Infective endocarditis caused by *Streptococcus tigurinus*-like organisms

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Abstract

Streptococcus species are important causes of infective endocarditis but species identification remains challenging. We report two cases of infective endocarditis due to *Streptococcus tigurinus*-like organisms, which were first identified by 16S ribosomal RNA gene sequence analysis and subsequently confirmed using phylogeny based on the analysis of the *shetA* gene encoding exfoliative toxin.

Keywords: 16S rRNA, endocarditis, PCR, *shet A* gene, *Streptococcus tigurinus*
Original Submission: 18 February 2016; **Revised Submission:** 11 May 2016; **Accepted:** 20 May 2016
Article published online: 26 May 2016

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Introduction

Streptococcus tigurinus is a recently described species [1]. It belongs to the *Streptococcus mitis* group, which includes commensal species such as *Streptococcus oralis* and *S. mitis*, which have been recognized as important agents in infective endocarditis (IE). Strain identification at the species level is challenging because of the similarities to *S. oralis* and *S. mitis*. Here, we present two cases of IE caused by *S. tigurinus*-like organisms, identified by molecular tools.

Case report 1

In May 2013, a 62-year-old woman was admitted to the emergency ward for weakness. Her medical history included colonic polyps treated surgically and an aortic valve replacement with a bioprosthetic heart valve. Fever was observed on admission. Cardiac auscultation was normal. The electrocardiogram revealed an atrial fibrillation. Transthoracic echocardiography did not show any sign of IE but transoesophageal echocardiography highlighted a mobile aortic image compatible with vegetation. No periprosthetic abscess was noticed and other valves were exempt from vegetation. The IE was complicated by a splenic embolism. Dental panoramic and sinus X-rays were normal. Peripheral blood cultures grew Gram-positive cocci arranged in chains with α-haemolysis on blood agar after 24 h of incubation. The strain 5171713 was identified as *S. mitis* using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF, Microflex LT; Bruker Daltonics, Bremen, Germany) as previously reported [2] with the non-reliable score of 1.7 (see Supplementary material, Fig. S1). The strain 5171713 was then identified using 16S rRNA sequencing (see ref. [3], and see Supplementary material, Appendix S1). Analysis of the 1471 bp primer-less 16S rRNA sequence (GenBank Accession no. KT780461) using the 'Quick BioInformatic Phylogeny of Prokaryotes' website [4] showed that this isolate was closely related to *S. tigurinus* type strain AZ_3a and the NCBI BLAST program revealed 99% identity with this type strain in a 1456-nucleotide overlap. Since *rpoB*-based and *groEL*-based identifications were not conclusive, the *shetA* gene encoding exfoliative toxin was then amplified and subsequently sequenced (see Supplementary material, Appendix S1). The 640-bp primer-less *shetA* sequence analysis also placed this strain near the *S. tigurinus* type strain (Fig. 1). The strain was susceptible to penicillin and rifampicin and showed a low-level resistance to gentamicin. The patient was treated with intravenous amoxicillin (12 g/day) for 6 weeks with gentamicin (500 mg/day) during the first 2 weeks, then relayed by rifampicin (20 mg/kg/day). She improved clinically with blood culture clearing and resolution of the inflammatory syndrome. At the end of antibiotherapy, trans-oesophageal echocardiography showed a voluminous periprosthetic aortic valve abscess with fistula formation in the left ventricle and severe paraprosthetic

http://dx.doi.org/10.1016/j.nmni.2016.05.012

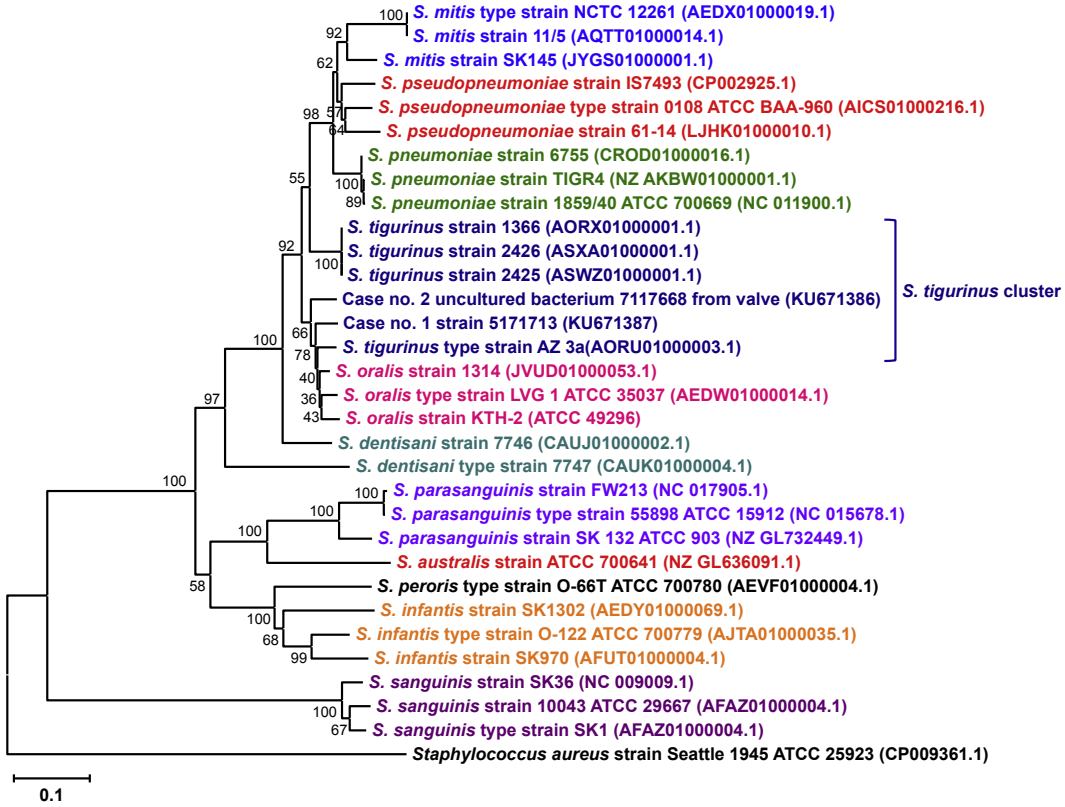

FIG. 1. Evolutionary relationship of *Streptococcus* taxa based on the *shetA* gene. The phylogeny presented is based on the alignment of approximately 640 nucleotides of *shet*A. The nucleotide sequences were aligned using the multiple alignment options on ClustalW. The phylogenetic tree topology of nucleotide alignment was constructed using the MEGA (Molecular Evolutionary Genetics Analysis) software version 6.06 [9] and the neighbour-joining method. The optimal tree with the sum of branch lengths = 3.15836692 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is not rooted and drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura two-parameter method and are represented in the units of the number of base substitutions per site. The analysis included 32 amino acid sequences. All ambiguous positions were removed for each sequence pair. There were a total of 652 positions in the final data set. All sequences are labelled by species, strain name and/or collection and the accession number in brackets. A *Staphylococcus aureus shetA* sequence was used as the outgroup sequence.

leakage. The patient underwent surgery with aortic valve and supra-coronary aortic replacements and fistula closure. The postoperative course was uncomplicated and antibiotherapy with amoxicillin and rifampicin was prolonged for 6 weeks. Culture and molecular analysis of the bioprosthetic valve remained negative.

Case report 2

In October 2014, a 70-year-old woman who had medical history of breast cancer, cholecystectomy and gingival hyperplasia, presented severe dyspnoea with cough and paroxysmal fever. Due to the suspicion of pneumonia, she was treated with ceftriaxone without clinical recovery. In December, she received clarithromycin for 5 days. She deteriorated and was admitted to hospital for sepsis (C-reactive protein level at 40 mg/L; leucocytosis 14.7 g/L and fever). Physical examination revealed an aortic diastolic murmur and signs of left heart failure. Transthoracic echocardiography showed voluminous mobile aortic vegetation with aortic valve regurgitation, supporting the diagnosis of aortic valve endocarditis. Emergency surgery was performed and the patient underwent an aortic valve replacement with a bioprosthetic valve. Empirical postoperative antibiotherapy with amoxicillin (6 g/day) and amoxicillin/clavulanate (6 g/day) was started for 6 weeks. Gentamicin (420 mg/day) was added during the first week. Computerized tomography scans showed possible small renal and cerebral

septic embolisms. Dental consultation revealed that two teeth required extraction. As she was improving, the antibiotics were not changed.

Three peripheral blood cultures collected during surgery remained sterile. As culture of the excised valve was negative, molecular analysis by 16S rRNA gene sequencing was performed (see above). The BIBI software [4] positioned the 16S rDNA sequence (GenBank Accession no. KT780462) near that of *S. tigurinus* type strain, both sequences presenting 99% identity in a 1456-nucleotide overlap using the NCBI BLAST program. The phylogeny based on *shetA* gene analysis also placed the sequence near that of *S. tigurinus* type strain (Fig. 1).

It should be noted that, although the 16S rDNA sequences of both cases presented 99% identity in a 1471-nucleotide overlap, BIBI revealed that case no. 2 was more distant from the *S. tigurinus* type strain than case no. 1. This observation was also confirmed using *shetA* phylogeny (Fig. 1). The *S. tigurinus*-like strain was identified as the causative agent of IE. A saliva specimen, obtained at the end of the antibiotherapy, was cultured and revealed *Enterococcus faecium* and *Candida albicans* (both identified with reliable score by MALDI-TOF) but no conclusive results were obtained with *rpoB*- and *shetA*-based identifications.

Discussion

Streptococcus tigurinus was first documented as the causative agent of IE in multiple blood cultures and aortic valve specimens [1]. It has also been identified as the causative agent of spondylodiscitis, meningitis, prosthetic joint infection, thoracic empyema and bacteraemia [5] but seems to be mainly associated with IE [5–7]. This bacterial species is a prevalent organism of the oral microbiome [8] but was not detected in the saliva of two reported cases of IE, probably because of previous antibiotherapy [5].

These two case reports highlight the importance of molecular analysis in culture-negative IE and for the accurate identification of *Streptococcus* sp. belonging to the *S. mitis* group. Indeed, phenotypic biochemical methods have a limited capacity to differentiate between these species compared with methods based on genetic discrimination and may result in incorrect identification in >50% of cases. In addition, it has been recently shown that accurate identification within the *S. mitis* group by mass spectrometry is limited and such results should be interpreted with caution [6]. However, *S. tigurinus* was not included in any of the MALDI-TOF databases studied, as this species was described so recently.

The strain isolated from the first patient was susceptible to all antibiotics tested, except doxycycline. Similar results were previously reported for 13 *S. tigurinus* strains, all susceptible to penicillin and none displaying high-level gentamicin resistance [5].

Few case reports of IE caused by *S. tigurinus* have been published and, to the best of our knowledge, this is the first report of prosthetic valve endocarditis caused by an *S. tigurinus*-like organism. Accurate identification of this bacterium requires a molecular analysis. In terms of routine practice, identification of streptococcal endocarditis at the species level is important for the clinical management of patients, to understand the pathogenic mechanism of the particular species and also to monitor trends in antimicrobial susceptibility.

Appendix A. Supplementary data

Additional Supporting Information may be found in the online version of this article can be found at http://dx.doi.org/10.1016/j.nmni.2016.05.012.

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