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Erythromycin Resistance Genes in Group A Streptococci in Finland

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Streptococcus pyogenes **isolates (group A streptococcus) of different erythromycin resistance phenotypes were collected from all over Finland in 1994 and 1995 and studied; they were evaluated for their susceptibilities to 14 antimicrobial agents (396 isolates) and the presence of different erythromycin resistance genes (45 isolates). The erythromycin-resistant isolates with the macrolide-resistant but lincosamide- and streptogramin B-susceptible phenotype (M phenotype) were further studied for their plasmid contents and the transferability of resistance genes. Resistance to antimicrobial agents other than macrolides, clindamycin, tetracycline, and chloramphenicol was not found. When compared to our previous study performed in 1990, the rate of resistance to tetracycline increased from 10 to 93% among isolates with the inducible resistance (IR) phenotype of macrolide, lincosamide, and streptogramin B (MLS_B) resistance. Tetracycline resistance was also found among 75% of the MLS_B-resistant isolates with the constitutive resistance (CR) phenotype. Resistance to chloramphenicol was found for the first time in *S. pyogenes* in Finland; 3% of the isolates with the IR phenotype were resistant. All the chloramphenicol-resistant isolates were also resistant to tetracycline. Detection of erythromycin resistance genes by PCR indicated that, with the exception of one isolate with the CR phenotype, all M-phenotype isolates had the macrolide efflux (*mefA*) gene and all the MLS_B-resistant isolates had the erythromycin resistance methylase (*ermTR*) gene; the isolate with the CR phenotype contained the *ermB* gene. No plasmid DNA could be isolated from the M-phenotype isolates, but the *mefA* gene was transferred by conjugation.**

From 1973 (13) to 1996 (31) target-site modification was the only erythromycin resistance mechanism known in streptococci. It is caused by a methylase encoded by erythromycin resistance methylase (*erm*) genes. Methylases cause conformational change in the prokaryotic ribosome leading to reduced binding of macrolide, lincosamide, and streptogramin B (MLS_B) antibiotics to the target site in the 50S ribosomal subunit (17). The phenotypic expression of MLS_B resistance can be inducible (IR) or constitutive (CR). In streptococci, MLS_B resistance is commonly mediated by genes belonging to the *ermAM* (*ermB*) class of genes (29). Recently, we have characterized a novel *erm* gene, *ermTR*, present in a Finnish erythromycin-resistant clinical isolate of *Streptococcus pyogenes* (group A beta-hemolytic streptococcus). The sequence of the *ermTR* gene shares only 58% identity with the sequence of *ermAM* (*ermB*) gene but shares 82% identity with the sequence of the *ermA* of *Staphylococcus aureus* (29). The *erm* determinants of streptococci are usually chromosomal in location (10), but they may also be located on plasmids (2, 8, 18, 19, 20, 24).

When an increase in erythromycin resistance in *S. pyogenes* was recognized at the beginning of the 1990s in Finland (27), a previously unknown erythromycin resistance phenotype (phenotype M) was identified (28). Strains with this phenotype were shown to be resistant only to 14- and 15-membered macrolide compounds, and therefore, a different mechanism of resistance was suspected for these isolates (28). Recently, Sutcliffe et al. (31) identified a drug efflux mechanism in strains with a similar phenotype, and Clancy et al. (7) showed that it was caused by a novel gene, *mefA*, which encodes a membrane-associated protein.

In Finland, the proportion of erythromycin-resistant isolates with the M phenotype increased among isolates collected from different parts of the country, from 40% in 1990 (28) to 80% in 1994 (15). In 1990, the only additional resistance present in erythromycin-resistant *S. pyogenes* isolates was that to tetracycline.

In the study described here we have investigated the antibiotic resistance patterns and epidemiology of known erythromycin resistance genes of *S. pyogenes* collected throughout Finland in 1994 and 1995. We also studied whether the *mefA*-mediated erythromycin resistance determinants were mobilized by conjugation and investigated the genetic locations of the resistance determinants in the M-phenotype isolates. The results show a predominance of the *mefA* gene among the M-phenotype isolates and a predominance of the *ermTR* gene among the MLS_B-resistant isolates.

MATERIALS AND METHODS

Group A streptococcal isolates. The isolates studied were selected from 1,546 erythromycin-resistant isolates of *S. pyogenes* consecutively collected from pharyngeal and pus specimens in 1994 and 1995 throughout Finland by 22 regional microbiology laboratories (15, 21). Control strains were selected from 8,173 erythromycin-susceptible *S. pyogenes* isolates that were consecutively collected during the same time period from the same areas. The isolates were identified by a commercial latex agglutination technique (Streptex; Wellcome, Dartford, United Kingdom), and erythromycin susceptibility was determined by the screening-plate method with Mueller-Hinton agar (Oxoid, Basingstoke, United Kingdom) as described previously (27).

Determination of erythromycin resistance phenotypes. The resistance phenotypes of erythromycin-resistant *S. pyogenes* isolates were determined by the double-disk test with erythromycin and clindamycin disks as described previously

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(28). Blunting of the clindamycin zone of inhibition proximal to the erythromycin disk indicated an inducible type of MLS_B resistance (IR), and resistance to both erythromycin and clindamycin indicated a constitutive type of MLS_B resistance (CR). Susceptibility to clindamycin with no blunting indicated the new erythromycin resistance phenotype (M phenotype).

Determination of MICs. The MICs of different antimicrobial agents were determined for a group of erythromycin-resistant *S. pyogenes* isolates of each phenotype and a group of erythromycin-susceptible isolates, both groups of which were randomly selected from each laboratory. Thus, 396 erythromycin-resistant isolates, including 150 IR-phenotype isolates, 242 M-phenotype isolates, and 4 CR-phenotype isolates, were studied. For comparison, 141 erythromycin-susceptible isolates were included.

The MICs of erythromycin, clindamycin, cephalothin, tetracycline, ciprofloxacin, chloramphenicol, penicillin, vancomycin (Sigma Chemical Co., St. Louis, Mo.), roxithromycin (Roussel Uclaf, Paris, France), azithromycin (Pfizer, Espoo, Finland), spiramycin, quinupristin-dalfopristin (Rhône-Poulenc Rorer, Vitry-sur-Seine, France), clarithromycin (Abbott Scandinavia AB, Kista, Sweden), and josamycin (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan) were determined by the agar dilution method according to the recommendations of National Committee for Clinical Laboratory Standards (23) as described previously (28). The breakpoints for resistance by the National Committee for Clinical Laboratory Standards were as follows: erythromycin and clindamycin, ≥1 μg/ml; azithromycin, ≥2 μg/ml; ciprofloxacin and penicillin, ≥4 μg/ml; tetracycline, ≥8 μg/ml; chloramphenicol, ≥16 μg/ml; and cephalothin and vancomycin, ≥32 μg/ml (23). Breakpoints which have not been published but which were derived from the distribution of the MICs for the isolates in the present study were as follows: clarithromycin, ≥0.5 μg/ml; roxithromycin, spiramycin and josamycin, ≥2 μg/ml; and quinupristin-dalfopristin, ≥4 μg/ml. *S. pyogenes* ATCC 10389, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 29213 were used as controls in MIC determinations.

Detection of erythromycin resistance genes. Erythromycin-resistant *S. pyogenes* isolates that were previously shown to be of different clonal origins either by T serotyping or randomly amplified polymorphic DNA analysis (15) as well as isolates with the same T serotype but of different geographical origins (15) were subjected to PCR-based detection of erythromycin resistance genes. PCR was thus performed with 19 isolates with the M phenotype, 24 isolates with the IR phenotype, 2 isolates with the CR phenotype, and, as a control, 8 erythromycin-susceptible *S. pyogenes* isolates that were of different epidemiological origins.

For the identification of the different erythromycin resistance genes in the selected 45 isolates by PCR, total DNA was extracted as described previously (30). The DNAs of the erythromycin-resistant isolates were amplified with primers specific for the *ermA* (32), *ermB* (32), *ermC* (32), and *mefA* (7) genes; PCR conditions for the primer sets were as described previously (7, 31). For the detection of the *ermTR* gene, primers TR_1 and TR_2 (5′-ATAGAAATTGGGT CAGGAAAAGG-3′ and 5′-TTGATTTTTAGTAAAAAG-3′, respectively) were designed on the basis of the *ermTR* sequence (GenBank accession no. AF002716) (29); and the PCR mixture, PCR conditions, and restriction endonuclease analysis of the PCR product were as described previously (16, 29). DNA from *S. aureus* RN2864 (22), *Clostridium perfringens* CP592 (3), *S. aureus* IHT 62242, *S. pyogenes* A200 (29), and *S. pyogenes* A569 were used as positive controls in the PCR-based detection of the *ermA*, *ermB*, *ermC*, *ermTR*, and *mefA* genes, respectively. Eight erythromycin-susceptible *S. pyogenes* isolates were used as negative controls. After the amplification, the detection and visualization of the PCR products were performed as described previously (26). Amplification of the DNAs from the positive controls with the corresponding primers produced PCR products of the expected sizes; *ermA*, *ermB*, and *ermC* were 640 bp, *ermTR* was 530 bp, and *mefA* was 1.4 kb. Digestion of the PCR product with the *Hin*fI endonuclease (Promega Co., Madison, Wis.) obtained with the TR_1 and TR_2 primers from the control strain containing the *ermTR* gene produced bands with lengths of 355, 128, and 54 bp, as expected.

Southern hybridization and plasmid isolation. Southern blotting and plasmid isolation experiments were performed for the 19 M-phenotype *S. pyogenes* isolates in order to study the genetic location of the *mefA* gene. Samples of DNA from the M-phenotype isolates were digested with the restriction endonucleases *Hin*dIII and *Eco*RI. DNA fragments were separated in a 0.7% agarose gel, denatured, and transferred onto a nitrocellulose membrane filter, as described by Ausubel et al. (1). The 1.4-kb *mefA*-specific PCR product labeled with the Prime-a-Gene Labeling System (Promega) was used as the probe. The filters were prehybridized for 2 h at 37°C in a buffer composed of 1× Denhardt's solution, 0.5% sodium dodecyl sulfate, 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]), 250 μg of denatured sonicated salmon sperm DNA per ml, and 50% formamide. Hybridization was performed in the same buffer for 18 h at 37°C. In this procedure *S. pyogenes* A569 served as a positive control and various erythromycin-susceptible *S. pyogenes* isolates served as negative controls.

Plasmids were isolated from 125-ml overnight cultures (Todd-Hewitt broth supplemented with 1% yeast extract) of the M-phenotype isolates by using the Wizard Plus Maxipreps DNA Purification System (Promega) as described previously (29). *S. pyogenes* 13 234 containing the 17.5-MDa MLS_B resistance plasmid pERL1 (18) was used as a positive control.

Conjugation experiments. The transferability of erythromycin resistance genes from the 19 M-phenotype isolates to *E. faecalis* JH2-2 (14) and *S. pyogenes* BM137 (12) was studied by filter matings (a modification of the method described by Scott et al. [25]). Both recipients were susceptible to erythromycin and were resistant to rifampin and fusidic acid. The selective-antibiotic agar plates contained 5 μg of erythromycin per ml and 4 μg of rifampin per ml. Putative transconjugants were further studied for their susceptibilities to erythromycin, rifampin, and fusidic acid with E-test strips (AB Biodisk, Solna, Sweden) and for the presence of the *mefA* gene by the PCR protocol described above.

RESULTS

Antimicrobial susceptibility patterns of *S. pyogenes* isolates with different erythromycin resistance phenotypes. All erythromycin-resistant isolates were resistant to the 14-membered macrolide compounds erythromycin, clarithromycin, and roxithromycin and the 15-membered macrolide compound azithromycin (Table 1). All 242 M-phenotype isolates were susceptible to 16-membered macrolides, but 63 and 60% of the 150 IR-phenotype isolates were resistant to the 16-membered macrolides spiramycin and josamycin, respectively. All four CR-phenotype isolates were also resistant to spiramycin and josamycin. The M- and IR-phenotype isolates were susceptible to clindamycin, but all CR-phenotype isolates were highly resistant to clindamycin. All M-phenotype isolates were susceptible to tetracycline, whereas 93% of the IR-phenotype isolates were tetracycline resistant. Of the 141 erythromycin-susceptible isolates, 16% were resistant to tetracycline. Chloramphenicol resistance was found in 3% of the IR-phenotype isolates but not in isolates of the other phenotypes. Of the erythromycin-susceptible isolates, 4% were, however, resistant to chloramphenicol. All chloramphenicol-resistant isolates were also resistant to tetracycline. Resistance to other antimicrobial agents was not detected (Table 1).

Erythromycin resistance genes of *S. pyogenes* isolates with different erythromycin resistance phenotypes. All 24 IR-phenotype isolates were positive with primers specific for the *ermTR* gene (Table 2). Of the two CR-phenotype isolates, one isolate was positive with primers specific for the *ermTR* gene and the other isolate was positive with primers specific for the *ermB* gene. Of the 19 M-phenotype isolates, all isolates were positive with primers specific for the *mefA* gene. The eight erythromycin-susceptible *S. pyogenes* isolates were not positive with any of the primers tested.

Southern hybridization and plasmid isolation. For all 19 M-phenotype isolates, the *mefA* gene probe hybridized to DNA fragments of the same size. When the DNAs were digested with *Eco*RI the probe hybridized to an approximately 1.9-kb DNA fragment of each isolate. When the DNAs were digested with *Hin*dIII, the probe hybridized to two DNA fragments of approximately 1.8 and 2.1 kb for every isolate. No plasmid DNA could be isolated from any of the 19 M-phenotype isolates.

Conjugation experiments. Transfer of the erythromycin resistance determinants by conjugation from M-phenotype isolates to *S. pyogenes* BM137 and/or *E. faecalis* JH2-2 was successful for 8 of the 19 M-phenotype isolates at frequencies of 10^{-7} to 10^{-4}. All the transconjugants were resistant to erythromycin, rifampin, and fusidic acid, as determined with the E-test strips, and harbored the *mefA* gene.

DISCUSSION

The results of this study showed that in Finland the *mefA* gene is predominant among erythromycin-resistant M-phenotype isolates and the *ermTR* gene is predominant among isolates with MLS_B resistance phenotypes. A comparison of susceptibilities between erythromycin-resistant *S. pyogenes* isolates in this study and isolates collected in 1990 (28) showed no changes in the antibiotic resistance patterns of the M- and

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TABLE 1. MICs of 14 antibiotics for 396 erythromycin-resistant and 141 erythromycin-susceptible *S. pyogenes* isolates

Erythromycin resistance phenotype (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)[a]			% Resistant
		50%	90%	Range	
M (242)	Erythromycin	8	8	2–16	100
	Clindamycin	0.064	0.064	0.032–0.125	0
	Clarithromycin	4	8	0.5–16	100
	Roxithromycin	16	16	2–64	100
	Azithromycin	4	8	2–16	100
	Spiramycin	0.25	0.5	0.064–0.5	0
	Josamycin	0.25	0.5	0.064–1	0
	Cephalothin	0.125	0.25	0.064–0.5	0
	Tetracycline	0.25	0.25	0.064–16	0
	Chloramphenicol	2	4	0.5–4	0
	Vancomycin	0.5	0.5	0.25–0.5	0
	Penicillin	0.008	0.008	0.008–0.016	0
	Ciprofloxacin	0.5	1	0.25–2	0
	Quinupristin-dalfopristin	0.25	0.5	0.125–1	0
IR (150)	Erythromycin	4	8	1–>64	100
	Clindamycin	0.125	0.5	0.064–16	1
	Clarithromycin	2	4	0.5–>64	100
	Roxithromycin	16	32	2–>64	100
	Azithromycin	16	32	2–>64	100
	Spiramycin	4	8	0.25–>64	63
	Josamycin	4	8	0.125–>64	60
	Cephalothin	0.25	0.5	0.125–0.5	0
	Tetracycline	32	32	0.125–64	93
	Chloramphenicol	4	4	2–32	3
	Vancomycin	0.5	0.5	0.25–0.5	0
	Penicillin	0.016	0.016	0.008–0.016	0
	Ciprofloxacin	0.5	0.5	0.25–1	0
	Quinupristin-dalfopristin	0.5	0.5	0.016–0.5	0
CR (4)	Erythromycin	>64	>64	4–>64	100
	Clindamycin	>64	>64	>64	100
	Clarithromycin	>64	>64	2–>64	100
	Roxithromycin	>64	>64	16–>64	100
	Azithromycin	>64	>64	32–>64	100
	Spiramycin	>64	>64	32–>64	100
	Josamycin	>64	>64	8–>64	100
	Cephalothin	0.125	0.5	0.125–0.5	0
	Tetracycline	32	64	0.25–64	75
	Chloramphenicol	4	4	2–4	0
	Vancomycin	0.5	0.5	0.25–0.5	0
	Penicillin	0.016	0.016	0.008–0.016	0
	Ciprofloxacin	0.5	0.5	0.25–0.5	0
	Quinupristin-dalfopristin	0.5	0.5	0.25–0.5	0
Erythromycin-susceptible isolates (141)	Erythromycin	0.032	0.064	0.032–0.125	0
	Clindamycin	0.064	0.064	0.032–0.125	0
	Clarithromycin	0.032	0.064	0.032–0.064	0
	Roxithromycin	0.125	0.25	0.064–0.25	0
	Azithromycin	0.125	0.25	0.064–0.25	0
	Spiramycin	0.25	0.5	0.125–0.5	0
	Josamycin	0.25	0.25	0.125–0.5	0
	Cephalothin	0.125	0.5	0.125–2	0
	Tetracycline	0.25	32	0.125–64	16
	Chloramphenicol	2	4	1–32	4
	Vancomycin	0.5	0.5	0.25–0.5	0
	Penicillin	0.008	0.016	0.008–0.016	0
	Ciprofloxacin	0.5	1	0.25–2	0
	Quinupristin-dalfopristin	0.25	0.5	0.125–1	0

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

CR-phenotype isolates. However, two interesting changes were seen in the antibiotic resistance pattern of the IR-phenotype isolates. In 1990, the proportion of the IR-phenotype isolates that were resistant to tetracycline was 10% (28), which was only a little more than the proportion found among erythromycin-susceptible *S. pyogenes* isolates (4%). However, in 1994 and 1995 (this study), 93% of the IR phenotype isolates were resistant to tetracycline, which is comparable to the 82 and

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TABLE 2. Distribution of erythromycin resistance genes among 45 erythromycin-resistant *S. pyogenes* isolates, including 19 M-phenotype isolates, 24 IR-phenotype isolates, and 2 CR-phenotype isolates

Erythromycin resistance phenotype and T-antigen type	No. of isolates tested	Presence of the following gene:				
		mefA	*ermA*	*ermB*	*ermC*	*ermTR*
M						
4+	12[a]	+	−	−	−	−
Nontypeable+	6	+	−	−	−	−
13/B3264−	1	+	−	−	−	−
IR						
28+	19[b]	−	−	−	−	+
9+	2	−	−	−	−	+
5/27/44+	1	−	−	−	−	+
9−	1	−	−	−	−	+
28−	1	−	−	−	−	+
CR						
4−	1	−	−	+	−	−
28+	1	−	−	−	−	+

[a] Eight of 12 isolates were shown to be of different clonal origin by randomly amplified polymorphic DNA analysis.
[b] Isolates of different geographical origins.

100% rates of resistance found among the CR-phenotype isolates in 1990 (28) and 1994 and 1995, respectively, and considerably more than the 16% rate of resistance found among erythromycin-susceptible isolates in 1994 and 1995. In addition, resistance to chloramphenicol was detected for the first time in *S. pyogenes* isolates from Finland; although the M- and CR-phenotype isolates remained susceptible to chloramphenicol, 3% of the IR-phenotype isolates were chloramphenicol resistant. Interestingly, resistance to chloramphenicol was also found in 4% of erythromycin-susceptible *S. pyogenes* isolates. Furthermore, all isolates that were resistant to chloramphenicol were also resistant to tetracycline. It has been shown that tetracycline and chloramphenicol resistance genes, whether they are located on resistance plasmids (14, 20) or in the chromosome (10), are often associated with the MLS_B resistance genes. Thus, it is possible that isolates with MLS_B resistance in Finland were becoming multidrug resistant with time, as happened in Japan during the 1970s (20). Except for resistance to the MLS_B antibiotics, tetracycline, and chloramphenicol, resistance to the other antimicrobial agents tested was not detected.

The *mefA* drug efflux gene has been detected among *S. pyogenes* isolates from different sources (31), and in this study we found it in all M-phenotype *S. pyogenes* isolates. Therefore, it is a predominant erythromycin resistance gene in *S. pyogenes* in Finland. The *mefA* gene was recently cloned and sequenced in *S. pyogenes* (7). It encodes a hydrophobic 44.2-kDa protein with homology to membrane-associated pump proteins (33), and the prokaryotic cell actively pumps 14- and 15-membered macrolide compounds out (31). A resistance mechanism similar to that caused by *mefA* has been found in *Streptococcus pneumoniae*, in which it is mediated by the *mefE* gene (33). The *mefE* gene has also been found in group B beta-hemolytic streptococcus, viridans group streptococci (33), and *E. faecium* (9). The *mefE* gene is ~90% identical to the *mefA* gene, and the primers used in this study to detect the *mefA* gene may detect either of the genes. In this study we have not further elucidated which of the genes was actually detected because these genes may be considered to be the same.

The *ermTR* gene is a new erythromycin resistance methylase gene that we recently characterized from an erythromycin-resistant clinical strain of *S. pyogenes* (A200) isolated in Finland (29). The *ermTR* gene is the first sequence of an *erm* gene other than *ermAM* (*ermB*) that mediates MLS_B resistance in *S. pyogenes*, and its nucleotide sequence is 82.5% identical to the *ermA* sequence; *ermA* was previously found, e.g., in *S. aureus* and coagulase-negative staphylococci (29). In this study, we found that all the IR-phenotype isolates harbored the *ermTR* gene. Furthermore, of the two CR-phenotype isolates one had the *ermTR* gene, and the other had the *ermB* gene (Table 2). Therefore, the *ermTR* gene is currently the predominant *erm* gene among *S. pyogenes* and has spread all over Finland.

So far, erythromycin resistance genes other than *ermB*, *ermTR*, and *mefA* have not been identified in *S. pyogenes*. In Finland, these erythromycin resistance genes have also been detected in group C and G streptococci (16), among which all the IR- and M-phenotype isolates had the *ermTR* and *mefA* or *mefE* genes, respectively. Besides these erythromycin resistance genes found in beta-hemolytic streptococci, a novel efflux resistance gene, *mreA*, which is distinct from both the *mef* and the *erm* gene families, was recently cloned and characterized from a strain of *Streptococcus agalactiae* (6).

Although the *mefA* gene was transferable from *S. pyogenes* M-phenotype isolates to erythromycin-susceptible *S. pyogenes* and *E. faecalis* isolates, we were not able to detect extrachromosomal DNA in these isolates. This is in accordance with studies indicating that in streptococci, including *S. pyogenes*, most antibiotic resistance determinants appear to be carried on the chromosome, and they are often associated with conjugative transposons (10, 11, 34). The results of the hybridization studies indicate that in all the different clones of *S. pyogenes* M-phenotype isolates collected in Finland in 1994 and 1995, the DNA structure of the regions close to the *mefA* gene is similar to the corresponding regions cloned by Clancy et al. (7). The reason for this may be that the different clones are derived from the same ancestor (15), or it is possible that there is an insertion element, e.g., a transposon, which is inserted into the same locus in different strains. It is thus possible that *mefA* resides on a chromosomal conjugative transposon; this hypothesis is supported by its relatively high transfer frequency.

We have not found plasmids in either *S. pyogenes* A200 (29) or other *S. pyogenes* isolates containing the *ermTR* gene. However, elsewhere plasmids carrying determinants for MLS_B resistance have been isolated from *S. pyogenes* with MLS_B resistance (2, 8, 18, 19, 20, 24). In general, most streptococcal plasmids carrying antibiotic resistance genes are conjugative and have been shown to transfer by conjugation between streptococcal species (4, 5, 14) and especially among *S. pyogenes* isolates by transduction (13).

In conclusion, in Finland erythromycin resistance in *S. pyogenes* is caused by two prevalent genes: the *mefA* gene among the M-phenotype isolates and the *ermTR* gene among isolates with the MLS_B resistance phenotype.

APPENDIX

The Finnish Study Group Members are as follows: Ahonen Esa, Central Hospital of Kainuu, Kajaani; Erkki Eerola, University of Turku, Turku; Pirkko Hirvonen, Central Hospital of Keski-Suomi, Jyväskylä; Henrik Jägerroos, Central Hospital of Lappi, Rovaniemi; Marja-Leena Katila, Kuopio University Hospital, Kuopio; Maritta Kauppinen, Central Hospital of Etelä-Karjala, Lappeenranta; Marja-Liisa Klossner, Central Hospital of Satakunta, Pori; Outi Kirsi, Central Hospital of Pohjois-Karjala, Joensuu; Jukka Korpela, Central Hospital of Kanta-Häme, Hämeenlinna; Markku Koskela, Oulu University Hospital, Oulu; Anja Kostiala-Thompson, Jorvi Hospital, Espoo; Päivi Kärkkäinen, Central Hospital of Mikkeli, Mikkeli, and Central Hos-

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