Susceptibility of *Streptococcus mutans* to Antimicrobial Agents

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Fifty strains of *Streptococcus mutans*, including defined strains and clinical isolates, were tested for susceptibility to 20 different antimicrobial agents. Minimal inhibitory concentrations were determined by a liquid microtiter procedure. Antibiotics that were most effective in concentrations below 0.1 μg/ml included penicillin, ampicillin, erythromycin, cephalothin, and methicillin. Antibiotics effective in concentrations between 0.1 μg and 10 μg/ml included rifampin, lincomycin, thioestrepton, spectinomycin, vancomycin, streptolydigan, novobiocin, tetracycline, chloramphenicol, spectinomycin, and gentamicin. Antibiotics effective at higher concentrations ranging from 10 μg/ml to 400 μg/ml included the aminoglycosides kanamycin, neomycin, streptomycin, and kasugamycin. Although most antibiotics exhibited inhibitory effects in a narrow range of concentrations, antibiotics such as tetracycline, thioestrepton, and spectinomycin had a 1,000-fold range from the lowest to highest concentrations required for growth inhibition.

Interest in the organism *Streptococcus mutans* is closely associated with its involvement in dental caries. This organism is also involved as a causative agent of endocarditis (1, 3). Knowledge of the antibiotic susceptibility pattern of *S. mutans* is of importance for proper therapy in the cases of endocarditis because *S. mutans* endocarditis has been in some instances confused with group D enterococcal endocarditis (3). In one recent report, approximately 5% of streptococcal endocarditis isolates studied were *S. mutans* (2).

Baker and Thornsberry (2) have reported the patterns of susceptibility of *S. mutans*, isolated from patients with endocarditis, to 11 antibiotics. In this study we have determined the susceptibility of 50 strains of *S. mutans*, isolated from dental plaque, to 20 different antimicrobial agents.

**MATERIALS AND METHODS**

Bacterial strains. The bacterial strains used in this study were obtained from the dental clinics of the University of Oklahoma College of Dentistry and from C. Schaechtele, the University of Minnesota. Of the 50 strains of *S. mutans*, 6 were defined strains representing each known serotype and included the following: LM7, SL-1, 6715, Ingbritt, BHT, and OMZ-61. The remaining strains were isolated from samples of dental plaque and were identified according to (i) colony morphology on Mitis Salivarius agar, (ii) fermentation of mannitol and sorbitol, and (iii) extracellular polysaccharide production in 5% sucrose broth.

Antibiotics. The antibiotics used and the sources from which they were obtained were: erythromycin, kanamycin, neomycin, novobiocin, and tetracycline (Sigma Chemical Co., St. Louis, Mo.); ampicillin, kasugamycin, and methicillin (Bristol Laboratories, Syracuse, N.Y.); lincomycin, spectinomycin, and streptolydigan (Upjohn Co., Kalamazoo, Mich.); cephalothin and vancomycin (Eli Lilly & Co., Indianapolis, Ind.); penicillin and streptomycin (Nutritional Biochemical Corp., Cleveland, Ohio); rifampin (Becton-Dickinson & Co., Rutherford, N.J.); chloramphenicol (Parke, Davis & Co., Detroit, Mich.); spectinomycin (Rhodia Inc., New York); gentamicin (Schering Laboratories, Bloomfield, N.J.); and thioestrepton (E. R. Squibb & Sons, New York).

Media. The standard liquid medium used in this study was proteose peptone broth. It contained 6% proteose peptone no. 3 (Difco), 0.3% NaCl, and 0.38% Na₂HPO₄. After sterilization the broth was completed by the addition of three more components at the following final concentrations: 0.02% CaCl₂, 0.05% glucose, and 5% normal horse serum (GIBCO). Trypticase soy broth (BBL) was also used in the initial portions of the study.

Susceptibility tests. Each strain of *S. mutans* was streaked onto Mitis Salivarius agar plates, incubated at 37°C in an anaerobic jar for 24 h, and then allowed to incubate at 37°C aerobically for an additional 24 h. Several colonies with similar morphology were picked and inoculated into proteose peptone broth. After overnight incubation at 37°C, 0.1 ml of each culture was inoculated into a tube containing 10 ml of proteose peptone broth. To sterile
microdilution plates containing appropriate serial dilutions of antibiotic in proteose peptone broth. 0.05 ml of the overnight diluted culture was added. Each plate was covered with clear plastic and incubated at 37°C for 48 h. Unless otherwise specified, all incubations were performed aerobically. In instances when anaerobic conditions were maintained, anaerobic Gas-Pak (BBL) jars were utilized. The minimal inhibitory concentration (MIC) was the lowest concentration of antibiotic that caused complete inhibition of growth.

RESULTS

Growth of the various strains of S. mutans was supported best in proteose peptone broth containing 5% horse serum. In comparison with other media, including Trypticase soy broth, growth of all bacterial strains was sufficient in 24 h to determine the MIC of a particular antibiotic within one dilution. To insure that maximal growth occurred, however, final MICs were routinely determined after 48 h of incubation.

There was some question whether there were any differences in growth or inhibition by antibiotics when the organism was incubated under aerobic or anaerobic conditions. To answer this question the MICs of several antibiotics (cephalothin, methicillin, and vancomycin) were determined with 50 different strains of S. mutans incubated under both aerobic and anaerobic conditions. The results from these experiments are given in Table 1. The average MICs obtained for each antibiotic were essentially identical with either aerobic or anaerobic incubation and indicated that the atmosphere had little effect on the growth of the organism or its susceptibility to antibiotics.

The MICs for inhibition of the 50 strains, plotted as a function of the percentage of susceptible strains, are shown in Fig. 1 through 3. In Fig. 1 the concentration of antibiotics ranged from 0.0005 to 2 μg/ml and included penicillin, ampicillin, erythromycin, cephalothin, methicillin, rifampin, and lincomycin. The inhibitory concentrations of most of the antibiotics covered a 10-fold range from the lowest to highest concentration required for growth inhibition. With two of the antibiotics, rifampin and lincomycin, there was a 250-fold range from the lowest to highest concentration required for growth inhibition.

In Fig. 2 the concentration of antibiotics ranged from 0.01 to 10 μg/ml and included streptomycin, thiomycycin, spectinomycin, kanamycin, and novobiocin. A 1,000-fold range in the concentration for inhibition of growth was observed with thiomycycin and spectinomycin.

In Fig. 3 the concentration of antibiotics ranged from 0.05 to 400 μg/ml and included tetracycline, chloramphenicol, streptomycin, gentamicin, kanamycin, neomycin, spectinomycin, and kasugamycin. A 1,000-fold range in the concentrations of tetracycline required for inhibition of growth was observed, whereas there was a more narrow range of inhibition for all other antibiotics.

DISCUSSION

The purpose of this investigation was to test the susceptibility of strains of S. mutans iso-
endocarditis strains due to prior chemotherapy. Further, it excludes antimicrobial susceptibility patterns as contributory to the ability of plaque-forming organisms to establish infection in the heart. The results of this study also increase the number of antimicrobial agents with known efficacy against these organisms.

Antimicrobial agents that were particularly effective at low concentrations (below 0.1 μg/ml) included penicillin, ampicillin, erythromycin, cephalothin, and methicillin. Each of these antibiotics inhibited all of the strains tested in a narrow concentration range. Two antibiotics that inhibited S. mutans at low concentrations, rifampin and lincomycin, had a 250-fold concentration range of effectiveness. Even more striking were the wide ranges of effectiveness of tetracycline, thiostrepton, and spiromycin. Each of these antibiotics had a 1,000-fold range from the lowest to highest concentrations required for growth inhibition.

Among group A streptococci there has been a world-wide increase in the number of tetracycline-resistant strains observed (4, 6). Since tetracycline is a frequently used antibiotic, the incidence of tetracycline resistance among S. mutans may also be increasing. However, the other two antibiotics with wide ranges of MICs, thiostrepton and spiromycin, are not frequently used and one would not expect much increase in the incidence of resistant organisms. There was no evidence of multiple drug resistance among any of the strains of S. mutans used in this study.

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