Identification of Bacteria Directly from Positive Blood Culture Samples by DNA Pyrosequencing of 16S rRNA

Maiko Motoshima\textsuperscript{a}, Katsunori Yanagihara\textsuperscript{a}\textsuperscript{*}, Yoshitomo Morinaga\textsuperscript{a}, Junichi Matsuda\textsuperscript{a}, Hiroo Hasegawa\textsuperscript{a}, Shigeru Kohno\textsuperscript{b,c} and Shimeru Kamihira\textsuperscript{a}

\textsuperscript{a}Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
\textsuperscript{b}Second Department of Internal Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
\textsuperscript{c}Global COE Program, Nagasaki University, Nagasaki, Japan

\textit{Running title:} Pyrosequencing of 16S rRNA from blood culture samples

\textit{Contents Category:} Diagnostics, typing and identification

\textit{Address correspondence to:} Katsunori Yanagihara, MD, Ph.D.
Department of Laboratory Medicine
Nagasaki University Graduate School of Biomedical Sciences
1·7·1 Sakamoto, Nagasaki 852·8501, Japan
Tel: +81·95·819·7418; Fax: +81·95·819·7257; E-mail: k-yanagi@nagasaki-u.ac.jp
ABSTRACT

Rapid identification of causative bacteria in patients with sepsis can contribute to appropriate selection of 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 of bacterial identification based on polymerase chain reaction and pyrosequencing of V1 and V3 gene of 16S rRNA using DNA directly from positive blood culture samples. One hundred and two culture positive blood culture bottles from 68 patients were randomly selected and the contained bacteria were identified by phenotyping and pyrosequencing. Pyrosequencing identification displayed 84.3% and 64.7% concordance with phenotypical identification at the genus and species levels, respectively. In the monomicrobial samples, the concordance at the genus level was 87.0%. Pyrosequencing identified one isolate in 60% of polymicrobial samples that were confirmed by culture analysis. Of the pyrosequencing-identified samples, the result of V1 and V3 were consistent in 55.7% and the other samples were identified based on the results of V1 (12.5%) or V3 (31.8%). One isolate was erroneously identified by pyrosequencing due to a highly similar sequence with another isolate. Pyrosequencing identified one isolate that was not detected by phenotyping. The process of pyrosequencing identification can be completed within approximately 4 h. The information provided by DNA-pyrosequencing identification of microorganism isolates in positive blood culture bottles is accurate and could provide a rapid and useful tool in standard laboratory practice.

Key words: genetic identification, pyrosequence, 16S rRNA
INTRODUCTION

Blood stream infections such as severe sepsis and septic shock result in high mortality. Detection and identification of causative microorganisms 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 microorganisms, and diagnostic laboratories are required to detect such microorganisms 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, sometimes difficult, and does not always accurately identify target microorganisms. In addition, interpretation of the results obtained using phenotypic methods can involve substantial subjective judgment (Stager & Davis, 1992).

Genotypic identification of microorganisms is an emerging technology that may provide an alternative or a 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 and nine variable regions (Woese, 1987). V1 and V3 are two distinct variable regions included in the \(16S\ rRNA\) gene that have been used as targets for a sequencing-based identification assay (Luna \textit{et al.}, 2007). This assay 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 \(16S\ rRNA\) genes.
sequences, allowing for 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 et al., 1996). 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 enzymatic 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 identification of bacteria in the field of microbiology (Jonasson et al., 2002; Luna et al., 2007; Ronaghi & Elahi, 2002). It has also been predicted that pyrosequencing of the 16S rRNA gene will be a useful tool for the identification of bacteria, and may function as a “molecular gram stain” (Jordan et al., 2005).

For patients with sepsis, the rapid identification of causative bacteria is important, however, the conventional phenotyping-based identification requires an extra day after blood culture become positive. Therefore, the rapidity of pyrosequencing-based identification is an attractive advantage for diagnosis. In fact, Jordan et al. reported that the combination methods of real-time polymerase chain reaction (PCR) and pyrosequencing rapidly identified bacteria from positive blood culture samples and provided highly-concordant results with the phenotypic identification (Jordan et al., 2009).

To rapidly identify clinical isolates from positive blood culture samples, we evaluated a rapid protocol for microorganism identification using PCR and pyrosequencing of 16S rRNA. Using clinical samples from our hospital, we compared our bacterial identification protocol with conventional culture identification.
METHODS

Sample collection

This study was performed at the Nagasaki University Hospital that is a tertiary hospital with about 850 beds and approved by the ethics committee of Nagasaki University Hospital. The positive blood culture samples were randomly selected from the blood culture bottles during 2010 that were submitted for the usual microbiological testing from both pediatrics and adults. The blood sampling was performed according to the recommended methods in our hospital and 5-10 mL of blood were collected into the each bottle. Bottles containing samples from the same patient but at different time points were excluded.

Blood culture and phenotypical identification

Blood samples that were collected in BacT/ALERT FA or BacT/ALERT FN bottles (bioMerieux, Hazelwood, MO) at the Nagasaki University Hospital were cultured using BacT/ALERT 3D (bioMerieux, Hazelwood, MO), 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 after the bottle went positive 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 (bioMerieux, Hazelwood, MO) or the Phoenix100 system (Becton Dickinson, Franklin Lakes, NJ).

DNA extraction and amplification

Bacterial DNA was extracted directly from 1mL of the blood culture fluid using the
BiOstic bacteremia DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer’s instructions. Chromosomal DNA was eluted in a final volume of 50 μL of elution buffer. The V1, amplicon size 115bp, and V3, amplicon size 81bp, regions of 16S rRNA genes were amplified according to a previously published method (Luna et al., 2007). Nucleotide positions refer to positions in the *Escherichia coli* 16S rRNA gene. The Bio-pBR5 (5’-biotin-GAAGAGTTTGATCATGGCTCAG-3’) and pBR-V1 (5’-TTACTCACCGTGCCGCACCCCTCAG-3’) primers were used for V1 amplification, and the Bio-B-V3 (5’-biotin-AACGACAGGACATGCACCTCAG-3’) and pJBS.V3 (5’-GCAACGCGAAGAACCTTACC-3’) primers were used for V3 amplification. Each 50 μL reaction mixture contained 25 μL of Ampdirect (Shimadzu Co., Kyoto, Japan), 0.2 M of each primer, 1.25 U AmpliTaq Gold DNA polymerase LD (Life Technologies, Carlsbad, CA) and 5 μL of DNA template. PCR was performed using the GeneAmp PCR system 9700 (Life Technologies, Carlsbad, CA) with the following cycling parameters: 10 min at 95°C, 35 cycles of 95°C for 40 s, 55°C for 40 s and 72°C for 60 s, followed by a single cycle of 72°C for 60 s. The DNA extracted from the clinical isolates including *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* and sterile water were used as positive controls and a negative control for PCR, respectively. Each PCR product was verified by agarose gel electrophoresis. The samples without single band were amplified after 10- or 100-fold dilution and reconfirmed by agarose gel analysis.

DNA pyrosequencing

The amplified V1 and V3 products were prepared for pyrosequencing by using the recommended protocol for the vacuum prep tool (Qiagen, Valencia, CA). 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 of binding buffer and 3 μL of streptavidin beads. Double-stranded DNA was then denatured to single-stranded DNA using a
0.2 M NaOH. Subsequently, single-stranded DNA was resuspended in 40 µL of annealing buffer with 0.3 µM sequencing primer and then annealed to the sequencing primer at 80°C for 2 min. The primers pBR-V1 and pJBS.V3 as described above were used as DNA sequencing primers for the V1 and V3 regions, respectively. Pyrosequencing was performed on the PyroMark ID instrument (Qiagen, Valencia, CA) with 8 cycles of a repetitive ACTG dispensation. Sequence homology of PCR products was compared using the DDBJ search program (www.ddbj.nig.ac.jp) and a strain with >99% sequence homology was considered as an isolated strain.

RESULTS

Culture results

In this study, 102 samples collected from 68 patients were cultured and 112 bacteria and 1 fungus (*Candida albicans*) were isolated. Two types of microorganisms were isolated in each of 10 (9.8%) samples from seven cases. The cultured bacteria included 15 genera and 28 species. One isolate was not identified and is referred to as an anaerobic gram-positive rod.

Detection and identification of microorganisms by DNA-pyrosequencing

All 102 samples were successfully amplified by PCR targeted on V1 or V3. 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%) and 68 (66.7%) strains were detected to the genus and species level, respectively, by DNA pyrosequencing. These bacteria were separated into 16 genera and 19 species. The strains included 41 gram-positive cocci, 9 gram-positive bacilli, 34 gram-negative bacilli and 4 anaerobic organisms. Of 68 cases, isolates from 61 (89.7%) and
49 (72.1%) cases were detected to the genus and species level, respectively.

Culture results and pyrosequencing identification of V1 and V3 gene

The pyrosequencing results corresponding to each culture-based organism were analyzed. In the monomicrobial samples (Table 1), 21 strains were completely agreed with the culture-based identification and both the two pyrosequencing identifications at species level. These completely-concordant strains were observed in *Staphylococcus aureus*, *S. epidermidis*, *Corynebacterium striatum*, *Escherichia coli* and *Pseudomonas aeruginosa*. The other concordant isolates at species level were dependent on either target (V1, 20 isolates; V3, 22 isolates). In two isolates, pyrosequencing presented different strains from the culture-based identification. One of these resulted from the consistent identification of V1 and V3 and the other from the V3 sequencing.

In the polymicrobial samples (Table 2), pyrosequencing failed to completely identify all of the bacteria that were identified by the culture method. However, one organism was identified in six (60%) of these samples and three of the six strains were identified to species level by pyrosequencing. One was based on both results of V1 and V3 sequence and the others were based on V1 sequencing.

However, in some bacteria, each target of 16S rRNA could not successfully identify the bacterium at both species and genus levels. Genus *Enterobacter*, *Bacteroides fragilis*, *Fusobacterium nucleatum*, *Bifidobacterium scardovii* were not detected by the sequencing of V1. In contrast, the pyrosequencing of V3 failed to detect *Citrobacter freundii* and Genus *Clostridium*.

Concordance rate of sequence-based identification

The percentage of concordance between culture-based and pyrosequence-based identification was calculated (Table 3). Of the 92 monomicrobial samples identified by
culture, 80 (87.0%) samples at genus level and 63 (68.5%) samples 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.

Of the 10 polymicrobial samples confirmed by culture, pyrosequencing identified one microorganism in 6 samples at genus level with concordance and in 3 samples at species level. Pyrosequencing did not detect two or more microorganisms in all samples.

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

Analysis of discordant results

Two samples displayed discordant identification between 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 *S. epidermidis* and the Phoenix100 system and VITEK2 system analysis identified this isolate as *Staphylococcus capitis* with 99% probability. The sequence homology of the 16S rRNA of *S. epidermidis* and *S. capitis* is 99%. The pyrosequencing result was 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 confirming the reproducibility.

**DISCUSSION**

Rapid identification of causative bacteria in patients with sepsis can lead to the
appropriate selection of antibiotics (Barenfanger et al., 1999) and the improvement of 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 isolate information obtained by pyrosequencing agreed with the information obtained using the culture method for 84.3% and 64.7% of isolates at the genus and species level, respectively. A previous report of DNA pyrosequencing identification that used pure-cultured isolates reported approximately 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 two methods that we observed 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 identification of bacteria. These results suggest that DNA-pyrosequencing identification of bacteria in positive blood culture samples will be useful for evaluation of clinical samples and can contribute to appropriate antimicrobial treatment and benefit patient outcome.

Previously, Jordan et al. reported a highly-accurate pyrosequencing identification from the positive blood culture bottles (Jordan et al., 2009). In Jordan’s report, the 23S rRNA gene was used as the targets to improve the identification efficiency of some specific bacteria such as *Enterobacteriae* and *Streptococcus* species, and the agreement between pyrosequencing- and culture-based identification reached 97.8%. In the present study, the concordant rate was lower than Jordan’s report. This was partly because a larger number of polymicrobial samples was included than Jordan’s report. Furthermore, the relatively large number of undetected specific strains such as Genus *Enterobacter* could also decrease the concordant rate of this study.

In this study, the sequences of V1 and V3 represented similar results in many samples but also showed different characteristics in some specific bacteria. V1 can effectively
classify genus *Enterococcus* into *E. faecalis* or *E. faecium* and V3 can have advantages of
detecting *S. epidermidis* and *E. coli*. These suggested that the sequencing V1 and V3
improved the accuracy of diagnosis. However, the best combination of variable regions of
*16S rRNA* for 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* sequencing was
previously reported to detect relevant isolates of nonfermenting gram-negative bacilli at high
rates compared to phenotypic identification. 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 of gram-negative bacteria. In this study, *B.
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
phenotypical identification.

Although the method described in this study is considered to be a useful and
convenient procedure for rapid identification of microorganisms, it also has some limitations
in terms of efficacy of identification. First, pyrosequencing can fail to separate distinct
bacteria which have similar sequences because it only reads short sequence lengths. The
genera *Aeromonas*, *Bacillus* and *Staphylococcus* are typical genera which have similar
sequences in the target gene in each genus. Therefore, organisms which belong to these
genera were not effectively identified at the species level, but showed good agreement with
culture results at the genus level. Other specific sequencing targets will be required to
identify the correct species of these bacteria. However, with the exception of *Staphylococci*, these genera are rarely isolated and their antibiotic resistance has not become problematic. Therefore, it is considered that genetic methods to identify these bacteria at the species level may not be necessary. Second, in polymicrobial infections, pyrosequencing may not identify all of the bacteria. Thus, when a sample for pyrosequencing contains polymicrobial genes, the result obtained from sequencing can consist of a mix of sequences from those organisms. Therefore, pyrosequencing may not effectively detect organisms in patients with polymicrobial infection. Among bacteria that were undetectable by pyrosequencing, some species such as *E. cloacae*, *E. faecalis*, *B. fragilis* and *B. 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 directly from positive blood cultures by using MALDI-TOF MS has been also attempted and 74.3 – 98.0 % of bacteria were correctly identified to the species level (Christner *et al*., 2010; Wimmer *et al*., 2012; Wuppenhorst *et al*., 2012). However, some bacteria including *E. coli* and *Shigella* spp. are known as indistinguishable bacteria by MALDI-TOF MS. Especially, *Streptococcus* spp. is not reliably identified to the species level in both MALDI-TOF MS and 16S RNA pyrosequencing. Therefore, these rapid protocols require the additional procedures to identify these bacteria correctly.

The process of pyrosequencing identification of bacteria that was used in this study including sample preparation, the sequencing reaction and analysis of the results, can be completed within approximately 4 h. Repeated sequencing from the same sample bottle
provided consistent results. This method would therefore be relatively easy to fit into a
standard routine work and obtaining information regarding the isolate within a day would be
of great help in improving the outcome of sepsis.

ACKNOWLEDGMENTS

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Technology, and a grant from the Global Centers of Excellence Program, Nagasaki
University.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.
REFERENCES


<table>
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<tr>
<th>Strains</th>
<th>No. of isolates</th>
<th>Concordant Species level</th>
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<td></td>
<td></td>
<td>V1+V3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>V1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>V3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
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<td>0</td>
<td>2</td>
<td>0</td>
</tr>
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<td>0</td>
<td>2</td>
<td>0</td>
</tr>
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<td>15</td>
<td>9</td>
<td>4</td>
<td>2</td>
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| *Staphylococcus capitis*  | 2              | 0                       | 0          | 0          | 0          | 1<sup>d</sup> | 1
| *Staphylococcus capitis*  | 2              | 0                       | 0          | 0          | 0          | 0          |
| *Staphylococcus epidermidis* | 9            | 4                       | 0          | 3          | 2          | 0          | 0
| *Staphylococcus haemolyticus* | 2            | 0                       | 1          | 0          | 1          | 0          | 0
| *Staphylococcus hominis*  | 1              | 0                       | 0          | 1          | 0          | 0          | 0
| *Staphylococcus schleiferi* | 1            | 0                       | 0          | 0          | 1          | 0          | 0
| *Staphylococcus simulans* | 2              | 0                       | 0          | 0          | 2          | 0          | 0
| *Staphylococcus agalactiae* | 3            | 0                       | 2          | 1          | 0          | 0          | 0
| Gram-positive bacilli     |                |                        |            |            |
| *Bacillus cereus*         | 3              | 0                       | 1          | 0          | 2          | 0          | 0
| *Bacillus thuringiensis*  | 1              | 0                       | 0          | 0          | 1          | 0          | 0
| *Corynebacterium striatum* | 2              | 2                       | 0          | 0          | 0          | 0          | 0
| Gram-negative bacilli     |                |                        |            |            |
| *Aeromonas hydrophila*    | 1              | 0                       | 0          | 0          | 1          | 0          | 0
| *Aeromonas sobria*        | 3              | 0                       | 0          | 0          | 3          | 0          | 0
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<tr>
<th>Genus</th>
<th>V1</th>
<th>V2</th>
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<th>V5</th>
<th>V6</th>
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<td>0</td>
<td>3</td>
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<td>0</td>
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<tr>
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<td>0</td>
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<td>Anaerobic gram-positive rod</td>
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<td>92</td>
<td>21</td>
<td>20</td>
<td>22</td>
<td>17</td>
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<td>10</td>
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</table>

*a* Consistent results of V1 and V3 sequence. *b* Identification by V1 sequence. *c* Identification by V3 sequence. *d* This isolate was misidentified as *Staphylococcus epidermidis* by both V1 and V3 sequencing. *e* This isolate was identified as *Bifidobacterium scardovii* by V3 sequencing.
Table 2. Pyrosequencing identification in polymicrobial samples

<table>
<thead>
<tr>
<th>Strains</th>
<th>No. of samples</th>
<th>Phenytypical identification</th>
<th>Pyrosequencing identification</th>
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<td>Genus Bacillus</td>
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<td>0</td>
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<tr>
<td>Enterobacter cloacae / Staphylococcus aureus</td>
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<td>Staphylococcus aureus</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Enterococcus faecium / Staphylococcus hemolyticus</td>
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<td>Enterococcus faecium</td>
<td>2&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Staphylococcus aureus / Candida albicans</td>
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<td>undetected</td>
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</tr>
</tbody>
</table>

<sup>a</sup> When at least one isolate was the same result, the sample was considered concordant.

<sup>b</sup> Identification based on V3 sequence.  
<sup>c</sup> Identification based on V1 sequence.  
<sup>d</sup> Identification based on both V1 and V3 sequence.  
<sup>e</sup> Identification based on V1 sequence.
Table 3. Summary of the concordance of DNA pyrosequencing identification with phenotypical identification

<table>
<thead>
<tr>
<th>Results of pyrosequencing identification</th>
<th>Monomicrobial (n = 92)</th>
<th>Polymicrobial* (n = 10)</th>
<th>All (n = 102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detected</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concordant</td>
<td>80 (87.0)</td>
<td>6 (60.0)</td>
<td>86 (84.3)</td>
</tr>
<tr>
<td>Discordant</td>
<td>2 (2.2)</td>
<td>0 (0.0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Undetected</td>
<td>10 (10.9)</td>
<td>4 (40.0)</td>
<td>14 (13.7)</td>
</tr>
<tr>
<td>Species level</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Detected</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Concordant</td>
<td>63 (68.5)</td>
<td>3 (30.0)</td>
<td>66 (64.7)</td>
</tr>
<tr>
<td>Discordant</td>
<td>2 (2.2)</td>
<td>0 (0.0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Undetected</td>
<td>27 (29.3)</td>
<td>7 (70.0)</td>
<td>34 (33.3)</td>
</tr>
</tbody>
</table>

* When at least one isolate was the same result, the sample was considered concordant.
FIGURE LEGENDS

Figure 1. A representative pyrogram of V1 and V3 gene. A representative pyrogram of targeted V1 (A) and V3 (B) gene. The sequence results were shown at the bottom of each pyrogram. This sample was identified as *P. aeruginosa* after homology search.
Figure 1

A

GAATCCAGGA GCAAGCCCCT TCCTATCGCC TCGACTGCTG ACT

B

TGGCCTTGAC ATGCTGAGAA CTTTCCAGAG ATGGATTGGT GCCTTC