

## Antimicrobial Susceptibility of *Streptococcus mutans* Isolated from Patients with Endocarditis

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Forty-one strains of *Streptococcus mutans* (34 from blood specimens from patients with endocarditis and 7 from stock cultures) were tested for susceptibility to penicillin, ampicillin, methicillin, erythromycin, cephalothin, vancomycin, chloramphenicol, tetracycline, gentamicin, streptomycin, and kanamycin. Minimal inhibitory and bactericidal concentrations were determined by a broth microdilution procedure. Most of the strains were very susceptible to ampicillin, penicillin, and erythromycin, with most strains having minimal inhibitory concentrations of 0.08  $\mu\text{g/ml}$  or less. Most of the strains were also susceptible to cephalothin, methicillin, chloramphenicol, tetracycline, and vancomycin. Gentamicin was the most effective aminoglycoside. The antimicrobial susceptibility patterns are similar to those of other viridans streptococci. *S. mutans* strains have proven to be difficult for some microbiologists to identify. But when organisms suggesting *S. mutans* are isolated from patients with endocarditis, they should be at least identified as nonenterococcal streptococci so that appropriate therapy can be initiated.

*Streptococcus mutans* was first isolated from a caries lesion by Clarke in 1924 (2). In 1928, Abercombie and Scott (1) reported this organism to be the cause of endocarditis. In recent years there has been increased interest in the role of this organism in dental caries (6). Increased interest is also indicated by a report concerning *S. mutans* which was made in 1971 by the International Subcommittee on Nomenclature of Bacteria, Subcommittee on Streptococci and Pneumococci (7).

Studies in our laboratory (M. Plaut et al., submitted for publication) and those of de Stoppeelaar (9) have confirmed that *S. mutans* may cause endocarditis. During a 4-year period, approximately 5% of the streptococcal endocarditis isolates studied in our laboratories were *S. mutans*.

The purpose of this study was to determine the susceptibility of 41 strains of *S. mutans* (34 from cases of endocarditis) to 11 antibiotics.

### MATERIALS AND METHODS

**Cultures.** The streptococcal strains were received in the Streptococcus Unit, Center for Disease Control (CDC), through state health departments, and from certain individual investigators by special arrangements (John A. Washington, Mayo Clinic, Rochester, Minn., and Phillip I. Lerner, V.A. Hospital, Cleveland, Ohio). Of the 41 strains used in this study, 7 were stock cultures and the remaining 34 were from

cases of endocarditis. Only one isolate per case was included.

The cultures were identified by R. R. Facklam of the Staphylococcus and Streptococcus Unit, CDC, by methods previously described (M. Plaut et al., submitted for publication). These organisms are members of the viridans group of streptococci and can be speciated on the basis of three typical reactions: (i) acid in mannitol broth, (ii) crystalline-like adherent colonies on 5% sucrose agar, and (iii) sticky gelatinous deposits in a tube of 5% sucrose broth.

**Antibiotics.** The eleven antibiotics used in this study and their sources were ampicillin, methicillin, and kanamycin from Bristol Laboratories; erythromycin, cephalothin, and vancomycin from Eli Lilly & Co., penicillin from Wyeth Laboratories; gentamicin from Schering Corp.; streptomycin and tetracycline from Pfizer Laboratories; and chloramphenicol from Parke, Davis & Co.

Stock solutions of the antibiotics were made with appropriate diluents. Further dilutions were made in Trypticase soy broth (TSB) to a concentration twice that of the highest concentration to be tested. Serial twofold dilutions were made in microtiter plates either manually or automatically with an Autotiter (Canalco). The plates containing the diluted antibiotics were stacked on top of each other in groups of six plates. Each stack was covered with an empty plate and sealed in a plastic bag. These plates containing diluted antibiotics were then stored at  $-65\text{ C}$ . The plates were removed as needed and were allowed to come to room temperature before inoculation.

**Susceptibility tests.** Cultures were streaked onto

Trypticase soy blood agar plates (containing 4% defibrinated rabbit blood) and incubated overnight in a candle jar at 35 C. Four to five similar colonies from the agar plate were inoculated into a tube containing 5 ml of TSB and were incubated overnight at 35 C. The overnight culture was diluted in TSB to contain  $10^4$  to  $10^6$  colony-forming units per ml. The antibiotic-containing microtiter plates were removed from the freezer and allowed to come to room temperature. They were inoculated with 0.05 ml of the diluted culture. The addition of the 0.05 ml of broth made another twofold dilution and thus gave the desired concentration of antibiotic. Each plate was covered with clear adhesive tape and incubated for 24 to 48 h at 35 C. The minimal inhibitory concentration (MIC) was the least concentration of antibiotic that caused complete inhibition of growth, as judged by the unaided eye. To determine the minimal bactericidal concentration (MBC), subcultures were made from the last well showing growth and the next five wells showing no growth. A volume of 0.025 ml from each well was transferred to blood agar plates which were subsequently incubated in a candle jar at 35 C for 48 h. The MBC was the least concentration of antibiotic which completely inhibited growth as indicated by no growth on the subculture.

## RESULTS

These organisms grow slowly, and on solid media they require an atmosphere of  $CO_2$ . Growth was usually adequate within 24 h to permit reading of the tests, but a few organisms required 48 h. Three cultures were judged not to grow well enough in 48 h for susceptibility testing. These were dropped from the study.

The MICs obtained for the 41 cultures of *S. mutans* are shown in Tables 1 and 2. Most of these strains were very susceptible to ampicillin, penicillin, and erythromycin, with the vast majority of strains having MICs of 0.08  $\mu\text{g/ml}$  or less. On the basis of their MICs, most of them were also susceptible to cephalothin, methicillin, vancomycin, chloramphenicol, and tetracycline. The latter two bacteriostatic antibiotics would not be recommended for therapy of endocarditis, although erythromycin has been. Gentamicin was the most effective aminoglycoside, with 42% of the strains having MICs of 3.9  $\mu\text{g/ml}$  or less, levels that are achievable in the serum.

The MBCs of nine of the antibiotics for these organisms are shown in Tables 3 and 4. Of the three bacteriostatic antibiotics, only erythromycin is included in these tables, because it has been recommended for use in the treatment of some cases of endocarditis. Penicillin appeared to be most active, on a weight basis, with 68% of the strains having MBCs of 0.08  $\mu\text{g/ml}$  or less. Gentamicin was the most active aminoglycoside with 31% of the strains having MBCs of 7.8  $\mu\text{g/ml}$  or less. Although erythromycin is a bacteriostatic antibiotic, the MBCs were quite low, with 36% of the strains having MBCs of less than 1  $\mu\text{g/ml}$ .

## DISCUSSION

Streptococci that are harbored in oral cavities are possible etiologic agents of endocarditis (4).

TABLE 1. Minimal inhibitory concentrations of six antibiotics for 41 strains of *S. mutans*<sup>a</sup>

Antibiotic	Percentage of strains with MIC of:							
	≤0.08	0.16	0.31	0.63	1.3	2.5	5.0	>5.0
Ampicillin	78	12	5	5	0	0	0	0
Penicillin	94	0	3	0	3	0	0	0
Erythromycin	85	9	3	3	0	0	0	0
Cephalothin	32	46	14	3	0	0	0	5
Methicillin	29	20	31	7	5	3	0	5
Vancomycin	0	0	0	17	65	3	3	12

<sup>a</sup> Minimal inhibitory concentration (MIC) is expressed as micrograms per milliliter.

TABLE 2. Minimal inhibitory concentrations of five antibiotics for 41 strains of *S. mutans*<sup>a</sup>

Antibiotic	Percentage of strains with MIC of:							
	≤0.48	0.97	1.9	3.9	7.8	16	31	≥63
Chloramphenicol	27	22	29	12	10	0	0	0
Tetracycline	61	22	7	3	0	3	0	4
Gentamicin	5	0	12	25	29	10	12	7
Streptomycin	0	0	0	0	5	19	44	32
Kanamycin	0	0	0	0	2	10	27	61

<sup>a</sup> Minimal inhibitory concentration (MIC) is expressed as micrograms per milliliter.

TABLE 3. Minimal bactericidal concentrations of six antibiotics for strains of *S. mutans*<sup>a</sup>

Antibiotic	Percentage of strains with MBC of:							
	≤0.08	0.16	0.31	0.63	1.3	2.5	5.0	>5.0
Penicillin	68	6	0	10	10	3	0	3
Ampicillin	32	11	14	4	25	7	4	3
Methicillin	5	16	26	11	16	0	0	26
Vancomycin	0	0	0	3	42	3	10	42
Cephalothin	10	12	21	3	12	18	9	15
Erythromycin	8	8	8	12	40	20	4	0

<sup>a</sup> Minimal bactericidal concentration (MBC) is expressed as micrograms per milliliter.

TABLE 4. Minimal bactericidal concentration of aminoglycosides for strains of *S. mutans*<sup>a</sup>

Antibiotic	Percentage of strains with MBC of:							
	0.97	1.9	3.9	7.8	16	31	63	≥125
Streptomycin	0	0	0	0	2	20	24	54
Kanamycin	0	0	0	0	0	2	20	78
Gentamicin	2	0	7	22	32	17	7	13

<sup>a</sup> Minimal bactericidal concentration (MBC) is expressed as micrograms per milliliter.

Our studies and those of others (9) have confirmed that *S. mutans* may cause endocarditis. Although these organisms are classified as viridans streptococci, they are more difficult to identify and to test for antimicrobial susceptibility than are most other members of this group of bacteria.

Most of the laboratories that referred the cultures to the CDC could not identify the organism. Two laboratories identified the organisms correctly, and in a few cases the organisms were identified as possible viridans streptococci. But in most cases they were called diphtheroids, gram-positive bacilli, or coccobacilli, or one of several genera other than streptococci.

de Stoppelaar (9) reports that some of the organisms that were isolated from blood in previous years and identified as *Streptococcus bovis* have been restudied and identified as *S. mutans*. *S. mutans* has also been confused with enterococci. It may be important clinically that streptococci isolated as causes of endocarditis be classified correctly, because the nonenterococcal streptococci are much more susceptible to appropriate antibiotics than are enterococci (3, 11; C. Thornsberry et al., submitted for publication). Although *S. mutans* organisms may share some characteristics with group D streptococci, such as a positive bile-esculin test, they will not grow in broth containing 6.5% NaCl; they can therefore be classified as nonenterococcus streptococci on this basis. The sus-

ceptibility data for these strains of *S. mutans* are similar to those for other viridans streptococci and are different from those for enterococci (C. Thornsberry et al., submitted for publication).

Performing susceptibility tests on *S. mutans* presents some technical problems. These organisms grow slowly and will not grow on solid media unless the incubation atmosphere contains 5 to 10% CO<sub>2</sub>. They grow very poorly or not at all in Mueller-Hinton broth (commonly used for susceptibility tests), but they grow better in TSB. Therefore, the latter broth was used in these tests. Even when TSB and a CO<sub>2</sub> atmosphere were used, growth was still relatively slow. With a few strains, growth was considered to be inadequate for susceptibility tests even after 48 h. These strains were not tested further. Agar diffusion disk susceptibility tests should not be used for *S. mutans* because of its slow growth. Although growth up to the disk (i.e., complete resistance) may be useful clinical information, zones of inhibition cannot be correlated with clinical susceptibility.

There was considerable variation in the MBCs obtained for all of the antibiotics tested, but for penicillin, the antibiotic that is most often used to treat streptococcal endocarditis (often in combination with an aminoglycoside), most of the MBCs were low. Seventy percent of the strains had MBCs for penicillin of 0.08 μg/ml or less; 85% were less than 1.0 μg/ml. Streptomycin is the aminoglycoside most often

used in conjunction with penicillin to achieve a synergistic action on the bacteria. However, based on these *in vitro* data, it is possible that gentamicin may offer an advantage over streptomycin, because the MBCs for gentamicin are, for many strains, lower than for streptomycin. This view is supported by the results of other investigators (10).

Preliminary studies in our laboratory have shown that MBC determinations are very method dependent and are also greatly influenced by the definition of the end point. MBC tests were performed in the present study by transferring 0.025 ml of a total volume of 0.1 ml to blood agar plates which were then incubated for 48 h at 35 C in a candle jar. The end point was defined as total inhibition of growth. If we had used a different end point as, for example, either 10 colonies or less or 99.9% reduction in viable cells, the MBCs (those that were greater than the MICs) would have been lower in almost all cases.

It has been suggested that it would be useful to clinicians if group D streptococci isolated from cases of endocarditis were separated into enterococcal and nonenterococcal (viridans) groups, in terms of both therapy and prognosis (3; W. L. Hoppes and P. I. Lerner, Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 13th, Washington, D.C., abstr. no. 211, 1973). Because *S. mutans* strains are viridans streptococci, they fall into the non-enterococcal group, even though they share some characteristics with enterococci. *S. mutans* and the nonenterococci in general are much more susceptible to penicillin and some other antibiotics than are enterococci. It has been reported that nonenterococcal (*S. bovis*) endocarditis may be successfully treated with penicillin alone (W. L. Hoppes and P. I. Lerner, Abstr. Intersci. Conf. Antimicrob. Ag. Chemother., 13th, Washington, D.C., abstr. no. 211, 1973; R. C. Moellering, personal communication). However, it should be emphasized that speciation alone is not adequate information on which to base a therapeutic regimen because some nonenterococcal streptococci have relatively high MICs to pen-

icillin (8). Therefore, an antimicrobial susceptibility test should be performed on all isolates. These tests should be done either by broth or agar dilution. If an MBC is desired, broth dilution should be used. Agar diffusion tests should not be used to test streptococci from endocarditis. If the nonenterococcal isolate is relatively resistant to penicillin, the therapeutic regimen probably should be the same as that for enterococci (5).

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