yuen, KY</td>
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S K P Lau, K H L Ng, P C Y Woo, K-t Yip, A M Y Fung, G K S Woo, K-m Chan, T-I Que and K-y Yuen

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SHORT REPORT

Identification of anaerobic Gram positive bacilli in clinical microbiology laboratories by phenotypic methods is often difficult. Comparison of the gene sequences of bacterial species has shown that the 16S ribosomal RNA (rRNA) gene is highly conserved within a species and among species of the same genus. Hence, it can be used as the new standard for classification and identification of bacteria. Recently, we reported the application of this technique for identifying this group of bacteria. The Microseq 500 16S rDNA bacterial identification system (Perkin-Elmer Applied Biosystems Division, Foster City, California, USA) has been designed for rapid and accurate identification of bacterial pathogens, using the first 527 bp fragment of the 16S rRNA gene. It has been shown that the system is useful for the identification of unusual aerobic pathogenic Gram negative bacilli, coryneform bacteria, mycobacterium, and nocardia species, and various bacterial strains with ambiguous biochemical profiles. In our study, we evaluate the usefulness of this system in the identification of 20 non-duplicating anaerobic Gram positive bacilli isolated from blood cultures.

 **MATERIALS AND METHODS**

**Bacterial strains**

The bacterial strains were isolates from blood cultures of patients hospitalised at the Queen Mary Hospital in Hong Kong during a four year period (January 1998 to December 2001). Isolates were identified as *Clostridium perfringens* and *Propionibacterium acnes* by phenotypic methods. One isolate each of *C. perfringens* and *P. acnes* and all isolates other than *C. perfringens* and *P. acnes* were subjected to 16S rRNA gene sequencing. One isolate for each species was selected for DNA sequencing of the first 527 bp fragment of the 16S rRNA gene and analysis by the MicroSeq 16S rDNA bacterial identification system, in addition to identification by three commercially available identification systems for anaerobes: the Vitek System (ANI: bioMerieux Vitek, USA, Hazelwood, Missouri, USA), the RapID ANA II system (Innovative Diagnostic Systems, Atlanta, Georgia, USA), and the API system (20A; bioMerieux Vitek). Each isolate was categorised as clinically significant or a contaminant (pseudobacteraemia) by criteria described previously.

**Conventional 16S rRNA gene sequencing**

Polymerase chain reaction amplification and DNA sequencing of the full 16S rRNA genes were performed according to our previous publications. Strains 1–13, 15–17, and 20 were amplified with primers LPW58 (5’-AGGCCCGGG AACGTATTGCAC-3’) and LPW81 (5’-TGGCGAACGGGTGATG GTA-3’), strains 14 and 19 with primers LPW55 (5’-AGTTGTGATCCTGGCTCAG-3’) and LPW325 (5’-CGGATACCTGTTAGCTAC-3’), and strain 18 with primers LPW55 (5’-AGTTGTGATCCTGGCTCAG-3’) and LPW205 (5’-CGGGACCTGTTACCGCC-3’). The sequences of the polymerase chain reaction products were compared with known 16S rRNA gene sequences in the GenBank by multiple sequence alignment using the CLUSTAL W program.

**Identification by the MicroSeq 500 16S rDNA bacterial identification system**

Bacterial DNA extracts were amplified with 0.5 μM primers (005F and 531R) according to the manufacturer’s instructions. The DNA sequences were analysed using the database provided by the system.

**RESULTS**

**Patient characteristics**

Twenty strains, representing 20 non-duplicating anaerobic Gram positive bacilli, were selected for further analysis by the MicroSeq 16S rDNA bacterial identification system and identification by three commercially available identification systems. Table 1 summarises the characteristics of the 20 patients. The clinical details of patients 14 and 18 have been described previously.

**Conventional 16S ribosomal RNA gene sequencing**

Table 1 shows the results of 16S rRNA gene sequence analysis. For all the 20 isolates, there was < 2% difference between the 16S rRNA gene sequences of the isolates and the most closely matched sequence in the GenBank.

Abbreviation: rRNA, ribosomal RNA
**Table 1** Identification of anaerobic Gram positive bacterial isolates by conventional 16S rRNA gene sequencing, commercially available bacterial identification systems, and the Microseq 500 16S rDNA bacterial identification system

<table>
<thead>
<tr>
<th>Patient/strain no.</th>
<th>Patient characteristics</th>
<th>Conventional 16S rRNA gene sequencing</th>
<th>Commercially available bacterial identification systems</th>
<th>Microseq 500 16S rDNA bacterial identification system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitek ANI</td>
<td>RapID ANA II</td>
</tr>
<tr>
<td>1</td>
<td>M/1 m</td>
<td>Pseudobacteraemia</td>
<td>Bifidobacterium pseudocatenulatum/ catenulatum</td>
<td>Unidentified</td>
</tr>
<tr>
<td>2</td>
<td>F/94</td>
<td>Pseudobacteraemia</td>
<td>Clostridium barati</td>
<td>Unidentified</td>
</tr>
<tr>
<td>3</td>
<td>M/1 m</td>
<td>Necrotising enterocolitis</td>
<td>Clostridium difficile</td>
<td>94% C difficile</td>
</tr>
<tr>
<td>4</td>
<td>F/75</td>
<td>Primary bacteraemia</td>
<td>Clostridium disporicum</td>
<td>99% C. barati</td>
</tr>
<tr>
<td>5</td>
<td>F/77</td>
<td>Pseudobacteraemia</td>
<td>Clostridium indolis</td>
<td>89% Cladobacterium tetani</td>
</tr>
<tr>
<td>6</td>
<td>M/80</td>
<td>Pseudobacteraemia</td>
<td>Clostridium innocuum</td>
<td>61% Corynebacterium jeikeum, 37% Lactobacillus jensenii</td>
</tr>
<tr>
<td>7</td>
<td>F/24</td>
<td>Pseudobacteraemia</td>
<td>Clostridium orisoidens</td>
<td>45% C subterminde, 39% Cladobacterium histolyticum, 11% C. jeikeum</td>
</tr>
<tr>
<td>8</td>
<td>M/6 m</td>
<td>Intussusception</td>
<td>Clostridium paraputreficium</td>
<td>81% Cladobacterium septicum</td>
</tr>
<tr>
<td>9</td>
<td>F/44</td>
<td>Acute cholangitis</td>
<td>Clostridium perfringens</td>
<td>99% C. perfringens</td>
</tr>
<tr>
<td>10</td>
<td>M/54</td>
<td>Primary bacteraemia</td>
<td>Clostridium ramosum</td>
<td>83% C. barati, 11% Lactobacillus catenulatum</td>
</tr>
<tr>
<td>11</td>
<td>F/40</td>
<td>Neutropenic leucer</td>
<td>Clostridium septicium</td>
<td>54% C. septicium, 42% Cladobacterium paraputreficium</td>
</tr>
<tr>
<td>12</td>
<td>F/45</td>
<td>Pseudobacteraemia</td>
<td>Clostridium sporopharyoides</td>
<td>83% C. jeikeum, 7% Cladobacterium histolyticum, 7% C. jeikeum</td>
</tr>
<tr>
<td>13</td>
<td>F/78</td>
<td>Pseudobacteraemia</td>
<td>Clostridium tertium</td>
<td>71% C. tertium, 22% Cladobacterium claudobacterium</td>
</tr>
<tr>
<td>14</td>
<td>F/87</td>
<td>Infected bed sore</td>
<td>Esterhella lenta</td>
<td>83% C. jeikeum, 7% Cladobacterium histolyticum, 7% C. jeikeum</td>
</tr>
<tr>
<td>15</td>
<td>F/85</td>
<td>Primary bacteraemia</td>
<td>Eubacterium tenue</td>
<td>81% Propionibacterium granulosum, 13% Actinomyces odontolyticus</td>
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<tr>
<td>16</td>
<td>F/41</td>
<td>Primary bacteraemia</td>
<td>Lactobacillus casei/paracasei</td>
<td>Unidentified</td>
</tr>
<tr>
<td>17</td>
<td>M/50</td>
<td>Primary bacteraemia</td>
<td>Lactobacillus rhamnosus</td>
<td>50% Lactobacillus jensenii, 46% Actinomyces odontolyticus</td>
</tr>
<tr>
<td>18</td>
<td>M/70</td>
<td>Acute cholecystitis</td>
<td>Lactobacillus salivarius</td>
<td>Unidentified</td>
</tr>
<tr>
<td>19</td>
<td>M/43</td>
<td>Acute cholangitis</td>
<td>Olsenella uli</td>
<td>81% P. granulosum, 11% Corynebacterium pseudotuberculosis</td>
</tr>
<tr>
<td>20</td>
<td>F/1 m</td>
<td>Pseudobacteraemia</td>
<td>Propionibacterium oram</td>
<td>99.9% P. acnes</td>
</tr>
</tbody>
</table>

*In years or months (m).
Identification by the MicroSeq 500 16S rDNA bacterial identification system

The identities of 13 strains were consistent with those obtained by conventional 16S rRNA gene sequencing (table 1). For the remaining seven sequences, four isolates were misidentified at the genus level (strain 6, *C innocuum* misidentified as *Eubacterium dolichum*; strain 7, *C orbiscindens* misidentified as *Ruminococcus productus*; strain 15, *E tenue* misidentified as *C tenuce*; and strain 19, *Olsenella uli* misidentified as *Atopobium rima*), whereas three were misidentified at the species level (strain 4, *C disporicum* misidentified as *C paraputerificum*; strain 5, *C indolis* misidentified as *C innocuum*; and strain 11, *C septicum* misidentified as *C tertium*).

Identification by commercially available bacterial identification systems

The Vitek ANI system was able to identify 10 and four of the 20 isolates, the RapID ANA II system nine and nine isolates, and the API 20A system nine and nine isolates to the genus and species levels with >70% confidence, respectively (table 1).

**DISCUSSION**

Although the MicroSeq 500 16S rDNA bacterial identification system was better than the three commercially available systems in the identification of the 20 anaerobic Gram positive bacilli tested in our present study, its accuracy is still suboptimal. Using conventional 16S rDNA sequencing as the gold standard, the MicroSeq 500 16S rDNA bacterial identification system was able to identify 16 of the 20 (80%) isolates to the genus level, and only 13 (65%) of the isolates to the species level in our present study, compared with the corresponding figures of 86.5% and 81.1% in our previous study on bacterial strains of more diverse genera and species,12 and 97.2% and 89.2% in a study on unusual aerobic Gram negative bacilli.11

"The database of the MicroSeq 500 16S rDNA bacterial identification system needs to be expanded to improve its accuracy in the identification of anaerobic Gram positive bacilli"  

The most common reason for the MicroSeq 500 16S rDNA bacterial identification system to fail to identify a bacterium was a lack of the 16S rRNA gene sequence of the particular bacterium in the database, which is in line with results from our previous study.13 The 16S rRNA gene sequences of five of the misidentified isolates were not included in the system database, probably because they are rarely encountered. When the same 527 bp DNA sequences of these seven misidentified isolates were compared with the known 16S rRNA gene sequences in the GenBank database, six yielded the correct identity, with good discrimination between the best and second best match sequences (table 2). Thus, the database of the MicroSeq 500 16S rDNA bacterial identification system needs to be expanded to improve its accuracy in the identification of anaerobic Gram positive bacilli.

**ACKNOWLEDGEMENTS**

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*Authors’ affiliations*

S K P Lau, P C Y Woo, A M Y Fung, G K S Woo, K-m Chan, K-Y Yuen, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong K H L Ng, K-t Yip, T-l Que, Department of Clinical Pathology, Tuen Mun Hospital, Hong Kong

Correspondence to: Dr K-Y Yuen, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Pokfulam Road, Hong Kong hkumicro@hkuc.hku.hk

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