Case Report

Streptococcus sinensis: an emerging agent of infective endocarditis

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The identification by conventional methods of viridans streptococcal species, which are rarely encountered clinically, requires confirmation by genomic methods. We characterized a strain of *Streptococcus sinensis* responsible for infective endocarditis by sequencing both the 16S rRNA and the manganese-dependent superoxide dismutase genes.

Case report

In April 2005, a 55-year-old white man complained of fatigue, night sweats and weight loss over the previous 3 months. Fever (38–39 °C) persisted after a 5 day course of oral amoxicillin. He had a history of arterial hypertension and cardiac systolic murmur never explored before. On admission, his oral temperature was 38.4 °C and a pansystolic heart murmur of 3–4/6 was present at the apex, radiating to the axilla. He had levels of 11.8 g haemoglobin dl⁻¹, 5690 leukocytes µl⁻¹ (74.8 % neutrophils), 232 000 platelets µl⁻¹, 113 mg C-reactive protein l⁻¹ and 1238 µg ferritin l⁻¹. All three blood cultures (BACTEC 9240; Becton Dickinson) grew within 24 h with Gram-positive cocci arranged in chains. Echocardiography showed severe mitral valve regurgitation due to posterior leaflet prolapse and with a regurgitant surface area >40 mm² by the proximal isovelocity surface area method. Valvular dystrophy was observed. Transoesophageal echocardiography showed cord rupture without evidence of vegetations. An intravenous treatment of 12 g amoxicillin per day and 240 mg gentamicin per day for 4 and 2 weeks, respectively, was started. Stable apyrexia was obtained on day 6. Stomatological examination revealed a cavity of the first molar, which was extracted. Elective mitral valve replacement with a Saint Jude no. 32 prosthesis was performed after 5 weeks of apyrexia. The cultures of the valve remained negative, although Gram-positive cocci were observed on microscopic examination. The postoperative course was uneventful and the patient was discharged with

oral amoxicillin treatment for 2 weeks, and long-term antivitamin K treatment with fluindione. On day 14 after surgery, the patient developed neutropenia (2300 leukocytes µl⁻¹, 245 neutrophils µl⁻¹), which normalized after discontinuing all treatments except the anticoagulants; fluindione was replaced with warfarin treatment. On follow-up as an outpatient, he remains well.

The *Streptococcus* strain HDP 2005-0155 (GenBank accession no. EF371928) grew on horse blood agar as tiny non-haemolytic colonies when incubated for 24 h at 37 °C in ambient air. Larger colonies were obtained in anaerobic atmosphere and in 5 % CO₂. Lancefield serogrouping using Streptex (Murex Biotech) was negative for group A, B, C, D, F and G antigens. The strain grew in 6.5 % NaCl broth, but not on bile aesculin agar (bioMérieux). The identification based on morphology, growth characteristics, negativity of the catalase test, absence of pyrrolidonyl arylamidase production, positivity of the leucine aminopeptidase test, and other biochemical reactions determined on both the API 20 Strep and the API Rapid ID 32 Strep systems (bioMérieux) confirmed that the strain belonged to the genus *Streptococcus*, but did not fit into patterns of any species included in the databases (v 7.0 and v 3.0, respectively). When compared to the characteristics of recently described streptococcal species, HDP 2005-0155 and the strains of *Streptococcus sinensis* described by Woo *et al.* (2002, 2004), including the type strain HKU4, and the more recently reported strain by Uçkay *et al.* (2007) shared the same phenotypical characteristics, except the positivity of the tagatose test and the negativity of the raffinose test (Table 1).

These results prompted us to determine the 16S rRNA gene sequence of the isolate as described by Edwards *et al.*

Abbreviation: IE, infective endocarditis.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of HDP 2005-0155 is EF371928.

Table 1. Comparison of biochemical characteristics of HDP 2005-0155 with those of published *S. sinensis* strains HKU4, HKU5 and HKU6 described by Woo *et al.* (2002, 2004), and one strain described by Uçkay *et al.* (2007)

Biochemical reaction or enzyme	Results by different testing methods		
	HDP 2005-0155		Published <i>S. sinensis</i> strains*
	API 20 STREP	Rapid ID 32 STREP	ID 32 STREP
Arginine hydrolysis	+	+	+
β -Glucosidase		+	+
β -Galactosidase	+	+	V
β -Glucuronidase	—	—	—
α -Galactosidase	+	+	V
Alkaline phosphatase	—	—	—
Voges-Proskauer test	—	—	V
Alanine-phenylalanine-proline arylamidase		—	V
Pyrrolidonyl arylamidase	—	—	—
<i>N</i> -Acetyl- β -glucosaminidase		—	—
Glycyltryptophan arylamidase		—	—
β -Mannosidase		—	—
Hippurate hydrolysis	—	—	—
Utilization of:			
Ribose	—	—	—
Mannitol	—	—	—
Sorbitol	—	—	—
Lactose	+	+	+
Trehalose	—	—	+
Raffinose	+	+	V
Sucrose		+	+
L-Arabinose	—	—	—
D-Arabitol		—	—
Cyclodextrin		—	—
Glycogen	—	—	—
Pullulan		+	+
Maltose		—	V
Melibiose		—	V
Melezitose		—	—
Methyl- β D-glucopyranoside		—	+
Tagatose		+	—
Inulin	—		V
Starch	—		V
Urease		—	—
Aesculin hydrolysis	+		+
Leucine arylamidase	+		+

V, Variable results according to the different strains tested.

*Woo *et al.* (2002, 2004); Uçkay *et al.* (2007).

(1989) and Janvier & Grimont (1995). Briefly, the 16S rRNA gene was amplified by PCR with the primers Al (5'-AGRGTTYGATYCTGGCTCAGGAYG-3') and rJ (5'-GGTTACCTTGTTACGACTT-3'). A total of 1490 continuous nucleotides were determined. The complete 16S rRNA gene sequence of the isolate was compared to the bacterial sequences available from the GenBank database by using the BLAST program (<http://www.ncbi.nih.gov/BLAST/BLAST.cgi>) and showed 99.8 % identity to the sequence of the type strain of *S. sinensis* (GenBank accession no. AF432856) (Woo *et al.*, 2002). It differed to the

level of 4 % or more from the 16S rRNA gene sequences of the type strains of the closest species of the viridans group of streptococci, including *Streptococcus gordonii* (4 %), *Streptococcus sanguinis* (5 %), *Streptococcus intermedius* (5 %) and *Streptococcus constellatus* (5 %) (Fig. 1).

The manganese-dependent superoxide dismutase gene sequence (*sodA*) of the isolate was determined as described by Poyart *et al.* (2002). Briefly, the bacterial genomic DNA was extracted using InstaGene matrix (Bio-Rad). The *sodA*

Fig. 1. Phylogenetic tree constructed with the 16S rRNA gene sequences of the 12 viridans *Streptococcus* strains, including two identical isolates HDP 2005-0155 and HDP 2005-0156 from the patient's blood cultures and representative of the closest species of viridans streptococci (GenBank accession numbers are shown in parentheses). The sequences were aligned with CLUSTAL V, using MEGALIGN, a program of the Lasergene package (DNASTar).

degenerate primers d1 (5'-CCITAYICITAYGAYGCIYTIG-ARCC-3') and d2 (5'-ARRTARTAIGCRTGYTCCCAIAC-RTC-3') were used to amplify an internal fragment, representing approximately 85% of the *sodA* genes. Nucleotide sequences were analysed with Perkin-Elmer software programs (Sequence Analysis, Sequence Navigator and Autoassembler). Multiple alignment of *sod* genes was carried out by the CLUSTAL_X program (Jeanmougin *et al.*, 1998), and the construction of the unrooted phylogenetic tree was performed by the neighbour-joining method (Saitou & Nei, 1987). The *sodA* gene sequence of strain HDP 2005-0155 showed 95.6 and 99.3% identity to two available sequences of *S. sinensis* including the type strain (Fig. 2). Susceptibility testing was performed using the standard disc-diffusion method, on Mueller-Hinton agar (bioMérieux), according to the instructions of the 'Antibiogram Committee' of the French Society for Microbiology (<http://www.sfm.asso.fr/>). The isolate was

sensitive to penicillin, amoxicillin, cefotaxime, imipenem, erythromycin, rifampicin, tetracycline and vancomycin, and exhibited low-level resistance to aminoglycosides.

Strain HDP 2005-0155 isolated from blood cultures of a patient with infective endocarditis (IE) has been speciated as *S. sinensis* on the results of sequence analysis of the 16S rRNA and *sodA* genes. Phenotypically, strain HDP 2005-0155 belongs to the viridans streptococci group, which includes several leucine arylamidase-positive and pyrrolidonyl arylamidase-negative species (Facklam, 2002). The strain is similar to the few strains of *S. sinensis* described by Woo *et al.* (2002, 2004) or more recently reported by Uçkay *et al.* (2007). Its identification as *S. sinensis* is based on both the 16S rRNA and the *sodA* gene sequences. The striking distance between *S. sinensis* strains and the closest genospecies of viridans streptococci is visible on both phylogenetic trees (Fig. 1 and 2). The homology levels between

Fig. 2. Phylogenetic tree constructed using the neighbour-joining method, with the *sodA* gene sequences of 14 strains of *Streptococcus* (GenBank accession numbers are shown in parentheses). T, Type strain.

the *sodA* sequences of HDP 2005-0155 and of the other *S. sinensis* strains range from 95.6 to 99.3 % (EF585234 sequence, Uçkay *et al.*, 2007). Although a species threshold of 3 % was proposed for coagulase-negative staphylococci by Sivadon *et al.* (2004), a higher level (3.9 %) of intraspecies divergence was observed by Poyart *et al.* (1998) on viridans streptococci. Different taxonomic groups could have different rates of change in the *sodA* sequence, and as more clinical specimens are sequenced, interspecies genetic variability may become more obvious, as indicated for the 16S rRNA gene sequence by Clarridge (2004). Moreover, in the GenBank database, another sequence (from an unpublished case), AY049738, of the 16S rRNA gene amplified from an aortic valve from a patient with IE also shares 99 % identity with strain HDP 2005-0155. *S. sinensis* was also identified by a molecular technique in the subgingival plaque of two subjects with severe chronic periodontitis (De Lillo *et al.*, 2006). As two of the patients with IE caused by *S. sinensis* had dental problems, this species might be part of the oral flora. This newly recognized species has been reported in patients with IE; however, the phylogeny between *S. sinensis* and members of both the *mitis* and the *anginosus* groups of viridans streptococci suggests it might be further identified as causative of various infectious diseases, particularly among immunocompromised patients (Chen *et al.*, 2005; Woo *et al.*, 2006).

To the best of our knowledge, this is only the second report of isolation of *S. sinensis* outside Asia. Owing to the difficulties in identifying viridans streptococci by biochemical methods, a genetic characterization of these organisms isolated in severe infections should be performed to ascertain the identity of the strain and the epidemiology of each species.

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