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Prevalence and Mechanisms of Macrolide Resistance in *Streptococcus pyogenes* in Santiago, Chile

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Thirty-two macrolide-resistant *Streptococcus pyogenes* isolates were found among 594 clinical isolates collected from 1990 to 1998 in Santiago, Chile, for an overall prevalence of 7.2%. Among the 32 resistant isolates, 28 (87.5%) presented the M phenotype and 4 (12.5%) presented the MLS_B phenotype. Serotyping and pulsed-field gel electrophoresis analysis showed genetic diversity among the resistant isolates.

Three different phenotypes have been described for erythromycin-resistant *Streptococcus pyogenes* isolates according to their susceptibilities to clindamycin: susceptible, inducibly resistant, and constitutively resistant. Isolates of the two last phenotypes have the conventional MLS_B type of resistance encoded by the *erm* genes (*ermAM* or *ermTR*) (6). Erythromycin-resistant but clindamycin-susceptible strains have the M type of resistance encoded by the *mef* gene, which codes for a macrolide efflux mechanism (13).

In this study we evaluated the in vitro activities of erythromycin and clindamycin against clinical isolates of *S. pyogenes* isolated in Santiago, Chile, from 1990 to 1998, identified the mechanisms of macrolide resistance, and investigated the genetic relatedness of the macrolide-resistant strains of *S. pyogenes*.

S. pyogenes strains isolated from 1990 to 1998 in the Clinical Microbiology Laboratory at the Hospital of the Universidad Catolica in Santiago, Chile, were studied. That laboratory received specimens from 10 outpatient centers distributed throughout the Santiago metropolitan area. Consecutive *S. pyogenes* isolates were saved and stored at −70°C and were later tested for their susceptibilities to penicillin, cefotaxime, erythromycin, clindamycin, and vancomycin by agar dilution with Mueller-Hinton agar (MHA) plates supplemented with 5% sheep blood according to the standards of the National Committee for Clinical Laboratory Standards (NCCLS) (8). The antibiotics were tested at doubling dilutions of from 0.03 to 32 μg/ml. The MIC breakpoints used were those published by NCCLS in supplement M100-S9 (9).

The three different phenotypes of the erythromycin-resistant strains (defined as MICs of >0.5 μg/ml) were differentiated by disk diffusion by the double-disk method. MHA plates with 5% sheep blood were inoculated with a 0.5 McFarland organism suspension, and 15-μg erythromycin and 2-μg clindamycin disks were placed 16 mm apart (edge to edge). Resistance to erythromycin with blunting of the clindamycin zone of inhibition on the side of the erythromycin disk indicated an inducible MLS_B phenotype, resistance to both erythromycin and clindamycin indicated a constitutive MLS_B phenotype, and susceptibility to clindamycin with no blunting of the erythromycin zone indicated an M phenotype.

Determination of the M serotypes and T-agglutination patterns was performed by standard techniques. The detection of resistance genes was performed by amplification of the *erm* and the *mef* genes by PCR. The PCR conditions and the specific primers for the *mef* and *erm* genes were used as described previously (14). The genetic relatedness of erythromycin-resistant strains was investigated by pulsed-field gel electrophoresis (PFGE), and the PFGE patterns were interpreted according to the criteria of Tenover et al. (15).

A total of 594 clinical isolates of *S. pyogenes* were studied. Susceptibility testing showed that all the *S. pyogenes* isolates tested were susceptible to penicillin, cefotaxime, and vancomycin. The MICs at which 50% of isolates were inhibited (MIC_{50}s) and MIC_{90}s were ≤0.03 and ≤0.03 μg/ml, respectively, for penicillin and cefotaxime and 0.125 and 0.5 μg/ml, respectively, for vancomycin. Thirty-two strains (7.2%) were erythromycin resistant (MICs, 2 to >32 μg/ml), while 562 strains were erythromycin susceptible (MICs, ≤0.03 to 0.06 μg/ml). However, resistance to erythromycin varied from year to year, with no resistant isolates being detected from 1990 to 1993 (Table 1). The different prevalence values obtained for each year may be due to the variation in the number of throat swab specimens (from which most of the resistant strains were isolated) processed each year. Other investigators reported a prevalence of erythromycin resistance of 10% in one area of Santiago from 1996 to 1998 (R. Camponovo, A. Sepulveda, O. Figueroa, and I. Heitmann, Abstr. XV Cong. Chil. Infect., abstr. CO-38, 1998).

A previous report evaluated the susceptibilities of *S. pyogenes* strains isolated from 1982 to 1987 in Santiago and found no resistance to macrolides (7). The present study confirmed the presence of erythromycin-resistant isolates of *S. pyogenes* in Santiago in 1994. The rate of usage of erythromycin remained constant during the last decade in Chile. Clarithromycin was

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TABLE 1. Annual distribution of erythromycin- and clindamycin-resistant *S. pyogenes* isolates in Santiago, Chile, 1990 to 1998

Yr	No. of isolates tested	No. of isolates resistant to:	
		Erythromycin	Clindamycin
1990	42	0	0
1991	41	0	0
1992	26	0	0
1993	25	0	0
1994	53	6	0
1995	108	5	1
1996	109	11	2
1997	100	5	0
1998	90	5	1
All yrs	594	32	4

introduced into clinical practice in 1991 and azithromycin was introduced into clinical practice in 1993, and usage of these two new macrolides soon exceeded that of erythromycin by more than threefold, which may be a factor in the emergence of macrolide-resistant strains not only of *S. pyogenes* but also of *Streptococcus pneumoniae*, for which the macrolide resistance rate is similar to that for *S. pyogenes* (4).

We found all three different macrolide resistance phenotypes described in streptococci: the MLS_B inducible, MLS_B constitutive, and M phenotypes. Among the 32 erythromycin-resistant isolates isolated from 1994 to 1998, 28 (87.5%) had the M phenotype, demonstrating that this phenotype is the predominant macrolide resistance phenotype among *S. pyogenes* strains isolated in Santiago. This finding is in concordance with the findings of other investigators (1, 2, 6, 10, 12), suggesting that the M phenotype is more common than the MLS_B phenotype in many parts of the world.

The erythromycin MIC_{90} for M-phenotype strains was 16 μg/ml, whereas it was >32 μg/ml for MLS_B-phenotype strains, while clindamycin MIC_{90}s were ≤0.03 and >32 μg/ml for strains of these two phenotypes, respectively. These findings are in agreement with the work of other investigators that M-phenotype strains have lower levels of resistance to erythromycin than MLS_B-phenotype strains (1, 5, 6, 10, 12). For the two strains with inducible clindamycin resistance, clindamycin MICs were within the susceptible range by agar dilution (0.06 and 0.12 μg/ml) after 24 h of incubation, but the clindamycin MICs for these two strains rose to >32 μg/ml after 48 h of incubation. However, these two strains were readily classified as being of the inducible MLS_B phenotype by disk diffusion after 24 h of incubation. These findings confirm our previous report for *S. pneumoniae* that disk diffusion by the double-disk method described above is the best method for the detection and characterization of macrolide-resistant strains (3). By this technique, strains with the constitutive MLS_B phenotype had no zone of inhibition around the erythromycin and clindamycin disks, while strains with the inducible MLS_B phenotype showed blunting of the clindamycin zone of inhibition on the side closer to the erythromycin disk.

All 28 M-phenotype strains had the *mefA* gene but did not have the *ermB* gene, demonstrating that the mechanism of macrolide resistance in these strains is due to the drug efflux system. None of the MLS_B-phenotype isolates amplified the *mefA* gene. Three isolates did, however, amplify the *ermTR* gene. One MLS_B-phenotype strain did not amplify any of the primers tested, and its mechanism of resistance is under investigation.

Serotyping was performed for 26 of the 28 M-phenotype strains, and 19 (73%) were found to be M type 2 (Table 2). The T-agglutination patterns of these M type 2 strains varied slightly, with 13 (68.5%) giving a T2 agglutination pattern and 5 (31.5%) giving a T2/28 agglutination pattern. M type 75 appeared for the first time in 1996 and accounted for 15% of the M-phenotype strains in the present study. These results suggest that erythromycin-resistant *S. pyogenes* M type 2 isolates emerged in Santiago in 1994. During 1994 and 1995 all of the M-phenotype strains were M type 2, and from 1996 to 1998 they constituted more than half of all the M-phenotype strains. M type 75 was the most frequent type observed among macrolide-resistant strains in the United States (5). Perez-Trallero et al. (10) found that type T4 was the most frequent T-agglutination pattern in a region of Spain between 1991 and 1996, followed by type T8/25. Type T4 was also the most frequent T-agglutination pattern in Finland (11), Canada (2), and Ohio (E. L. Fasola, S. Bajaksouzian, P. C. Appelbaum, and M. R. Jacobs, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C87, 1996). However, we did not detect any strains with the type 4 T-agglutination pattern among macrolide-resistant *S. pyogenes* isolates in Santiago.

The molecular studies by PFGE showed that each of the four MLS_B-phenotype strains had a unique electrophoretic pattern, suggesting that they were not genetically related. Fifteen different electrophoretic patterns were observed among the 28 M-phenotype strains (Table 2). However, 14 strains had one of the two more frequent electrophoretic patterns (patterns A and B) obtained in this study. During 1994, all four M2 T2 strains and the two M2 T2/28 strains had identical PFGE patterns, suggesting that all six strains were genetically related. The same PFGE pattern was found for the strains isolated in

TABLE 2. Distribution of M serotypes, T-agglutination patterns, and PFGE patterns among the 28 M-phenotype strains, by year of isolation

PFGE pattern	M and T types	No. of isolates				
		1994	1995	1996	1997	1998
A	M2, T2	4	2	2		
A	M2, T2/28	2	1			
B	M75, T25			2		
B	M75, T8/25			1		
C	M2, T2/28		1			
D	M2, T2			1		
E	M2, T2			1		
F	M2, T2/28			1		
G	Nontypeable			1		
H	M2, T2/28				1	
I	M2, T2				1	
J	M1, T1				1	
K	M22, T12/8				1	
L	M2, T2				1	1
M	M75, T8/25					1
N	Not done					1
O	Not done					1
Total		6	4	9	5	4

1995 and 1996, but beginning in 1995 additional unique PFGE patterns were found. These findings suggest that one clone of M-phenotype erythromycin-resistant strains emerged in 1994 but that subsequently many clones were present in Santiago, including both M- and MLS_B-phenotype strains.

In conclusion, our study demonstrates the presence of erythromycin-resistant *S. pyogenes* strains in Santiago, with the M phenotype being the most frequent phenotype present. The macrolide-resistant strains emerged as one clone that soon spread, and several clones of macrolide-resistant *S. pyogenes* are now present in Santiago.

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