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Resistance to Erythromycin and Telithromycin in *Streptococcus pyogenes* Isolates Obtained between 1999 and 2002 from Greek Children with Tonsillopharyngitis: Phenotypic and Genotypic Analysis

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Since the late 1990s, the prevalence of erythromycin-resistant *Streptococcus pyogenes* has significantly increased in several European countries. Between January 1999 and December 2002, 1,577 isolates of *S. pyogenes* were recovered from children with tonsillopharyngitis living in various areas of Western Greece. Erythromycin resistance was observed in 379 (24%) of the 1,577 isolates. All erythromycin-resistant strains along with 153 randomly selected erythromycin-susceptible *S. pyogenes* isolates were tested for their antimicrobial susceptibility, resistance phenotypes, and genotypes. Representative isolates underwent *emm* gene sequence typing. Isolates with reduced susceptibility to telithromycin (MIC, ≥2 μg/ml) were studied for multilocus sequence type, L22, L4, and 23S rRNA mutations. Of the total 379 erythromycin-resistant isolates, 193 (50.9%) harbored the *mef*(A) gene, 163 (43%) *erm*(A), 1 (0.3%) *mef*(A) plus *erm*(A), and 22 (5.8%) the *erm*(B) gene. Among the erythromycin-susceptible isolates, *emm* 1 (25%), *emm* 2 (12.5%), and *emm* 77 (12.5%) predominated. Furthermore, among the erythromycin-resistant isolates, *emm* 4 (30.6%), *emm* 28 (22.2%), and *emm* 77 (12.5%) prevailed. Resistance to telithromycin was observed in 22 (5.8%) of the erythromycin-resistant isolates. Sixteen (72.7%) of the 22 isolates appeared to be clonally related, since all of them belonged to *emm* type 28 and multilocus sequence type 52. One of the well-known mutations (T2166C) in 23S rRNA, as well as a new one (T2136C), was detected in erythromycin- and telithromycin-resistant isolates. High incidence of macrolide resistance and clonal spread of telithromycin resistance were the characteristics of the Greek *S. pyogenes* isolates obtained from 1999 to 2002.

Streptococcus pyogenes is the most common bacterial agent implicated in acute tonsillopharyngitis and can also cause a variety of skin and soft tissue infections and severe invasive disease (20). Macrolides constitute the alternative choice for the treatment of streptococcal tonsillopharyngitis and other respiratory tract infections, valuable especially in patients allergic to β-lactams.

Since the late 1990s, resistance to erythromycin and other 14- and 15-membered ring macrolides has been increasingly detected in *S. pyogenes* in several European countries (1, 10, 16) and other parts of the world, such as Korea (24). The main known mechanisms of macrolide resistance in *S. pyogenes* are a 14- and 15-membered ring macrolide-specific efflux mechanism (M phenotype) (21), encoded by the *mef*(A) gene (6), as well as the modification of the ribosomal target by a methylase encoded by the *erm*(B) (26) or the *erm*(TR) gene (18); the latter is currently referred to as *erm*(A) or *erm*(A), subclass *erm*(TR) gene (17). Methylation results in reduced binding of and coresistance to 14-, 15-, and 16-membered ring macrolide, lincosamide, and streptogramin B (MLS) antibiotics. Methylase can be expressed either constitutively (cMLS phenotype) or inducibly (iMLS phenotype). Three subtypes of the iMLS macrolide resistance phenotype have been distinguished: iMLS-A, iMLS-B, and iMLS-C (10). Susceptibility to 16-membered ring macrolides was of particular importance in distinguishing these three subtypes. The iMLS-A subtype was characterized by high-level constitutive resistance to 16-membered ring macrolides. In contrast, the iMLS-B and iMLS-C strains were susceptible to the 16-membered ring macrolide josamycin, but after induction they became high-level and low-level resistant, respectively.

Apart from the above, in a few clinical isolates of *S. pyogenes*, macrolide resistance has been attributed to changes clustered in a highly conserved sequence of L4 (2, 12) and in nucleotide residues of domain V of 23S rRNA (11, 12), which have a key role in macrolide binding (25).

Telithromycin is a ketolide developed specifically for the treatment of community-acquired respiratory tract infections. Some of the constitutively resistant *erm*(B)-positive *S. pyogenes* isolates were found to be telithromycin resistant, although telithromycin retains activity against strains possessing the other macrolide resistance genotypes, such as *erm*(A) and *mef*(A) (10, 16).

The aim of the present study was to investigate in pharyngeal *S. pyogenes* isolates recovered from Greek children with acute tonsillopharyngitis over a 4-year study period (i) the phenotypes and genotypes of erythromycin-resistant isolates, (ii) the predominant *emm* types in erythromycin-susceptible and -resistant isolates, (iii) the in vitro activity of telithromycin in comparison with that of other antibiotics used for the treatment of respiratory tract infections, and (iv) the molecular

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characteristics of isolates with reduced susceptibility to telithromycin.

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MATERIALS AND METHODS

Subjects and specimens. Between January 1999 and December 2002, *S. pyogenes* isolates were recovered from children with tonsillopharyngitis living in Western Greece. Isolates were recovered in various areas of the prefectures of Preveza, Etoloakarnania, Achaia, Ilia, and Messinia. The study population consisted of children between 2 and 16 years of age, with signs and symptoms of acute tonsillopharyngitis confirmed by a positive throat culture for *S. pyogenes*. The study was performed in collaboration with 14 practicing pediatricians, who participate in our working group, the Hellenic Antibiotic-Resistant Respiratory Pathogens (HARP) Study Group. From November 2000 through December 2002, these pediatricians enrolled children with tonsillopharyngitis on clinical studies of different treatment regimens (G. A. Syrogiannopoulos, I. N. Grivea, N. G. Beratis, and the HARP Study Group, 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. G-436, 2002; G. A. Syrogiannopoulos, I. N. Grivea, D. Kritikou, and the HARP Study Group, 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. G-1547, 2003). One *S. pyogenes* isolate from each child was included in the study. Throat cultures were performed at the Laboratory of the Division of Pediatric Infectious Disease of the University of Patras.

Isolates were identified as *S. pyogenes* by colony morphology, β-hemolysis on sheep blood agar, and Lancefield grouping by using a commercially available agglutination technique (Slidex, Streptokit; bioMérieux, Marcy l'Etoile, France) and by using the pyrrolidonyl-arylamidase test.

S. pyogenes isolates were screened for susceptibility to erythromycin by both the disk diffusion method and the Etest method (AB Biodisk, Sweden). All the erythromycin-resistant and randomly sampled erythromycin-susceptible (n = 153) *S. pyogenes* isolates were studied for their antimicrobial susceptibility. MIC testing was performed by using the broth microdilution method as recommended by CLSI (formerly the National Committee for Clinical Laboratory Standards [NCCLS]) (13). Commercially manufactured microtiter plates (Micronaut-S; Merlin Diagnostics, Bornheim, Germany) containing penicillin G, amoxicillin, cefotaxime, erythromycin, clindamycin, telithromycin, tetracycline, gatifloxacin, ciprofloxacin, vancomycin, and teicoplanin and cation-adjusted Mueller-Hinton broth (Oxoid, Wesel, Germany) plus 5% lysed horse blood (Oxoid) were used. The final inoculum was 5×10^5 CFU/ml (equivalent to a 0.5 McFarland standard). MICs were determined after incubation at 35°C for 24 h in ambient air. *S. pneumoniae* strain ATCC 49619 was used as a control strain. Current NCCLS interpretive criteria were used to define antimicrobial resistance (14). For telithromycin, *S. pneumoniae* breakpoints of ≤1 and ≥4 μg/ml were used for sensitivity and resistance, respectively.

Determination of macrolide resistance phenotype. The macrolide resistance phenotypes and their subtypes were determined on the basis of the pattern of susceptibility to erythromycin and clindamycin and confirmed by the triple-disk (erythromycin plus clindamycin and josamycin) test. The triple-disk test was set up to facilitate the laboratory discrimination of the 3 subtypes (iMLS-A, iMLS-B, and iMLS-C) of the iMLS macrolide resistance phenotype, as described previously (10).

Macrolide resistance determinants. *S. pyogenes* isolates showing resistance to erythromycin were tested by PCR for the presence of *erm*(A), *erm*(B), or *mef*(A) macrolide resistance determinants (3). Isolates with reduced susceptibility to telithromycin (MIC, ≥2 μg/ml) were further analyzed for mutations in 23S rRNA and ribosomal proteins L4 and L22. Nucleotide sequences for 23S rRNA and L4 and L22 ribosomal proteins in *Escherichia coli* and *S. pneumoniae* were obtained from the Institute for Genome Research website (http://www.tigr.org). Specific oligonucleotide primers were designed from these sequences. Primer sequences and conditions for PCR amplifications were those described by Canu et al. (4). The following primers were used: for *rplV* (L22), 5′-GCAGACGACAAGAAAACACG-3′ and 5′-GCCGACACGCATACCAATTG-3′; for *rplD* (L4), (i) 5′-AAAGGTAACGTACCAGGTGC-3′ and 5′-GCGTGGTGGTGGTGTTG-3′ and (ii) 5′-CACGAGTGTCAACTTCAAATAC-3′ and 5′-GAGCGTCTACAGCTACG-3′; for *rrl* (23S rRNA domain II), 5′-CGGCGAGTTACGATTATGATGC-3′ and 5′-CTCTAATGTCGACGCTAGCC-3′; and for *rrl* (23S rRNA domain V), (i) 5′-CTGTCTCAACGAGAGACTC-3′ and 5′-CTTAGACTCCTACCTATCC-3′ and (ii) 5′-GTATAAGGGAGCTTGACTG-3′ and 5′-GGGTTTCACACTTAGATG-3′. Since better discrimination between mutated alleles was obtained for denatured DNA fragments between 150 and 500 bp, portions of the *rrl* gene (domains II and V of 23S rRNA), the entire *rplV* gene and two overlapping fragments of the L4 gene (*rplD*) were amplified. The three fragments amplified from *rrl*, two for domain V and one for domain II, included bases that are essential for the development of erythromycin resistance: G2057, A2058, A2062, G2505, C2611, and A752 (4).

Quinolone resistance determinants. Isolates with reduced susceptibility to ciprofloxacin were tested by PCR for the presence of mutations in the GyrA subunit of the DNA gyrase and/or the ParC subunit of topoisomerase IV.

***emm* types of *S. pyogenes* isolates.** A randomly selected sample of 104 erythromycin-susceptible and -resistant *S. pyogenes* isolates were studied for their *emm* type according to the method of Podbielski et al. (15). Similarity searching was performed by using the N-terminal hypervariable region of the M gene based on the latest information from the Centers for Disease Control and Prevention website (http://www.cdc.gov/ncidod/-biotech/strep/strains/emmtypes.html). *S. pyogenes* CS101 (*emm* 49) was used as a reference strain.

Multilocus sequence typing. Telithromycin-resistant isolates were analyzed by multilocus sequence typing as described by Enright et al. (8).

Statistical analysis. The standard chi-square test was used for comparison of proportions between groups of isolates, employing Yates' continuity correction in 2 by 2 tables. In the event of low expected frequencies, exact P values were obtained from SPSS version 11 (SPSS Inc., Chicago, Ill). A two-tailed P value of ≤0.05 was considered significant.

TABLE 1. Year of recovery and macrolide resistance phenotypes of the 379 erythromycin-resistant isolates

Macrolide resistance phenotype (n)	No. of isolates for the corresponding yr (%)			
	1999 (n = 23)	2000 (n = 82)	2001 (n = 200)	2002 (n = 74)
M (193)	8 (34.8)	49 (59.8)	87 (43.5)	49 (66.2)
iMLS				
iMLS-A (2)	0	1 (1.2)	1 (0.5)	0
iMLS-B (34)	0	5 (6.1)	27 (13.5)	2 (2.7)
iMLS-C (129)	15 (65.2)	27 (32.9)	67 (33.5)	20 (27)
cMLS (21)	0	0	18 (9)	3 (4.1)

RESULTS

Between January 1999 and December 2002, 1,577 consecutive clinical strains of *S. pyogenes* were isolated from children with tonsillopharyngitis living in Western Greece. *S. pyogenes* infections were mainly seen among children 5 to 10 years of age (64% of cases). The distribution of isolates during the study period was the following: 70 in 1999, 200 in 2000, 823 in 2001, and 484 in 2002.

Resistance to erythromycin was found in 379 (24%) of these 1,577 isolates. Out of a total of 379 erythromycin-resistant isolates, 50.9% had the M phenotype, 0.5% the iMLS-A, 9% had the iMLS-B, 34% the iMLS-C, and finally 5.6% had the cMLS phenotype (Table 1). There was phenotype and genotype agreement in >99% of the erythromycin-resistant isolates. The 379 tested erythromycin-resistant isolates harbored the *mef*(A) (50.9%), *erm*(A) (43%), *mef*(A) plus *erm*(A) (0.3%), or *erm*(B) (5.8%) gene (Table 2).

The antimicrobial susceptibility results for the 153 randomly sampled erythromycin-susceptible and the 379 erythromycin-resistant isolates are presented in Table 3. One isolate, not included in the table, had intermediate resistance to erythromycin. The molecular analysis of this isolate did not reveal the presence of any macrolide resistance determinant. The highest rates of reduced susceptibility to

TABLE 2. Macrolide resistance phenotypes of the 379 erythromycin-resistant isolates

Macrolide resistance genotype (*n*)	No. of isolates for the following macrolide resistance phenotype:				
	M (*n* = 193)	iMLS			cMLS (*n* = 21)
		iMLS-A (*n* = 2)	iMLS-B (*n* = 34)	iMLS-C (*n* = 129)	
mef(A) (193)	193	0	0	0	0
erm(A) (163)	0	1	34	128	0
erm(A) plus *mef*(A) (1)	0	0	0	1	0
erm(B) (22)	0	1	0	0	21

tetracycline were noted among isolates harboring the *erm*(A) gene and expressing the iMLS-B (38.2%) and iMLS-C (53.1%) subtypes.

In addition txxxo the antimicrobial agents presented in Table 3, good activity against all isolates was seen with cefotaxime (MICs, ≤0.016 μg/ml), vancomycin (MICs, 0.125 to 0.5 μg/ml), and teicoplanin (MICs, ≤0.03 to 0.25 μg/ml). Among the *S. pyogenes* isolates tested, one had relatively reduced susceptibility to ciprofloxacin (MIC = 2 μg/ml) and 22 were telithromycin resistant.

All the isolates that showed the cMLS phenotype and harbored the *erm*(B) gene had high-level resistance to erythromycin and clindamycin (MICs, ≥32 μg/ml), except one isolate that had an erythromycin MIC of 2 μg/ml and clinda-

TABLE 3. Antimicrobial susceptibility of erythromycin-susceptible and erythromycin-resistant *S. pyogenes* isolates with different resistance genotypes

Macrolide resistance genotype (*n*)	Antimicrobial agent	MIC (μg/ml)			No. of strains (%)		
		Range	50%[a]	90%	Susceptible	Intermediate	Resistant
Susceptible (153)	Penicillin G	≤0.016	≤0.016	≤0.016	153 (100)	0	0
	Amoxicillin	≤0.016–0.03	≤0.016	≤0.016	153 (100)	0	0
	Erythromycin	≤0.06–0.125	≤0.06	≤0.06	153 (100)	0	0
	Clindamycin	≤0.06–0.125	≤0.06	≤0.06	153 (100)	0	0
	Telithromycin	≤0.016–0.5	0.03	0.03	153 (100)	0	0
	Tetracycline	≤0.016–≥32	0.25	0.5	138 (90.2)	2 (1.3)	13 (8.5)
	Gatifloxacin	≤0.06–0.5	0.125	0.25	153 (100)	0	0
	Ciprofloxacin	0.125–2	0.5	0.5	153 (100)	0	0
mef(A) (193)	Penicillin G	≤0.016	≤0.016	≤0.016	193 (100)	0	0
	Amoxicillin	≤0.016–0.03	≤0.016	≤0.016	193 (100)	0	0
	Erythromycin	2–≥32	8	16	0	0	193 (100)
	Clindamycin	≤0.06–0.125	≤0.06	≤0.06	193 (100)	0	0
	Telithromycin	0.03–1	0.5	0.5	193 (100)	0	0
	Tetracycline	≤0.06–16	0.125	0.25	190 (98.5)	1 (0.5)	2 (1)
	Gatifloxacin	≤0.06–0.25	0.125	0.25	193 (100)	0	0
	Ciprofloxacin	0.125–1	0.5	0.5	193 (100)	0	0
erm(A) (163)	Penicillin G	≤0.016	≤0.016	≤0.016	163 (100)	0	0
	Amoxicillin	≤0.016–0.03	≤0.016	≤0.016	163 (100)	0	0
	Erythromycin	1–≥32	2	≥32	0	0	163 (100)
	Clindamycin	≤0.06–0.25	≤0.06	≤0.06	163 (100)	0	0
	Telithromycin	≤0.016–8	0.03	0.03	163 (99.4)	0	1 (0.6)
	Tetracycline	0.125–≥32	2	16	82 (50.3)	19 (11.7)	62 (38)
	Gatifloxacin	≤0.06–0.25	0.125	0.25	163 (100)	0	0
	Ciprofloxacin	0.25–1	0.5	0.5	163 (100)	0	0
erm(A) plus *mef*(A) (1)	Penicillin G	≤0.016	≤0.016	≤0.016	1 (100)	0	0
	Amoxicillin	≤0.016	≤0.016	≤0.016	1 (100)	0	0
	Erythromycin	2	N/A	N/A	0	0	1 (100)
	Clindamycin	≤0.06	N/A	N/A	1 (100)	0	0
	Telithromycin	0.03	N/A	N/A	1 (100)	0	0
	Tetracycline	4	N/A	N/A	0	1 (100)	0
	Gatifloxacin	0.25	N/A	N/A	1 (100)	0	0
	Ciprofloxacin	0.25	N/A	N/A	1 (100)	0	0
erm(B) (22)	Penicillin G	≤0.016	≤0.016	≤0.016	22 (100)	0	0
	Amoxicillin	≤0.016	≤0.016	≤0.016	22 (100)	0	0
	Erythromycin	2–≥32	≥32	≥32	0	0	22 (100)
	Clindamycin	0.25–≥32	≥32	≥32	1 (4.5)	0	21 (95.5)
	Telithromycin	0.06–16	8	8	1 (4.5)	0	21 (95.5)
	Tetracycline	0.125–≥32	0.125	0.25	20 (90.9)	0	2 (9.1)
	Gatifloxacin	0.125–0.25	0.25	0.25	22 (100)	0	0
	Ciprofloxacin	0.25–0.5	0.5	0.5	22 (100)	0	0

[a] N/A, not applicable.

TABLE 4. *emm* type distribution in 32 erythromycin-susceptible and 72 erythromycin-resistant *S. pyogenes* isolates with different macrolide resistance genotypes

emm type	Susceptible (n = 32) (%)	Macrolide resistance genotype			
		mef(A) (n = 28)	*erm*(A) (n = 22)	*erm*(A) plus *mef*(A) (n = 1)	*erm*(B) (n = 21)
1	8 (25)	1 (3.6)	3 (13.7)	0	0
2	4 (12.5)	0	1 (4.5)	0	0
4	2 (6.2)	19 (67.8)	3 (13.7)	0	0
11	1 (3.1)	1 (3.6)	0	0	0
12	3 (9.4)	3 (10.7)	0	0	2 (9.5)
22	0	0	4 (18.2)	0	0
25	0	0	0	1 (100)	0
28	0	0	0	0	16 (76.2)
75	0	2 (7.1)	0	0	1 (4.8)
77	4 (12.5)	0	9 (40.9)	0	0
89	0	0	1 (4.5)	0	2 (9.5)
102	1 (3.1)	1 (3.6)	0	0	0
110	2 (6.2)	0	0	0	0
Others[a]	7 (22)	1 (3.6)	1 (4.5)	0	0

[a] *emm* types 3 (n = 1), 6 (n = 1), 15 (n = 1), 27a=1/27b=2 (n = 1), 44 (n = 1), 49 (n = 1), 65 (n = 1), 68 (n = 1), and [PT 3875(M88)] (n = 1).

mycin MIC of ≥32 μg/ml, but was susceptible to telithromycin (MIC, 0.06 μg/ml).

***emm* types of *S. pyogenes* isolates.** Three *emm* types, 1, 2, and 77, accounted for 50% of the 32 typed erythromycin-susceptible isolates (Table 4). Among the 72 typed erythromycin-resistant isolates, *emm* 4 (30.6%), *emm* 28 (22.2%), and *emm* 77 (12.5%) predominated. *emm* types 4, 12, 75, and 1 accounted for 86.2% of the *mef*(A)-positive isolates.

emm type 1 accounted for 8 (25%) of the 32 erythromycin-susceptible isolates and 4 (5.6%) of the 72 erythromycin-resistant strains (P = 0.007). *emm* type 4 accounted for 2 (6.2%) of the 32 erythromycin-susceptible isolates and 22 (30.6%) of the 72 erythromycin-resistant strains (P = 0.006). *emm* type 28 was not observed in any of the erythromycin-susceptible isolates compared to 16 (22.2%) of the 72 erythromycin-resistant strains (P = 0.002).

Telithromycin-resistant isolates. In 18 of the 22 telithromycin-resistant isolates, one point mutation was detected in the 23S rRNA (Table 5). Specifically, a T2136C mutation was present in 15 isolates and a T2166C mutation in three. Sixteen (72.7%) of the 22 isolates showed an identical multilocus sequence type (ST 52) and *emm* type (*emm* 28), indicating clonal relatedness of most of the isolates. These 16 isolates were recovered in different areas of the prefecture of Etoloakarnania over a 16-month period.

Isolate with relatively reduced susceptibility to ciprofloxacin. The isolate with the relatively reduced susceptibility to ciprofloxacin had the wild type of the GyrA subunit of the DNA gyrase, whereas a mutation was found in the ParC subunit of topoisomerase IV. Specifically, in regions of the ParC, which determine quinolone resistance, a TCC(S)-79-GCC(A) mutation was detected.

DISCUSSION

Prospectively collecting pediatric tonsillopharyngitis isolates, we were able to create a quite large collection of clinical *S. pyogenes* isolates from Western Greece over a 4-year period, from 1999 to 2002. One-fourth (24%) of the 1,577 Greek isolates were erythromycin resistant. In Athens, Greece, 15.2% of the *S. pyogenes* isolates recovered between August 1996 and July 1997 and 23.9% of those isolated between October 1997 and September 1998 were erythromycin resistant (19, 23).

In this study, the M phenotype was encountered in 50.9% of the erythromycin-resistant isolates. Throughout the 4-year study period, the predominant subtype of the iMLS phenotype was iMLS-C. In the present collection, only two isolates exhibited the iMLS-A subtype; one isolate harbored the *erm*(A) and the other one the *erm*(B) gene. Both of the isolates with the iMLS-A subtype have been found to show constitutive resistance to the 16-membered ring macrolide miocamycin (MIC > 16 μg/ml) (G. A. Syrogiannopoulos, B. Bozdogan, I. N. Grivea, L. Ednie, G. D. Katopodis, D. Kritikou, N. G. Beratis, and P. C. Appelbaum, 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1984, 2002).

Resistance to tetracycline appears to be common in the erythromycin-resistant *S. pyogenes* strains (9, 10). Among the Greek *S. pyogenes* isolates obtained from 1999 to 2002, variable rates of resistance to tetracycline were associated with the carriage of different macrolide resistance determinants. The highest rate (49.7%) of reduced susceptibility to tetracycline was noted among the isolates carrying the *erm*(A) gene, whereas the lowest (1.5%) was in strains harboring the *mef*(A) gene. Concomitant resistance to erythromycin and tetracycline has been attributed to the presence in the same transposon of *mef*(A), *erm*(A), or *erm*(B) gene as well as *tet*(M) or *tet*(O) gene encoding resistance to tetracycline (5, 9).

Our study showed that the erythromycin-resistant isolates belonged to a limited number of *emm* types. *emm* types 4, 12, 75,

TABLE 5. Characteristics of the 22 telithromycin-resistant *S. pyogenes* isolates

No. of strains	Date of isolation (mo/day/yr)	MIC (μg/ml)[a] (n)			Resistance genotype (n)	*emm* type	MLST[b]	L22	L4	23S rRNA (n)
		Ery	Cli	Teli						
16	29/1/2001 to 15/4/2002	≥32	≥32	8 (15), 16 (1)	*erm*(B)	28	52	WT[c]	WT	T2136C (13), T2166C (3)
3	14/12/2000 to 26/5/2002	≥32	0.125 (1), 0.25 (1), ≥32 (1)	8	*erm*(A) (1), *erm*(B) (2)	89	101	WT	WT	WT
2	19/7/2001 and 18/12/2002	≥32	≥32	8	*erm*(B)	12	36	WT	WT	T2136C (2)
1	29/10/2001	≥32	≥32	8	*erm*(B)	75	49	WT	WT	WT

[a] Ery, erythromycin; Cli, clindamycin; Teli, telithromycin.
[b] MLST, multilocus sequence type.
[c] WT, wild type.

and 1 accounted for 86.2% of *mef*(A)-positive erythromycin-resistant *S. pyogenes* isolates. The same types accounted for 68.2%, 77.1%, and 100% of *mef*(A)-positive erythromycin-resistant *S. pyogenes* isolates in Italy, North America, and France, respectively (1, 7, 22). In the present study, *emm* type 28 was found to be predominant among the *erm*(B)-positive erythromycin-resistant *S. pyogenes* isolates. The same type accounted for 50% and 70% of *erm*(B)-positive erythromycin-resistant *S. pyogenes* isolates in North America and France, respectively (1, 22).

Our findings indicate that over the last few years in Western Greece, 5.8% of erythromycin-resistant *S. pyogenes* isolates had exhibited resistance to telithromycin. In addition, it should be noted that 72.7% of the telithromycin-resistant *S. pyogenes* isolates belonged to a single *emm* type (*emm* 28) and multilocus sequence type (ST 52), conferring clonal relatedness of isolates.

Sequencing data of telithromycin-resistant isolates showed unique results. In 18 of the 22 isolates one point mutation was detected in the 23S rRNA. Specifically, in 15 isolates a T2136C mutation was detected and in 3 isolates, a T2166C mutation was detected. The T2136C is a new mutation, whereas the T2166C mutation has been described in telithromycin-resistant *S. pyogenes* isolates recovered in Germany (16). The relevance between these mutations and the development of macrolide resistance in *S. pyogenes* needs to be confirmed by further transformation experiments. Most information available today is based on in vitro selection studies showing that certain structures involving domains II and V of 23S rRNA participate in the binding of macrolides (4). In clinical isolates, most of the point mutations were identical to those found in in vitro selection studies, but new mutations were also observed (11, 12, 16). The A2058G and A2058U substitutions confer the highest level of MLS resistance (11, 12).

In summary, 24% of the 1,577 *S. pyogenes* isolates recovered in Western Greece from 1999 to 2002 were erythromycin resistant. Moreover, 5.8% of the erythromycin-resistant isolates also had resistance to telithromycin. The erythromycin- and telithromycin-resistant isolates showed one of the well-known mutations in the 23S rRNA, but they also exhibited a new mutation in the 23S rRNA. Finally, it should be noted that the Greek *S. pyogenes* isolates were characterized by the clonal spread of telithromycin resistance.

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