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Globicatella bacteraemia identified by 16S ribosomal RNA gene sequencing

S K P Lau, P C Y Woo, N K H Li, J L L Teng, K-W Leung, K H L Ng, T-L Que and K-Y Yuen

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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 subjected 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 0.3% 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.

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, the Vitek System (gram positive identification; GPI) (bioMérieux Vitek, Hazelwood, MO, USA) and the ATB expression system (ID32 Strep) (bioMérieux 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. 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. 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
deteriorate and succumbed on the next day. Blood cultures were performed. However, she continued to have a creatinine 155 μmol/l, alkaline phosphatase 115 IU/l, and alanine aminotransferase 87 IU/l.

Her total leukocyte count was 36.3 × 10⁹/l, haemoglobin level 9.8 g/dl, and platelet count 457 × 10⁹/l.

15 minutes, rinsed, and photographed under 312 nm ultraviolet light. 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 MgCl₂, and 0.01% gelatin), 200 μmol/l of each dNTP and 1.0 U Taq polymerase (Boehringer Mannheim, Germany). The mixtures were amplified 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 (×174 HaeIII 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 (http://www.ncbi.nlm.nih.gov) by multiple sequence alignment using the Clustal W program. Phylogenetic tree construction was performed using Clustal X (version 1.81) 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 being treated with 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 × 10⁹/l, haemoglobin level 9.8 g/dl, and platelet count 457 × 10⁹/l. Her serum urca 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% CO₂ and anaerobic environment. It also grew on MacConkey agar but did not grow in 6.5% NaCl. It was positive for pyrrolidonyl 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, E, 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), intermediate-susceptible to penicillin (MIC 2 μg/ml), resistant to and susceptible to 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 × 10⁹/l, haemoglobin level 11.1 g/dl, and platelet count 95 × 10⁹/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 cefazidime 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 phenotype characteristics similar to isolate 1 (table 1). It was susceptible to vancomycin (MIC 0.25 μg/ml) and clindamycin, intermediate-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. S0214), 3.69% nucleotide difference from Facklamia hominis (GenBank accession no. Y10772), 4.9% difference from Aerophora tuatra (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. Phylogenetically, they resembled G. sanguinis in the production of pyrrolidonylarylaminidase and the negative H₂S 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.

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

\section*{DISCUSSION}

We describe two cases of \textit{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 \textit{Globicatella} species after 16S rRNA gene sequencing. In retrospect, the phenotypic characteristics of the two blood culture isolates actually closely resembled those of \textit{Globicatella} (table 1). They are facultative anaerobic, catalase negative, \textalpha-\textipa{9}haemolytic, gram positive cocci. Similar to \textit{G. sanguinis} and \textit{G. sulfidifaciens}, arginine is not hydrolysed, urease not produced, and Voges-Proskauer test negative for the two isolates. While both \textit{G. sanguinis} and \textit{G. sulfidifaciens} grow in 6.5% NaCl, one of our isolates did not. Identification of more isolates of \textit{Globicatella} would help delineate the phenotypic characteristics and variations within the genus. As \textit{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.\textsuperscript{61 32 4}

\begin{table}[h]
\centering
\caption{Phenotypic characteristics of the two blood culture isolates, \textit{Globicatella sulfidifaciens}, and \textit{Globicatella sanguinis}}
\begin{tabular}{|l|c|c|c|}
\hline
Characteristics & Isolate 1 & Isolate 2 & \textit{G. sulfidifaciens}\textsuperscript{19} & \textit{G. sanguinis}\textsuperscript{19 19} \\
\hline
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\textsubscript{2}S & - & - & - & - \\
Utilisation of: & & & & \\
Hemicellulase & - & - & - & - \\
Dextrose & + & + & + & + \\
Lactose & - & - & - & - \\
Mannitol & - & - & - & - \\
Raffinose & - & - & - & - \\
Salicin & + & + & + & + \\
Sorbitol & - & + & + & + \\
Sucrose & + & + & + & + \\
Trehalose & + & + & + & + \\
Arabinose & - & - & - & - \\
Pyrurate & - & - & - & - \\
Pullulan & - & + & + & + \\
Inulin & - & + & + & + \\
Melibiose & - & + & + & + \\
Melezitose & - & - & - & - \\
Cellubiose & + & - & - & - \\
Ribose & + & + & + & + \\
Xylose & - & - & - & - \\
Maltose & + & + & + & + \\
Glycogen & - & + & + & + \\
D-Arabitol & - & - & - & - \\
Methyl-B-D-glucopyranoside & + & + & + & + \\
Tagatose & - & - & - & - \\
Cyclodextrin & - & - & - & - \\
Pyrollidonylarylamidase & + & - & + & + \\
\textalpha-galactosidase & + & + & + & + \\
\textbeta-glucuronidase & + & - & + & + \\
\textbeta-galactosidase & + & - & + & + \\
\textbeta-glucosidase & - & - & + & + \\
Alanine-phenylalanine-proline & + & + & + & + \\
arylamidase & - & - & - & - \\
N-acetyl-\textbeta-glucosaminidase & - & - & - & - \\
Glycyl-tryptophane arylamidase & - & - & - & - \\
\textbeta-mannosidase & - & - & - & - \\
Alkaline phosphatase & - & - & - & - \\
\hline
\end{tabular}
\end{table}
Globicatella

- 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.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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