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Molecular analyses of bacterial DNA in extirpated heart valves from patients with infective endocarditis

R. Nomura[1], K. Nakano[1], H. Nemoto[1], T. Mukai[2], H. Hata[3], K. Toda[3], H. Yoshioka[2], K. Taniguchi[3], A. Amano[4], T. Ooshima[1]

[1]Department of Pediatric Dentistry, Division of Oral Infection and Disease Control, Osaka University Graduate School of Dentistry, [2]Departments of Dentistry and Oral Surgery, [3]Cardiovascular Surgery, Osaka Rosai Hospital, and [4]Department of Oral Frontier Biology, Division of Oral Biology and Disease Control, Osaka University Graduate School of Dentistry, Osaka, Japan

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Background/aims: Infective endocarditis (IE) is caused by a microbial infection of the endothelial surface of the heart. Although blood culture examinations are commonly used to determine the associated bacterial species, molecular techniques, which enable rapid identification of targeted bacterial species, have recently been applied in clinical cases.
Methods: Nine heart valve specimens from IE patients (six subacute cases and three acute cases) were extirpated and collected, then bacterial DNA was extracted. Bacterial species in the specimens were determined by two different molecular methods and the results were compared with those from a conventional blood culture technique. In addition, a comparison between the two molecular methods was carried out using known numbers of six streptococcal species.
Results: The conventional blood culture method revealed the bacterial species in eight cases, while one was found to be negative. Multiple species were identified in most of the cases by both molecular methods; however, those specified by one method were not always consistent with those specified by the other. Furthermore, the species determined by the blood culture technique were not always identified by the molecular methods. We also found that the two molecular methods used in the present study were extremely sensitive to detect from 1 to 100 cells of individual oral streptococcal species.
Conclusion: Our results suggest that species specified by molecular methods may have disseminated incidentally into the bloodstream, so interpretation of such results should be carefully undertaken in clinical situations.

Key words: 16S rRNA; blood culture; broad-range polymerase chain reaction; infective endocarditis; molecular approach

Kazuhiko Nakano, Department of Pediatric Dentistry, Osaka University Graduate School of Dentistry, 1-8 Yamada-oka, Suita, Osaka 565-0871, Japan
Tel.: +81 6 6879 2963;
fax: +81 6 6879 2965;
e-mail: nakano@dent.osaka-u.ac.jp

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Infective endocarditis (IE), caused by microbial infection of the endothelial surface of the heart, is a life-threatening disease with a rate of incidence of approximately 2–12 cases per 100,000 individuals per year (6). Recent studies of IE in Japan have shown that the most common causative microorganisms are streptococci (approximately 50%), followed by staphylococci (32–37%) (20, 22). Dental manipulation is considered to be an important factor for the onset of IE so dentists are warned to be cautious to prevent its occurrence in patients with cardiac disorders who are predisposed for IE (27, 29).

Oral streptococci, major members of the oral flora, frequently cause bacteremia and IE. Among them *Streptococcus mutans*, a major causative bacterium of dental caries, has been isolated from the blood of patients with IE (3, 23, 30, 31), although the mitis group of streptococci (*Streptococcus mitis* and *Streptococcus*

sanguinis) have been more frequently identified than *S. mutans* (6, 32). Although only a limited number of studies have demonstrated the presence of *S. mutans* in blood, we recently reported that *S. mutans* DNA was much more frequently detected in cardiovascular specimens than *S. sanguinis* (17).

Recent developments in molecular techniques have enabled the rapid identification of targeted bacterial species in various specimens, including broad-range polymerase chain reaction (PCR) and sequencing methods using primers targeting the common region of 16S ribosomal RNA (rRNA) alignment among eubacterial species (1–5, 9–14, 24, 26). Such molecular approaches are thought to provide improved diagnostic outcomes and to reduce laboratory errors because they are not affected by phenotypic variations or technological bias (2, 24). In the present study, we analyzed nine IE cases using these two molecular methods for the determination of bacterial species and compared the results with those of blood culture examinations.

Material and methods

Patients and specimens

All procedures used in the present study were approved by the Ethics Committee of Osaka Rosai Hospital. Nine heart valve specimens collected during heart valve replacement procedures under diagnoses of suspected IE at the Department of Cardiovascular Surgery of Osaka Rosai Hospital, Sakai, Osaka, Japan, from August 2005 to May 2007 were studied (Table 1). Clinical examinations of all cases including echocardiography revealed functional failure or destruction of the affected valve, vegetation formation, embolic events as a result of detached vegetation, or abscess formation. In addition, all of the patients had valvular diseases that predisposed for IE and prominent fever was identified. The blood culture examinations were carried out as follows. Briefly, each blood specimen was divided equally into two bottles with aerobic or anaerobic culture medium (BacT/ALERT FA and BacT/ALERT FN, respectively; Sysmex-Biomerieux, Tokyo, Japan) and then placed into an automated microbial detection device (BacT/Alert system, Sysmex-Biomerieux), which detected the growth using a colorimetric sensor that changed color as the level of carbon dioxide changed. The bottles were cultured for 7 days at 35–37°C. Once bacterial growth was detected, identification of the species was carried out. The blood culture examinations specified the bacterial species present in eight of the nine cases because no specific species were identified in Case 2. Six of the patients were diagnosed with subacute IE and the remaining three were diagnosed with acute IE. Results of the blood culture examinations and values for serum concentrations of C-reactive protein are included in Table 1. The extirpated heart valves with vegetation-like lesions were placed in sterile phosphate-buffered saline (PBS) immediately after collection during the operations. In addition, dental plaque specimens were collected in sterile PBS from three of the nine patients who visited the Department of Dentistry and Oral Surgery before valve replacement surgery.

Specification of bacterial species in infected heart valve specimens by molecular methods

Broad-range PCR methods targeting 16S rRNA and sequencing analysis were carried out to identify the bacterial species in the specimens, as described previously (13, 17, 26). The 16S rRNA gene was amplified by PCR with AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA, USA) using two sets of broad-range 16S rRNA primers; 536f (5′-CAG CAG CCG CGG TAA TAC-3′) and 1050r (5′-CAC GAG CTG ACG ACA-3′) (26), and PA (5′-AGA GTT TGA TCC TGG CTC AG-3′) and PD (5′-GTA TTA CCG CGG CTG CTG-3′) (13). The PCR products were separated by electrophoresis on 1.5% agar gels and the amplified fragments were extracted with QIAEX (QIAGEN Sciences, Düsseldorf, Germany). DNA was directly cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA), for which the nucleotide sequences of 50 clones per specimen were determined by a dye-terminator reaction with a DNA sequencing system (373-18 DNA sequencer; Applied Biosystems) and an ABI PRISM kit. Data analysis was performed with GENE WORKS software (IntelliGenetics, Mountain View, CA, USA). The 16S rRNA sequences obtained were compared with those available in the GenBank, EMBL, and DDBJ databases using the gapped BLASTN 2.0.5 program obtained from the National Center for Biotechnology Information server (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Identification to species level was defined as a 16S rRNA sequence similarity of more than 99% with that of the GenBank prototype strain sequence.

Comparison of two molecular methods

To consider the differences between the two molecular methods in the bacterial species identified (13, 26), we analyzed their specificity and sensitivity. First, the 16S rRNA amplified regions were compared using the nucleotide sequences of *S. mutans* NCTC25175 (GenBank accession no. AY188348) and *S. sanguinis* NCTC7865 (GenBank accession no. D38483) to determine the specificity of the two methods. Then, the sensitivity of the two methods was evaluated by simultaneous PCR assays using the genomic DNA extracted from known numbers of bacterial cells from six streptococcal species; *S. mutans* MT8148, *Streptococcus sobrinus* 6715, *S. sanguinis* ATCC10556, *Streptococcus oralis* ATCC10557, *Streptococcus gordonii* ATCC10558, and *Strep-*

Table 1. Case descriptions for infective endocarditis (IE)

Case	Age/gender	Affected valve	Diagnosis	Blood culture examination	CRP values (mg/dl)[1]
1	91/F	Mitral	Subacute IE	*Staphylococcus aureus*	15.24
2	69/M	Aortic	Subacute IE	ND[2]	4.70
3	41/M	Tricuspid	Subacute IE	*Staphylococcus aureus*	14.17
4	60/M	Tricuspid	Subacute IE	Group B streptococcus	1.09
5	40/M	Mitral	Subacute IE	*Streptococcus constellatus*	5.87
6	72/F	Aortic	Subacute IE	*Methicillin-resistant coagulase negative staphylococci*	4.19
7	46/F	Mitral	Acute IE	*Staphylococcus aureus*	8.82
8	67/F	Aortic	Acute IE	*Streptococcus mitis*	12.43
9	52/M	Mitral	Acute IE	*Staphylococcus aureus*	17.98

[1]Standard range: less than 0.30.
[2]Specific species were not identified.

tococcus salivarius HHT. In addition, two broad-range PCR assays were carried out with genomic DNA extracted from these six species used as templates. Ten clones were sequenced and the bacterial species were specified, then the results were compared with those for clones from which DNA was extracted. Furthermore, five mixtures with various ratios of bacterial cells of *S. mutans* MT8148 and *S. sanguinis* ATCC10556 were analyzed. Briefly, 19.1 μg and 36.0 μg of genomic DNA were extracted from 5×10^8 colony-forming units (CFU) of *S. mutans* MT8148 and 5×10^7 CFU of *S. sanguinis* ATCC10556, respectively. Then, the amount of DNA equivalent to 1×10^6 CFU for each species was given a value of 1, after which five mixture ratios (1 : 9, 1 : 1, 9 : 1, 19 : 1 and 49 : 1) of genomic DNA were prepared. Broad-range PCR was carried out using the two methods with the mixtures used as templates; 20 clones per sample were sequenced and the bacterial species were specified based on the sequences available in the database.

Results

Comparisons of species identified by blood culture and molecular methods

Table 1 lists the species identified by the blood culture examinations. Bacterial species were specified in eight of nine cases; specific species were not identified in one of the cases. Three cases (Cases 2, 3, and 6) had both dental plaque and heart valve specimens available, and were analyzed by the method of Rovery et al. (26), giving the results listed in Table 2. Various oral streptococcal species, such as *S. mutans* and *S. sanguinis*, as well as periodontitis-related species (*Capnocytophaga* and *Prevotella* species) were identified in the dental plaque specimens, whereas several kinds of oral streptococci and staphylococci were identified in the heart valve specimens.

Staphylococcus aureus was identified by blood culture examinations in four of the nine cases (Cases 1, 3, 7, and 9), while the molecular methods also showed that *S. aureus* was the most numerous among 50 clones in three cases (Cases 3, 7, and 9) (Tables 2 and 3). On the other hand, *S. mutans* was the only species identified in Case 1 using the method of Rovery et al. (26), whereas approximately 70% of the clones were found to be *S. mutans* when the method of Marques da Silva et al. (13) was employed. In Case 2, the blood culture examination did not find any specific species but the broad-range PCR analyses showed that approximately half of the clones had the 16S rRNA sequences of *S. salivarius*, *Streptococcus thermophilus*, or *Streptococcus vestibularis*. These three species could not be specified as a single species because the clone sequences had the same similarities.

In Case 4, *S. mutans* was identified in the largest number of clones by both molecular methods, while the blood culture examination detected only Group B Streptococcus species. In the molecular analyses, *Streptococcus agalactiae*, a Group B Streptococcus species, was identified in only three or four of the 50 clones examined. The blood culture examination detected *Streptococcus constellatus* in Case 5, which was not identified by either molecular method. Furthermore, coagulase-negative staphylococci were specified in the blood culture examination of Case 6, indicating that the isolates were staphylococci, except for *S. aureus*, while the molecular methods showed that only 6% of the clones from the heart valve specimen in this case were non-*S. aureus* staphylococci. In Case 8, *Streptococcus pneumoniae* was identified by both molecular methods, which differed from the blood culture method.

Specificity and sensitivity of the broad-range PCR and sequencing methods

Determination of the 16S rRNA nucleotide alignments of known species was performed using the two molecular methods, which specifically identified genomic DNA from *S. mutans*, *S. sobrinus*, and *S. sanguinis* as each corresponding single species (Table 4). On the other hand, multiple species were identified by both methods when genomic DNA extracted from *S. gordonii* and *S. salivarius* was analyzed. *S. oralis* was the only species specified by the method of Marques da Silva et al. (13), while four species were identified by that of Rovery et al. (26). The amplified regions estimated by these two methods based on the nucleotide alignments of *S. mutans* NCTC25175 (GenBank accession no. AY188348) and *S. sanguinis* NCTC7865 (GenBank accession no. D38483) are shown in Fig. 1. The method of Marques da Silva et al. (13) amplified the 5′ one-third region of 16S rRNA, while the middle region was amplified by the method of Rovery et al. (26).

The minimum numbers of bacterial cells identified by the two methods using the

Table 2. Comparisons of bacterial profiles in heart valve and dental plaque specimens from the same patients specified with primers designed by Rovery et al. (26)

Case	Heart valves	Dental plaque
2	*Streptococcus salivarius*/*Streptococcus thermophilus*/ *Streptococcus vestibularis* (26)	*Capnocytophaga sputigena* (17)
	Streptococcus mitis (9)	*Lautropia mirabilis* (10)
	Streptococcus mutans (8)	*Fusobacterium nucleatum*/ *Fusobacterium canifelinum* (9)
	Others (7)	*Prevotella melaninogenica* (6)
		Others (8)
3	*Staphylococcus aureus*/*Staphylococcus haemolyticus*/ *Staphylococcus simiae* (31)	*Capnocytophaga gingivalis* (19)
	S. salivarius/*S. thermophilus*/*S. vestibularis* (7)	*L. mirabilis* (8)
	S. mutans (6)	*P. melaninogenica* (8)
	Others (6)	*S. sanguinis* (6)
		Others (9)
6	*S. mutans* (36)	*L. mirabilis* (27)
	Streptococcus sanguinis (4)	*S. mutans* (15)
	Staphylococcus epidermidis/*Staphylococcus saccharolyticus*/ *Staphylococcus capitis* (3)	Others (8)
	Others (7)	

Values in parentheses indicate the number of clones for each species. Only species with more than three clones detected are listed.

Table 3. Bacterial detection by broad-range polymerase chain reaction and sequencing with two different sets of primers

Case	Primers designed by Rovery et al. (26)	Primers designed by Marques da Silva et al. (13)
1	*Streptococcus mutans* (50)	*S. mutans* (34) Beta *Proteobacterium* (3) Gamma *Proteobacterium* (3) *Stenotrophomonas maltophilia* (3) Others (7)
4	*S. mutans* (23) *Streptococcus pneumoniae/Streptococcus oralis/ Streptococcus mitis/Streptococcus sanguinis* (7) *Streptococcus salivarius/Streptococcus thermophilus/ Streptococcus vestibularis* (7) *Streptococcus parasangunis* (3) *Streptococcus agalactiae* (3) *Streptococcus gordonii/Streptococcus mitis* (3) Others (4)	*S. mutans* (19) *S. mitis* (5) *S. sanguinis* (5) *Streptococcus sobrinus* (4) *S. agalactiae* (4) *S. gordonii* (4) *S. salivarius* (3) Others (6)
5	*S. salivarius/S. thermophilus/S. vestibularis* (12) *S. sanguinis* (10) *Delftia tsuruhatensis* (8) *S. mutans* (7) *S. mitis/S. oralis* (3) Others (10)	*S. sanguinis* (21) *S. gordonii* (12) *S. mutans* (5) *S. salivarius* (4) Others (8)
7	*Staphylococcus aureus/Staphylococcus haemolyticus/ Staphylococcus croceolyticus* (42) *Staphylococcus epidermidis/Staphylococcus pasteuri/ Staphylococcus hominis/S. aureus/S. haemolyticus/ Staphylococcus warneri/Staphylococcus capitis/ Staphylococcus lugdunensis/S. croceolyticus* (8)	*S. aureus* (48) Others (2)
8	*S. pneumoniae* (50)	*S. pneumoniae* (48) Others (2)
9	*S. aureus* (33) *S. mutans* (11) Others (6)	*S. aureus* (40) *S. mutans* (4) Others (6)

Values in parentheses indicate the number of clones for each species. Only species with more than three clones detected are listed.

Table 4. Determination of DNA from known streptococcal bacterial species by two methods of broad-range polymerase chain reaction and sequencing

		BLAST results	
Species	Strains	536f and 1050r	PA and PD
S. mutans	MT8148	*S. mutans* (100)	*S. mutans* (100)
S. sobrinus	6715	*S. sobrinus* (100)	*S. sobrinus* (100)
S. sanguinis	ATCC10556	*S. sanguinis* (100)	*S. sanguinis* (100)
S. oralis	ATCC10557	*S. oralis* (99)/*S. mitis* (99)/*S. pnuemoniae* (99)/ *S. sanguinis* (99)	*S. oralis* (99)
S. gordonii	ATCC10558	*S. gordonii* (100)/*S. mitis* (100)	*S. gordonii* (100)/ *S. mitis* (100)
S. salivarius	HHT	*S. salivarius* (100)/*S. thermophilus* (100)/ *S. vestibularis* (100)/*Enterococcus faecium*(100)	*S. salivarius* (99)/ *S. mitis* (100)

Values in parentheses indicate the highest homology for the reference strains shown by the BLAST program.

genomic DNA from known cell numbers of six streptococcal species were approximately 1–100 with the method of Rovery et al. (26) and 1–10 with that of Marques da Silva et al. (13). The sensitivity of identification was shown to be dependent on the species, with the highest sensitivity found for *S. sanguinis* (one to five cells) and the lowest for *S. gordonii* (10–100 cells). The results of assays using mixtures of various ratios of bacterial cells of *S. mutans* and *S. sanguinis* are shown in Table 5. Only *S. sanguinis* was identified when the mixture of genomic DNA was extracted from the same bacterial cell numbers (1×10^6 CFU) of *S. sanguinis* and *S. mutans*. On the other hand, increasing the ratio of *S. mutans* cells in the mixture led to identification of higher numbers of *S. mutans*.

Discussion

Isolation of IE-associated microorganisms using a blood culture method is generally conducted when diagnosing IE in a clinical setting (15). However, several types of bacterial species are difficult to isolate and identification of pathogenic bacteria in patients who have taken antibiotics is challenging. Molecular techniques, such as those used in the present study, that enable identification of bacteria even in difficult cases have been developed. It has also been reported that bacterial DNA can be detected by a PCR method after the cultures have become sterile, because DNA is sufficiently stable and can be amplified for long periods after the bacte-

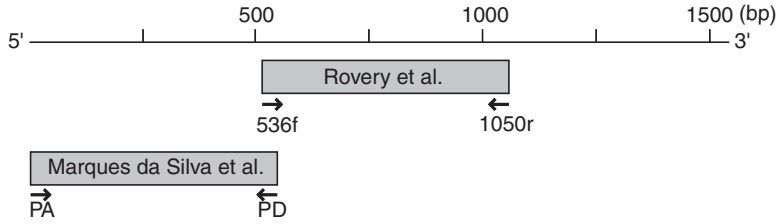

Fig. 1. Illustration of locations of the amplified 16S ribosomal RNA (rRNA) sequences of *Streptococcus mutans* and *Streptococcus sanguinis* by two broad-range polymerase chain reaction methods. Arrows indicate the binding regions for each primer [536f and 1050r; Rovery et al. (26), PA and PD; Marques da Silva et al. (13)]. Base pair scales corresponding to the 16S rRNA sequence of *S. mutans* NCTC25175 (GenBank accession no. AY188348) and *S. sanguinis* NCTC7865 (GenBank accession no. X53653) are shown at the top.

Table 5. Total number of clones containing *Streptococcus mutans* and *Strepotococcus sanguinis* DNA in mixtures of the two species analyzed by broad-range polymerase chain reaction (PCR) and sequencing

	Broad-range PCR and sequencing			
Bacterial mixture[1]	Primers designed by Rovery et al. (26) (n = 20)		Primers designed by Marques da Silva et al. (13) (n = 20)	
S. mutans : *S. sanguinis*	*S. mutans*	*S. sanguinis*	*S. mutans*	*S. sanguinis*
1 : 9	0	20	0	20
1 : 1	0	20	0	20
9 : 1	2	18	3	17
19 : 1	16	4	15	5
49 : 1	19	1	20	0

[1]Ratio indicates the number of cells for each species.

ria are no longer viable (9, 26). In our previous study, a surprising result was that *S. mutans* DNA was frequently identified in cardiovascular specimens (26). To compare the bacterial species identified by a method constructed by another group, we decided to use the method of Marques da Silva et al. (23) in the present study. These molecular methods target different regions of the 16S rRNA sequence (13, 26) and were performed to identify bacterial species present in extirpated heart valve specimens from nine cases of IE.

In our previous studies, bacterial DNA from 35 heart valve specimens, extirpated under diagnoses of aortic regurgitation, mitral regurgitation, or tricuspid regurgitation, were analyzed using the method of Rovery et al. (26), which showed that *S. mutans* was the most frequently detected species at a rate of 77.8% (17). Since all of those heart valve specimens were from non-IE cases, it is reasonable to speculate that one of the major reasons for bacterial detection is the transient or prolonged bacteremia caused by an oral infection. That hypothesis is supported by the results of another study, in which professional dental treatments as well as daily oral practices such as tooth brushing, flossing, and food chewing, were considered to be associated with the development of bacteremia (27). It is reasonable to speculate that *S. mutans* strains are able to invade the bloodstream, leading to incidental identification in the infected heart valves in these cases.

Dental plaque specimens were collected from three of the present patients, who were referred to the Department of Dentistry and Oral Surgery before their cardiovascular operations. It is unfortunate that the number of cases with dental plaque specimens is so small, making it impossible to speculate regarding the correlation of species in dental plaque and heart valve specimens. In fact, an analysis of the association of bacterial profiles between heart valve and dental plaque specimens in these three patients did not show a clear correlation (Table 2). However, most of the species detected in the heart valve specimens were oral streptococci, which would be derived from the oral cavity. Dental plaque specimens are regarded as representing the bacterial profiles of the site of collection, which might be why no species were identified in the heart valve specimens. On the other hand, saliva specimens are generally considered to represent the bacterial profiles of the entire oral cavity. Further analyses should focus on bacterial profiles in saliva specimens collected from subjects.

In the present study, *S. mutans* was the most frequently identified species in Cases 1, 4 and 6, which indicates the possibility that it was the pathogenic bacterium that caused IE in those cases. Several animal experiments, in which the association of the cell surface antigens and onset of IE were analyzed, have shown that *S. mutans* causes IE (8, 16, 21, 25, 28). In our previous study, analyses of four blood isolates of *S. mutans* found uncommon cell surface polysaccharides and protein antigens (18, 19). In addition, rare *S. mutans* strains with a defect of all three types of glucosyltransferases were isolated from an infected heart valve in another (23). Since there are a limited number of case reports describing IE caused by *S. mutans*, additional studies of the surface structures of *S. mutans* related to IE using the clinical specimens would be helpful.

Using blood culture techniques, the mitis group of streptococci (*S. mitis* and *S. sanguinis*) have been more frequently identified than *S. mutans* in IE cases (6, 32). *S. mutans* was not identified by the blood culture examinations performed in any cases in the present study, while *S. mitis* was detected in one (Case 8). In contrast, molecular analysis targeting the 16S rRNA sequence frequently identified *S. mutans* at a detection rate higher than that of *S. sanguinis* (Tables 2 and 3). *S. sanguinis* was detected in genomic DNA extracted from fewer cells than *S. mutans* when analyzing the genomic DNA mixtures containing various cell numbers

of the tested species (Table 5). We therefore speculated that *S. mutans* DNA from viable or non-viable organisms was more frequently present in the heart valves at the time of extirpation than *S. sanguinis*.

The sensitivity of the different methods must be discussed when comparing molecular approaches. Lang et al. (10) reported that the sensitivity threshold of the method constructed in their study was 5×10^4 cells per 25 μl PCR mixture. In the present study, the sensitivity threshold of the two methods was shown to be significantly lower at 1–100 cells per 20 μl PCR mixture. One of the possible reasons for the detection of various bacterial species in the specimens in the present study as compared with others might be because of the high sensitivity shown with the amplified PCR products. This speculation is supported by the results of the study by Marques da Silva et al. (13), in which multiple species were identified in all the aortic aneurysm specimens examined. The ability to detect extremely low numbers of bacterial species in lesions is an advantage, whereas high sensitivity may result in the detection of species with incidental dissemination in the bloodstream.

Positive identification of species by molecular methods is generally defined as a 16S rRNA sequence similarity of greater than 99% with the sequence of the corresponding species in the GenBank database. Oral streptococci are regarded as major pathogens for IE (15, 29), and the 16S rRNA alignments of several species have been shown to be similar (7). It is possible that the sequences of several different species are occasionally found to be the same using different broad-range PCR methods, which are unable to specify a single species. In the present study, molecular analysis of the samples containing *S. oralis* genomic DNA using the method of Rovery et al. (26) detected *S. oralis* and three other species (Table 4). On the other hand, the method of Marques da Silva et al. (13) detected only *S. oralis*, which was probably because of the different regions of 16S rRNA amplified by the two methods (Fig. 1). Therefore, we concluded that the method of Marques da Silva et al. (13) is better for discriminating oral streptococcal species.

Bacterial species found in IE patients have been analyzed by molecular approaches, though no details regarding the total number of clones for the determined sequence have been described. In our study, 50 clones per specimen were sequenced, which identified multiple species in each. In Cases 1 and 8, only one species was identified by one of the molecular methods, while the other detected multiple species (Table 3). As for Cases 3, 7, and 9, *S. aureus* was identified by the blood culture method, while more than 60% of the clones showed *S. aureus* in the results of the molecular methods. In addition, the molecular analyses and blood culture examinations were not consistent in the other six cases. It has been reported that the specificity and sensitivity of molecular methods were adequate to recommend them for clinical application (14). In contrast, several researchers have stated that these methods should be used under controlled conditions to prevent false-positive results (5, 9, 12). Interpretation of the results from molecular methods should be carefully undertaken in clinical situations.

In summary, the molecular methods used in the present study revealed bacterial species present in the bloodstream with high sensitivity, and made it possible to compare the bacterial profiles of dental plaque and diseased valvular tissues. Additional studies should be conducted to accumulate results that could lead to the elucidation of virulent strains that pose a risk for certain kinds of cardiovascular diseases, including IE.

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