

Resistance Determinants and Clonal Diversity in Group A Streptococci Collected during a Period of Increasing Macrolide Resistance

Stefania Cresti,^{1,2} Maria Lattanzi,¹ Alessandra Zanchi,¹ Francesca Montagnani,¹ Simona Pollini,² Carla Cellesi,¹ and Gian Maria Rossolini^{2*}

Clinica delle Malattie Infettive¹ and Sezione di Microbiologia,² Dipartimento di Biologia Molecolare, Università degli Studi di Siena, I-53100 Siena, Italy

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Susceptibility to macrolides and lincosamides was investigated with 299 consecutive nonduplicate *Streptococcus pyogenes* clinical isolates collected over a 6-year period (1992 to 1997) from an area of central Italy. During this period, macrolide resistance rates steadily increased (from 9% in 1992 to 53% in 1997; $P < 0.001$). The increase was caused by isolates with a macrolide-lincosamide-streptogramin B resistance phenotype, carrying mostly *erm*(B) but also *erm*(TR) genes, that were not detected in the first 2 years and were detected with increasing prevalence (8, 5, 26, and 37%, respectively) during the following 4 years. During the same period, the prevalence of isolates with a macrolide resistance phenotype, carrying *mef*(A) determinants, did not vary significantly; on average it was 13%, with modest rate fluctuations in different years and no definite trend. Molecular typing revealed a remarkable clonal diversity among susceptible and resistant isolates and a notable heterogeneity of the genetic environment of the resistance genes. The analysis of clonal diversity in relation with resistance phenotypes and genotypes revealed that increased macrolide resistance rates were due to a complex interplay of different mechanisms, with a relevant contribution played by an “epidemic” spread of genetic elements carrying the *erm*(B) gene among the circulating streptococcal population.

Streptococcus pyogenes (group A streptococcus) remains one of the leading bacterial pathogens worldwide. Superficial infections caused by group A streptococci, such as pharyngitis and impetigo, are usually mild and self-limiting but ubiquitous and extremely common. On the other hand, the occurrence of severe invasive infections and of nonsuppurative sequelae, although less common, make of *S. pyogenes* a major public health concern (see reference 3 and references therein).

Macrolide antibiotics are among the preferred drugs for the treatment of group A streptococcal pharyngitis and are largely used in community medicine for empirical chemotherapy of respiratory tract infections, due to their clinical efficacy, good compliance, and low toxicity (34). Resistance to macrolides in *S. pyogenes* can be caused by two different mechanisms: (i) active drug efflux via a transmembrane pump encoded by horizontally acquired *mef* genes (7, 35) and (ii) modification of the 23S rRNA target by rRNA adenine methylases encoded by horizontally acquired *erm* genes (see references 19 and 38 and references therein). The Mef efflux system operates only with 14- and 15-membered ring macrolides (M resistance phenotype) (7), while ribosomal modification by Erm methylases prevents the binding of macrolides, lincosamides, and streptogramins B, leading to resistance to all these compounds (MLS resistance phenotype) (19). The MLS resistance phenotype can be expressed either constitutively (cross-resistance MLS [cMLS] resistance phenotype) or upon induction (inducible

MLS [iMLS] resistance phenotype), with variable patterns of activation by different compounds (19, 38, 39).

Since the first report in the 1950s (20), a large number of epidemiological surveys on macrolide resistance in *S. pyogenes* have been carried out. Results of these studies overall revealed a remarkable variability of resistance rates at different times and in different epidemiological settings (for examples, see references 1, 8, 9, 11, 25, 31, 37, and 41). A clear relationship between resistance rates and the extent of macrolide usage in community medicine was also demonstrated, with relatively rapid variations of the former in response to modifications of prescription policies (6, 11, 30, 31).

Recently, increased macrolide resistance rates in clinical isolates of *S. pyogenes* have been reported in several countries (1, 6, 8, 25). This phenomenon, which likely reflects the vast popularity of the last generation of macrolides (such as azithromycin, clarithromycin, and roxithromycin) in community medicine, is a cause of considerable concern for antimicrobial chemotherapy.

In this study we investigated, at the level of resistance genes and of clonal diversity of the microbial population, the dynamics underlying the rapid increase of macrolide resistance rates observed in clinical isolates of *S. pyogenes* from an area of central Italy.

MATERIALS AND METHODS

Group A streptococcal isolates. The 299 *S. pyogenes* organisms analyzed in this study were consecutive nonduplicate clinical isolates collected at the Laboratory of Clinical Bacteriology of the Institute of Infectious Diseases, University of Siena, Italy, from 1 January 1992 to 31 December 1997. Most isolates (279; 93%) were from pharyngeal swabs of patients with pharyngitis (in some cases complicated with scarlet fever). The remaining ones were from skin swabs of patients

* Corresponding author. Mailing address: Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, Policlinico “Le Scotte,” 53100 Siena, Italy. Phone: 39 577 233327. Fax: 39 577 233325. E-mail: rossolini@unisi.it.

TABLE 1. Susceptibility patterns and resistance determinants of the 299 *S. pyogenes* isolates analyzed in this study

Susceptibility category ^a (no. of isolates)	MIC range ($\mu\text{g/ml}$) of:			Resistance phenotype ^b (no. of isolates)	Resistance determinants ^c (no. of isolates)
	Erythromycin	Josamycin	Clindamycin		
Susceptible (214)	$\leq 0.06\text{--}0.25$	$\leq 0.06\text{--}0.5$	$\leq 0.06\text{--}0.5$	NA ^d	None ^e
Erm ^r Jos ^r Cli ^r (21)	>64	>64	>64	cMLS (21)	<i>erm</i> (B) (19); <i>erm</i> (TR) (2)
Erm ^r Jos ^r Cli ^s (21)	>64	>64	0.12–0.5	iMLS (21)	<i>erm</i> (B) (19); <i>erm</i> (TR) (2)
Erm ^r Jos ^s Cli ^s (43)	2–>64	$\leq 0.06\text{--}0.5$	$\leq 0.06\text{--}0.5$	M (40) iMLS (3)	<i>mef</i> (A) (40) <i>erm</i> (TR) (3)

^a According to the results of the broth microdilution assay. Superscripts: S, susceptible; R, resistant.

^b According to the results of the triple-disk diffusion assay (see text for more details).

^c The presence of resistance determinants was investigated by colony blot hybridization with specific probes and confirmed by PCR followed by restriction analysis of the amplification products (see text for more details).

^d NA, not applicable.

^e The presence of resistance determinants was screened by colony blot hybridization in 70 (33%) randomly selected susceptible isolates.

with impetigo (18; 6%) or from invasive infections (one from the lower respiratory tract and one from blood). The streptococcal isolates were identified as belonging to group A according to standard methods (27).

In vitro susceptibility testing. MICs were determined by a broth microdilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (22). The breakpoints for susceptibility classification were those recommended by the NCCLS (23). *S. pyogenes* ATCC 10383 was used for quality control of susceptibility testing. Erythromycin and clindamycin were from Sigma Chemical Co. (St. Louis, Mo.). Josamycin was from ICN Biomedicals (Costa Mesa, Calif.). The triple-disk diffusion test to determine the phenotypes of erythromycin-resistant isolates was performed as described previously (13), except that the disks were placed at the edges of a triangle, spaced from each other by a distance of 20 mm (center to center). Results were interpreted as follows: resistance to only erythromycin indicated an M phenotype; resistance to the three antibiotics indicated a cMLS phenotype; resistance to erythromycin and josamycin associated with a blunting of the clindamycin zone of inhibition or resistance to erythromycin associated with a blunting of the josamycin and clindamycin zones of inhibition indicated an iMLS phenotype.

DNA hybridization experiments. Colony blot hybridization was performed with cells grown directly on nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) layered onto Columbia blood agar plates supplemented with 20 mM glycine. The streptococcal cell wall was lysed by placing the filter onto filter paper soaked with lysis solution I (0.5 M Tris-HCl [pH 8.0], 50 mM EDTA, 50 μg of hen egg white lysozyme [Sigma, grade I]/ml) for 1 h at 37°C. Otherwise, filter processing and hybridization conditions were as described previously (28). Southern blot hybridization was carried out with nitrocellulose membranes (Schleicher & Schuell) as described previously (28). DNA was extracted from *S. pyogenes* as described previously (5). The *erm*(B), *erm*(TR), and *mef*(A) probes were amplicons containing partially or entirely the respective genes, generated as described below (see "PCR experiments"). Probes were labeled with ³²P by the random priming technique using a commercial kit (Roche Biochemicals, Mannheim, Germany). *S. pyogenes* 8B27, A200 (32), and 1A77 were included as positive hybridization controls for the *erm*(B), *erm*(TR), and *mef*(A) probes, respectively.

PCR experiments. PCR for the *erm*(B) gene was carried out using primers ERMAM-up (5'-CACTTCAGGAGTGATTACATGAA) and ERMAM-dn (5'-CTCATAGAATTATTTCTCCCGT), targeting amplification of a 765-bp region covering the entire *erm*(B) open reading frame (ORF), and the following cycling conditions: 94°C for 20 s, 54°C for 60 s, and 72°C for 30 s, repeated for 40 cycles. PCR for the *erm*(TR) gene was carried out using primers ERMTR-f (5'-CCCGAAAATACGCAAAATTTTCAT) and ERMTR-r (5'-CCCTGTTTA CCCATTATAAACG), targeting amplification of a 590-bp region internal to the *erm*(TR) ORF (32) and the following cycling conditions: 94°C for 20 s, 48°C for 60 s, and 72°C for 30 s, repeated for 40 cycles. PCR for the *mef*(A) gene was carried out using primers MEFA-up (5'-GACCAAAAGCCACATTGTGGA) and MEFA-dn (5'-CCTCTGTCTATAATCGCATG), targeting amplification of a 1,432-bp region covering the entire *mef*(A) ORF and some flanks, as described previously (24). Restriction analysis was carried out with *Rsa*I and *Hinc*II for the *erm*(B) amplicons (expected sizes of the restriction fragments, 339 + 175 + 98 + 78 + 75 bp and 628 + 137 bp, respectively) and for the *erm*(TR) amplicons (expected sizes of the restriction fragments, 307 + 203 + 80 bp and 447 + 143 bp, respectively) and with *Cl*A1 and *Bam*HI for the *mef*(A) amplicons (expected sizes of the restriction fragments, 812 + 620 bp and 1,209 + 223 bp, respectively). Restriction enzymes were from Roche Biochemicals.

Analysis of the chromosomal DNA macrorestriction patterns by PFGE. Chromosomal DNA for macrorestriction analysis was extracted from *S. pyogenes* isolates as described previously (33), with minor modifications (the PIV buffer contained Tris-HCl [pH 8] and 1 M NaCl; the ESP solution contained EDTA [pH 9] and 0.1% [wt/vol] sarkosyl). Pulsed-field gel electrophoresis (PFGE) separation of the restriction fragments was carried out using a CHEF-DRIII PFGE apparatus (Bio-Rad, Hercules, Calif.) and the following electrophoretic parameters: run time, 23 h; voltage, 6 V/cm; switch time ramp, 10 to 30 s at included angle of 120°. Analysis of the macrorestriction patterns was carried out with the help of the Diversity Database software (version 2.2.0; Bio-Rad), using an electronic database of images of restriction patterns. Clonal relatedness was inferred in consideration of the criteria proposed by Tenover et al. (36).

Statistical analysis. The significance of the differences of the observed rates of M-type and MLS-type resistant isolates during the study period was evaluated with the χ^2 test. Clustering of isolates according to the macrorestriction profiles was carried out according to the Dice coefficient in combination with the UPGMA clustering method. For clustering analysis the band intensity was not weighted.

RESULTS

Antibiotic susceptibility of *S. pyogenes* isolates. Two hundred ninety-nine nonduplicate clinical isolates of *S. pyogenes*, consecutively collected during a 6-year period (from January 1992 to December 1997) at the Laboratory of Clinical Bacteriology of the Institute of Infectious Diseases of the University of Siena (central Italy), were investigated in this study.

In vitro susceptibilities to erythromycin (a 14-membered ring macrolide), josamycin (a 16-membered ring macrolide), and clindamycin (a lincosamide) were evaluated by microdilution assay for all the isolates. Of these, 214 (72%) were found to be susceptible to all three antibiotics, 21 (7%) were resistant to all of them, 21 (7%) were resistant to erythromycin and josamycin but not to clindamycin, and 43 (14%) were resistant to erythromycin only (Table 1).

The 85 erythromycin-resistant isolates were then analyzed by a triple-disk diffusion test to determine the nature of their resistance phenotype. Results of this test assigned (i) a cMLS phenotype to the 21 isolates resistant to the three antibiotics, (ii) an iMLS phenotype to the 21 isolates resistant to erythromycin and josamycin and to 3 isolates resistant to erythromycin only, and (iii) an M phenotype to the remaining 40 isolates resistant to erythromycin only (Table 1).

The MICs of macrolides and clindamycin for the susceptible isolates were always ≤ 0.5 $\mu\text{g/ml}$. The M-type resistant isolates showed a low-level resistance to erythromycin (MICs, 2 to 16 $\mu\text{g/ml}$) and were susceptible to josamycin and clindamycin,

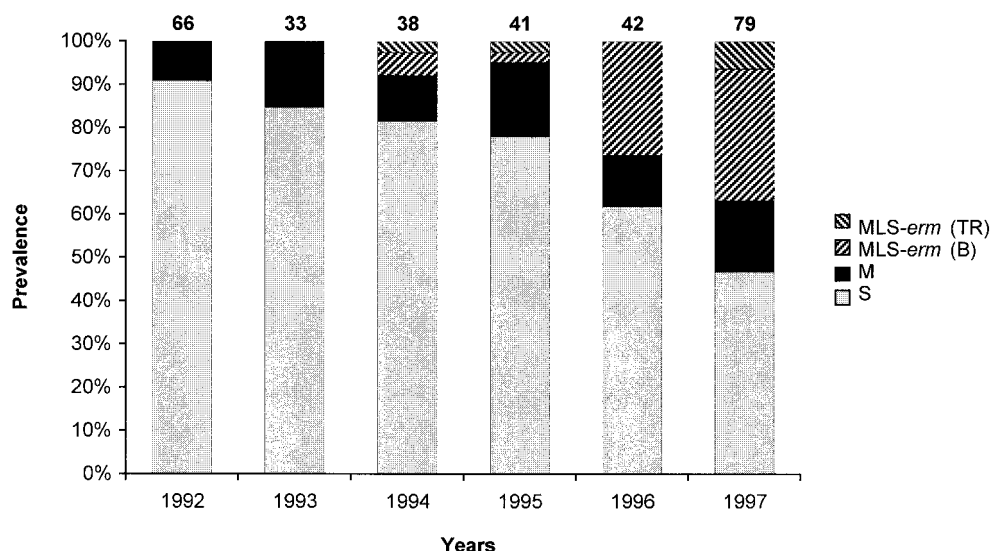


FIG. 1. Prevalence of macrolide resistance phenotypes and genotypes observed during the study period in the 299 *S. pyogenes* isolates. S, susceptible phenotype (□); M, M resistance phenotype (■); MLS-erm(B), MLS resistance phenotype with an *erm*(B) gene (▨); MLS-erm(TR), MLS resistance phenotype with an *erm*(TR) gene (▩). The number above each column represents the total number of isolates in the indicated year. The prevalence differences observed during the study period were not significant for the M-type resistant isolates and were statistically significant ($P < 0.001$) for the MLS-type resistant isolates.

with MICs similar to those of the susceptible isolates. The cMLS-type resistant isolates always exhibited a high-level resistance to both macrolides and clindamycin ($>64 \mu\text{g/ml}$). The iMLS-type resistant isolates showed a high-level resistance to the inducing compounds (erythromycin and, usually, also josamycin [$>64 \mu\text{g/ml}$]), while the MICs of the noninducing antibiotics (clindamycin and, occasionally, josamycin) were similar to those for the susceptible isolates (Table 1).

Resistance determinants. The occurrence of genomic sequences related to the *erm*(B), *erm*(TR), and *mef*(A) genes, which are the macrolide resistance determinants so far identified in group A streptococci, was investigated in all the resistant isolates by means of colony blot hybridization and confirmed by PCR experiments.

In colony blot hybridization the *erm*(B) probe recognized 38 MLS-type resistant isolates (19 cMLS and 19 iMLS, all of which had a resistance phenotype inducible by erythromycin and josamycin), while it did not recognize any of the M-type resistant isolates. The *erm*(TR) probe recognized seven MLS-type resistant isolates (two cMLS and five iMLS, of which two were inducible by erythromycin and josamycin and three were inducible by erythromycin only), while it did not recognize any of the M-type resistant isolates. Hybridization of the same MLS-type resistant isolate with both *erm* probes was never observed. The *mef*(A) probe recognized all the isolates with an M phenotype, while it did not recognize any of the MLS-type resistant isolates. The three probes were also tested with 70 (33%) randomly selected susceptible isolates and always yielded negative results (Table 1).

PCR amplification of the resistance determinants, carried out with specific sets of primers as described in Materials and Methods, yielded amplification products of the expected sizes [765 bp for *erm*(B), 590 bp for *erm*(TR), and 1,432 bp for *mef*(A)] in the various resistant isolates. Results were always consistent with those of the colony blot assay. Restriction anal-

ysis of the amplicons revealed, in all cases, a pattern compatible with that of the respective sequences (data not shown), suggesting that the *erm*(B), *erm*(TR), and *mef*(A) determinants carried by the resistant isolates were either identical or closely related, at the sequence level, to those previously described (14, 32, 35).

Variation of the resistance rates, phenotypes, and determinants during the study period. The prevalence of erythromycin-resistant isolates increased steadily, from 9% in 1992 to 53% in 1997, during the study period (Fig. 1).

The resistance phenotypes showed a different chronological distribution. Isolates with an M phenotype were encountered throughout the study period with modest rate fluctuations and no definite trend (Fig. 1). On the other hand, the isolates with an MLS phenotype have been encountered only since 1994 and their prevalence underwent a remarkable increase from 1996 onward (Fig. 1). The progressive increase of resistance rates observed during the study period, therefore, was essentially contributed by a net increase of MLS-type resistant isolates.

Concerning the distribution of the resistance determinants, both the *erm*(B) and the *erm*(TR) genes have been detected since 1994, but the *erm*(B)⁺ isolates were by far the most prevalent and constituted the principal cause for the increased resistance rates observed during the last 2 years (Fig. 1).

Clonal diversity of the group A streptococcal isolates. Clonal relationships were investigated, by comparison of the PFGE profiles of genomic DNAs digested with *Sma*I, with all the resistant isolates and with a sample of 49 susceptible isolates collected at different times (14 of those collected in 1997 and 7 per year of those collected in the other years, selected at random). A readable restriction profile was obtained with all the 45 MLS-type resistant isolates, with 26 of the 40 M-type resistant isolates, and with 48 of the 49 susceptible isolates. The numbers of detectable bands ranged from 5 to 12, and their sizes ranged from 45 to 380 kb (data not shown). In the

were isolated over prolonged periods; (iii) the M- and MLS-type isolates were found to be clonally unrelated to each other except in one case (clone P); (iv) among the MLS-type isolates, the *erm(B)* and *erm(TR)* genes were sometimes found in clonally related isolates (e.g., clones D, XXI, and XXIII); (v) susceptible isolates clonally related to resistant ones carrying either an *erm(B)*, or an *erm(TR)*, or an *mef(A)* determinant were found (e.g., clones M, W, I, II, XX, XXII, and XXIII); (vi) the net increase of *erm(B)*⁺ isolates observed during the last two years was apparently contributed both by a phenomenon of clonal expansion and by the appearance of new clones.

Among the 14 M-type isolates whose chromosomal DNA was resistant to *Sma*I, the *Sfi*I macrorestriction profiles revealed the presence of several different clones (data not shown).

Analysis of the genetic environment of the resistance determinants. In streptococci, the *mef(A)* and *erm(B)* genes are known to be carried on transposable elements that are usually chromosome borne and have been variously characterized (4, 12, 17, 18, 21, 29), while no information is currently available on the genetic support of *erm(TR)*. The genetic environments of the *mef(A)* and *erm(B)* genes carried by the resistant isolates were analyzed by Southern blot experiments in which the genomic DNAs, digested with *Eco*RI [for *mef(A)*⁺ isolates] or with *Hind*III [for *erm(B)*⁺ isolates], were hybridized to the respective probes. The above enzymes were selected since they do not cut into the respective resistance genes but cut within the mobile elements carrying the respective genes that have thus far been characterized among streptococci (12, 14, 18, 21, 29, 35).

In the *mef(A)*⁺ isolates the probe always recognized a single *Eco*RI fragment, although it varied in size (2.0, 3.7, or 10.2 kb) (data not shown). The 2.0-kb hybridization pattern was found in the 14 isolates whose genomic DNA was resistant to digestion with *Sma*I, as well as in 5 typeable isolates belonging to three clones (P, IV, and VI). The 3.7-kb hybridization pattern was found in eight isolates belonging to the same three clones (although, usually, in different clonal variants) and in eight isolates belonging to six additional clones (G, L, O, II, XII, and XIII). These two patterns were the most prevalent and were found during the whole study period. The 10.2-kb hybridization pattern was less common, being found in isolates of a separate clone (XXII) detected since 1996. The 2.0-kb hybridization pattern could be consistent with the structure of Tn1207.1, an *mef(A)*-containing genetic element recently characterized from *Streptococcus pneumoniae* (29).

In the *erm(B)*⁺ isolates the Southern blot profiles were more heterogeneous. In most isolates the probe recognized a single *Hind*III fragment whose sizes were quite variable (2.8, 6.7, 9.7, 17, or 28 kb), while in a limited number of isolates it recognized two fragments (2.8 + 9.7 or 2.8 + 5.8 kb) (data not shown). No clear relationship was evident between clonality and the hybridization patterns, with identical patterns being consistently found in clonally unrelated isolates and different patterns in clonally related ones (although in this case the different patterns were usually found in different clonal variants). The 6.7- and 2.8-kb single-banded patterns were the first to be detected (in 1994 and in 1995, respectively) and persisted during the following years. The number of circulating patterns increased in the last 2 years, with four different patterns over a

total of 11 *erm(B)*⁺ isolates in 1996 and seven different patterns over a total of 24 *erm(B)*⁺ isolates in 1997. Some hybridization patterns (5.8, 6.7, and 17 kb) could be consistent with the structures of some *erm(B)*-containing transposons previously identified in streptococci (Tn1545, Tn3872, and Tn3701, respectively) (4, 17, 21).

DISCUSSION

Resistance determinants and clonal relatedness among macrolide-resistant isolates of *S. pyogenes* have been recently investigated in several studies (2, 13, 15, 16, 37, 40), but a combined analysis of these aspects in consecutive clinical isolates from a defined epidemiological setting while macrolide resistance rates were rapidly increasing was not reported previously.

Analysis of the resistance phenotypes and determinants revealed that, in our area, the increased macrolide resistance rates observed in *S. pyogenes* were essentially contributed by MLS-type isolates, while the rates of M-type isolates did not vary significantly during the study period. Such findings were different from those observed elsewhere which showed that the increased resistance rates were mostly or almost uniquely contributed by M-type resistant isolates (16, 25, 26, 40), and this discrepancy underscores the variability that can occur in different epidemiological settings. The reasons for this variability, which could be relevant to the formulation of guidelines for empirical chemotherapy, remain as yet poorly understood. Different conditions of selective pressure (due to the use of different antimicrobials in clinical practice) and/or a variable linkage of the macrolide resistance determinants with other resistance or virulence genes (10) could be among the causes. Similar reasons could also account for the fact that, notwithstanding that both types of *erm* determinants have been detected since 1994, *erm(TR)* remained considerably less common than *erm(B)* during the following years and could account for the simultaneous presence of *mef* and *erm* genes in the same isolate, an occurrence which was never detected in this study but was reported elsewhere (2, 9, 13, 16).

The resistance phenotypes could always account for the presence of known resistance determinants: *mef(A)* was detected in all the M-type isolates, while *erm(B)* or the more recently described *erm(TR)* was found in the MLS-type isolates, with the possibility of constitutive or inducible expression for either type of methylase gene. The presence of cryptic *erm* or *mef* determinants was never observed in susceptible isolates from the same area, suggesting that a molecular approach based on detection of the resistance genes could have been highly predictive for testing macrolide resistance in group A streptococci. Analysis of the genetic environment of the *mef(A)* and *erm(B)* genes revealed a notable heterogeneity, especially for *erm(B)*, suggesting a remarkable diversity of the cognate genetic elements present in the group A streptococcal population. Similar findings have also been reported for *erm(B)*-containing elements from pneumococci (21), although in that case the strains had originally been selected to represent different clones.

PFGE genotyping showed a remarkable clonal diversity in the group A streptococci circulating during the study period, in spite of a relatively low-stringency criterion adopted for the

definition of clonal relatedness. This finding is consistent with those reported in other studies on isolates from either defined (9, 25, 26) or diverse (16) epidemiological settings and confirms the notion that the group A streptococcal populations circulating in human communities tend to be highly polyclonal. On the other hand, the finding of clonally related isolates at different times indicates that members of some lineages can circulate for years among the population of a certain area.

When microbial drug resistance is caused by acquired resistance determinants, as in this case, two mechanisms can variably contribute to increased resistance rates: (i) a clonal spread of strains that acquired the resistance determinants; (ii) an epidemic spread of the resistance determinants among the circulating microbial population by horizontal transfer. Analysis of clonal diversity in relation with resistance phenotypes and genotypes provided some insight on the mechanisms underlying the increased macrolide resistance rates observed among group A streptococci in our setting. Resistant strains were, at least in part, generated following the acquisition of resistance genes by circulating susceptible strains, as suggested by the consistent finding (for any type of resistance determinant) of resistant isolates that were clonally related to susceptible ones circulating during the same period. In fact, since only a fraction of the susceptible isolates were analyzed, a similar phenomenon could have been relatively common, although it is also possible that some resistant strains could have been de novo introduced into the circulating population from external sources. A clonal spread of resistant strains was also apparent, especially with some of the *erm(B)*⁺ strains, suggesting that a similar mechanism could have played a relevant role in the increase of resistance rates observed during the last 2 years. However, (i) the diversity of the *erm(B)* genetic environments, (ii) the heterogeneity at the subclonal level observed within the "expanded" clones, and (iii) the consistent finding of identical genetic environments in clonally unrelated strains suggest that the observed clonal spread most likely resulted from the acquisition of different elements carrying the resistance gene by members of a circulating susceptible clone, rather than from the spreading of a single strain that had originally acquired the resistance determinant. It could also be possible that the different *erm(B)* genetic environments observed within the same clone were the result of genetic rearrangements which occurred after the acquisition of the resistance determinant, but the finding of discrete patterns of genetic environments in different clones does not favor this mechanism as a major cause for the remarkable intraclonal diversity of the genetic elements bearing the *erm(B)* gene. In fact, the remarkable diversity of the genetic elements carrying *erm(B)* genes among the resistant isolates points to a scenario in which different mobile elements have been recruited in the circulating population of susceptible clones from external sources rather than to one in which a single mobile element arrived in the population of group A streptococci and then spread among the population. In conclusion, the rapid increase of macrolide resistance rates observed in our setting was likely due to a complex interplay of different mechanisms, among which an epidemic spread of genetic elements carrying the *erm(B)* gene among the circulating streptococcal population was apparently a relevant one. An investigation into the nature of these elements is currently under way.

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S.C. and M.L. contributed equally to this work.

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