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# NOTES

## 16S rRNA Gene Sequencing in Routine Identification of Anaerobic Bacteria Isolated from Blood Cultures[▽]

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**A comparison between conventional identification and 16S rRNA gene sequencing of anaerobic bacteria isolated from blood cultures in a routine setting was performed ($n$ = 127). With sequencing, 89% were identified to the species level, versus 52% with conventional identification. The times for identification were 1.5 days and 2.8 days, respectively.**

Anaerobic bacteria isolated from blood cultures can originate from multiple locations, e.g., the oral cavity, lower intestine, or female genital tract. If species identification is not performed, important information concerning the recognition of the primary site of infection could be lost and appropriate treatment, e.g., surgical drainage, may be delayed. Also, the diagnosis of recurrent bacteremia will be more difficult and may result in unnecessary reconsideration of the primary site of infection. Conventional phenotypic identification of anaerobic bacteria to the species level can be difficult and laborious in the clinical microbiology laboratory. Commercial phenotypic systems like the Rapid ID 32A or Vitek 2 ANC card (bioMérieux, Marcy-l'Etoile, France) are faster but limited by the taxa included in the system database and, in most cases, only reliable to the genus level (6, 8, 11, 12, 14). In the last few years, the use of 16S rRNA gene sequencing as an alternative method for species identification has increased, while the time and costs (≈$35 to $40) have been reduced (7, 8, 14, 15). The method has been applied for anaerobic bacteria, including isolates from blood cultures, in some studies but not in a routine prospective design (8, 10, 15, 16). To decide in which specific diagnostic situations 16S rRNA gene sequencing could be of benefit in a routine setting with anaerobic bacteria isolated from blood cultures, we performed a comparison between conventional identification and 16S rRNA gene sequencing. The foci of the study were specificity, time, and material cost of the identification.

(The preliminary results from this study were presented at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington DC, 25 to 28 October, 2008 [8a].)

During a 1-year period, 16S rRNA gene sequencing was applied to pure culture material from blood cultures (BACTEC Plus anaerobic/F bottle and BACTEC 9240 automated instrument; Becton Dickinson Diagnostic Instrument Systems, Franklin Lakes, NJ) as soon as anaerobic bacteria, including *Lactobacillus* spp. and *Actinomyces* spp., were suspected (no corresponding aerobic growth). The MicroSeq 500 system (Perkin-Elmer, Applied Biosystems Division, Foster City, CA) was used for the entire period (8, 11, 14). Conventional anaerobic identification was performed simultaneously, using the identification tables in the Manual of Clinical Microbiology, and was based on Gram stain and wet mount characteristics; hemolysis; fluorescence; susceptibility to metronidazole, vancomycin, kanamycin, colistin, and bile; and biochemical testing, including fermentation of carbohydrates and enzyme tests using commercially available substrates and diagnostic tablets/discs (Statens Serum Institut Diagnostica, Copenhagen, Denmark, and Rosco Diagnostica, Taastrup, Denmark) (13). All isolates were tested, including if the same anaerobic bacteria were isolated from more than one blood culture bottle from the same patient (double isolate). The primary outcome parameter was the specificity of the result, i.e., at what level the method was capable of identifying the isolate (species, genus, descriptive, or no result). Identification to the species level was accepted if there was a ≥99% match with the 16S rRNA gene sequence and at the genus level if there was a ≥97 to 99% match according to CLSI guideline MM18-A (3). Consensus sequences were primarily compared with the MicroSeq ID 2.0 500-bp library. If there were no matches at the species level, the sequences were compared with the EzTaxon server, followed by the NCBI BLAST search engine (2). Secondary conventional identification was done retrospectively in cases of incomplete or conflicting primary conventional identification to determine to what extent conventional identification was in accordance with 16S rRNA gene sequence identification. Secondary conventional identification was supplemental testing and/or retesting of isolates based on the same tests as the primary conventional identification. The secondary outcome parameter was the use of resources, i.e., time and material cost. Standardized time and costs were calculated for 16S rRNA gene sequencing, and the time and material costs of conventional identification were

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TABLE 1. Anaerobic bacteria identified to the species or genus level by 16S rRNA gene sequencing ($n = 123$)[a]

| Bacterium (no. of isolates identified[b]) |
|---|
| Gram positive |
| *Actinomyces europaeus* (1/0/0/1/0) |
| *Actinomyces funkeii* (2/0/2/0/0) |
| *Clostridium cadaveris* (1/0/1/0/0) |
| *Clostridium hathewayi* (4/0/4/0/0) |
| *Clostridium paraputrificum* (1/0/1/0/0) |
| *Clostridium perfringens* (5/5/0/0/0) |
| *Clostridium ramosum* (2/0/2/0/0) |
| *Clostridium scindens* (1/0/0/1/0) |
| *Clostridium septicum* (1/1/0/0/0) |
| *Clostridium sordelli* (3/2/1/0/0) |
| *Clostridium symbiosum* (1/0/1/0/0) |
| *Clostridium tertium* (2/2/0/0/0) |
| *Clostridium* sp. (1/0/1/0/0) |
| *Gemella morbillorum* (2/0/0/0/2) |
| *Lactobacillus rhamnosus* (4/0/4/0/0) |
| *Lactobacillus sakei* (1/0/0/1/0) |
| *Lactobacillus salivarius* (1/0/1/0/0) |
| *Peptoniphilus* sp. (1/0/1/0/0) |
| *Propionibacterium acnes* (19/19/0/0/0) |
| *Robinsoniella* sp. (3/0/3/0/0) |
| *Solobacterium moorei* (3/0/0/3/0) |
| *Staphylococcus saccharolyticus* (1/0/0/1/0) |
| *Turicibacter sanguinis* (2/0/0/0/2) |
| Gram negative |
| *Bacteroides dorei* (7/0/0/0/7) |
| *Bacteroides fragilis* (26/26/0/0/0) |
| *Bacteroides nordii* (1/0/1/0/0) |
| *Bacteroides ovatus* (2/0/2/0/0) |
| *Bacteroides thetaiotaomicron* (6/5/1/0/0) |
| *Bacteroides vulgatus* (4/4/0/0/0) |
| *Bacteroides xylanisolvens* (1/0/0/0/1) |
| *Bacteroides* sp. (1/0/1/0/0) |
| *Fusobacterium gonidiaformans* (2/0/2/0/0) |
| *Fusobacterium nucleatum* (2/2/0/0/0) |
| *Fusobacterium* sp. (1/0/1/0/0) |
| *Prevotella* sp. (2/0/0/2/0) |
| *Sneathia sanguinegens* (2/0/0/2/0) |
| *Veillonella dispar* (2/0/2/0/0) |
| *Veillonella rodentium* (1/0/1/0/0) |
| *Veillonella* sp. (1/0/1/0/0) |

[a] Four isolates had no match at ≥97%. The closest matches using the NCBI BLAST search engine (October 2009) were *Butyricimonas virosa* (3/0/0/2/1 [see footnote *b*]) with 87.3% (428/490), 87.5% (435/497), and 87.4% (432/494) identity (GenBank accession no. AB443949.1), and *Actinomyces lingnae* (1/0/0/1/0), with 93.7% (429/458) identity (GenBank accession no. AJ271894.1).

[b] The values in parentheses are total number of isolates/number positive with conventional identification to species level/number positive with conventional identification to genus level/number positive by descriptive criteria/number misidentified by conventional identification in comparison to 16S rRNA gene sequencing result.

estimated using the worksheets from our local microbiology laboratory database. *Bacteroides* spp., *B. fragilis*, *Clostridium* spp., and *Propionibacterium acnes* were also evaluated separately, as they usually represent the majority of anaerobic blood culture isolates.

One hundred and twenty-seven isolates (36 double isolates), corresponding to 91 unique isolates, were identified in 84 patients (Table 1). With 16S rRNA gene sequencing, 113 isolates (89%) were identified to the species level with a ≥99% match and another 7 (5.5%) with a ≥97% match. Three isolates were characterized as *Clostridium* sp. with a match of <97% but were reclassified after the end of the study to a newly described genus, *Robinsoniella* (4). Four isolates had no match of ≥97%. With primary conventional identification, 66 (52%) isolates were identified to the species level and another 34 (26.8%) to the genus level, including the three *Robinsoniella* spp. Fourteen isolates (11%) were only characterized by morphology and Gram stain. Thirteen isolates (10.2%) were misidentified (wrong genus or species) as compared to the results of 16S rRNA gene sequencing. The corresponding results without double isolates ($n = 91$) were as follows: with 16S rRNA gene sequencing, 87.9% identification to species and 6.6% to genus, and with conventional identification, 51.6% to species and 27.5% to genus, 12.1% descriptive, and 8.8% misidentified.

After retrospective secondary conventional identification, another 30 isolates were identified to the species level in accordance with the results of 16S rRNA gene sequencing. Only one isolate, which was suspected to be anaerobic, was misclassified. An isolate primarily identified as a *Peptostreptococcus* sp. by conventional identification was identified as *Staphylococcus caprae* (usually considered to be facultative) with 16S rRNA gene sequencing (data not included) (13). Time to identification was 1.5 days with 16S rRNA gene sequencing and a mean (range) of 2.8 days (1 to 7 days) with conventional identification. Material costs were $35 to $40 for 16S rRNA gene sequencing and a mean (range) of $10 ($2 to $23) for conventional identification. Only *P. acnes* could be identified to the species level faster (1.3 days) (100% specificity) and at a lower cost ($4) with conventional identification. Time and material costs were 2.4 days and $12.70 for *Bacteroides* spp., 2.2 days and $12.70 for *B. fragilis*, and 3.2 days and $15.70 for *Clostridium* spp.

The results for identification to the species and genus level by conventional methodology in this study are comparable to those of other studies (15). In our laboratory, species like *Sneathia sanguinegens*, *Solobacterium moorei*, and *Turicibacter sanguinis* would not have been identified to the species level in a reasonable time with conventional methodology. The identification of *Sneathia sanguinegens* and *Solobacterium moorei* are good examples of the importance of species identification with prompt recognition of the primary site of infection (17). *Sneathia sanguinegens* (female genital tract) was isolated from a patient with postpartum fever, and *Solobacterium moorei* (oral cavity) from a patient with a dental abscess (5, 9). The recognition of contaminants is also important to avoid unnecessary diagnostic testing. *Propionibacterium acnes* is very often considered a contaminant when isolated from blood cultures, and indeed, in our study, none of the *P. acnes* isolates were associated with true infection. All other isolates were considered clinically significant pathogens, although *Turicibacter sanguinis* and *Robinsoniella* have just recently been described and only a single case report exists on each species (1, 4). Hopefully, species identification with 16S rRNA gene sequencing will further clarify the clinical significance and association between the primary site of infection of both old and new anaerobic bacteria.

In the study, only material costs were calculated and not labor or equipment costs. Conventional identification material costs were lower than those of 16S rRNA gene sequencing. However, the time for identification was longer, with a decrease in specificity. Furthermore, if attempts had been made primarily to identify isolates to the species level with conven-

tional methodology, this would have further prolonged the time, workload, and costs of identification. Undoubtedly, the clinical microbiology specialists used less time per isolate with 16S rRNA gene sequencing, and it is an attractive alternative to conventional identification of anaerobic bacteria from blood cultures. Laboratory technician workload would depend on the number of isolates that were sequenced simultaneously, as more isolates would result in less time per isolate. Only *P. acnes* was identified faster and at a lower cost with conventional identification. Since the end of the study, the time for 16S rRNA gene sequencing has been reduced with the use of a fast-cycle program, and it can be performed within 8 h if required.

Although material costs are relatively high, 16S rRNA gene sequencing is now applied in our laboratory for anaerobic bacteria from blood cultures and other sterile body sites, e.g., pleural fluid, bones, and joints (except for cases where *P. acnes* is suspected). However, it should be emphasized that the use of 16S rRNA gene sequencing of anaerobic bacteria from blood cultures and other sterile body sites alone is probably not cost effective if equipment costs are included. Consequently, we run several 16S rRNA gene sequencing tasks (other difficult-to-identify bacteria and culture-negative clinical specimens) simultaneously to maximize cost benefit.

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