The incidence of infective endocarditis is about 50 cases per million citizens per year in western countries (11). Despite the apparently low number of cases, the disease must be regarded very seriously because of the high mortality of 30 to 50% (5, 14, 20). On the basis of cultivation of blood samples from endocarditis patients, up to 63% of the cases can be attributed to streptococci (1, 4, 16), and among those most frequently encountered are viridans streptococci, which are normal inhabitants of the oral cavity.

Streptococcal blood isolates from endocarditis patients probably originate in the oral cavity, but identity between blood isolates and corresponding isolates from the oral cavity has not been shown. Therefore, the purpose of the present study was to isolate streptococcal strains from the oral cavities of streptococcal endocarditis patients and compare the strains biochemically and genetically with the corresponding streptococcal blood isolates.

Two patients were included in the study. Both patients fulfilled the “Duke criteria” for definite diagnosis of infective endocarditis (6). Patient 1 was a 72-year-old male who had a persistent fever (38 to 39°C) for 4 weeks, and no history of cardiac disease except amyocardial infarction 2 years earlier. On admission, the patient was found to be chronically ill with a temperature of 38.1°C, and sinus rhythm with no murmur was recognized by stethoscopy. Two sets of blood cultures were drawn with a 4-h interval, and cultures yielded positive growth of nonhemolytic streptococci after 48 h of incubation. A systolic murmur was recognized 2 days after admission, and transthoracic echocardiography showed vegetations on the septal leaflets of the aortic valve.

Before antibiotic therapy was instituted in the two patients, samples of gingival plaque and saliva were obtained. Gingival plaque was sampled with a curette from sites with gingival inflammation and immediately transferred to 2 ml of sterile phosphate-buffered saline. Two to three milliliters of unstimulated saliva was collected into a sterile glass tube.

Plaque and saliva samples and blood isolates were cultivated on mitis salivarius (M-S) agar (Difco Laboratories, Detroit, Mich.) at 37°C under anaerobic conditions (70% N₂, 20% H₂, 10% CO₂) for 2 days followed by 2 days of aerobic incubation. After 4 days, the colonies on the M-S agar plates were examined under a stereomicroscope (magnification, ×10 to ×40). Five to ten colonies from plaque and saliva with a colony morphology similar to that of the blood isolate on M-S agar were subcultivated on M-S agar until purity was obtained. All isolates including the blood isolate were Gram stained and characterized in accordance with the following criteria: catalase; fermentation of sorbitol, mannitol, salicin, inulin, and amygdalin; acetoin and H₂O₂ production; hydrolysis of arginine and esculin; dextrin formation from sucrose; and activity of β-glucosaminidase, α-l-fucosidase, and acid and alkaline phosphatases (15). For patient 1, the blood isolate and a plaque isolate were identical, and according to the characteristics examined they were identified as *Streptococcus mutans*. The diagnosis of *S. mutans* was confirmed in the second blood sample. For patient 2, the blood isolate and a saliva isolate were identified as *S. oralis* or *S. mitis*. The diagnosis was verified in the three separate blood cultures.

Blood isolates and corresponding plaque and saliva isolates were ribotyped. The following reference strains and clinical isolates from healthy subjects were included in the study and ribotyped: *S. mutans* ATCC 25175 (type strain); *S. mutans* 6715; *S. mutans* IB; *S. oralis* ATCC 35037 = NCTC 11427 (type strain); *S. mitis* ATCC 33399 (type strain); *S. mutans* 40, 54, 70, 74, 80, 84, and 88 (8); *S. oralis* SK105 and SK109 (15); *S. mitis* SK145 (15); and *S. oralis*-*S. mitis* 53 (8).

The ribotyping comprised the following steps: (i) DNA extraction by a modification of the method of Marmur (9, 18); (ii) digestion with restriction endonucleases BamHIII, CfrI, EcoRI, HindIII, PstI, HaeIII, and Sau3A (Boehringer GmbH, Mannheim, Germany) in accordance with the manufacturer’s instructions; (iii) agarose gel electrophoresis (7); and (iv) Southern blotting and hybridization as described by Gerner-Smidt (9). Hybridization was carried out with a digoxigenin 11-dUTP
FIG. 1. Ribotypes of blood and plaque isolates from patient 1 and for three *S. mutans* reference strains after digestion of chromosomal DNAs with *EcoRI*, *PstI*, or *HindIII*.

FIG. 2. Ribotypes of blood and saliva isolates from patient 2 and for the *S. oralis* and *S. mitis* type strains after digestion of chromosomal DNAs with *EcoRI*, *PstI*, or *HindIII*. 
copy DNA probe of 16S and 23S rRNAs from *Escherichia coli* (Boehringer). For each restriction enzyme, the ribotyping was repeated twice.

The band patterns for the isolates examined differed between endonucleases, but the two pairs of blood and plaque-saliva isolates always showed identical patterns with each of the different enzymes. Figure 1 shows the ribotypes of blood and plaque isolates from patient 1 and three *S. mutans* reference strains after digestion with *Eco*RI, *Pst*I, or *Hin*dIII. The blood and plaque isolate pairs are identical to each other but different from the reference strains. The *S. mutans* reference strains also differ from each other, except for strains ATCC 25175 and 6715 after digestion with *Pst*I, where the band patterns seem identical. The observations after digestion with *Bam*HI, *Cla*I, *Hae*III, and *Sal*I were similar to the above-mentioned findings. The ribotypes of seven clinical isolates of *S. mutans* after digestion with each of the seven endonucleases examined were different. For patient 2, comparisons similar to those for patient 1 were made. Figure 2 shows ribotypes of a blood isolate, a saliva isolate, and the type strains of *S. oralis* and *S. mitis* after digestion with *Eco*RI, *Pst*I, or *Hin*dIII. For each enzyme, the band patterns for the two isolates were identical to each other but different from those of the type strains. Similar results were observed when the other four endonucleases were used. The ribotypes of four clinical isolates of *S. oralis* and *S. mitis* after digestion with each of the endonucleases examined were different.

In the present study, samples from the oral cavity were obtained immediately after it was confirmed that endocarditis was caused by nonhemolytic streptococci, presumably by a viridans streptococcus. Isolation of the viridans streptococci from M-S agar was carried out on the basis of colony morphology. Identity between the blood isolate and single colonies of samples from the oral cavity. For the two patients examined, total identity between the streptococcal blood isolate and an oral isolate was observed on the basis of both conventional microbiological methods and ribotyping.

Ribotyping has proved useful in distinguishing between different strains of many species (3, 10, 12, 13, 17, 21). This is the first report which describes a direct comparison between blood isolates and corresponding oral cavity isolates in endocarditis patients. The identity between the corresponding pairs of isolates was verified by digestion of chromosomal DNA with seven different endonucleases. Previous investigation has demonstrated a high discriminatory power of ribotyping with three or more restriction enzymes and established the value of this method as an epidemiological tool (2). The reference strains and clinical isolates of the relevant streptococcal species from other, healthy individuals showed different ribotypes after digestion of their DNAs with the same endonucleases; this documents the genetic diversity within *S. mutans* and *S. oralis-S. mitis*, which has previously been observed for mutants streptococci (19), and strengthens the point of view that the streptococcal species isolated in blood and identified in the two endocarditis cases originated in the oral cavity.

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REFERENCES