First Human Case of Fastidiosipila sanguinis Infection

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Fastidiosipila sanguinis is a Gram-positive anaerobic coccus. We report the first case of osteitis implicating this species. The strain was accurately identified by 16S rRNA sequence analysis, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification having failed. The reservoir remained unclear; an endogenous origin is suspected.

CASE REPORT

A 84-year-old woman presented a purulent collection at the left 4th toe, excised the day before admission; amoxicillin-clavulanate (3 g/day) was instituted. Clinical examination revealed an open wound, edema, erythema, and heat and pain in the left 4th toe and dorsal face. An X ray detected osteitis. There was no fever or history of occlusive arterial disease or type 2 diabetes. Hematology found 1,370 g/liter hemoglobin, 344 × 10⁹/liter platelets, 9.95 × 10⁹/liter leukocytes, and 5.5 mg/liter C-reactive protein. After debridement and sampling, amoxicillin (3 g/day) was administered for 1 week intravenously and then 11 weeks orally. After 3 months, antibiotic therapy could be stopped, and the patient was discharged.

Subcutaneous tissue, capsular tissue, cortical bone, cancellous bone, and synovial membrane were sampled. After 2 min of grinding (Retsch MM401 bead-mill) followed by Gram staining, samples were inoculated into brain heart infusion (BHI) broth (AES, Bruz, France), incubated for 24 h (37°C), and then subcultured onto chromogenic agar (CPS agar), chocolate agar with PolyViteX (PVX), and Columbia agar with 5% sheep blood (COS agar) (bioMérieux, Marcy l’Etoile, France). The CPS agar was incubated at 37°C under aerobic conditions, the PVX agar at 37°C under a 5% CO₂-enriched atmosphere, and the COS agar under anaerobic conditions at 37°C and examined daily for 7 days. The subculture results are summarized in Table 1.

All specimens were negative on direct microscopy after Gram staining. After 24 h, cancellous bone was positive for Streptococcus agalactiae on all 3 subculture agar plates. After 96 h, pinpoint gray colonies (Fastidiosipila sanguinis BRE20130822) were detected on PVX agar and COS agar in capsular tissue, cortical bone, and synovial membrane. F. sanguinis BRE20130822 showed small Gram-positive coccoid chains and was catalase negative. Microflex LT mass spectrometry (MALDI-Biotyper 2.0 software, MBT-BDAL-5627 MSP library; Bruker Daltoniks GmbH, Bremen, Germany) gave Clostridium halophilum as the best match (log score, 2.022). Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) results were discordant with the phenotype, notably with the Gram-staining features revealing cocci. Discrepancies were resolved molecularly. Complete 16S rRNA gene sequencing of F. sanguinis BRE20130822, as previously described (1), revealed 99% homology with GenBank F. sanguinis CCUG 47711 (CIP 108292).

Antibiotic susceptibility was investigated by disk diffusion and MICs by Etest (AES). Following the guidelines of the French Society for Microbiology Antibiogram Committee (http://www.sfm-microbiologie.org/), susceptibilities were found to benzylpenicillin (MIC, <0.064 µg/ml), amoxicillin (MIC, <0.064 µg/ml), vancomycin, imipenem, rifampin, and clindamycin, and resistance was found to metronidazole (MIC, >256 µg/ml), levofloxacin (MIC, >32 µg/ml), and ciprofloxacin.

Fastidiosipila was proposed as a genus in 2005, comprising a single species, F. sanguinis (2), described as a Gram-positive, non-spor-forming, small coccus, showing growth under anaerobic and 2- to 6% O₂ conditions. Likewise, we found an anaerobic but

### Table 1. Summary of bacterial subculture characteristics for diagnosis of Fastidiosipila sanguinis osteitis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result for medium and culture conditions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPS agar, 37°C, aerobic</td>
</tr>
<tr>
<td>Subcutaneous tissue</td>
<td>Sterile</td>
</tr>
<tr>
<td>Capsular tissue</td>
<td>Sterile</td>
</tr>
<tr>
<td>Cortical bone</td>
<td>Sterile</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>S. agalactiae (1 day)</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>Sterile</td>
</tr>
</tbody>
</table>

*The number of days of culture is shown in parentheses. CPS agar, CPS ID3 chromogenic agar; PVX agar, chocolate agar plus PolyViteX; COS agar, Columbia agar plus 5% sheep blood.
aerotolerant isolate of fastidious growth, requiring 96 h for pinpoint colonies. F. sanguinis grows poorly in fastidious anaerobic broth (2); attempts to obtain the MIC by microdilution using Mueller-Hinton broth supplemented with lysed horse blood failed. However, susceptibility testing did not follow recommendations, and results are not definitive.

Initially (2), 2 F. sanguinis strains were isolated from elderly male human clinical blood samples; the origin of bacteremia was undetermined, and the species habitat was unknown. No further isolates have been described. After identifying F. sanguinis strain BRE20130822 from 3 deep sites (capsular tissue, cortical bone, and synovial membrane), we report the first case of F. sanguinis osteitis. Gram-positive anaerobic cocci like Fastidiosipila are frequently present in deep-seated anaerobic soft tissue infections, bone and joint infections (3, 4), and acute and chronic wounds (5). They constitute a heterogeneous group of bacteria, with many taxonomic revisions over the last few years (6, 7).

The MALDI-TOF MS identification as C. halophilum was erroneous. 16S rRNA analysis identified the 3 isolates approximating F. sanguinis (99% homology) to the species level: the results for Clostridium and Fastodiosipila were very close (see an unrooted tree based on 16S rRNA sequencing in Fig. 1). For Falsen et al., Fastidiosipila displayed the closest relatedness to the Clostridium subphylum, which is highly distinct from all other recognized genera of Gram-positive anaerobic cocci (2).

BLAST search revealed greater homology (100%) with 16S rRNA gene sequences of uncultivated bacteria from microbiome analysis of skin and ileous samples than with F. sanguinis CIP 108292 (8–10). The results suggested an endogenous origin for the F. sanguinis isolate in the present case of osteitis. Moreover, Gram-positive anaerobic cocci prevail in healthy subjects’ microbiomes, colonizing skin, mouth, and upper airway mucosa and gastrointestinal and female genitourinary tracts (3).

In conclusion, Fastidiosipila is difficult to identify and isolate: its pathogenicity to humans has been underestimated, and the organism requires genomic analysis for accurate identification and complex sample detection.

Nucleotide sequence accession number. The 16S rRNA sequence from strain BRE20130822 has been deposited in GenBank under accession no. KJ419955.

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REFERENCES


