In Vitro Susceptibility of *Streptococcus mutans* to Chlorhexidine and Six Other Antimicrobial Agents

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The susceptibility of *Streptococcus mutans* to chlorhexidine and to six commonly used, systemic antibacterial agents (amoxicillin, cefuroxime, penicillin, sulfamethoxazole-trimethoprim, tetracycline, and erythromycin) was studied for 424 clinical isolates from 116 children and students. The MIC of chlorhexidine for all isolates was ≤1 μg/ml. No resistance to the other antimicrobial agents was detected. Although widely exposed to various antimicrobial agents, *S. mutans* has remained susceptible to common antimicrobial agents, most importantly to chlorhexidine.

Streptococcus mutans is considered one of the most important cariogenic species of the human oral microbial flora (12). There is ample evidence for the association between *S. mutants* and dental caries (22). The suppression of *S. mutans* by antimicrobial agents, especially locally administered chlorhexidine, is consequently of clinical importance (18, 19). For example, chlorhexidine gel used twice daily for 2 weeks three or four times yearly significantly decreases the caries rate (11, 22).

In 1987, approximately 130,000 chlorhexidine rinses (0.2%) were given for dental indications in Finland (population, about 5 million). The gel formulation (1% chlorhexidine) is also widely used as an anticariogenic agent in Finland (16), but the number of gel treatments is not available.

The resistance of *Staphylococcus aureus* and *Streptococcus sanguis* to chlorhexidine has been documented (20, 21), and the resistance of *S. aureus* to chlorhexidine has been shown to be plasmid mediated. Although the susceptibility of *S. mutans* to a number of antimicrobial agents has been studied, the susceptibility of the organism to chlorhexidine has not been systematically evaluated (5, 8, 14). Increased MICs of chlorhexidine for *S. mutans* have been reported only once, in abstract form 10 years ago (3).

Because the use of chlorhexidine is rising, both for caries prophylaxis and in hospital hygiene (2, 4, 13), we studied the susceptibility of *S. mutans* from salivary samples collected from healthy subjects to chlorhexidine.

Paraffin-stimulated whole saliva samples were collected from 70 healthy school children aged 11 years and from 46 healthy dental students aged 22 to 32 years within 1 month during February and March of 1991. Although exact numbers were unavailable, a majority (70%) of the test subjects or their parents had received chlorhexidine for dental indications; *S. mutans* infection often spreads within a family, usually from mother to child (9).

One hundred microliters of whole saliva was transferred immediately after collection into a tube containing 1 ml of tryptic soy broth (Oxoid, Basingstoke, United Kingdom) supplemented with 20% glycerol and stored at −40°C. Samples were cultured on mitis salivarius-bacitracin agar, composed of mitis salivarius agar base (Difco Laboratories,

Detroit, Mich.), 15% sucrose, and 0.1 U of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml. From each cultured saliva sample, four different *S. mutans*-like colonies were chosen for further susceptibility studies. Isolates were subcultured in brain heart infusion broth (Difco) and identified by positive fermentation tests (glucose, melibiose, mannitol, raffinose, sorbitol, and *N*-acetylglucosamine) and the negative dextran agglutination test (1, 6). A total of 424 *S. mutans* isolates were identified.

The susceptibility of the isolates to the following agents was tested: amoxicillin (Astra, Mölndal, Sweden; MIC, 0.03 to 32 μg/ml), chlorhexidine diacetate (Fluka BioChemika, Buchs, Switzerland; MIC, 0.25 to 128 μg/ml), cefuroxime (Sigma; MIC, 0.063 to 16 μg/ml), benzylpenicillin (Sigma; MIC, 0.008 to 16 μg/ml), sulfamethoxazole-trimethoprim (Sigma; ratio, 19:1; MIC, 0.016 to 16 μg/ml), tetracycline (Sigma; MIC, 0.063 to 32 μg/ml), and erythromycin base (Orion Co., Vantaa, Finland; MIC, 0.063 to 64 μg/ml). Inoculum preparation and susceptibility testing of the isolates were done by the plate dilution method (15). Bacteria were cultured on Mueller-Hinton agar supplemented with 5% sheep blood and doubling concentrations of antimicrobial agents (15). The plates were incubated at 35°C in a 5% CO_2 atmosphere and read after 24 h of incubation; at 48 h of incubation, the results were the same.

The chlorhexidine MICs for the control isolates were as follows: *Streptococcus pyogenes* ATCC 10389, 0.25 to 1 μg/ml; *S. mutans* ATCC 25175, 0.25 to 1 μg/ml; and *Streptococcus sobrinus* ATCC 33478, 0.5 to 2 μg/ml. Quality control isolates were used at each run.

Chlorhexidine was highly effective against all the *S. mutans* isolates; the MIC did not exceed 1 μg/ml for any of the isolates (Table 1). The distribution of the MICs of the other antimicrobial agents revealed only susceptible populations; no resistant isolates were found.

The susceptibility of *S. mutans* to chlorhexidine has apparently not been studied systematically before. Hennessey (8) studied the susceptibility of only one isolate of *S. mutans* to chlorhexidine; the MIC was 0.19 μg/ml. Meurman et al. (14) found all 28 clinical isolates of *S. mutans* tested to be chlorhexidine susceptible (MICs for 50 and 90% of isolates, 4 and 8, respectively). Regarding the resistance of *S. mutans* to other antimicrobial agents, our results are in agreement with those of Liebana et al. (10), who found that *S. mutans* remained susceptible for at least 5 years to all 10

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TABLE 1. In vitro susceptibility of 424 clinical isolates of
S. mutans to chlorhexidine and six other antimicrobial agents

Antimicrobial agent	MIC (μg/ml)		
	Range	50%	90%
Chlorhexidine	0.25–1	0.5	1
Amoxicillin	0.031–0.063	0.063	0.063
Penicillin	0.016–0.031	0.031	0.031
Cefuroxime	0.063	0.063	0.063
Erythromycin	0.063–0.25	0.13	0.13
Tetracycline	0.063–1	1	1
Sulfamethoxazole-trimethoprim	0.5–4	2	4

antimicrobial agents studied, although there was a tendency for increased resistance. Our study differs, however, from the one by Peros and Gibbons (17), who reported slightly higher MICs for tetracycline (MIC, 2 μg/ml, compared with 0.063 to 1.0 μg/ml in the present study) and penicillin (MIC, 0.13 μg/ml, compared with 0.016 to 0.031 μg/ml in the present study). The difference may be due to the fact that they incubated the cultures in an anaerobic atmosphere, while we used a 5% CO_2 atmosphere.

In conclusion, although there is an increasing and continuing selection pressure on S. mutans by commonly used antimicrobial agents (7), especially chlorhexidine, oral S. mutans has remained susceptible in Finland. However, continuing follow-up and vigilance are needed to detect the resistance of S. mutans, particularly to chlorhexidine, in time.

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