ORIGINAL ARTICLE

Globicatella bacteraemia identified by 16S ribosomal RNA gene sequencing

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Background: *Globicatella* are streptococcus-like organisms that have been rarely isolated from clinical specimens. Their epidemiology and clinical significance remain largely unknown.
Aims: To describe two cases of *Globicatella* bacteraemia identified by 16S ribosomal RNA (rRNA) gene sequencing.
Methods: Two unidentified streptococcus-like bacteria isolated from blood cultures of patients were subject to 16S rRNA gene sequencing.
Results: Two cases of *Globicatella* bacteraemia were identified by 16S rRNA gene sequencing. In the first case, a gram positive coccus was isolated from the blood culture of an 80 year old woman with diabetes mellitus and nosocomial sepsis, who died the day after developing the bacteraemia. The bacterium was unidentified by conventional phenotypic tests, the Vitek (gram positive identification) and the ATB expression (ID32 Strep) systems. In the second case, a similar bacterium was isolated from the blood culture of a 92 year old woman with polymicrobial acute pyelonephritis complicated by septic shock, who subsequently recovered after antibiotic treatment. 16S rRNA gene sequencing of the two isolates showed 0.5% nucleotide difference from that of *G. sulfidifaciens* and 0.7% nucleotide difference from that of *G. sanguinis*, indicating that they were *Globicatella* species.
Conclusions: Because *Globicatella* is rarely encountered in clinical microbiology laboratories, it may have been overlooked or misidentified in these cases. 16S rRNA gene sequencing is a useful tool to better characterise the epidemiology and clinical significance of *Globicatella*.

Since the recognition of the 16S ribosomal RNA (rRNA) gene as a new standard for classification and identification of bacteria,[1][2] most bacterial species have been subjected to 16S rRNA gene sequence analysis, with reclassification made and new species identified. The taxonomy of the genera *Streptococcus*, *Enterococcus*, *Lactococcus*, and related members have also undergone major revisions.[3–5] Recently, we reported the use of this technique for identifying and defining the clinical significance of streptococci[6–11] and streptococcus-like organisms;[12–14] and discovery of a novel *Streptococcus* species.[15][16]

The genus *Globicatella* was first described in 1992 when several unidentified streptococcus-like clinical isolates were characterised in the USA.[17] The isolates were isolated from blood cultures of patients with bacteraemia, urine of patients with urinary tract infections, and cerebrospinal fluid of a patient with meningitis, but the clinical details were not described. Based on their unique phenotypic characteristics and phylogenetic position by 16S rRNA gene sequence analysis, they were classified in a new genus *Globicatella*, as *G. sanguis*, which was later renamed as *G. sanguinis*. *G. sanguinis* was also later isolated from a lamb with meningoencephalitis in Spain.[18] Subsequently, a new species of the genus, *G. sulfidifaciens*, was described, when several animal isolates from Belgium with resemblance to *G. sanguinis* were studied. They comprised isolates from the lungs of calves and a lamb with purulent lung infections, and joint fluid of a pig and a calf with polyarthritis.[19] Although there was 99.2% similarity in their 16S rRNA gene sequences to those of *G. sanguinis*, they were classified as a new species based on differences in their whole cell protein patterns and biochemical profiles. Since then, there have been no further reports on the isolation of *Globicatella* from humans and the clinical significance of this rarely encountered genus remains

to be determined. In this report, we describe the application of 16S ribosomal RNA gene sequencing in characterising two cases of *Globicatella* bacteraemia. The difficulties in identifying the two isolates in clinical microbiology laboratories and their clinical significance are also discussed.

METHODS
Microbiological methods
Clinical specimens were collected and handled according to standard protocols. The BACTEC 9240 blood culture system (Becton Dickinson, MD, USA) was used. All isolates were identified by standard conventional biochemical methods,[20] the Vitek System (gram positive identification; GPI) (bioMerieux Vitek, Hazelwood, M), USA) and the ATB expression system (ID32 Strep) (bioMerieux Vitek). Antimicrobial susceptibility was tested by disk diffusion and E-test (AB Biodisk, Solna, Sweden) and results interpreted according to the NCCLS criteria for anaerobic bacteria.[21] All tests were performed in triplicate with freshly prepared media on separate occasions.

Extraction of bacterial DNA for 16S ribosomal RNA gene sequencing
Bacterial DNA extraction was performed as described previously.[6][15] Briefly, 80 µl of NaOH (0.05 mol/l) was added to 20 µl of bacterial cells suspended in distilled water and the mixture was incubated at 60°C for 45 minutes, followed by addition of 6 µl of Tris-HCl (pH 7.0), achieving a final pH of 8.0. The resultant mixture was diluted 100×, and 5 µl of the diluted extract was used for PCR.

Abbreviations: GPI, gram positive identification; rRNA, ribosomal RNA

PCR, gel electrophoresis, and 16S ribosomal RNA gene sequencing

PCR amplification and DNA sequencing of the 16S rRNA gene was performed according to our previous publications.[6][12] Briefly, DNase I treated distilled water and PCR master mix (dNTPs, PCR buffer, and *Taq* polymerase) were used in all PCR reactions by adding 1 U of DNase I (Pharmacia, Sweden) to 40 µl of distilled water or PCR master mix, incubating the mixture at 25°C for 15 minutes, and subsequently at 95°C for 10 minutes to inactivate the DNase I. The bacterial DNA extract and control were amplified with 0.5 µmol/l primers (LPW57 5′-AGTTTGATCCTGGCTCAG-3′ and LPW205 5′-CTTGTTACGACTTCACCC-3′; Gibco BRL, Rockville, MD, USA). The PCR mixture (50 µl) contained bacterial DNA, PCR buffer (10 mmol/l Tris-HCl pH 8.3, 50 mmol/l KCl, 2 mmol/l $MgCl_2$, and 0.01% gelatin), 200 µmol/l of each dNTP and 1.0 U *Taq* polymerase (Boehringer Mannheim, Germany). The mixtures were amplified in in an automated thermal cycler (Perkin-Elmer Cetus, Gouda, The Netherlands), using 40 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes, with a final extension at 72°C for 10 minutes. DNase I treated distilled water was used as the negative control. A 10 µl sample of each amplified product was electrophoresed in 1.5% (w/v) agarose gel, with a molecular size marker (ϕX174 *Hae*III digest,; Boehringer Mannheim, Germany) in parallel. Electrophoresis in Tris borate EDTA buffer was performed at 100 V for 1.5 hours. The gel was stained with ethidium bromide (0.5 µg/ml) for 15 minutes, rinsed, and photographed under 312 nm ultraviolet light illumination.

The PCR products were gel purified using the QIAquick PCR purification kit (QIAgen, Hilden, Germany). Both strands of the PCR product were sequenced twice with an ABI 377 automated sequencer according to manufacturers' instructions (Perkin-Elmer, Foster City, CA, USA), using the PCR primers. The sequences of the PCR products were compared with known 16S rRNA gene sequences in GenBank (hwww.ncbi.nlm.nih.gov) by multiple sequence alignment using the Clustal W program.[22] Phylogenetic tree construction was performed using Clustal X (version 1.81)[23] and the neighbour joining method with GrowTree (Genetics Computer Group Inc., San Diego, USA). In total, 1360 nucleotide positions were included in the analysis.

RESULTS
Patients and identification of the bacterial strains by conventional methods and commercially available systems
Patient 1

An 80 year old Chinese woman was admitted to hospital because of a 3 month history of chronic diarrhoea. She had been passing loose stool with mucus 2–3 times a day. She had diabetes mellitus, hypertension, and gouty arthritis, and was receiving oral hypoglycaemic and anti-hypertensive medications. On admission, she was afebrile. Abdominal examination and colonoscopy was unremarkable. She was found to have hypercalcaemia with adjusted serum calcium 3.14 mmol/l and was given intravenous fluid replacement, loop diuretics, and bisphosphonate with gradual normalisation of serum calcium. She developed sudden cardiac arrest 2 weeks after admission and was stabilised after initial resuscitation. Her total leukocyte count was 22×10^9/l, haemoglobin level 9.8 g/dl, and platelet count 457×10^9/l. Her serum urea was 19.3 mmol/l, creatinine 211 µmol/l, albumin 30 g/l, globulin 36 g/l, bilirubin 7 µmol/l, alkaline phosphatase 115 IU/l, and alanine aminotransferase 87 IU/l. Blood cultures were performed. However, she continued to deteriorate and succumbed on the next day.

On day 2 post-incubation, the aerobic blood culture bottle showed a gram positive coccus (isolate 1). It grew on sheep blood agar as greyish white, α-haemolytic colonies of 0.5 mm in diameter after 24 hours of incubation at 37°C in ambient air. There was enhancement of growth in 5% CO_2 and anaerobic environment. It also grew on MacConkey agar but did not grow in 6.5% NaCl. It was positive for pyrolidonyl arylamidase and esculin, and negative for catalase, arginine, or hippurate hydrolysis, bile aesculin, and Voges-Proskauer tests. It was non-groupable with Lancefield groups A, B, C, D, F, or G antisera. Both the Vitek and ATB expression systems showed that it was "unidentified" (table 1). It was susceptible to vancomycin (MIC 0.25 µg/ml), intermediately susceptible to penicillin (MIC 2 µg/ml), and resistant to and cefotaxime (MIC 4 µg/ml), erythromycin, clindamycin, and neomycin.

Patient 2

A 92-year-old Chinese woman was admitted to hospital because of fever and productive cough with yellow sputum for one day. She had dementia, congestive heart failure, and history of recurrent urinary tract infection. She was bedridden and was put on nasogastric tube feeding. On admission, her oral temperature was 39.5°C. Physical examination did not reveal an obvious focus of infection. Her total leukocyte count was 36.3×10^9/l, haemoglobin level 11.1 g/dl, and platelet count 95×10^9/l. Her renal and liver function tests were within normal limits. Blood cultures were performed. She went into septic shock soon after admission and empirical intravenous cefuroxime was administered. Urine microscopy showed the presence of numerous leucocytes and bacteria. Urine culture recovered *Pseudomonas aeruginosa* with bacterial count >100 000 cfu/ml. Ultrasonography of the kidneys only showed the presence of a renal stone on the right side with no evidence of obstructive uropathy. Antibiotic was switched to intravenous ceftazidime. Her fever responded and she was discharged after 2 weeks of antibiotics.

On day 2 post-incubation, the aerobic blood culture bottle turned positive with a gram negative bacillus and two gram positive cocci. The gram negative bacillus and one of the gram positive cocci were identified as *Pseudomonas aeruginosa* and *Streptococcus sanguis* respectively. The other gram positive coccus (isolate 2) possessed phenotypic characteristics similar to isolate 1 (table 1). It was susceptible to vancomycin (MIC 0.25 µg/ml) and clindamycin, intermediately susceptible to penicillin (MIC 1 µg/ml) and cefotaxime (MIC 2 µg/ml), and resistant to erythromycin and neomycin.

16S ribosomal RNA gene sequencing and phylogenetic analysis

PCR of the 16S rRNA gene of both isolates showed bands at about 1450 bp. The 16S rRNA gene sequences of the two isolates were identical and had 0.5% nucleotide difference from *G. sulfidifaciens* (GenBank accession no. AJ297627), 0.7% nucleotide difference from *G. sanguinis* (GenBank accession no. S50214), 3.69% nucleotide difference from *Facklamia hominis* (GenBank accession no. Y10772), 4.9% difference from *Aerosphaera taetra* (GenBank accession no. AJ279038), and 5.5% difference from *F. languida* (GenBank accession no. Y18053), indicating that the two isolates belong to the genus *Globicatella* (fig 1).

Based on their phenotypic and genotypic data, the two isolates could not be assigned to a particular species of the genus *Globicatella*. Phenotypically, they resembled *G. sanguinis* in the production of pyrrolidonylarylamidase and the negative H_2S and β-glucuronidase production. On the other hand, they resembled *G. sulfidifaciens* in that they did not use mannitol and did not produce N-acetyl-β-glucosaminidase.

Table 1 Phenotypic characteristics of the two blood culture isolates, *Globicatella sulfidifaciens*, and *Globicatella sanguinis*

Characteristics	Isolate 1	Isolate 2	G. sulfidaciens[19]	G. sanguinis[17 19]
Catalase	−	−		−
Lancefield grouping	Non-group A, B, C, D, F, or G	Non-group A, B, C, D, F, or G		
Resistance to bacitracin	−	−		
Resistance to optochin	−	−		
Growth in 6.5% NaCl	−	+	+	+
Growth in 10% bile	−	+		
Growth in 40% bile	−	−		
Esculin hydrolysis	+	−	+	+
Hippurate hydrolysis	−	−	−	+
Arginine hydrolysis	−	−	−	−
Urease	−	−	−	−
Voges−Proskauer test	−	−	−	−
Tetrazolium reduction	−	−		−
Resistance to novobiocin	−	−		
Production of H$_2$S	−	−	+	−
Utilisation of:				
Hemicellulase	−	−		
Dextrose	+	+	+	
Lactose	−	−	−	V
Mannitol	−	−	−	+
Raffinose	−	+	+	+
Salicin	+	+		+
Sorbitol	−	−	−	V
Sucrose	+	+	+	+
Trehalose	+	+	+	+
Arabinose	−	−	−	
Pyruvate	−	−		−
Pullulan	−	+		V
Inulin	−	+	+	−
Melibiose	−	+	+	+
Melezitose	−	−	−	
Cellobiose	+	+	+	
Ribose	+	−	−	+
Xylose	−	−	−	
Maltose	+	+	+	+
Glycogen	−	+	+	+
D-Arabitol	−	−		
Methyl-B-D-glucopyranoside	+	+		
Tagatose	−	−		
Cyclodextrin	−	−		
Pyrrolidonylarylamidase	+	+	−	+
α-galactosidase	+	+	+	+
β-glucuronidase	−	−	+	−
β-galactosidase	+	+	V	+
β-glucosidase	+	−		
Alanine-phenylalanine-proline arylamidase	+	+		+
N-acetyl-β-glucosaminidase	−	−	−	+
Glycyl-tryptophane arylamidase	−	−		
β-mannosidase	+	−		
Alkaline phosphatase	−	−	−	

However, like *G. sanguinis*, isolate 1 used ribose, while isolate 2. like *G. sulfidifaciens*, did not (table 1). Genotypically, the 16S rRNA gene sequences of the two isolates were close to both *G. sulfidifaciens* and *G. sanguinis*, which possess highly homologous 16S rRNA sequences with only 0.8% difference. Therefore, the identification of more strains of *Globicatella* and correlation of their phenotypic characteristics with 16S rRNA gene sequences should help in defining the number of different species within the genus.

DISCUSSION

We describe two cases of *Globicatella* bacteraemia characterised by 16S rRNA gene sequencing. The two isolates were unidentified by conventional biochemical tests and commercial identification systems, and were only confirmed to be *Globicatella* species after 16S rRNA gene sequencing. In retrospect, the phenotypic characteristics of the two blood culture isolates actually closely resembled those of *Globicatella* (table 1). They are facultative anaerobic, catalase negative, α-haemolytic, gram positive cocci. Similar to *G. sanguinis* and *G. sulfidifaciens*, arginine is not hydrolysed, urease not produced, and Voges-Proskauer test negative for the two isolates. While both *G. sanguinis* and *G. sulfidifaciens* grow in 6.5% NaCl, one of our isolates did not. Identification of more isolates of *Globicatella* would help delineate the phenotypic characteristics and variations within the genus. As *Globicatella* is rarely encountered in clinical laboratories, most technicians and microbiologists are not familiar with their phenotypic characteristics and identification. As a result, the bacterium may be overlooked when isolated or reported as unidentified streptococcus-like organisms. 16S rRNA gene sequencing will continue to be useful in the characterisation of rarely encountered bacteria and defining their clinical significance.[6 13 24] As PCR and sequencing techniques are becoming
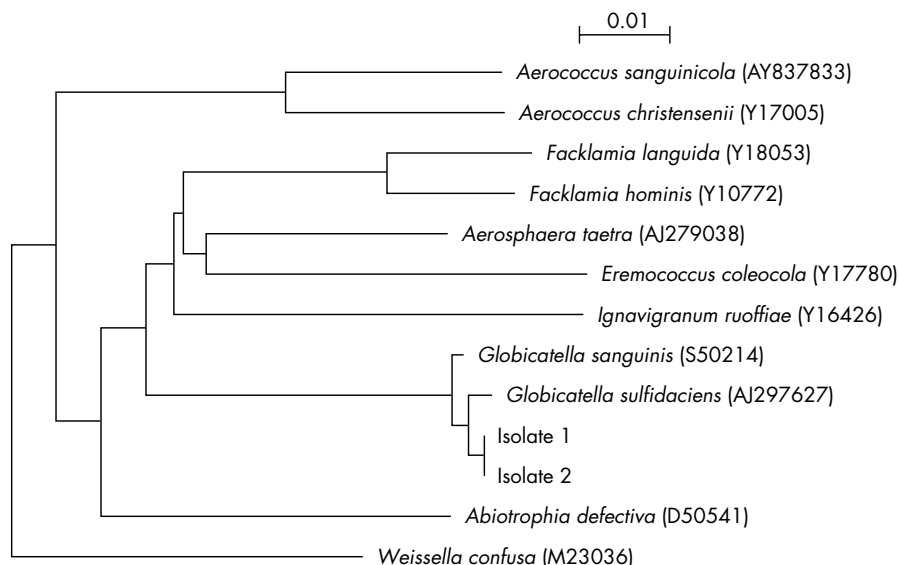
Figure 1 Phylogenetic tree showing the relationships of the two blood culture isolates to *Globicatella* and members of other related genera. The tree was constructed by using the neighbour joining method. The scale bar indicates the estimated number of substitutions per 100 bases using the Jukes-Cantor correction. Names and accession numbers are given as cited in the GenBank database.

more readily available in clinical laboratories, this molecular tool is probably the most practical approach to identify these bacteria.

The clinical significance of the two isolates in our two patients is evident by their isolation from blood in association with the development of fever and neutrophilia. Their infections have been severe because the first patient died soon after the bacteraemia and the second was complicated by septic shock. While the source of the bacteraemia in the first patient could not be identified, the second case probably represents a case of polymicrobial acute pyleonephritis complicated by bacteraemia. *S. sanguis* and *P. aeruginosa*, which were concomitantly isolated from the blood of the second patient, are known pathogens of the urinary tract and can reside in the gastrointestinal tract.[25] Moreover, *G. sanguis* has been isolated from the urine of patients with urinary tract infections.[17] Therefore, *Globicatella* is likely to be an uncommon cause of urinary tract infections and may have originated from the gut. However, only *P. aeruginosa* was recovered in her urine. This may be explained by the slower growth of *Globicatella* and *S. sanguis* as opposed to the larger colonies of *P. aeruginosa*. Nevertheless, as the second patient had mixed infections, the role of *Globicatella* in this case still remains to be determined. Further studies are required to investigate the role of *Globicatella* in human infections.

TAKE HOME MESSAGES

- *Globicatella* are streptococcus-like organisms that have been rarely isolated from clinical specimens, whose epidemiology and clinical significance remain largely unknown.
- Using 16S rRNA sequencing, two cases were identified as *Globicatella* spp.
- The infections were severe, leading to death in one case and septic shock in the other.
- Further studies are required to investigate the role of *Globicatella* in human infections.

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