Macrolide-Resistant *Streptococcus pyogenes* in Norway: Population Structure and Resistance Determinants

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A 2.7% prevalence of macrolide resistance in 1,657 Norwegian clinical *Streptococcus pyogenes* isolates was primarily due to *erm*(TR) (59%) and *mef*(A) (20%). Four clonal complexes comprised 75% of the strains. Macrolide resistance in *S. pyogenes* in Norway is imported as resistant strains or locally selected in internally disseminated susceptible clones.

The emergence of macrolide resistance in one of the most challenging human bacterial pathogens, *Streptococcus pyogenes* (group A streptococci [GAS]), is an increasing problem worldwide (11, 14, 27, 36). Although penicillin is the drug of choice, macrolides are used for patients who are allergic to beta-lactams and in combination therapy for severe infections. The principal mechanisms of macrolide resistance in GAS include target site modification encoded by *erm* genes and active drug efflux due to *mef* genes (34, 37, 39). Two classes of methylase genes, *erm*(B) and *erm*(A) subclass *erm*(TR) [hereafter designated *erm*(TR)], (29) have been described in GAS and encode cross-resistance to macrolides, lincosamides, and streptogramin B (MLS_B). They are expressed either constitutively (cMLS_B phenotype) or inducibly (iMLS_B phenotype). Efflux-encoding *mef* genes have been assigned to a single class of *mef*(A) genes (29), but recent work distinguishes between *mef*(A) (first identified in GAS) and *mef*(E) (originally described in *S. pneumoniae*) because of important genetic differences (6). Two new subclasses of *mef* genes have recently been reported: *mef*(I) in pneumococci (4) and *mef*(O) in GAS (30). Giovanetti et al. have described a putative novel efflux system, which contributes to high-level macrolide resistance in some *erm*(TR)-positive GAS strains (12), and *msr*(D) has also proved necessary for drug efflux (5). The aim of the present study was to examine the prevalence of macrolide-resistant GAS in Norway and perform a population structure analysis of resistant strains and their resistance determinants.

A total of 1,657 GAS from clinical infections were consecutively collected within three separate periods from 1993 to 2002 in Norway (Table 1). Forty-four strains (2.7%) showed reduced susceptibility to erythromycin (MIC $\geq$ 1 mg/liter) and were further investigated. Antimicrobial susceptibility testing

and determination of macrolide resistance phenotype and genotype and tetracycline resistance genotype was performed as previously described (9, 16, 25, 32, 33, 37, 38). Subtyping of *mef* was achieved by BamHI digestion of the 346-bp *mef* amplicon (22). *mef* genes were DNA sequenced and compared using BLAST as previously described (30). T-typing, opacity factor analysis, *emm* typing, and multilocus sequence typing (MLST) were performed as previously described (18) and according to published protocols (<http://www.cdc.gov/ncidod/biotech/strep/protocols.htm> and <http://spyogenes.mlst.net>).

Of 1,657 *S. pyogenes* isolates, 44 (2.7%) were macrolide resistant (Table 1). The prevalence of resistance was significantly higher in isolates from wounds (5.8%) than in isolates from the respiratory tract (2.3%) ($P = 0.002$). iMLS_B dominated among the 33 of 44 (75%) strains displaying an MLS_B phenotype encoded by *erm*(TR) ($n = 26$), *erm*(B) ($n = 6$), or *erm*(TR) and *mef*(E) ($n = 1$). M phenotype strains ($n = 11$) harbored either *mef*(A) ($n = 9$) or a recently described *mef* gene ($n = 2$) (30), which is hereafter designated *mef*(O). Tetracycline resistance (MIC $\geq$ 8 mg/liter) was found in 31 of 44 (70%) strains, predominantly MLS_B strains (29 of 33 [88%]), and was encoded by either *tet*(M) ($n = 23$) or *tet*(O) ($n = 8$). *erm*(B) and *mef*(O) were exclusively associated with *tet*(M), whereas *erm*(TR) was also found in strains possessing *tet*(O) (Table 2). The iMLS_B, *erm*(TR)-carrying strains harboring *tet*(M) had lower erythromycin MICs compared to strains carrying *tet*(O) (data not shown).

The *emm* types were all present in the Centers for Disease Control and Prevention database, and 31 of 44 strains (70%) had sequence types (STs) previously defined in the MLST database (Table 2) (10, 20, 28, 36). A total of 13 strains revealed new allele combinations and were assigned to new STs (Table 3).

The prevalence of resistance was low (2.7%), which is comparable to rates in Denmark (3%) and Canada (4.7%), but in contrast to rates in Finland (7.4%), Poland (12%), Germany (14%), Spain (29.7%), and Greece (38%) (2, 3, 23, 27, 35, 36, 40). *erm*(TR) dominated in our material as in Poland, Bulgaria, and Canada (8, 36, 40) but in contrast to reports from Spain,

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TABLE 1. Epidemiological and clinical origin of 44 Norwegian macrolide-resistant *S. pyogenes* isolates included in the study

Study period (yr)	Norwegian population coverage (%)	No. of strains	No. of macrolide-resistant strains (%)		Reference
			Respiratory tract	Wounds	
1993	15.3	181	3 (1.6)		15
1997	27.9	513	6 (1.2)		15
2002 ^a	100	568	12 (2.1)		24
2002 ^a	100	395		23 (5.8)	24
Total		1,657	21 (1.3)	23 (1.4)	

^a Wounds and respiratory tract specimens were consecutively collected in 2002.

Belgium, Greece, Argentina, and Finland where M type resistance encoded by *mef*(A/E) predominates (2, 7, 14, 17, 35).

A significant association between macrolide usage and macrolide resistance in *S. pyogenes* has been documented in Finland (31). Albrich et al. found a direct association between the antibiotic selection pressure and the prevalence of macrolide resistant *S. pyogenes* on a national level in 16 industrialized countries from 1994 to 2000 (1). Total macrolide usage in Norway is comparable to Finland, but the prevalence of macrolide resistance in *S. pyogenes* has remained lower in our country. Goossens et al. reported that azithromycin usage is widespread in Finland, whereas erythromycin is more commonly used in Norway (13). Whether this observation can explain the differences in macrolide resistance rates warrants further investigation. Coresistance was primarily associated with tetracycline, and *tet*(M) was the most common determinant. Since coresistance to tetracycline predominated among MLS_B strains in our study, it is likely that tetracycline resis-

TABLE 3. Allelic profiles of new STs in macrolide-resistant *S. pyogenes* from Norway

ST	No. of isolates	Allelic profile ^a						SLV ^b in MLST database
		<i>gki</i>	<i>gtr</i>	<i>murL</i>	<i>mutS</i>	<i>recP</i>	<i>xpt</i>	
ST253	1	16	2	11	3	1	13	1
ST340	4	2	2	8	3	2	3	2
ST368	1	94	8	1	1	1	3	4
ST369	6	13	6	2	3	23	3	62
ST371	1	4	6	2	54	21	7	1

^a Alleles that are different from those found in the MLST database are in boldface.

^b SLV, single locus variant of ST.

tance determinants were genetically linked to *erm* genes rather than to *mef*(A)-containing elements.

Population structure analysis revealed four clonal complexes (CCs) defined by *emm* typing, T typing, and MLST (33 of 44 [75%]). Three of the CCs displayed the *erm*(TR)-encoded MLS_B phenotype and were coresistant to tetracycline. ST46 in CC1 has been associated with *erm*(B), *mef*(A/E), or macrolide susceptibility in other countries (20, 28, 36), whereas ST63 in CC2 contained *erm*(TR) in Poland and Germany in accordance with our findings (28, 36). The remaining six strains in CC2 belonged to ST369, which is a single locus variant (SLV) of ST63 not previously registered in the MLST database. CC3 comprised *erm*(TR)-positive ST340, which is a novel SLV of the Australian ST205 but carrying a *mef* gene (21). CC4 was monoresistant to macrolides due to *mef*(A). This clone has previously been observed in Finland, Germany, Spain, and England (14, 20, 26, 28).

McGeer et al. have recently described local selection and clonal spread as two fundamentally different evolutionary

TABLE 2. Macrolide resistance genotypes and phenotypes, tetracycline resistance genotypes, T-types, *emm* types, and MLST of 44 *S. pyogenes* isolates

No. of isolates	Macrolide resistance		Tetracycline resistance (genotype)	T-type	<i>emm</i>	ST	CC ^b
	Genotype ^a	Phenotype					
6	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T12,3/13/B3264	22.0	46	CC1
2	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T12,3/13	22.0	46	CC1
1	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T12,3	22.0	46	CC1
1	<i>erm</i> (TR)/ <i>mef</i> (E)	cMLS _B		T8/25/imp19	22.0	46	CC1
1	<i>erm</i> (TR)	iMLS _B		T12,3/13/B3264	22.0	368	CC1
4	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T8/25/imp19	53.2	340	CC3
1	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (O)	T8/25/imp19	77.0	63	CC2
1	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (O)	T28	77.0	63	CC2
4	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (O)	T28	77.0	369	CC2
2	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (O)	T28,13	77.0	369	CC2
2	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T8/25/imp19	58.0	176	
1	<i>erm</i> (TR)	cMLS _B		T8/25/imp19	75.0	150	
1	<i>erm</i> (TR)	iMLS _B	<i>tet</i> (M)	T3/13/B3264	94.1	89	
2	<i>erm</i> (B)	cMLS _B	<i>tet</i> (M)	T3/13/B3264	73.0	331	
1	<i>erm</i> (B)	iMLS _B	<i>tet</i> (M)	T12	79.1	362	
1	<i>erm</i> (B)	iMLS _B	<i>tet</i> (M)	T14, 8/25/imp19	49.0	371	
1	<i>erm</i> (B)	iMLS _B	<i>tet</i> (M)	T9,14, 8/25/imp19	9.0	75	
1	<i>erm</i> (B)	cMLS _B		T11, 8/25/imp19	78.0	253	
9	<i>mef</i> (A)	M-type		T4	4.0	39	CC4
1	<i>mef</i> (O)*	M-type	<i>tet</i> (M)	T3/13/B3264,8/25/imp19	33.0	3	
1	<i>mef</i> (O)*	M-type	<i>tet</i> (M)	T5,8/25/imp19	stNS1033	205	CC3

^a New *mef* alleles. See the text.

^b CC, clonal complex. CC1 to -4 define four different CCs.

mechanisms for the emergence and dissemination of antimicrobial resistance (19). Our findings elucidate the relationship between these two mechanisms in Norway. Most isolates (43 of 44 [98%]) were indistinguishable or closely related to internationally reported clones, suggesting import and clonal spread. However, several STs (ST340, ST369, and ST46) displayed differences in their content of resistance genes. This may indicate that they have acquired their determinants in response to local antibiotic selection, but the paucity of macrolide susceptible strains in our study precludes any definite conclusion on this issue. The regional dissemination of the M type ST39 clone in both high- and low-consumption countries is intriguing, and the selective advantage of this clone warrants further studies. Furthermore, *mef(A)* was recently shown to predominate among macrolide resistant *S. pneumoniae* in Norway (16). The evolutionary mechanism(s) for the successful spread of this efflux mechanism between streptococci remain(s) to be elucidated.

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