Identification of bacteria directly from positive blood culture samples by DNA pyrosequencing of the 16S rRNA gene

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Received 26 June 2012
Accepted 14 August 2012

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Rapid identification of the causative bacteria of sepsis in patients can contribute to the selection of appropriate antibiotics and improvement of patients’ prognosis. Genotypic identification is an emerging technology that may provide an alternative method to, or complement, established phenotypic identification procedures. We evaluated a rapid protocol for bacterial identification based on PCR and pyrosequencing of the V1 and V3 regions of the 16S rRNA gene using DNA extracted directly from positive blood culture samples. One hundred and two positive blood culture bottles from 68 patients were randomly selected and the bacteria were identified by phenotyping and pyrosequencing. The results of pyrosequencing identification displayed 84.3 and 64.7 % concordance with the results of phenotypic identification at the genus and species levels, respectively. In the monomicrobial samples, the concordance between the results of pyrosequencing and phenotypic identification at the genus level was 87.0 %. Pyrosequencing identified one isolate in 60 % of polymicrobial samples, which were confirmed by culture analysis. Of the samples identified by pyrosequencing, 55.7 % showed consistent results in V1 and V3 targeted sequencing; other samples were identified based on the results of V1 (12.5 %) or V3 (31.8 %) sequencing alone. One isolate was erroneously identified by pyrosequencing due to high sequence similarity with another isolate. Pyrosequencing identified one isolate that was not detected by phenotypic identification. The process of pyrosequencing identification can be completed within ~4 h. The information provided by DNA-pyrosequencing for the identification of micro-organisms in positive blood culture bottles is accurate and could prove to be a rapid and useful tool in standard laboratory practice.

INTRODUCTION

Bloodstream infections causing severe sepsis and septic shock result in high mortality. Detection and identification of the causative micro-organisms of sepsis are crucial for selection of the appropriate antimicrobial agents. Blood culture is an important method for the growth and subsequent identification of causative micro-organisms and diagnostic laboratories are required to detect such micro-organisms as rapidly as possible. Accurate identification of bacterial isolates is also an essential task of the clinical microbiology laboratory. While traditional phenotypic identification is universally used in clinical laboratories, this method has some disadvantages, for example, it is time consuming, is sometimes difficult and does not always accurately identify target micro-organisms. In addition, interpretation of the results obtained using phenotypic methods can be subjective (Stager & Davis, 1992).

Genotypic identification of micro-organisms is an emerging technology that may provide an alternative or complementary method to established phenotypic identification procedures. Sequence analysis of the 16S rRNA gene is a widely accepted tool for molecular identification of bacteria (Kolbert & Persing, 1999; Patel, 2001; Woese, 1987). Bacterial 16S rRNA genes consist of eight highly conserved regions and nine variable regions (Woese, 1987).
V1 and V3 are two distinct variable regions of the 16S rRNA gene that have been used as targets for sequencing-based identification assays (Luna et al., 2007). This method capitalizes on the highly conserved nature of 16S rRNA genes by positioning amplification and sequencing primers in the conserved regions that flank the variable regions (specifically V1 and V3), thereby allowing primers to theoretically amplify most bacterial pathogens. Public databases such as GenBank, the Nucleotide Sequence Database at the European Molecular Biology Laboratory (EMBL-Bank), the DNA Data Bank of Japan (DDBJ) and the Ribosomal Database Project II (RDP II) contain a vast number of bacterial 16S rRNA gene sequences, allowing rapid analysis and providing phylogenetically meaningful information (Bosshard et al., 2006).

Pyrosequencing is a DNA sequencing technique that is based on the detection of pyrophosphate that is released during DNA synthesis and was introduced as a rapid alternative to traditional Sanger DNA sequencing (Ronaghi & Elahi, 2002). The DNA base sequence is determined by measuring the strength of visible light that is generated in proportion to the number of incorporated nucleotides in a cascade of enzymic reactions (Ronaghi, 2001). The main advantage of pyrosequencing is its rapidity and lower price compared to conventional sequencing. Although the length of the sequence that can be obtained by pyrosequencing is fairly short and limited to about 30–60 bases, carefully designed applications can provide information that is sufficient for the differentiation of gene sequences. Pyrosequencing has already been applied to the identification of bacteria (Jonasson et al., 2002; Luna et al., 2007; Ronaghi & Elahi, 2002). It has also been predicted that pyrosequencing of the 16S rRNA gene may function as a ‘molecular Gram stain’ for the identification of bacteria (Jordan et al., 2005). In order to effectively treat patients with sepsis, the rapid identification of causative bacteria is important; however, conventional phenotyping-based identification requires an extra day after blood culture tests become positive. Therefore, the rapidity of pyrosequencing-based identification is an attractive advantage for diagnosis. In fact, Jordan et al. (2009) reported that the combination of real-time PCR and pyrosequencing methods rapidly identified bacteria from positive blood culture samples and provided highly concordant results with those obtained using phenotypic identification.

To rapidly identify clinical isolates from positive blood culture samples, we evaluated a rapid protocol for the identification of micro-organisms using PCR and pyrosequencing of 16S rRNA genes. Using clinical samples from our hospital, we compared this molecular bacterial identification protocol with conventional culture identification techniques.

**METHODS**

**Sample collection.** This study was performed at the Nagasaki University Hospital, a tertiary hospital with about 850 beds, and was approved by the ethics committee of the Nagasaki University Hospital. Positive blood culture samples from both paediatrics and adults were randomly selected from blood culture bottles that were submitted during 2010 for routine microbiological testing. Blood sampling was performed according to the recommended methods in the hospital and 5–10 ml blood was collected into each bottle. Bottles containing samples from the same patient but at different time points were excluded.

**Blood culture and phenotypic identification.** Blood samples collected in BacT/ALERT FA or BacT/ALERT FN bottles (bioMérieux) at the Nagasaki University Hospital were cultured using BacT/ALERT 3D (bioMérieux), which is an automated microbial detection system that displays a positive result if microbial growth is detected by a fluorescent sensor. Each bottle was removed from the blood culture instrument within 12 h following a positive result and >1 ml samples were immediately extracted from the bottle. The sample was Gram stained and subcultured on the appropriate agar-based culture plates. All samples were identified according to standard biochemical identification methods using the VITEK 2 system (bioMérieux) or the Phoenix100 system (Becton Dickinson).

**DNA extraction and amplification.** Bacterial DNA was extracted directly from 1 ml blood culture fluid using the BiOtic bacteremia DNA isolation kit (MoBio Laboratories) according to the manufacturer’s instructions. Chromosomal DNA was eluted in a final volume of 50 μl elution buffer. The V1 (amplicon size 115 bp) and the V3 (amplicon size 81 bp) regions of 16S rRNA genes were amplified according to a previously published method (Luna et al., 2007). Nucleotide positions refer to positions in the 16S rRNA gene of Escherichia coli. Primers Bio-pBRS (5′-biotin-GAAGAGTTGATCATTGCTCAG-3′) and pBR-V1 (5′-TTACTCACCCGTCGCCGACT-3′) were used for amplification of the V1 region and Bio-V-V3 (5′-biotin-ACGACAGGCGATGGACGACTC-3′) and pBVS.V3 (5′-GGACGCGGAAGAACCCTTACC-3′) were used for amplification of the V3 region. Each 50 μl reaction mixture contained 25 μl Ampdirect (Shimadzu), 0.2 M each primer, 1.25 U AmpliTaq Gold DNA polymerase LD (Life Technologies) and 5 μl DNA template. PCRs were performed using the GeneAmp PCR system 9700 (Life Technologies) with the following cycling parameters: 95 °C for 10 min, followed by 35 cycles of 95 °C for 40 s, 55 °C for 40 s and 72 °C for 60 s, with a final elongation step at 72 °C for 60 s. PCR products were purified by Agarose gel electrophoresis using the Ribosomal Database Project II (RDP II) contain a vast number of bacterial 16S rRNA gene sequences, allowing rapid analysis and providing phylogenetically meaningful information (Bosshard et al., 2006).

**DNA pyrosequencing.** The amplified products of the 16S rRNA V1 and V3 regions were prepared for pyrosequencing by using the vacuum prep tool (Qiagen) following the recommended protocol. For the preparation of each reaction, 40 μl of the biotinylated PCR product was used. To prepare the sequencing plate, purified PCR products were resuspended in 43 μl binding buffer and 3 μl streptavidin beads. Double-stranded DNA was then denatured to single-stranded DNA using 0.2 M NaOH. Subsequently, single-stranded DNA was resuspended in 40 μl annealing buffer with 0.3 μM sequencing primer and then annealed to the sequencing primer at 80 °C for 2 min. The primers pB-R-V1 and pBVS.V3 were used as described above as DNA sequencing primers for the V1 and V3 regions, respectively. Pyrosequencing was performed on a PyroMark ID instrument (Qiagen) with eight cycles of a repetitive ACTG dispensation. Sequence similarities of the PCR products were determined using the DDBJ search program (www.ddbj.nig.ac.jp) and a strain with >99% sequence similarity was considered as an isolated strain.
RESULTS

Culture results

In the present study, 102 samples collected from 68 patients were cultured and 112 bacteria and one fungus (Candida albicans) were isolated. Two different microorganisms were isolated from each of 10 (9.8%) samples collected from a total of seven cases. The cultured bacteria included a total of 15 genera and 28 species. One isolate was not identified and is referred to as an anaerobic Gram-positive rod.

Detection and identification of micro-organisms by DNA-pyrosequencing

All 102 samples were successfully amplified by PCR, targeting the V1 or V3 region of the 16S rRNA gene. Four samples required dilution for amplification of the products because of inhibition. DNA pyrosequencing-based identification was then performed using these PCR products. From the 102 samples, 88 (86.3%) strains were detected to the genus level and 68 (66.7%) strains were detected to the species level by DNA pyrosequencing, comprising 16 genera and 19 species, including 41 Gram-positive cocci, nine Gram-positive bacilli, 34 Gram-negative bacilli and four anaerobic organisms. Of the 68 patient cases, isolates from 61 (89.7%) cases were detected to the genus level and isolates from 49 (72.1%) cases were detected to the species level.

Identification of isolates based on culture and pyrosequencing of V1 and V3 regions

Pyrosequencing results corresponding to each culture-based organism were analysed. Of the 92 monomicrobial samples, 21 strains identified by pyrosequencing showed complete agreement with the results of culture-based identification with both V1 and V3 targeted pyrosequencing methods (Fig. 1) providing identification to the species level (Table 1), identifying strains of Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus capitis, Staphylococcus haemolyticus, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylococcus sciuri, Staphylo-

However, in some samples, the 16S rRNA gene targets could not be used to successfully identify the bacterium at both the species and genus levels. Strains of the genus Enterobacter as well as strains of Bacteroides fragilis, Fusobacterium nucleatum and Bifidobacterium scardovii were not detected by the sequencing of the V1 region. In contrast, pyrosequencing of the V3 region failed to detect Citrobacter freundii and strains of the genus Clostridium.

Concordance rate of sequence-based identification

The percentage of concordance between the results of culture-based and pyrosequence-based identification was calculated (Table 3). Of the 92 monomicrobial samples identified by culture, 80 (87.0%) samples identified at genus level and 63 (68.5%) samples identified at species level were concordant with pyrosequence-based identifications. Two (2.2%) samples showed discordant results and 10 (10.9%) samples were unidentified by pyrosequencing.

Ten samples were confirmed as polymicrobial by culture-based identification and pyrosequencing identified one micro-organism at the genus level in six of these samples and one micro-organism at species level in three of these samples, all of which were concordant with the results of culture-based identification. Pyrosequencing did not detect two or more micro-organisms in any of the samples.

The overall agreement between the results of culture- and pyrosequence-based identification was 84.3% (86/102) at the genus level and 64.7% (66/102) at the species level.

Analysis of discordant results

Two samples displayed discordant results following identification by DNA pyrosequencing and phenotyping. In one sample, the isolate was determined as Staphylococcus epidermidis by pyrosequencing; however, the characteristics of this isolate were inconsistent with biochemical data of Staphylococcus epidermidis and the Phoenix100 and VITEK2 system analyses identified this isolate as Staphylococcus capitis with 99% probability. 16S rRNA gene sequence similarity between Staphylococcus epidermidis and Staphylococcus capitis is 99%. The pyrosequencing result was therefore interpreted as a false-positive result.

In the other sample, the isolate was identified as Bifidobacterium scardovii by pyrosequencing but the conventional culture method identified it simply as an anaerobic Gram-positive rod that could not be further classified because of poor data regarding morphological and biochemical characteristics. This isolate was ultimately determined as B. scardovii after confirmation following reproducibility testing.

DISCUSSION

Rapid identification of the causative bacteria of sepsis in patients allows the appropriate antibiotics to be selected
(Barenfanger et al., 1999) and improves prognosis (Barenfanger et al., 2001). Bacterial identification based on genetic methods can provide information that is useful for the selection of targeted antibiotics.

The overall results for the identification of isolates by pyrosequencing and culture-based methods agreed in 84.3% and 64.7% of samples for identification at the genus and species levels, respectively. A previous study of DNA pyrosequencing identification using pure-cultured isolates reported ~90% agreement between the isolates identified by the two methods (Luna et al., 2007). Considering that DNA was extracted directly from blood culture bottle fluids, we believe that the concordance between the results of the two methods used in the present study is reasonable. In addition, pyrosequencing resulted in only one error in sample identification, which was due to very high sequence similarity between two bacteria, implying that DNA-pyrosequencing is a very accurate method for the identification of bacteria. These results suggest that the identification of bacteria in positive blood culture samples by DNA-pyrosequencing will be useful for the evaluation of clinical samples and may influence the choice appropriate antimicrobial treatment, benefitting patient outcome.

Highly accurate pyrosequencing identification of bacteria from positive blood culture bottles has been reported previously (Jordan et al., 2009). In this report, the 23S rRNA gene was used as the target to improve the accuracy of identification of some specific bacteria, such as members of the family Enterobacteriaceae and the genus Streptococcus, and the agreement between the results of pyrosequencing- and culture-based identification reached 97.8%. In the present study, the rate of concordance between the two techniques was lower. This was partly because a larger number of polymicrobial samples were included in the present study than were present in the study by Jordan et al. (2009). Furthermore, the relatively large number of specific strains that went undetected, such as members of the genus Enterobacter, could also decrease the rate of concordance of this study.

In the present study, pyrosequencing of the V1 and V3 regions produced similar results in many samples but highlighted different characteristics in some specific groups of bacteria. The V1 region can effectively be used to identify members of the genus Enterococcus and distinguish between E. faecalis and E. faecium; conversely, using the V3 region can have advantages in detecting S. epidermidis and E. coli. This suggests that sequencing both the V1 and V3 regions improves the accuracy of diagnosis. However, the optimal combination of variable regions of the 16S rRNA gene for use in diagnosis has been a controversial issue (Sundquist et al., 2007; Wang et al., 2007).

Conventional biochemical testing, especially for difficult-to-identify pathogens, may result in incorrect pathogen identification, resulting in inconsistent information for the physician (Downes et al., 1998; Stager & Davis, 1992). Molecular methods provide novel strategies for bacterial pathogen identification (Tang et al., 1998). 16S rRNA gene sequencing has previously been reported to detect relevant isolates of non-fermenting Gram-negative bacilli at high rates compared to phenotypic identification techniques. The reason for the low rate of phenotypic identification was that nearly half of the isolates that corresponded to species based on sequencing data were not included in the databases of conventional phenotypic identification systems (Bosshard et al., 2006). Molecular methods are considered useful for identification of Gram-positive bacteria or anaerobes as well as Gram-negative bacteria. In the present study, Bifidobacterium scardovii was identified by the genetic method but not by the usual laboratory procedures. Therefore, the genetic method described in this study may complement current methods of phenotypic identification.

Although the method described in this study is considered to be a useful and convenient procedure for the rapid identification of micro-organisms, it also has some limitations in terms of efficacy of identification. First, pyrosequencing can fail to separate distinct bacteria that have similar sequences because it provides only short sequence lengths. Members of the genera Aeromonas, Bacillus and Staphylococcus typically have similar sequences in the target gene to other members within their respective genus. Therefore, organisms that belong to these genera were not
effectively identified at the species level by pyrosequencing but showed good agreement with the results of culture-based identification at the genus level. Other specific sequencing targets are required to identify the correct species of these bacteria. However, with the exception of the genus *Staphylococcus*, members of these genera are rarely isolated and their antibiotic resistance has not become problematic. Therefore, genetic methods to identify these bacteria at the species level may not be considered necessary.

Second, in polymicrobial infections, pyrosequencing may not identify all of the different bacteria. Thus, when a sample for pyrosequencing contains polymicrobial genes, the result obtained from sequencing can consist of a mix of sequences from those micro-organisms. Therefore, pyrosequencing may not effectively detect organisms in patients with polymicrobial infections. Among the bacteria that were undetectable by pyrosequencing, some species, such as *Enterobacter cloacae, Enterococcus faecalis, Bacteroides fragilis*

### Table 1. Distribution of pyrosequencing identification results in monomicrobial samples

Values represent numbers of isolates identified by pyrosequencing, targeting either V1 + V3, V1 or V3 regions, to the species level or to the genus level. Isolates identified only to the genus level by pyrosequencing and those results that were discordant or undetected by pyrosequencing were assigned to a species based on the results of culture-based identification.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>No. of isolates</th>
<th>Number of concordant samples</th>
<th>Number of discordant samples</th>
<th>Undetected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Species level</td>
<td>Genus level</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>V1 + V3  V1  V3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>2</td>
<td>0  2  0  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>3</td>
<td>0  2  0  0 0 0</td>
<td>0</td>
<td>0  1 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
<td>9  4  2  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus capitis</em></td>
<td>2</td>
<td>0  0  0  0 0 0</td>
<td>0</td>
<td>1*  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>9</td>
<td>4  0  3  2 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus haemolyticus</em></td>
<td>2</td>
<td>0  1  0  1 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus hominis</em></td>
<td>1</td>
<td>0  0  1  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus schleiferi</em></td>
<td>1</td>
<td>0  0  0  1 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus simulans</em></td>
<td>2</td>
<td>0  0  0  2 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Staphylococcus agalactiae</em></td>
<td>3</td>
<td>0  2  1  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td>Gram-positive bacilli</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>3</td>
<td>0  1  0  2 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Bacillus thuringiensis</em></td>
<td>1</td>
<td>0  0  0  1 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td><em>Corynebacterium striatum</em></td>
<td>2</td>
<td>2  0  0  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
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<tr>
<td>Gram-negative bacilli</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
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<td>0  0  0  1 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
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<tr>
<td><em>Aeromonas sobria</em></td>
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<td>0  0  0  3 0 0</td>
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<td>0  0 0 0 0 0</td>
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<tr>
<td><em>Citrobacter freundii</em></td>
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<td>0  2  0  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
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<tr>
<td><em>Enterobacter aerogenes</em></td>
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<td>0  0  0  0 0 0</td>
<td>0</td>
<td>0  1 0 0 0 0</td>
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<tr>
<td><em>Enterobacter cloacae</em></td>
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<td>0  0  0  3 0 0</td>
<td>0</td>
<td>0  4 0 0 0 0</td>
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<tr>
<td><em>Escherichia coli</em></td>
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<td><em>Haemophilus influenzae</em></td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<td>3  0  0  0 0 0</td>
<td>0</td>
<td>0  0 0 0 0 0</td>
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<td><em>Pseudomonas putida</em></td>
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<td>0  0 0 0 0 0</td>
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<tr>
<td>Others (anaerobes)</td>
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<tr>
<td><em>Bacteroides fragilis</em></td>
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<td>0  0  1  0 0 0</td>
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<td>0  0 0 0 0 0</td>
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<td><em>Fusobacterium nucleatum</em></td>
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<td>0  0 0 0 0 0</td>
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<tr>
<td><em>Genus Veillonella</em></td>
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<td>Anaerobic Gram-positive rod</td>
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<td>1*</td>
<td>0  0 0 0 0 0</td>
</tr>
<tr>
<td>Total</td>
<td>92</td>
<td>21  20  22 17</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

*This isolate was misidentified as *Staphylococcus epidermidis* by both V1 and V3 sequencing.
†This isolate was identified as *Bifidobacterium scardovii* by V3 sequencing.
and *Bacillus thuringiensis*, were commonly observed and the samples that included these isolates were often polymicrobial. Most of the bacteria that were not detected by pyrosequencing in this study are commonly known as causative pathogens of intra-abdominal and urinary tract infections, in which polymicrobial infections are often observed (Reuben et al., 1989).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been used as an accurate identification tool with fast and cost-effective benefits. Identification of bacteria directly from positive blood cultures by using MALDI-TOF MS has also been attempted and 74.3–98.0% of bacteria were correctly identified to the species level (Christner et al., 2010; Wimmer et al., 2012; Wüppenhorst et al., 2012). However, some bacteria including *E. coli* and species of the genus *Shigella* are known to be indistinguishable by MALDI-TOF MS. Members of the genus *Streptococcus* are also not reliably identifiable to the species level by using MALDI-TOF MS or 16S rRNA pyrosequencing. Therefore, these rapid protocols require additional procedures to identify these bacteria correctly.

The process of pyrosequencing identification of bacteria that was used in the present study, including sample preparation, the sequencing reaction and analysis of the

<table>
<thead>
<tr>
<th>Table 2. Identification of micro-organisms in the 10 polymicrobial samples</th>
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<td><strong>Phenotypic identification</strong></td>
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<tr>
<td><strong>Result</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>Species level</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>Genus Clostridium</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>
| *When at least one of the micro-organisms was identified by both identification methods, to either the genus or the species level, the sample was considered concordant.†Identification based on V3 sequence.‡Identification based on V1 sequence.§Identification based on both V1 and V3 sequence.*

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The process of pyrosequencing identification of bacteria that was used in the present study, including sample preparation, the sequencing reaction and analysis of the

<table>
<thead>
<tr>
<th>Table 3. Summary of DNA pyrosequencing results and the concordance with results of phenotypic identification</th>
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</thead>
<tbody>
<tr>
<td><strong>Results of pyrosequencing identification</strong></td>
</tr>
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<td>Genus level</td>
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<td>Concordant</td>
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<td>Discordant</td>
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<tr>
<td>Species level</td>
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<td>Concordant</td>
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<tr>
<td>Discordant</td>
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<td>Undetected</td>
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| *When at least one isolate gave the same result, the sample was considered concordant.*
results, can be completed within ~4 h. Repeated sequencing from the same sample bottle provided consistent results. This method would therefore be relatively easy to fit into a standard laboratory routine and obtaining information regarding the isolate within a day would be of great help in improving the outcome of sepsis.

ACKNOWLEDGEMENTS

This research was partially supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science and Technology (no. 21591294) to K.Y, and a grant from the Global Centers of Excellence Program, Nagasaki University.

REFERENCES


