Phenotypic and Molecular Characterization of Solobacterium moorei Isolates from Patients with Wound Infection

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Though seldom reported, Solobacterium moorei, which was first described in 2000, has been identified in specimens from patients with root canals, periradicular lesions, periodontal disease, dentoalveolar abscesses, bacteremia, septic thrombophlebitis, and halitosis. In the present study, we describe 9 cases of mixed wound infection, from a pool of 400 surgical wound infections that we have studied, in which S. moorei was isolated or found in a clone library. All isolates of S. moorei were identified by 16S rRNA gene sequence analysis, and then six were examined for their physiological and biochemical characteristics and for antimicrobial susceptibility. The results of the present study indicate that Solobacterium moorei may be a significant component in some mixed surgical wound infections and that surgical management and antimicrobial therapy may be indicated when these bacteria are identified in significant situations.

Solobacterium moorei, a non-spor forming Gram-positive anaerobic bacillus previously known as “Bulleidia extructa” or “Bulleidia moorei,” was first reported from human feces in 2000 (4) and was considered an indigenous member of the intestinal microflora. The original B. moorei (now S. moorei, the only member of the genus Solobacterium) shows only 93% sequence similarity to B. extructa and has other differences as well; that is why a new genus was created for it. S. moorei has only 88% sequence similarity to Erysipelothrix rhusiopathiae and Holdemania filiformis. The involvement of S. moorei in human infections has been described in recent years; to date, it has been identified in specimens from patients with root canals (8), periradicular lesions (9), periodontal disease and dentoalveolar abscesses (2), bacteremia (1, 6, 7), and halitosis (3, 5). The cases of bacteremia occurred in a patient with acute proctitis and carcinoma of the cervix (6), a patient with multiple myeloma (1), and an intravenous drug user (IVDA) who had femoral vein thrombophlebitis with septic pulmonary embolism and whose blood cultures also yielded Fusobacterium nucleatum and Bacteroides ureolyticus in addition to Solobacterium moorei (7).

In our study, we found 9 cases of wound infection with mixed aerobic and anaerobic bacteria, among 400 cases studied, that included Solobacterium moorei; we cultured the specimens and then characterized the different pure colony types by 16S rRNA gene sequencing. We further characterized six of the isolates by physiological and biochemical tests and by antimicrobial susceptibility profiles.

MATERIALS AND METHODS

Samples from patients’ wounds were taken, transferred to the lab in anaerobic transport vials (Anaerobe Systems) within 2 h, and then processed within 24 h of collection. The specimens were processed by standard methods, using brucella blood agar, and aerobic and anaerobic bacteria were isolated for identification. The Solobacterium moorei type strain (CCUG 39336) was included in biochemical and antimicrobial susceptibility testing, for purposes of comparison. Isolated strains were maintained at ~70°C in double-strength skim milk before characterization.

Surgical management. Wounds were treated by drainage and debridement, as required, and by antimicrobial therapy.

Identification and physiological and biochemical characterization. Identification was performed on isolated colonies by 16S rRNA sequencing analysis as described previously (10). Solobacterium moorei grows well, though slowly and with small colonies, on brucella blood agar. Both the Rapid ID 32A and API ZYM systems (bioMérieux, Hazelwood, MO) were used for further biochemical and enzymatic activity examination according to the manufacturer’s instructions.

Antibiotic susceptibility testing. Susceptibility to 16 antimicrobials was determined by Etest (bioMérieux, Hazelwood, MO) according to the manufacturer’s instructions. Bacteroides fragilis ATCC 25285 and Bacteroides thetaiotaomicron ATCC 29741 were used as quality control strains, as recommended. Zones of inhibition were measured after 24 h of incubation, except for clindamycin, results for which were confirmed after 48 h.

Sequencing. Genomic DNA was extracted and purified from bacterial cells in the mid-logarithmic-growth phase by using a QIAamp DNA minikit (Qiagen, Inc., Chatsworth, CA). The 16S rRNA gene fragments were amplified by standard methods. Two subregions of the 16S rRNA gene were amplified by using two pairs of primers. The first part of the 16S rRNA gene was a region of approximately 800 bp between primers 8UA and 907B. The second part of the 16S rRNA gene was a region of approximately 700 bp between primers 8UA and 907B. Both were sequenced to obtain the complete 16S rRNA gene sequence. PCR was performed for 35 cycles of 30 s at 95°C, 30 s at 45°C, and 1 min at 72°C, with a final extension at 72°C for 5 min. The PCR products were excised from a 1% agarose gel after electrophoresis and were purified using a QIAquick gel extraction kit. The purified PCR products were sequenced directly with BigDye Terminator cycle-sequencing kits (Applied Biosystems, Foster City, CA) on an ABI Prism 3100-Avant genetic system (Applied Biosystems, Foster City, CA).

The sequencing data were analyzed by comparison of the consensus sequences with GenBank sequences using BLAST software, and the percentage of similarity to other sequences was determined. Closely related sequences were retrieved from GenBank and were aligned with the newly determined sequences by using the CLUSTAL W program.
TABLE 1. Clinical and cultural data on *Solobacterium moorei* infections

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Source</th>
<th>Comments</th>
<th>Bacteria (CFU counts) identified by 16S rRNA sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Left thigh swab</td>
<td>Spontaneous abscess of 3 wks’ duration; source unknown</td>
<td><em>Solobacterium moorei</em> (1 × 10⁸), <em>Actinomyces europaeus</em> (1 × 10⁹), <em>Enterococcus faecalis</em> (1.15 × 10⁷), <em>Stenotrophomonas maltophilia</em> (4.5 × 10⁸), <em>Corynebacterium spl. sp.</em> (2 × 10⁷), <em>Corynebacterium urealyticum</em> (1 × 10⁷)</td>
</tr>
<tr>
<td>22</td>
<td>Abdominal wound abscess</td>
<td>Perforated appendix</td>
<td><em>Peptostreptococcus micros</em> (8 × 10⁸), <em>Slackia exigua</em> (8 × 10⁸), <em>Solobacterium moorei</em> (1 × 10⁸), <em>Bacteroides fragilis</em> (1 × 10⁷), <em>Enterococcus avium</em> (1 × 10⁸), <em>Eikenella corrodens</em> (1 × 10⁷), uncultured bacterium (human fecal flora) (1 × 10⁷), <em>Escherichia coli</em> (1 × 10⁷), <em>Bilophila wadsworthia</em> (3 × 10³)</td>
</tr>
<tr>
<td>28</td>
<td>Furuncle, right axilla</td>
<td></td>
<td><em>Staphylococcus aureus</em> (3 × 10⁹), <em>Propionibacterium</em> (5 × 10⁸), clone library (27 clones, comprising 25 clones of <em>Staphylococcus aureus</em> and 1 clone each of <em>Solobacterium moorei</em> and <em>Corynebacterium amylolacticum</em>)</td>
</tr>
<tr>
<td>51</td>
<td>Abdominal wound infection after mesh placement for nonhealing ventral hernia repair</td>
<td></td>
<td><em>Prevotella nigrescens</em> (2.6 × 10⁹), <em>Parvimonas micra</em> (2 × 10⁹), <em>Streptococcus constellatus/Streptococcus anginosus</em> (1 × 10⁸), <em>Fusobacterium nucleatum</em> (1 × 10⁷), <em>Actinomyces turicensis</em> (1 × 10⁹), <em>Dialister pneumosintes</em> (1 × 10⁹), <em>Dialister pneumosintes</em> (1 × 10⁹), <em>Bacteroides ureolyticus</em> (1 × 10⁸), <em>Pseudomonas aeruginosa</em> (1 × 10⁸), <em>Bacteroides fragilis</em> (6 × 10⁸), <em>Solobacterium moorei</em> (1 × 10⁸), <em>Bacteroides ovatus</em> (1 × 10⁷)</td>
</tr>
<tr>
<td>91</td>
<td>Perirectal abscess</td>
<td>Intermittent 1-yr history of this problem; recently released from prison</td>
<td><em>Actinomyces turicensis</em> (1 × 10⁹), <em>Streptococcus anginosus</em> (1 × 10⁸), <em>Streptococcus constellatus</em> (1 × 10⁷), <em>Bacteroides species</em> (1 × 10⁸), <em>Prevotella disiens</em> (1 × 10⁹), <em>Megasphaera species</em> (8 × 10⁹), <em>Fusobacterium nucleatum</em> (2 × 10⁸), <em>Solobacterium moorei</em> (1 × 10⁷), uncultured bacterium (1 × 10⁷), <em>Porphyromonas species</em> (4 × 10⁸)</td>
</tr>
<tr>
<td>121</td>
<td>Perirectal abscess</td>
<td>Spontaneous; history of diabetes mellitus</td>
<td><em>Parvimonas micra</em> (1.3 × 10⁹), <em>Solobacterium moorei</em> (5 × 10⁸), <em>Actinomyces turicensis</em> (5 × 10⁹), <em>Bacteroides fragilis</em> (5 × 10⁹), <em>Fusobacterium equinus</em> (4 × 10⁸), <em>Streptococcus constellatus</em> (1 × 10⁷), <em>Staphylococcus aureus</em> (1 × 10⁷), <em>Escherichia coli</em> (1 × 10⁷)</td>
</tr>
<tr>
<td>152</td>
<td>Infected pilonidal cyst</td>
<td></td>
<td><em>Parvimonas micra</em> (1.02 × 10⁹), <em>Solobacterium moorei</em> (5 × 10⁹), uncultured <em>Clostridiales</em> bacterium (4 × 10⁸), <em>Streptococcus anginosus</em> (1 × 10⁹), <em>Streptococcus constellatus</em> (3 × 10⁷), bacterium N14-24 (4 × 10⁸), <em>Peptostreptococcus species</em> (2 × 10⁶), <em>Bilophila wadsworthia</em> (1 × 10⁸)</td>
</tr>
<tr>
<td>232</td>
<td>Right thigh</td>
<td>Abscess for 2–3 days; IVDA, heroin</td>
<td><em>Streptococcus anginosus</em> (5 × 10⁹), <em>Parvimonas micra</em> (2 × 10⁹), <em>Atopobium species</em> (9.3 × 10⁹), <em>Solobacterium moorei</em> (2.5 × 10⁹), <em>Streptococcus constellatus</em> (1 × 10⁷)</td>
</tr>
<tr>
<td>361</td>
<td>Pilonidal abscess</td>
<td>Recurrent × 5; family history of same problem</td>
<td><em>Parvimonas micra</em> (8 × 10⁹), <em>Solobacterium moorei</em> (8 × 10⁹), <em>Fusobacterium gordonii</em> (5 × 10⁹), <em>Atopobium minutum</em> (4 × 10⁹), <em>Slackia exigua</em> (2 × 10⁹), <em>Prevotella intermedia</em> (2 × 10⁹), <em>Tannerella forsythia</em> (1 × 10⁹), <em>Parvimonas micra</em> (1 × 10⁹), <em>Peptostreptococcus stornatis</em> (1 × 10⁹), <em>Bacteroides vulgatus</em> (1 × 10⁹), <em>Actinomyces turicensis</em> (8 × 10⁹)</td>
</tr>
</tbody>
</table>

**Clone libraries.** Two microliters of community DNA (extracted from original specimens, such as pus) was first amplified with primers 8UA and 907B and then purified. The purified products were ligated into PCRI.1-TOPO (Invitrogen) as specified by the manufacturer. Competent *Escherichia coli* cells were transformed with ligation products by a heat shock (30 s at 42°C). Recombinant *E. coli* cells were selected on Luria-Bertani medium with kanamycin (50 µg/ml) and with isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Colonies that contained a plasmid with an insert (which cannot produce β-galactosidase and consequently are white, because they cannot degrade X-Gal) were picked for sequencing. DNA preparations for sequencing were made with a QIAprep Spin plasmid kit (Qiagen). Plasmids were eluted with 35 µl of elution buffer, and the plasmid inserts were sequenced with an automated ABI 3100 sequencer (Applied Biosystems). Sequences were compared to those in GenBank using BLAST software.

**Nucleotide sequence accession numbers.** The 16S rRNA gene sequences obtained for the six *S. moorei* strains in this study have been deposited in the GenBank database under accession numbers GQ182979 to GQ182984.

**RESULTS**

Clinical and laboratory data are presented in Table 1. In all infections, *S. moorei* was present together with other anaerobes and/or aerobes, and isolates are listed from the most to the least dominant in quantity. For five of the nine patients (patients 21, 22, 121, 232, and 361), *S. moorei* was part of the dominant flora numerically (Table 1). For one patient (patient 28), *Solobacterium* was not cultured but was recovered in a clone library; only a single clone was recovered, and the *Solobacterium* was clearly not important in this infection. Two patients were hospitalized; the others were treated as outpatients. All infections resolved after routine surgical management and various antimicrobial regimens.

The 16S rRNA gene sequences were obtained for these six
strains and deposited in GenBank. The 16S rRNA gene sequence analysis results indicated that all nine isolates had 100% identity to each other, and also 100% sequence similarity to other well-characterized strains in GenBank (N407 0592, which was isolated from a patient with septicemia, and an oral isolate, AHP 13983).

By Rapid ID 32A testing, detectable enzyme activities found in all six S. moorei isolates tested and in the type strain (CCUG 39336) included arginine dihydrolase, α- and β-galactosidase, α-glucosidase, arginine arylamidase, proline arylamidase, and leucine arylamidase. N-Acetyl-β-glucosaminidase, alkaline phosphatase, and nitrate reduction were found only in the six clinical isolates, not in the type strain; β-galactosidase phosphatase was detected in just one of the six clinical isolates. The other 18 reactions were all negative for all clinical isolates and the type strain.

By API ZYM testing, detectable enzyme activities found in our isolates and the type strain included leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, and α-glucosidase. Acid phosphatase was found in the six clinical isolates but not in the type strain. In contrast, valine arylamidase was found only in the type strain, not in the six clinical isolates, and esterase (C4) was detected only in one of the clinical strains. None of the strains were positive by the remaining 12 tests.

Differences in results between different commercial kits are not uncommon and may be due to different amounts of the substrate or different reagents for detection. In this study, the differences related to variable results with certain reagents. We considered only results where all strains tested either positive or negative.

In vitro susceptibility test results demonstrated that all S. moorei strains were susceptible to all antimicrobials tested except for trimethoprim-sulfamethoxazole, to which all were resistant (MIC, >32 µg/ml). Virtually all S. moorei strains were susceptible to <1 µg/ml of all of the antimicrobials tested (ampicillin-sulbactam, amoxicillin-clavulanate, benzylpenicillin, cephalothin, cefotaxime, cefoxitin, clindamycin, ertapenem, imipenem, levofloxacin, meropenem, metronidazole, piperacillin-tazobactam, ticarcillin-clavulanate, and vancomycin), except for two strains with metronidazole MICs of 1.5 µg/ml, one strain with a cefoxitin MIC of 1.5 µg/ml, and one strain with a levofloxacin MIC of 1 µg/ml.

**DISCUSSION**

The procedures followed in our study were related to the research nature of the study and would not be indicated, or practical, for use by clinical laboratories.

*Solobacterium moorei* is described as an obligately anaerobic, non-spore-forming, Gram-positive bacillus. It grows slowly and produces relatively few positive biochemical reactions in commercially available identification kits. These characteristics and the fact that the phenotypic variations appear to be commonly exhibited by different strains of *S. moorei* make detection and identification of the bacterium, and hence understanding of its epidemiology and clinical significance, difficult. The results in our study demonstrated the same phenotypic variety as was shown in the literature. Clearly, phenotypic tests of the types studied to date are not adequate for the identification of the organism.

As a new genus, first described in 2000 (4), *Solobacterium* was classified on the basis of 16S rRNA gene sequence analysis. Thus far, all isolates of *S. moorei* have been identified exclusively by 16S rRNA gene sequencing, including the isolates from this study.

The difficulty of culture and identification of *S. moorei* has undoubtedly caused an underestimation of its clinical significance in the past. However, the number of reports involving *S. moorei* has increased in recent years (1–3, 5–9). Here we describe nine cases of surgical wound infection with mixtures of aerobic and anaerobic bacteria involving *S. moorei*; six of the strains isolated were studied. The significance of *S. moorei* in the infections we studied cannot be determined, but it was part of the numerically dominant flora in five of our patients and probably contributed to the pathology in these patients. It is noteworthy that Schirrmeister et al. (9) found that *Solobacterium moorei* and *Fusobacterium nucleatum* were the most common isolates in their study of 10 periradicular infections; these organisms were each found in six of the patients, and they were found in the same infections in five of these cases. In the case described by Martin et al., the two organisms were associated, along with *Bacteroides ureolyticus*, in bacteremia (7). In our series of nine cases, these two organisms were found together in two of the cases, and *Solobacterium* was found with two other species of *Fusobacterium* (*Fusobacterium equinum* and *Fusobacterium gondii/formans*) in one additional case each. These organisms may act synergistically, and/or each may supply growth factors for the other.

The literature review showed that *S. moorei* can cause serious infections, such as bacteremia. The data from our study suggest that *S. moorei* may be more common in clinical specimens than previously appreciated, but still it was found in only 9 of 400 surgical wound infections studied. However, this organism may well be more important at other sites and in other types of infection; the literature reports certainly indicate its significance in dental/oral infections. In our study, all six isolates studied were susceptible to the antimicrobial agents tested, except for trimethoprim-sulfamethoxazole. In terms of antimicrobial therapy, any regimen suitable for nonresistant anaerobic bacteria in general will be appropriate for *Solobacterium*. As with other anaerobic infections, debridement and drainage are commonly required.

**ACKNOWLEDGMENTS**

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**REFERENCES**