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Incidence of *mefA* and *mefE* Genes in Viridans Group Streptococci

Viridans group streptococci form the major part of the commensal flora of the human upper respiratory tract. However, these organisms are also the leading cause of infective endocarditis and an increasing source of bacteremia in neutropenic patients (1). Beta-lactam agents are the treatment of choice for these infections, but macrolides and related drugs are recommended for prophylaxis and alternative treatment in allergic patients (1). The two presently recognized mechanisms of resistance to macrolide antibiotics in streptococci are (i) target site modification mediated by erythromycin resistance methylases (Erm), which confer cross-resistance to macrolides, lincosamides, and streptogramin B components (MLS phenotype), and (ii) active-drug efflux pumps, encoded either by the *mefAE* genes or by the *mreA* gene (3, 4, 11). The efflux systems encoded by the *mef* genes cause resistance to 14- and 15-membered macrolide compounds only, and this phenotype is designated M (10). Phenotype M is widespread among beta-hemolytic streptococci and *Streptococcus pneumoniae* in a number of countries (6, 7, 10). During a survey of antimicrobial resistance in viridans streptococci, strains with the M phenotype were investigated.

A total of 90 consecutive strains of viridans group streptococci were isolated from 90 patients hospitalized in a French hospital (Haut-Lévêque, Pessac), between 1988 and 1995. These strains were identified with two commercial kits, API20 STREP and Rapid ID32 (Biomérieux): 57 isolates belonged to the *Streptococcus mitis* group, 24 to the *Streptococcus milleri* group, and 9 to the *Streptococcus salivarius* group. By the disk diffusion method, the strains were classified in three categories with regard to their MLS behavior: (i) 55 strains (61.1%) were susceptible, 27 (30%) had the MLS phenotype, and 8 (8.9%) had the M phenotype. The latter strains (five *S. mitis* strains, two *Streptococcus oralis* strains, and one *S. salivarius* strain) were susceptible to all other antibiotics, except for three which were additionally penicillin resistant. MICs of MLS antibiotics were determined by the agar dilution method on Mueller-Hinton medium supplemented with 5% horse blood. The eight isolates with the M phenotype exhibited low-level resistance to erythromycin and other 14- and 15-membered macrolides, although the intrinsically more active new ketolide HMR 3647 retained significant activity; in contrast, they remained fully susceptible to 16-membered macrolides, lincosamides, and streptogramins (Table 1). The DNAs of the eight isolates were amplified with primers specific to the *mefAE* genes (2). The PCR protocol consisted of a 5-min melt at 94°C, followed by 35 cycles (1-min melt at 94°C, 1-min primer-annealing step at 50°C, and 1-min extension step at 72°C), with a final extension step of 10 min at 72°C. All strains except for one (*S. mitis* 4) yielded a PCR product of the expected size (1.2 kb), whether no amplification was obtained with the DNAs of negative-control strains (sensitive or MLS phenotype strains). The amplicons were analyzed by restriction using four endonucleases designed to differentiate *mefA* and *mefE* (*Cla*I, *Hin*dIII, *Acc*I, and *Hha*I) (2). The results showed that six strains carried a *mefE* gene, while the remaining one (*S. oralis* 6) possessed a *mefA* gene (Table 1). Thus, *mefE* appears to be predominant in viridans streptococci with the M phenotype, as previously observed for *S. pneumoniae* (6) and *Streptococcus agalactiae* (2). With specific primers for *ermA*, *-B*, *-C* (9), *ermTR* (8), and *mreA* (4), no PCR amplification was obtained with *S. mitis* 4 under the above conditions (Table 1). These results suggest the existence of a novel erythromycin resistance gene or mechanism in these species.

The erythromycin resistance rate in viridans group streptococci was similar to those reported recently (around 40%) (1, 5), but the incidence of the M phenotype was lower than that reported elsewhere (about 20%) (1, 12). This is consistent with a lower incidence of beta-hemolytic streptococci and pneumococci with *mef* genes in France (<1%) (2). Commercially available 14- and 15-membered macrolides appear to be of limited value for chemoprophylaxis and therapy in viridans streptococcal infections.

TABLE 1. MICs of MLS antibiotics correlated with the presence of *mef* genes in streptococci

Strain	MIC (mg/liter)[a]													*mef* gene
	Macrolides								Lincosamides		Streptogramins			
	14 membered					15 membered	16 membered							
	E	ROX	CLA	DIR	HMR 3647	AZI	SP	JOS	L	CM	PT	IIA	IB	
1. *S. mitis*	1	2	0.2	4	0.02	1	0.2	0.2	0.2	0.05	0.1	8	2	*E*
2. *S. mitis*	1	2	0.2	4	0.05	1	0.2	0.2	0.5	0.05	0.2	32	4	*E*
3. *S. mitis*	1	2	0.5	4	0.05	1	0.1	0.1	0.1	0.02	0.05	2	2	*E*
4. *S. mitis*	4	16	4	32	0.2	4	0.1	0.1	0.2	0.02	0.1	1	2	
5. *S. oralis*	4	8	1	16	0.2	4	0.2	0.2	0.1	0.01	0.05	1	4	*E*
6. *S. oralis*	16	32	4	64	0.2	8	0.5	0.2	0.5	0.05	0.1	8	4	*A*
7. *S. salivarius*	8	32	4	64	0.2	4	0.2	0.2	0.5	0.02	0.2	8	4	*E*
8. *S. anginosus*	1	2	1	4	0.05	2	0.2	0.2	0.1	0.02	0.05	2	2	*E*
S.[b] *S. mitis*	0.05	0.05	0.02	0.2	0.01	0.1	0.5	0.5	0.5	0.05	0.1	16	2	
MLS. *S. mitis*	>512	512	512	512	0.02	512	512	256	256	128	0.1	8	32	

[a] E, erythromycin; ROX, roxithromycin; CLA, clarithromycin; DIR, dirithromycin; AZI, azithromycin; SP, spiramycin; JOS, josamycin; L, lincomycin; CM, clindamycin; PT, pristinamycin; IIA, pristinamycin IIA; IB, pristinamycin IB. Data were read at 24 h.
[b] S, susceptible strain.

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