Globicatella sanguinis meningitis associated with human carriage

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Abstract

*Globicatella sanguinis* is a rare cause of acute meningitis. We demonstrated human carriage of *Globicatella* by identifying cefotaxime-resistant strains in groin and rectal specimens nine months after the invasive infection. The pathogen strain isolated from the cerebrospinal fluid and the carriage strains were accurately identified by *sodA* gene sequence analysis.
A 56-year-old woman presented with vomiting and headache, associated with instability when walking, memory impairment and clinical signs of meningitis. Lumbar puncture revealed a turbid cerebrospinal fluid (CSF) with pleiocytosis (11800 $\times$ 10$^6$ white blood cells/liter with 94% polymorphonuclear cells), a glucose concentration of 0.59g/L, and a protein concentration of 2.32 g/L. Gram-positive cocci were observed, subsequently identified as *Globicatella sanguinis* strain HDP50220. Based on the results of antibiotic susceptibility tests, the primary antibiotic treatment which consisted of cefotaxime (100 mg/kg/day intravenously [i.v.]) combined with fosfomycin (200 mg/kg/day i.v.) was replaced by amoxicillin (200 mg/kg/day i.v.) for three weeks. Remission of meningitis was confirmed by the absence of bacterial growth from a follow-up CSF sample. Nine months later, *Globicatella* isolates HDP60616 and HDP60617 were retrieved from the groin and rectum, respectively.

The *Globicatella* CSF strain grew on blood and chocolate agar (Oxoid, Wesel, Germany) incubated for 18h at 37°C under atmosphere enriched in 5% of CO$_2$ as pinpoint α-hemolytic colonies; Gram-positive, catalase-negative cocci organized into short chains and clusters were observed. Biochemical characteristics of the strain obtained from the rapid ID32 STREP system corresponded to *G. sanguinis* (profile 623.761.733.50, certainty of 99.9%), whereas those from the API 20 STREP system corresponded to *Aerococcus viridans* (profile 7306773, certainty of 88.9%) (bioMérieux, Marcy l’Etoile, France) (Table 1).

The antibiotic susceptibility of the isolate was investigated by the disk diffusion method and MICs were determined with the E-test (AES, Combourg, France). The Antibiogram Committee of the French Society for Microbiology criteria for *Streptococcus* spp. were used for the interpretation of antimicrobial drug susceptibility (http://www.sfm.asso.fr). The strain was susceptible to benzylpenicillin (MIC = 0.125

3/11
µg/mL), amoxicillin (MIC ≤ 0.016 µg/mL), chloramphenicol, tetracycline, pristinamycin, rifampin, fosomycin, vancomycin, and teicoplanin, and resistant to cefotaxime (MIC ≥ 32 µg/mL), erythromycin (MIC = 32 µg/mL), and clindamycin (MIC ≥ 256 µg/mL). The presence of the mefA, ermB, and ermA genes was tested by multiplex PCR as previously described (7, 9). Amplification was obtained for ermA, but not for the other genes.

Swab samples from the nasal fossae, throat, armpit, groin, and rectum were plated on colistin-nalidixic blood agar plates, for the investigation of colonization (Oxoid). Cefotaxime-resistant α-hemolytic colonies growing around disks loaded with 30 µg of cefotaxime (Biorad, Marnes-La-Coquette, France) placed on the agar surface were selected for further identification. Two Globicatella strains, HDP60616 and HDP60617, were identified from the groin and the rectum, respectively. They were both resistant to cefotaxime (MICs ≥ 32 µg/mL).

Complete 16S rRNA gene sequencing of the three isolates from the patient and of the type strains G. sanguinis CIP 107044T (ATCC 51173T) and Globicatella sulfidifaciens CIP 107175T was carried out as previously described (3). The two type strains were found to be more than 99% similar. Species differentiation was achieved by sodA sequencing as described by Poyart et al. (5). The sodA sequences of the two type strains were 96.6% identical. The sodA sequences of HDP50220 CSF (GenBank accession no. EU649715) and HDP60616 groin isolates were 100% identical to each other. They were 99.8% and 96.3% identical to those of G. sanguinis CIP 107044T (GenBank accession no. EU649714) and G. sulfidifaciens CIP 107175T (GenBank accession no. EU649713), respectively. The HDP60617 rectal isolate (GenBank accession no. EU649716) sodA sequence was 99.8% identical to that of G. sulfidifaciens and 96.3% identical to that of G. sanguinis. Pulsed-field gel electrophoresis showed that the HDP50220 and HDP60616 isolates were closely related and clearly differed from the rectal isolate and the two type strains (Fig. 1).
Globicatella was proposed as a new genus, initially with only one species, *G. sanguinis* (2). A second species, *G. sulfidifaciens*, was subsequently described in animals (12). The few reports of *Globicatella* spp. isolation from humans concerned only cases of infection with *G. sanguinis* (1, 4, 6, 8). We report here the first case of human carriage of both species of *Globicatella* which was documented several months after the patient had recovered from an acute meningitis. A strain of *G. sanguinis* identical to the meningitis strain was isolated from groin, while a strain of *G. sulfidifaciens* was isolated from the rectum. These two bacterial species are difficult to distinguish from related catalase-negative gram-positive cocci. Caution is required in interpretation according to the previously described phenotypic identification system, as only four strains were investigated for the description of *G. sulfidifaciens* (12). Indeed, the various descriptions of *Globicatella* strains report different phenotypic reactions for the same species (Table 1). Among them, production of pyrrolidonyl-arylamidase and absence of production of leucine aminopeptidase might contribute to the presumptive identification of *Globicatella* spp. but do not distinguish them from *Aerococcus viridans*. Genotypic study is therefore required for accurate identification.  

16S rRNA gene analysis was useful for identifying the three isolates to the genus level, but not to the species level, due to the high degree of similarity of 16S rRNA sequences between the species (> 99%). By contrast, the *sodA* gene provided an accurate discrimination between the two species.

The pathogenic role of *Globicatella* spp. remains partially known because of the difficulties involved in identification and the small number of reports. *G. sanguinis* has been reported to cause meningitis or bacteremia in humans (1, 2, 4), whereas *G. sulfidifaciens* was thought to be a secondary agent of suppurative processes in animals (12). This report confirms the role of *G. sanguinis* as a human pathogen, and furthermore demonstrates that
Globicatella spp. are commensal organisms in humans. Indeed, both species were isolated from the patient in the absence of any symptom. The sites from which they were isolated, the rectum and groin, are consistent with an intestinal bacterial reservoir, as previously suggested (4). The close relationship between the CSF and groin isolates was primarily deduced from the identity of phenotypic characters (Table 1) and of sodA sequences. It was confirmed by similarities in PFGE patterns (Fig. 1). As a farmer, the patient has been exposed to the intestinal flora of animals. However, at the time of the carriage investigation, she was no longer keeping cows, therefore the animals with which she had previously been in contact could not be explored.

MICs determination confirmed the resistance to cefotaxime of both invasive and colonizing Globicatella strains. Cefotaxime resistance has been previously reported by Shewmaker et al. in 48% of 27 clinical isolates of G. sanguinis (8). This unexpected resistance to cefotaxime may be a useful marker for presumptive identification of this Streptococcus-like bacterium, although half of the strains tested by Shewmaker et al. were susceptible to cefotaxime (8). We found that the invasive strain HDP50220 was also resistant to erythromycin and clindamycin in relation to the presence of the ermA gene. The presence of the ermA gene has not previously been reported in Globicatella spp. Indeed previous reports have implicated only the mefA gene in the erythromycin resistance of Globicatella strains (6, 8, 12).

This case report highlights the difficulties involved in identifying Globicatella, resulting in underestimation of the potential role of this bacterium as a human pathogen, and the need of accurate genomic analysis.
Acknowledgments

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TABLE 1. Phenotypic characteristics of *Globicatella* isolates as compared to previous reports (2, 6, 8, 12)

<table>
<thead>
<tr>
<th>Phenotypic characteristics</th>
<th>G. sanguinis</th>
<th>G. sulfidifaciens</th>
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<tr>
<td></td>
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<td>HDP isolates</td>
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<tr>
<td></td>
<td>(2)</td>
<td>(8)</td>
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<tr>
<td>Hippurate hydrolysis</td>
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<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;S production</td>
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<td>nd</td>
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<td>Production of</td>
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<tr>
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<td>V</td>
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<td>-</td>
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<tr>
<td>β-galactosidase</td>
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<sup>a</sup>+ , 85% or more of the strains tested positive; -, 15% or less of the strains tested positive; V, 16 to 84% of the strains tested positive; +/-, test positive on API 20 STREP and negative on Rapid ID 32 STREP; nd, not determined; CSF, cerebrospinal fluid.

<sup>b</sup>HDP50220, HDP60616 and HDP60617 strains presented the common characteristics of *Globicatella* spp. They grew in 6.5% NaCl BHI broth. They hydrolyzed esculin and produced pyrrolidonyl-arylamidase; they were negative for leucine aminopeptidase, bile-esculin, urease, and Voges-Proskauer tests. No reaction of the Lancefield extracts with group A, B, C, D, F, or G antisera (Prolex<sup>TM</sup> Streptococcal Grouping Latex Kit, Pro-Lab, Strasbourg, France).

<sup>c</sup>including the 9 from Collins *et al.* (2).
FIG. 1. Pulsed-field gel electrophoresis of the three clinical strains of *Globicatella* from the patient and the type strains of *G. sanguinis* and *G. sulfidifaciens*. Patterns were obtained by *SmaI* macrorestriction, with a method adapted from that of van den Braak et al. (11). M, molecular weight marker: 48.5 to 970 kb (BioRad 170-3635). Lane 1, strain *G. sanguinis* CIP 107044<sup>T</sup>. Lane 2, strain *G. sulfidifaciens* CIP 107175<sup>T</sup>. Lane 3, strain HDP50220 (isolated from CSF). Lane 4, strain HDP60616 (isolated from the groin). Lane 5, strain HDP60617 (isolated from the rectum). According to Tenover et al., strain HDP50220 and strain HDP60616 are closely related, as their profiles differ by only three DNA fragments (10). These patterns differed from the pattern of the rectal strain and from the individual patterns of the type strains.