MAJOR ARTICLE







Globicatella sanguinis Osteomyelitis and Bacteremia: Review of an Emerging Human Pathogen with an Expanding Spectrum of Disease

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Background. Globicatella sanguinis is an uncommon pathogen that may be misdiagnosed as viridans group streptococci. We review the literature of *Globicatella* and report 2 clinical cases in which catalase-negative Gram-positive cocci resembling viridans group streptococci with elevated minimum inhibitory concentrations (MICs) to ceftriaxone were inconsistently identified phenotypically, with further molecular characterization and ultimate identification of *G sanguinis*.

Methods. Two clinical strains (from 2 obese women; 1 with a prosthetic hip infection and the other with bacteremia) were analyzed with standard identification methods, followed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry, 16S recombinant ribonucleic acid (rRNA), and *sodA* polymerase chain reaction (PCR). The existing medical literature on *Globicatella* also was reviewed.

Results. Standard phenotypic methods failed to consistently identify the isolates. 16S PCR yielded sequences that confirmed *Globicatella* species. *sodA* sequencing provided species-level identification of *G sanguinis*. The review of literature reveals *G sanguinis* as an increasingly reported cause of infections of the urine, meninges, and blood. To our knowledge, this is the first reported case of an orthopedic infection caused by *Globicatella sanguinis*. A review of the 37 known cases of *G sanguinis* infection revealed that 83% of patients are female, and 89% are at the extremes of age (<5 or >65 years).

Conclusions. Globicatella sanguinis, an uncommon pathogen with elevated minimum inhibitory concentrations to third-generation cephalosporins, is difficult to identify by phenotypic methods and typically causes infections in females at the extremes of age. It may colonize skin or mucosal surfaces. Advanced molecular techniques utilizing 16S rRNA with sodA PCR accurately identify *G sanguinis*.

Keywords. bacteremia; ceftriaxone; *Globicatella sanguinis*; prosthetic joint infection; *sodA*.

Globicatella sanguinis is a rare pathogenic Gram-positive coccus that has been sporadically reported as an unusual cause of human infections of the bloodstream, central nervous system (CNS), and urinary tract. The other member of the genus, Globicatella sulfidifaciens, is not a known human pathogen. Accurate identification of this organism is important, in part because of its unusual antimicrobial susceptibility pattern despite its resemblance to viridans group streptococci. The organism may be inaccurately identified by commercial phenotypic methods. In this study, we report species-level identification in two patients using

16S recombinant ribonucleic acid (rRNA) and *sodA* polymerase chain reaction (PCR), including the first known case of osteoarticular infection. We also further discuss and expand previous descriptions of *Globicatella* as a human pathogen.

CASE REPORTS

Case 1

A 72-year-old morbidly obese woman with a gastric lap band and lymphedema presented with progressive pain and dysfunction of a total hip arthroplasty. Five years earlier, open synovectomy had yielded sterile tissue cultures with unremarkable histopathological findings. Subsequent arthrocenteses were nondiagnostic, but symptoms persisted, so the patient underwent further surgical debridement.

Three intraoperative cultures of inflammatory synovial tissue grew aerobic, α-hemolytic, catalase-negative Grampositive cocci in pairs and short chains, which were identified with low certainty (<85%) as viridans group streptococci by MicroScan Pos Combo Panel type 29 (Siemens Healthcare Diagnostics, Tarrytown, NY). Of note, the MicroScan database

Received 1 November 2016; editorial decision 21 December 2016; accepted 2 January 2017. Correspondence: A. Miller, MD, Hospital for Special Surgery, 535 E. 70th St, New York, NY 10021 (milleran@hss.edu).

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does not include Globicatella. The isolates demonstrated an elevated minimum inhibitory concentration (MIC) to ceftriaxone (≥8 µg/mL) despite a low MIC to penicillin (≤0.12 µg/ mL), which prompted further investigation. API 20STREP (bioMérieux, Durham, NC) identified the organism with high certainty as Globicatella spp, and Vitek 2 Gram-Positive ID Card (bioMérieux) also obtained an identification of *G sanguinis* with high certainty. The organism was subsequently found to be hippurate positive, hydrogen sulfide negative, pyrrolidonylarylamidase (PYR) negative, mannitol positive, bile esculin negative, and leucine aminopeptidase (LAP) negative. This biochemical profile did not correspond to members of the viridans streptococci group: all biochemicals-with the exception of bile esculin—supported the identification of *G* sanguinis. An identification of G sanguinis (score 2.26) was obtained on the Bruker matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA) system using the research use only (RUO) MBT 6903 MSP library database. The antimicrobial susceptibility profile, as performed by Etest (bioMérieux), is listed in Table 1.

This patient was treated with vancomycin. Her course was complicated by prolonged wound drainage and secondary wound infection with enteric organisms (but not *G sanguinis*), requiring a lengthy hospitalization and discharge to a nursing facility. Six months after presentation, she presented with fever and markedly elevated blood monocyte cell counts: acute myelogenous leukemia was diagnosed via bone marrow biopsy. The patient died three months thereafter.

Table 1. Susceptibility Testing Results of Two Globicatella sanguinis Isolates as Performed by Etest (bioMérieux), Unless Otherwise Noted

	G sanguinis, Case 1	G sanguinis, Case 2		
Antimicrobial Agent	^a MIC in μg/mL	^a MIC in μg/mL		
Penicillin G	0.094	0.125		
Amoxicillin-clavulanate	0.023	N/A		
Ceftriaxone	2	N/A		
Ceftaroline	0.75	N/A		
Oxacillin	3	N/A		
Doripenem	1.5	N/A		
Ertapenem	1.5	N/A		
Meropenem	1.5	N/A		
Vancomycin	0.125	0.19		
Daptomycin	< 0.016	N/A		
Erythromycin	≥256	6 mm ^b		
Tetracycline	0.5	N/A		
Clindamycin	0.75	N/A		
Linezolid	1	1		
Gentamicin	1	N/A		

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration; N/A, not available.

Case 2

A 54-year-old obese woman with type II diabetes mellitus, history of gastric bypass, and recurrent episodes of cystitis, presented with fatigue, fever, and abdominal pain. Sterile pyuria was noted by urinalysis; peripheral leukocytosis was absent. Two sets of blood cultures grew Gram-positive cocci in pairs and short chains on day 1. Vancomycin was initiated.

The next day, α-hemolytic colonies were noted. The organism was negative for catalase and PYR and grew in 6.5% sodium chloride (NaCl). Various low-certainty identifications were obtained by phenotypic methods: *Leuconostoc* spp by Vitek 2; *Gemella haemolysans* by API 20STREP; and *Streptococcus oralis* by RapID Strep (bioMérieux). An identification of *G sanguinis* (score 2.22) was obtained on the Bruker MALDI-TOF MS system with the RUO MBT 6903 MSP library database. The antimicrobial susceptibility profile of this isolate as performed on a limited number of antimicrobials by Etest is listed in Table 1.

Vancomycin-resistant *Enterococcus faecium* concurrently grew from a single blood culture bottle. A 14-day course of linezolid was prescribed to treat both organisms. No vegetations were noted on transesophageal echocardiography. Abdominal imaging showed cholelithiasis and minimal nephrolithiasis. Repeat blood cultures after completion of antibiotic therapy were negative. No recurrent bacteremia occurred in four years of follow up.

METHODS

Molecular Diagnosis

Isolates from each patient underwent PCR and sequence analysis of the 16S rRNA gene and the manganese-dependent superoxide dismutase (Mn-SOD) gene sodA to establish species identity. To prepare the deoxyribonucleic acid (DNA) for 16S recombinant DNA (rDNA) and sodA sequencing, each isolate was selected from a sheep blood agar plate using a 1-µL sterile, disposable inoculating loop and placed into a tube containing 500 µL of sterile water and 0.05 mL of 0.1-mm silica beads (Biospec Products, Bartlesville, OK). Deoxyribonucleic acid was lysed by placing the tube in a 95°C heat block for 5 minutes and then mechanically lysed by vortexing for 2 minutes using a Disruptor Genie (Scientific Industries, Inc., Bohemia, NY). The near complete length of the 16S rRNA gene (1492 basepairs) was PCR amplified using the universal bacterial primers (16SF:5'-AGAGTTTGATCMTGGCTCAG-3' and 16SR: 5'-AAGGAGGTGATCCARCCGCA-3') [1, 2]. A pair of degenerate primers (d1: 5'-CCITAYICITAYGAYGCIYTIGARCC-3' and d2: 5'-ARRTARTAIGCRTGYTCCCAIACRTC-3') was used to amplify the sodA gene fragment [2]. Each PCR reaction was prepared with 45 μL of Platinum PCR SuperMix (ThermoFisher, Waltham, MA), 1 μL of each primer at 50 μM, and 5 μL of DNA. 16S rDNA PCR amplifications were performed on a Veriti 96 Well Thermocycler (Applied Biosystems, Foster City, CA) using the following program: an initial denaturation step of 5 minutes

^aMICs are provided without interpretation, because there are no CLSI interpretive guidelines for this genus.

^bSusceptibility testing performed by disk diffusion.

at 95°C followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 64°C, and 1 minute at 72°C, and ending with a final elongation step for 5 minutes at 72°C [1]. *SodA* PCR sequencing parameters were performed as previously described by Poyart et al [2]. To prepare amplified PCR products for cycle sequencing, the amplified products were purified using Exo-SAP IT (Affymetrix, Santa Clara, CA).

Direct sequencing of PCR fragments was performed using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (PE Applied Biosystems) and an ABI PRISM 3730xl DNA Analyzer. The Sequencher program (Gene Codes Corporation, Ann Arbor, MI; http://www.genecodes.com) was used to analyze the derived sequence data. Sequence data were compared with sequences available in GenBank (http://www.ncbi.nlm.nih.gov) using the BLASTN program and both the nucleotide collection (nr/nt) and 16S rRNA sequence databases. Sequencing of 16S rRNA identified the organisms as Globicatella with >99% similarity to each other, but it did not resolve the identification to species level. Subsequent sodA gene sequencing identified both organisms as G sanguinis; organisms were 99.5% similar to each other. Cases 1 and 2 matched published GenBank G sanguinis (EU649714) and G sulfidifaciens (EU649713) sodA gene sequences with 99.5% and 97.0% concordance and 100% and 97.0% concordance, respectively.

Literature Review

A search of all published medical literature available on PubMed and Google Scholar (all years until May 2016) containing the keyword "Globicatella" was performed, and all references were retrieved and reviewed. Available clinical data were abstracted from each relevant citation.

Review of Literature

In humans, *G sanguinis* has been described to cause bacteremia, endocarditis, CNS infections, and urinary tract infections (UTIs) [3–9]. The entire published clinical experience with *Globicatella* infections (37 cases) is summarized in Table 2: 83% of patients were female, and a majority of patients were either older than 65 (63%) or younger than 5 years (19%). Of these 37 reported human cases of *G sanguinis*, 27 presented with bacteremia (73%; one patient had endocarditis), five with CNS infections (of which at least two were post-neurosurgical), four with UTI, and one with the orthopedic infection described above. No obvious association between infection site and age or gender was apparent. To our knowledge, osteoarticular infection caused by *G sanguinis* has not been heretofore reported.

DISCUSSION

In 1992, on the basis of differences in ecology and 16S rRNA sequence, Collins et al [10] separated all human strains of *Streptococcus uberis* into a new genus and species, *G sanguinis*. *Streptococcus uberis*, a major cause of mastitis in cattle, was at

that time a rare cause of human disease [11]. A second species implicated in livestock infections, G sulfidifaciens, was later described [12, 13]. Although this species has not been found to cause human illness, it may colonize human skin surfaces [3]. The two members of the genus Globicatella are aerobic, α -hemolytic, catalase-negative, PYR-negative, Gram-positive cocci related to the streptococci and are within the Firmicutes phylum and the Aerococcaceae family. They have been found in the environment as part of the microbiota of maari, an indigenous African fermented condiment made from Baobab seeds [14], and in wastes from chicken farms [15].

Globicatella grow as small, a-hemolytic colonies of Grampositive cocci in chains. Certain characteristics can be helpful in differentiating Globicatella spp and viridans group streptococci [16, 17]: for instance, Globicatella strains often grow in 6.5% NaCl broth and are LAP negative [16]. Commercial phenotypic identification systems have difficulty distinguishing G sanguinis from other streptococci. Shewmaker et al [4] reported incorrect or no identification with the use of four commercial phenotypic panels performed on 15 strains of G sanguinis. Incorrect identifications obtained included aerococci, Streptococcus mutans, and enterococci, as well as no identification. An inability to obtain identification of Globicatella spp by commercial phenotypic panels was also noted elsewhere [5]. Current phenotypic methods are based on enzymatic and/or biochemical testing of isolates, but the strain may vary in its reactions leading to a variety of misidentifications with closely related isolates such as those mentioned above and illustrated by our cases. It is interesting to note that isolates from Case 1 and Case 2 were both run on the same Vitek2 system but resulted in different identifications, highlighting the fact that not all strains in a species may be positive for a common trait. Furthermore, databases of phenotypic systems may not include the current species. For instance, although the Vitek2, the BD Phoenix (BD Diagnostics, Sparks, MD), and API systems include Globicatella in their databases, other systems such as the RapID Strep and MicroScan do not. Globicatella is not included in the US Food and Drug Administration-cleared Vitek MS MALDI (bioMérieux) or Bruker MALDI databases. However, the recently updated Bruker MALDI RUO MBT 6903 MSP library includes both *G sanguinis* and *G sulfidifaciens*. Until further investigation is performed on RUO MALDI databases with different species of Globicatella, laboratories must be cautious when calling Globicatella to the species level until RUO and other databases can be expanded with more well characterized isolates of this genus.

Distinguishing *G sanguinis* from viridans group streptococci carries important therapeutic implications, because *Globicatella* typically demonstrates elevated MICs to ceftriaxone and low MICs to penicillin, a pattern considered unusual for most viridans group streptococci [16]. Because there are no Clinical and Laboratory Standards Institute (CLSI)-approved

Table 2. Published Human Cases of Globicatella Infection

Publication Number	Country	Age (Years)	Gender	Underlying Conditions	Presenting Signs and Symptoms	Site of Isolation	Infection
This publication	USA	72	F	Obesity, gastric lap banding, tobacco	Hip pain	Hip synovium	Prosthetic joint infection
This publication	USA	54	F	Obesity, DM, gastric bypass, tobacco	Fatigue and fever	Blood	Bacteremia
[3]	France	56	F	N/A	Meningitis	CSF	Meningitis
[4]	USA	N/A	F	N/A	N/A	Blood	Bacteremia
[4]	USA	N/A	F	N/A	N/A	Blood	Bacteremia
[4]	USA	N/A	М	N/A	N/A	Urine	Urinary
[4]	USA	N/A	N/A	N/A	N/A	Blood	Bacteremia
[4]	USA	N/A	N/A	N/A	N/A	Blood	N/A
[4]	USA	69	М	N/A	N/A	Urine	Urinary
[4]	USA	85	F	N/A	N/A	Urine	Urinary
[4]	USA	N/A	N/A	N/A	N/A	Blood	N/A
[4]	USA	1	М	N/A	N/A	CSF	Meningitis
[4]	USA	84	F	N/A	N/A	Blood	Sepsis
[4]	Canada	N/A	F	N/A	N/A	Urine	N/A
[4]	USA	90	F	N/A	N/A	Blood	Urosepsis
[4]	USA	68	F	N/A	N/A	Blood	N/A
[4]	Canada	82	F	N/A	N/A	Blood	N/A
[4]	Canada	79	F	N/A	N/A	Blood	N/A
[4]	USA	N/A	N/A	N/A	N/A	Blood	N/A
[4]	USA	1	М	N/A	N/A	Blood	Septicemia
[4]	USA	N/A	N/A	N/A	N/A	Blood	N/A
[4]	USA	58	М	N/A	N/A	Blood	Septicemia
[4]	USA	82	F	N/A	N/A	Blood	Septicemia
[4]	Canada	2	F	N/A	N/A	Blood	N/A
[4]	Canada	92	F	N/A	N/A	Blood	N/A
[4]	Canada	N/A	F	N/A	N/A	Blood	N/A
[4]	USA	70	F	N/A	N/A	Blood	Endocarditis
[4]	Canada	43	F	N/A	N/A	CSF	N/A
[4]	Canada	85	М	N/A	N/A	Blood	N/A
[4]	Canada	1	F	N/A	N/A	Blood	N/A
[4]	USA	3	F	N/A	N/A	Blood	Septicemia
[5]	Taiwan	80	F	Chronic diarrhea, DM	Cardiac Arrest	Blood	Bacteremia ^a
[5]	Taiwan	92	F	Dementia, chronic heart failure	Fever cough	Blood	Bacteremia ^a
[6]	India	70	М	Craniectomy	Meningitis	CSF	Meningitis
[7]	Denmark	23	F	Endocarditis	Pneumonia	Blood	Bacteremia
[8]	Germany	69	F	VPS	Meningitis	CSF	Meningitis
[9]	Japan	94	М	Dementia, CHF	Back pain, fever	Blood	Bacteremia

Abbreviations: CHF, chronic heart failure; CSF, cerebrospinal fluid; DM, diabetes mellitus; F, female; M, male; N/A, not available; THR, total hip replacement; USA, United States of America; VPS, ventriculoperitoneal shunt.

interpretive guidelines for *Globicatella*, laboratories are encouraged to report the MIC alone, if needed. *Globicatella sanguinis* typically, but not universally, demonstrates low MICs to penicillin (\leq 0.12 µg/mL) [17]. The largest survey to date of *G sanguinis* susceptibility testing demonstrated high cefotaxime MIC₅₀ of 1 µg/mL and MIC₉₀ of 4 µg/mL (MIC required to inhibit growth of 50% of tested isolates and 90% of testing isolates, respectively) [4]. The MIC₅₀ and MIC₉₀ for penicillin were low at 0.06 µg/mL and 0.12 µg/mL, respectively. The isolates were tested for susceptibility in the study using CLSI-approved broth microdilution with lysed horse blood and

cation-adjusted Mueller-Hinton broth. Elevated MICs were also seen for meropenem, erythromycin, clindamycin, tetracycline, and trimethoprim-sulfamethoxazole, whereas low MICs were demonstrated for penicillin, amoxicillin, chloramphenicol, and levofloxacin. Our susceptibility testing results were similar to those of Shewmaker et al [4], but cefotaxime was not tested on our case isolates. Vancomycin resistance has not been reported. Due to the unusual pattern of high MICs to cefotaxime and meropenem (≥ 2.0 and > 0.5 µg/mL, respectively) in the face of low MICs to penicillin (typically ≤ 0.06 µg/mL), a high MIC to cefotaxime has been proposed as a presumptive

^aSpecies-level identification inconclusive (Globicatella genus).

marker of *Globicatella* in a streptococcus-like bacterium [3]. This requires confirmation with further studies.

Globicatella sanguinis appears to cause sporadic disease with a predilection for older females, and it has been noted as a colonizer of the skin. Both patients reported in this study were also obese. We hypothesize that this organism may be part of the urogenital or lower gastrointestinal microbiome, with potential to cause disease in susceptible hosts; further evaluation of its presence in noninfected hosts is needed. Its low incidence may be in part due to difficulty in laboratory identification by phenotypic techniques, and it is possible that recognition of this pathogen will be improved as advanced technologies such as MALDI-TOF MS and next-generation sequencing emerge into common use.

CONCLUSIONS

In summary, *G sanguinis* is rare and difficult to identify by commercial phenotypic methods, but it should be considered when assessing a catalase-negative, α-hemolytic Gram-positive coccus with elevated MICs to third-generation cephalosporins. We report species-level identification in two patients using 16S rRNA with *sodA* PCR and an RUO version of the Bruker MALDI database. In addition to infections of the bloodstream, CNS, and urinary tract, *G sanguinis* causes osteoarticular infections, and it represents a rare and emerging pathogen worthy of careful attention.

Acknowledgments

Potential conflicts of interest. A. O. M. has served as a consultant to Theranos. S. P. B. has received royalties for real-time polymerase chain reaction (PCR) assays and/or methodologies not relevant to this study from Roche. T. J. W. has received research grants for experimental and clinical antimicrobial pharmacotherapeutics from Cubist, Theravance, Medicines Company, Allergan, and Merck. He has served as consultant to Actavis, ContraFect, Allergan, and Trius. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that

the editors consider relevant to the content of the manuscript have been disclosed

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