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# Different Erythromycin Resistance Mechanisms in Group C and Group G Streptococci

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**Different mechanisms of erythromycin resistance predominate in group C and G streptococcus (GCS and GGS, respectively) isolates collected from 1992 to 1995 in Finland. Of the 21 erythromycin-resistant GCS and 32 erythromycin-resistant GGS isolates, 95% had the *mefA* or *mefE* drug efflux gene and 94% had the *ermTR* methylase gene, respectively.**

Lancefield group C and G streptococci (GCS and GGS, respectively) may cause pharyngitis and a variety of severe infections in humans (9, 19). For penicillin-allergic patients, erythromycin has long been a good alternative in the treatment of streptococcal infections. Resistance to erythromycin in group A streptococci (GAS) has been reported from a number of countries (6, 11, 15), but there are few reports of erythromycin resistance in GCS and GGS (1, 20).

The two presently recognized mechanisms for resistance to macrolide antibiotics in streptococci are target-site modification and active-drug efflux. Target-site modification is mediated by an erythromycin resistance methylase (*erm*) that reduces binding of macrolide, lincosamide, and streptogramin B (MLS) antibiotics to the target site in the 50S ribosomal subunit (5). The phenotypic expression of MLS resistance can be inducible (IR) or constitutive (CR). In active-drug efflux, the protein encoded by the *mefA* or *mefE* (macrolide efflux) gene causes resistance to 14- and 15-membered macrolide compounds only (3, 18). This phenotype is called the M phenotype (17).

In the present study, we investigated the susceptibilities of GCS and GGS to erythromycin and three other antimicrobials and determined the erythromycin resistance mechanism in the isolates resistant to erythromycin.

From January 1992 through December 1995, a total of 579 clinical isolates of GCS and 911 clinical isolates of GGS were collected from throat swab and pus samples in the microbiological laboratory of the Central Hospital of Pohjois-Karjala in Joensuu, Finland, and sent to the Antimicrobial Research Laboratory of the National Public Health Institute, Turku, Finland. Identification of β-hemolytic isolates was performed by a commercial latex-agglutination technique (Streptex; Wellcome, Dartford, England). The MICs of erythromycin, clindamycin, tetracycline, and penicillin (Sigma Chemical Co. Ltd., St. Louis, Mo.) were determined by the plate dilution method according to the recommendations of the National Committee for Clinical Laboratory Standards (8) as described previously (12). The breakpoints for resistance were as follows: erythromycin and clindamycin, ≥1 μg/ml (8); penicillin, ≥4 μg/ml (8); and tetracycline, ≥8 μg/ml (8). The control strains were those described previously (11).

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The IR, CR, and M phenotypes of erythromycin-resistant GCS and GGS isolates were determined by the double-disk test by using erythromycin and clindamycin disks as described previously (12).

For the detection of different erythromycin resistance genes in the genomes of GCS and GGS by PCR, DNA was extracted as described previously (14). The DNAs of the erythromycin-resistant isolates were amplified with primers specific for the *ermA*, *ermB*, *ermC*, and *mefA* or *-E* genes; PCR conditions for the primer sets were as described previously (16, 3). Primers used for the detection of the *ermTR* gene were designed on the basis of the sequence of *ermTR* (13) as follows: 5′-ATAGAA ATTGGGTCAGGAAAAGG-3′ ($TR_1$) and 5′-TTGATTTTT AGTAAAAAG-3′ ($TR_2$). The PCR mixture was as described previously (13). A total of 35 cycles were carried out with a thermal reactor (model HB-TR1; Hybaid Ltd., Middlesex, United Kingdom) as follows: denaturation at 94°C for 30 s, annealing at 42°C for 60 s, and elongation at 72°C for 90 s. To confirm that $TR_1$ and $TR_2$ had amplified the *ermTR* gene, the PCR products obtained by using these primers were digested with *Hin*fI endonuclease (Promega Co., Madison, Wis.) under conditions recommended by the manufacturer. The DNAs containing erythromycin resistance genes from *Staphylococcus aureus* RN2864 (7), *Clostridium perfringens* CP592 (2), *Staphylococcus aureus* IHT 62242, *Streptococcus pyogenes* A200 (13), and *S. pyogenes* A569 were used as positive controls in the PCR-based detection of the *ermA*, *ermB*, *ermC*, *ermTR*, and *mefA* or *-E* genes, respectively. After the amplification, the detection and visualization of the PCR products were performed as described previously (10). Amplification of the DNAs from the positive controls with the corresponding primers produced PCR products of expected sizes; *ermA*, *ermB*, and *ermC* were 640 bp, *ermTR* was 530 bp, and *mefA* or *-E* was 1.4 kb. Digestion of the PCR products obtained with the $TR_1$ and $TR_2$ primers from the control strain produced bands with lengths of 355, 128, and 54 bp, as expected.

**Susceptibility results.** The proportions of the GCS and GGS isolates resistant to erythromycin (3.6 and 3.5%, respectively) and clindamycin (1.0 and 0.3%, respectively) were similar, but the proportion of GCS isolates resistant to tetracycline was clearly lower than that of GGS isolates (13 versus 43%). All isolates were susceptible to penicillin (Table 1). Tetracycline resistance was found in 2 (10%) and 16 (50%) of the 21 erythromycin-resistant GCS and 32 GGS isolates, respectively, which parallels the common tetracycline resistance level well.

TABLE 1. MICs of four antibiotics for 579 GCS isolates and 911 GGS isolates

| Organisms (no. of isolates tested) | Antimicrobial agent | MIC (μg/ml)[a] | | | % Resistant[b] |
|---|---|---|---|---|---|
| | | 50% | 90% | Range | |
| GCS (579) | Erythromycin | 0.064 | 0.125 | 0.064–64 | 3.6 |
| | Clindamycin | 0.064 | 0.125 | 0.032–2 | 1.0 |
| | Tetracycline | 0.5 | 16 | 0.032–64 | 13 |
| | Penicillin G | 0.008 | 0.032 | 0.008–0.5 | 0 |
| GGS (911) | Erythromycin | 0.064 | 0.064 | 0.064–>64 | 3.5 |
| | Clindamycin | 0.064 | 0.125 | 0.032–>64 | 0.3 |
| | Tetracycline | 2 | 64 | 0.125–>64 | 43 |
| | Penicillin G | 0.016 | 0.016 | 0.008–0.5 | 0 |

[a] 50% and 90%, MICs at which 50 and 90% of the isolates are inhibited.
[b] Breakpoints according to the National Committee for Clinical Laboratory Standards.

Hence, it seems that tetracycline resistance in GCS or GGS is not linked to erythromycin resistance.

Published figures of the frequencies of erythromycin resistance in GCS and GGS from other countries are scarce. In England in the middle of the 1980s, erythromycin resistance was found in 1.4 and 1.6% of GCS and GGS isolates, respectively (1). In Taiwan in 1993 to 1994, resistance to erythromycin was found in 41.7 and 23.5% of GCS and GGS isolates, respectively (19).

**Erythromycin resistance phenotypes and genes.** Nearly all (95%) of the erythromycin-resistant GCS isolates had the M phenotype, whereas 91 and 6% of the erythromycin-resistant GGS isolates had the IR and the CR phenotype, respectively.

All isolates with the M phenotype were positive with primers specific for the *mefA* or *-E* gene. Likewise, the *ermTR* gene was found in all IR phenotype isolates. Of the CR phenotype isolates, one had the *ermTR* gene and the other had the *ermB* gene. Hence, 20 (95%) of the 21 erythromycin-resistant GCS isolates had the *mefA* or *-E* gene, and the mechanism conveyed by this gene has been shown to be active-drug efflux (17). As 30 (94%) of the 32 erythromycin-resistant GGS isolates had the *ermTR* gene and one GGS isolate had the *ermB* gene, altogether 31 (97%) of the 32 GGS isolates had an *erm* gene, and the mechanism of resistance is therefore proposed to be target-site modification.

The *mefA* gene was first identified in GAS (16), and we have also found it in GAS with the M phenotype (4). The *ermTR* gene in GAS A200 was recently characterized by us (13), and we have found it to be common in Finland among GAS with the IR phenotype (4). This study is the first to show that the *ermTR* gene and the *mefA* or *-E* gene may exist in other β-hemolytic streptococcus species as well. Because the erythromycin resistance genes are the same in GCS, GGS, and GAS in Finland, it is possible that the genes have transferred among these species. Although there is an association between the active-efflux mechanism and GCS in Finland, it is possible that this association is not a universal phenomenon. In Taiwan the MIC results showed that 33.3% of the GCS isolates were resistant to clindamycin (20), which is related to MLS resistance (12).

In summary, different mechanisms of erythromycin resistance predominate in GCS and GGS isolates in Finland.

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