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Survey of *emm* Gene Sequences from Pharyngeal *Streptococcus pyogenes* Isolates Collected in Spain and Their Relationship with Erythromycin Susceptibility

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We conducted a nationwide survey of the variable 5′ *emm* (M protein gene) sequences from 614 pharyngeal *Streptococcus pyogenes* isolates susceptible (299 isolates) and resistant (315 isolates) to erythromycin that were isolated in Spain from 1996 to 1999. Almost 98% of these isolates had *emm* sequences in agreement with previously recorded M antigen association. We only identified a new 5′ *emm* sequence in 17 isolates. Nine different *emm* types accounted for 85% of the *S. pyogenes* isolates susceptible to erythromycin. By contrast, only 3 *emm* types accounted for 70% of the erythromycin-resistant isolates. Further characterization of these isolates by ribotyping and pulsed-field gel electrophoresis indicated that high frequency of erythromycin resistance in Spain is due to few clones.

Streptococcus pyogenes or group A streptococci (GAS) is an important human pathogen as the etiological agent of streptococcal sore throat, skin and soft tissue infections, and the postinfectious syndromes of glomerulonephritis and acute rheumatic fever.

Penicillin remains the drug of choice for the treatment of streptococcal pharyngitis (7); however, an increased failure of this treatment due to copathogenicity with β-lactamase-producing microorganisms has been reported (3). In these cases and in cases where patients are allergic to penicillin, other antibiotics not subject to inactivation by β-lactamases, i.e., amoxicillin-clavulanate, oral cephalosporins, or erythromycin, have been substituted for penicillin (3).

Resistance to erythromycin remained at low levels among *S. pyogenes* in most countries of the world (14); however, in the last years, a significant increase in erythromycin-resistant isolates in different countries has been reported (15, 20, 22, 23). In most of these studies, resistance to erythromycin was caused by a few clones and the mechanisms of resistance were mainly based on the presence of an active drug efflux by pumps encoded by the gene *mefA* (M phenotype) that take out of the cells 14- and 15-membered macrolides (25). By contrast, the other strategy described for erythromycin resistance, target site modification by *erm* methylase strains that express the macrolide-lincosamide-streptogramin B (MLS) phenotype, was not very common among the erythromycin-resistant *S. pyogenes* isolates.

In the last few years, in two different nationwide antimicrobial surveillance studies, an increasing resistance to erythromycin has been reported in *S. pyogenes* isolated in Spain, where the erythromycin resistance frequency has increased from 12 to 26% in the last 10 years, reaching 60% in some regions of the country (2, 19). Therefore, we decided to conduct an epidemiologic investigation to determine the reasons for the high frequency of erythromycin resistance in *S. pyogenes* observed in our country.

A serologic test for M protein antigens has long been a primary method for the identification and epidemiological study of *S. pyogenes* isolates. However, this method is dependent on the preparation of type-specific antisera and extraction of a protein identified as M protein on the surface of *S. pyogenes*, a process that is difficult and specialized. The 5′ ends of *emm* genes are highly heterogeneous and encode the serotype specificity used for the M typing system developed by Lancefield in 1928. Since, in 1995, Beall et al. demonstrated the usefulness of *emm* gene sequence analysis for the routine typing of GAS (5), few studies have used this typing method for monitoring *S. pyogenes* diversity (4, 5, 8, 9, 27).

In this study, we conducted an epidemiological survey of *emm* gene sequences from pharyngeal *S. pyogenes* isolates isolated over a period of 4 years in 8 Spanish hospitals and studied their relationship to erythromycin resistance.

MATERIALS AND METHODS

Strains. *S. pyogenes* clinical strains isolated from patients with community-acquired acute pharyngitis were collected at 8 different hospitals geographically distributed in Spain and belonging to the surveillance collections SAUCE I (May 1996 to April 1997) (2) and SAUCE II (November 1998 to October 1999) (19). For this study, we selected 315 *S. pyogenes* isolates resistant to erythromycin

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(MIC ≥ 1 μg/ml) from both surveillance collections (145 isolates from 1996 to 1997 and 170 isolates from 1998 to 1999). Additional 1:1 matched erythromycin-susceptible *S. pyogenes* strains were also selected. Hence, for every resistant isolate, the immediate next susceptible strain isolated in the same hospital from a patient belonging to the same age group was chosen in order to minimize temporal, geographical, or age-related bias. Two hundred ninety-nine isolates (133 from 1996 to 1997 and 166 from 1998 to 1999) fulfilled the above criteria. The organisms were isolated from patients from 7 cities (number of isolates and period of isolation): Córdoba (30 isolates, 1996 to 1997; 15 isolates, 1998 to 1999), Seville (26 isolates, 1996 to 1997; 47 isolates, 1998 to 1999), Granada (24 isolates, 1996 to 1997; 34 isolates, 1998 to 1999), Valencia (117 isolates, 1996 to 1997), Madrid (81 isolates, 1996 to 1997; 174 isolates, 1998 to 1999), San Sebastián (32 isolates, 1998 to 1999), and Santander (34 isolates, 1998 to 1999). Identification of *S. pyogenes* isolates was confirmed by serogroup A immunoagglutination assays (Streptex; Murex, Chantillon, France).

Susceptibility tests. Susceptibility testing was performed on all isolates by double dilution with the semiautomated microdilution method with custom-dried 96-well trays and Mueller-Hinton broth supplemented with 3% lysed horse blood according to the guidelines of the NCCLS (18) and with a final inoculum of 5×10^5 CFU/ml. Cultures were incubated for 24 h at 35°C in ambient air with erythromycin. The breakpoint used for erythromycin resistance was ≥1 μg/ml.

The mechanism of resistance to erythromycin was evaluated with a double-diffusion disk test as described elsewhere (24) with erythromycin (15 μg) and clindamycin (2 μg) disks placed 20 mm (edge to edge) apart on 5% defibrinated horse blood agar. After overnight incubation at 35°C, the presence of blunting in the zone of inhibition of the clindamycin disk was recorded (21). If the clindamycin inhibition zone was blunted toward the erythromycin disk, the strain was interpreted as clindamycin inducible. Resultant phenotype patterns were clindamycin-sensitive strains (M phenotype) and clindamycin-resistant or -inducible strains (MLS phenotype).

***emm* gene typing.** The *emm* gene type of *S. pyogenes* isolates was determined by amplification and sequencing of the *emm* gene as described by Beall et al. (5). Lysates of the *S. pyogenes* isolates were prepared with mutanolysin as described previously (1). Primers GASM1 and GASM2 were used in PCRs carried out according to the method described previously (5). PCR products were sequenced with primer GASM1 with the dye terminator mix (Applied Biosystems, Foster City, Calif.) and were subjected to automated sequence analysis on a 377 DNA sequencer (Applied Biosystems). DNA sequences were subjected to homology searches against the bacterial DNA database with BLASTN. Sequences were given the GenBank *emm* designations following the criteria previously described (4, 10).

Ribotyping analysis. Ten micrograms of purified genomic DNA, isolated following the protocol previously described (1), were digested with *Hin*dIII, *Sac*I, or *Xho*I according to the specifications of the manufacturer (Amersham-Pharmacia, Uppsala, Sweden). DNA fragments were separated on a 1% agarose gel. Southern blot analysis and *S. pyogenes* 16S rRNA gene probe labeling and detection were carried out with the ECL kit (Amersham-Pharmacia). Differences in banding patterns were documented by visual examination and indexed by small lettering.

PFGE analysis. Analysis of chromosomal DNA was carried out by pulsed-field gel electrophoresis (PFGE) analysis by following standard procedures (16). Chromosomal DNA was digested with *Sfi*I (Amersham-Pharmacia), and fragments were separated in a CHEF-DRIII apparatus (Bio-Rad Laboratories, Barcelona, Spain). Electrophoretic pulses were linearly distributed from 20 to 70 s, for a run time of 22 h. The voltage was 6 V/cm, and the temperature of the electrophoresis chamber was kept at 14°C. The gels were stained with ethidium bromide and photographed.

Differences in banding patterns were documented by visual examination and indexed by capital lettering. The interpretation of restriction fragment patterns was performed in accordance with recent consensus publications (28).

Nucleotide sequence accession number. The sequence of *emm*st2002 obtained in this study has been given GenBank accession number AJ515525.

RESULTS

Prevalent *emm* genes. Overall, 597 (97.2%) of 614 *S. pyogenes* clinical isolates included in our study had 5′ *emm* sequences ≥95% identical to the 160 first bases of one of the *emm* or *emm*-like genes deposited in GenBank. For most of these sequences, this high level of identity actually extended to 200 to 450 bases, without diminishing. The sequences of 522 (85%) isolates were ≥95% identical to the sequence of standard M type reference strain *emm* genes, and the sequences of 75 isolates were ≥95 identical to the sequence of st1815, a sequence not linked with known M typing specificity. The remaining 17 of 614 isolates had an undocumented *emm* gene sequence. This sequence was provisionally designated as st2002 and was only 85% identical to the *emm*104 (formerly st2035) sequence over the first 160 bases.

emm1, *emm3*, *emm12*, and *emm9* were the most prevalent *emm* sequences among *S. pyogenes* isolates susceptible to erythromycin (Table 1). They accounted for 12.4, 11.4, 10, and 9.4%, respectively, of these isolates. Besides these *emm* sequences, the following most common sequences were *emm28*, *emm44*, *emm4*, *emm6*, and the new *emm* gene sequence designated *emm*st2002, each one of which accounted for approximately ≤6% of the erythromycin-susceptible isolates. We did not detect substantial differences between both periods (1996 to 1997 versus 1998 to 1999) in the distribution of the prevalent *emm* sequences among GAS erythromycin-susceptible isolates (Table 1). Perhaps the most interesting difference was that most of the M type st2002 isolates were collected in the first period and their number decreased markedly in the second period.

The prevalent *emm* sequence encountered in the *S. pyogenes* isolates resistant to erythromycin was *emm4* followed by st1815, *emm12*, and *emm75* (Table 1). Altogether, these four M types represented 85% of the *S. pyogenes* isolates resistant to erythromycin while they only represented 19% of the susceptible isolates. Interestingly, M type 75 resistant isolates were almost absent in 1996 to 1997 and became predominant, together with M type 4 resistant isolates, in 1997 to 1998. By contrast, the frequency of M type st1815 resistant isolates was significantly reduced in 1998 to 1999 compared to the first time period. We found a strong association between the resistance to erythromycin and three different M types (Table 1). Among the erythromycin-resistant strains, up to 40.9% belonged to M type 4 isolates versus 5.0% among susceptible strains (odds ratio [OR], 13.13; 95% confidence interval [CI], 7.25 to 24.14). Likewise, 20.6% of the resistant strains were type st1815 versus only 3.3% among erythromycin-susceptible isolates (OR, 7.51; 95% CI, 3.64 to 15.92). For M type 75 isolates, this association was even stronger since this type comprised up to 11.7% of the overall resistant isolates (but 20.6% in the period 1998 to 1999) versus only 0.7% of the susceptible strains (OR, 19.76; 95% CI, 5.00 to 170.29).

We found that 298 (94.6%) of the erythromycin-resistant isolates expressed the M phenotype of resistance. All the M type 4, type st1815, and type 75 (except one M75 isolate) erythromycin-resistant isolates presented this phenotype of resistance. Only 17 (5.4%) resistant isolates (M types 22, 6, 77, and 28) displayed the classical MLS phenotype, including constitutive (5 isolates) and inducible (12 isolates) resistance.

Clonal diversity of the erythromycin-resistant *S. pyogenes* isolates. We investigated whether the erythromycin-resistant isolates represent only a few clonal types, since they belong mainly to only three different M types (M4, st1805, and M75), and all of them displayed the M phenotype. For this purpose, we randomly selected at least one *S. pyogenes* erythromycin-resistant isolate of each one of these M types from each hospital and from both national surveillance collections.

TABLE 1. *emm* sequences of pharyngeal *S. pyogenes* isolated in Spain (1996 to 1999)

No. of isolates	*emm* type	No. (%) of isolates resistant to erythromycin[a] collected in:			No. % of isolates susceptible to erythromycin collected in:		
		1996–1997 (n = 145)	1998–1999 (n = 170)	Total (n = 315)	1996–1997 (n = 133)	1998–1999 (n = 166)	Total (n = 299)
144	4	72 (49.7)	57 (33.5)	129 (41.0)	8 (6.0)	7 (4.2)	15 (5.0)
75	st1815	42 (29.0)	23 (13.5)	65 (20.6)	8 (6.0)	2 (1.2)	10 (3.3)
67	12	15 (10.3)	22 (12.9)	37 (11.7)	19 (14.3)	11 (6.6)	30 (10.0)
39	75	2 (1.4)	35 (20.6)	37 (11.7)	1 (0.8)	1 (0.6)	2 (0.7)
38	1		1 (0.6)	1 (0.3)	11 (8.3)	26 (15.7)	37 (12.4)
34	9	1 (0.7)	5 (2.9)	6 (1.9)	8 (6.0)	20 (12.0)	28 (9.4)
34	3				11 (8.3)	23 (13.9)	34 (11.4)
25	28	3 (2.1)	3 (1.8)	6 (1.9)	6 (4.5)	13 (7.8)	19 (6.4)
24	6		9 (5.3)	9 (2.9)	8 (6.0)	7 (4.2)	15 (5.0)
22	22	1 (0.7)	8 (4.7)	9 (2.9)	3 (2.3)	10 (6.0)	13 (4.3)
17	st2002				14 (10.5)	3 (1.8)	17 (5.7)
16	2	5 (3.4)		5 (1.6)	6 (4.5)	5 (3.0)	11 (3.7)
16	44				6 (4.5)	10 (6.0)	16 (5.4)
12	11				8 (6.0)	4 (2.4)	12 (4.0)
10	77		6 (3.5)	6 (1.9)		4 (2.4)	4 (1.3)
8	78					8 (4.8)	8 (2.7)
6	13				5 (3.8)	1 (0.6)	6 (2.0)
5	50	1 (0.7)		1 (0.3)	4 (3.0)		4 (1.3)
3	73					3 (1.8)	3 (1.0)
2	67		1 (0.6)	1 (0.3)		1 (0.6)	1 (0.3)
2	87				2 (1.5)		2 (0.7)
2	19					2 (1.2)	2 (0.7)
2	66				2 (1.5)		2 (0.7)
2	8					2 (1.2)	2 (0.7)
2	41	2 (1.4)		2 (0.6)			
2	fcrV	1 (0.7)		1 (0.3)	1 (0.8)		1 (0.3)
2	89				1 (0.8)	1 (0.6)	2 (0.7)
1	61				1 (0.6)	1 (0.3)	
1	58					1 (0.6)	1 (0.3)
1	33				1 (0.8)		1 (0.3)

[a] The breakpoint for erythromycin was ≥1 μg/ml.

Ribotyping analysis with genomic DNA from *S. pyogenes* isolates digested with *Sac*I and probed with an internal fragment of the 16S rRNA gene led to 13 different patterns, which are illustrated in Fig. 1A. Each strain was tested at least twice with *Sac*I and with two additional enzymes (*Xho*I and *Hin*dIII); however, *Sac*I-digested fragments were more discriminatory than the *Xho*I- and *Hin*dIII-digested fragments that only rendered two and three different patterns, respectively (data not shown). Type c and j were the most common types among M type 4 and M type st1815 isolates, respectively. For both M types, we observed more variability between the *S. pyogenes* isolated from 1996 to 1997 than in those isolated from 1998 to 1999, where types c and j were almost exclusive (Table 2). Similarly, all M type 75 *S. pyogenes* isolates that were collected only between 1998 and 1999 presented pattern j. All the other patterns were represented by only one strain.

Further genotypic characterization was carried out by genomic DNA macrorestriction with *Sfi*I and PFGE. Representative PFGE profiles of the erythromycin-resistant *S. pyogenes* isolates are shown in Fig. 1B. We identified 17 distinct PFGE types, one (type C) of them divided in 3 subtypes (C.1, C.2, and C.3; data not shown). We found a good correlation between ribotyping and PFGE patterns (Table 2). Thus, ribotypes c and j corresponded, for most of the isolates, to PFGE types C and J, respectively. Again, PFGE types C and J were prevalent, and all other ribotypes corresponded to PFGE types represented, again, by a single isolate.

In summary, results obtained by either ribotyping or PFGE suggest that *S. pyogenes* resistance to erythromycin in Spain between 1996 to 1999 was caused by a few clones belonging to three M types. For M types 4 and st1815, we observed more variability between the *S. pyogenes* isolated from 1996 to 1997 than in those isolated from 1998 to 1999, where all the isolates presented the same genotyping profile. Interestingly, clones c and j, prevalent from 1998 to 1999, were also present from 1996 to 1997.

DISCUSSION

The purpose of this study was to survey the genetic diversity of pharyngeal *S. pyogenes* isolates by using *emm* gene sequence analysis to better understand the increased level of resistance to erythromycin in Spain and to further investigate the epidemiology of these *S. pyogenes* erythromycin-resistant isolates.

In our study, we have determined the 5′ *emm* sequence from 614 pharyngeal *S. pyogenes* isolates taken from two nationwide surveillance collections conducted from 1996 to 1999. To our knowledge, this is the first survey study of *emm* typing conducted in Spain. As expected, nearly 100% of the strains could be genotyped by the *emm* typing system as described recently Facklam et al. (10). Only one newly encountered *emm* gene, provisionally designated *emm* st2002, from 17 isolates had a 5′ *emm* sequence with an identity ≤95% over the first 160 bases to *emm* sequences deposited in GenBank. However, according to Facklman et al. (10), further studies are required to validate

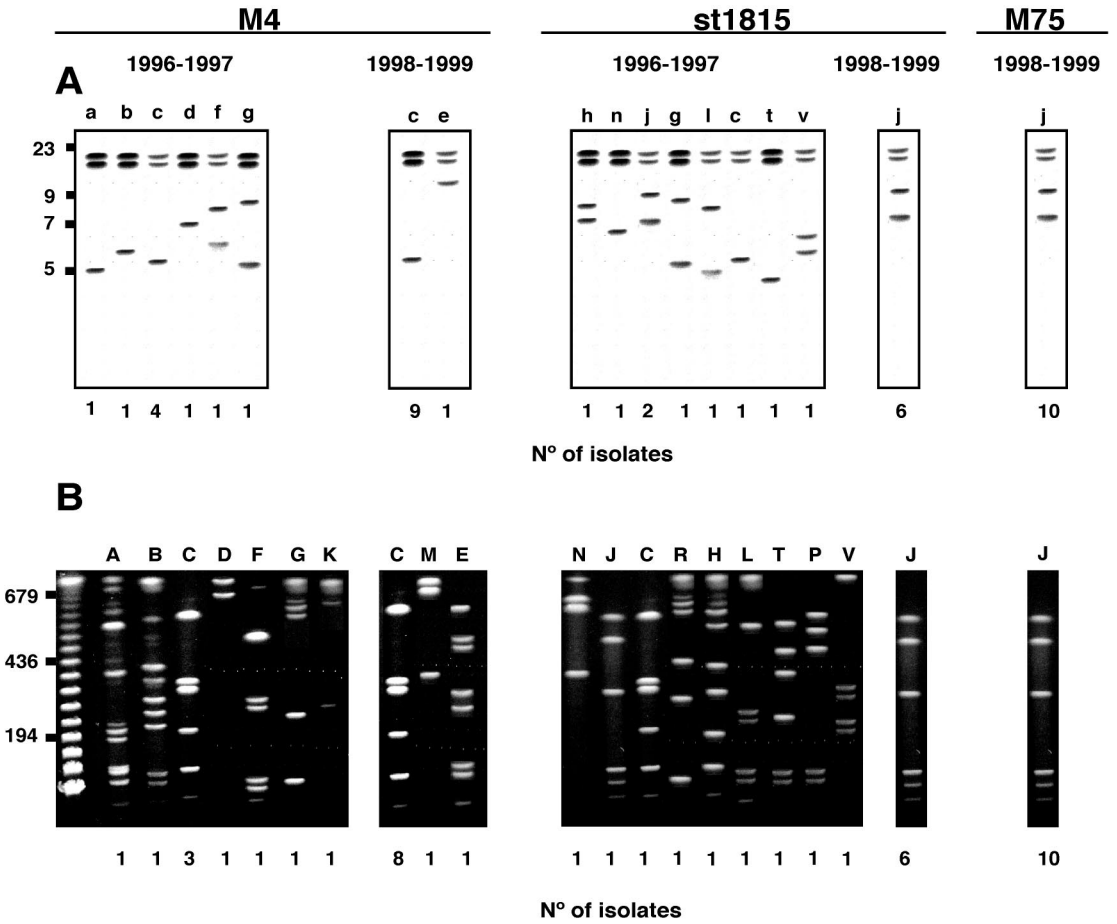

FIG. 1. Representative ribotypes (A) and PFGE types (B) of erythromycin-resistant *S. pyogenes* M types 4, st1815, and 75 isolated between 1996 to 1997 and 1998 to 1999 in Spain. (A) Southern blot of genomic *S. pyogenes* DNA digested with *Sac*I and probed with a GAS 16S rRNA gene probe as described in Materials in Methods. (B) PFGE analysis of genomic DNA restricted with *Sfi*I. Letters on the lanes refer to types, and numbers below each lane refer to the number of isolates of each type. Molecular size markers (in kilobases) are indicated on the left.

TABLE 2. Correlation between *emm* typing, ribotyping, and PFGE typing of erythromycin-resistant *S. pyogenes* isolated in two different time periods in Spain

emm type	Type (no.) of isolates collected in:			
	1996–1997		1998–1999	
	Ribotype	PFGE type	Ribotype	PFGE type
4	a (1)	A (1)		
	b (1)	B (1)		
	c (4)	C (3), K (1)	c (9)	C (8), E (1)
	d (1)	D (1)		
	f (1)	F (1)		
	g (1)	G (1)		
			e (1)	M (1)
st1815	h (1)	H (1)		
	n (1)	N (1)		
	j (2)	J (1), P (1)	j (6)	J (6)
	g (1)	R (1)		
	l (1)	L (1)		
	c (1)	C (1)		
	t (1)	T (1)		
	v (1)	V (1)		
75			j (10)	J (10)

this new *emm* type that shows only 85% identity over the first 160 bases with *emm*104 (formerly st2035) (11).

In our survey, M type 1 and M type 3 were the most prevalent M types among *S. pyogenes* isolates susceptible to erythromycin. Since resistance to erythromycin represents approximately 20 to 30% of the isolates (2, 19), we can conclude that these M types are probably the most common in Spain. This result is in agreement with other M typing studies conducted by other authors (4, 8, 13).

To investigate the reasons for the increased levels of resistance to erythromycin detected in Spain, we analyzed the *emm* gene sequence of 315 GAS isolates resistant to erythromycin collected simultaneously and at the same site to that of the erythromycin-susceptible isolates. Our results show that a few M types were responsible for up to 85% of the erythromycin-resistant isolates, suggesting that few clones caused this phenomenon. This hypothesis was supported by the fact that the M phenotype was the most common resistance mechanism, a feature previously reported by other authors (20, 23). However, genetic divergence among strains sharing the same *emm* gene sequence has been reported (5, 6, 17, 29). For this reason, *emm* sequencing must be supplemented with other approaches

such as PFGE to identify related GAS isolates (4). To further investigate the clonal diversity of the erythromycin-resistant GAS isolates, we randomly selected strains from each hospital geographically distributed all over Spain and from both surveillance collections. We used two genotyping techniques that yielded similar results. Ribotyping analysis of the GAS genomic DNA digested with *Hin*dIII and *Xho*I was poorly discriminatory as previously described (data not shown) (26). However, ribotyping by *Sac*I restriction polymorphism showed equivalent power for strain typing to that of PFGE in favor of labor and time benefit.

The data obtained by both approaches indicate that during the first period (1996 to 1997) many different clones sharing the same M type (M type 4 or M type st1815) were distributed all over Spain and responsible for the resistance to erythromycin detected between 1996 to 1997. By contrast, in the following two years (1997 to 1998), clonal diversity was reduced to a few clones that were the major clones identified in the years 1996 to 1997. These results suggest that these clones have been selected over a short period of time (only 2 years) and have become predominant in Spain. It is reasonable to believe that selection of these clones is related to the consumption of macrolides and that this may account for the geographical differences in the prevalence of resistance to erythromycin in Spain (12, 19).

Our results are in agreement with those of a previous study conducted by Pérez-Trallero et al. in Spain (20). In that study, erythromycin-resistant GAS isolates from Spain ($n = 437$, 1988 to 1997) were composed mainly of two clones designated clone B (*emm* type 4) and clone D (*emm* type 75) that were already isolated in Spain in 1991. Those clonal types showed same lineage to our *emm* types, corresponding to PFGE C and J types, respectively, suggesting that they are probably the same strains. Apart from the strains described in Spain, M type 4 strains with the M phenotype were isolated in Finland and Great Britain before 1991 (22, 23). We do not know whether the strains of clone C isolated in our study are the same as the erythromycin-resistant type 4 found in Finland and Great Britain, but it is probable. In addition to *emm* type 4, *emm* type st1815 accounted for 20% of the GAS resistant to erythromycin. This *emm* type was not detected by Pérez-Trallero et al. in their study, where they assayed only 14 *emm* types (20); however, it was one of the prevalent types in our study. Interestingly, the most common PFGE and ribotype pattern among st1815 *emm* type strains was identical to that of *emm* type 75 strains. A similar observation was previously reported by Whatmore et al. that demonstrated that highly divergent *emm* sequences were present in strains with identical multilocus electrophoretic types, suggesting horizontal transfer of *emm* genes between unrelated strains (29). Alternatively, it might be that *emm*75 and *emm*st1815 share identical PFGE types because *emm* sequence st1815 was generated by homologous excision between the tandem *emm* and *enn* sequences in an *emm*75 parental strain, as suggested in the *emm* sequence database (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm).

In summary, our results show that monitoring of GAS isolate diversity by *emm* gene typing is a useful approach for a better understanding of the epidemiology and origins of specific GAS strains. The application of this typing technique to nationwide multicenter surveillance has revealed that a few clones cause GAS erythromycin resistance in Spain.

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REFERENCES

1. **Albertí, S., C. D. Ashbaugh, and M. R. Wessels.** 1998. Structure of the *has* operon promoter and regulation of hyaluronic acid capsule expression in group A *Streptococcus*. Mol. Microbiol. **28:**343–353.
2. **Baquero, F., J. A. García-Rodríguez, J. García de Lomas, L. Aguilar, and the Spanish Surveillance Group for Respiratory Pathogens.**1999. Antimicrobial resistance of 914 beta-hemolytic streptococci isolated from pharyngeal swabs in Spain: results of a 1-year (1996–1997) multicenter surveillance study. Antimicrob. Agents Chemother. **43:**178–180.
3. **Bass, J. W.** 1991. Antibiotic management of group A streptococcal pharyngotonsillitis. Pediatr. Infect. Dis. J. **10:**543–549.
4. **Beall, B., R. Facklam, T. Hoenes, and B. Schwartz.** 1997. Survey of *emm* gene sequences and T-antigen types from systemic *Streptococcus pyogenes* infection isolates collected in San Francisco, California; Atlanta, Georgia; and Connecticut in 1994 and 1995. J. Clin. Microbiol. **35:**1231–1235.
5. **Beall, B., R. Facklam, and T. Thompson.** 1995. Sequencing *emm*-specific polymerase chain reaction products for routine and accurate typing of group A streptococci. J. Clin. Microbiol. **34:**953–958.
6. **Bessen, D. E., C. M. Sotir, T. L. Readdy, and S. K. Hollingshead.** 1996. Genetic correlates of throat and skin isolates of group A streptococci. J. Infect. Dis. **17:**896–900.
7. **Betriu, C., A. Sánchez, M. Gómez, A. Cruceyra, and J. J. Picazo.** 1993. Antibiotic susceptibility of group A streptococci: a 6-year follow-up study. Antimicrob. Agents Chemother. **37:**1717–1719.
8. **Brandt, C. M., B. Spellerberg, M. Honscha, N. D. Truong, B. Hoevener, and R. Lutticken.** 2001. Typing of *Streptococcus pyogenes* strains isolated from throat infections in the region of Aachen, Germany. Infection **29:**163–165.
9. **Dicuonzo, G., G. Gherardi, G. Lorino, S. Angeletti, M. de Cesaris, E. Fiscarelli, D. E. Bessen, and B. Beall.** 2001. Group A streptococcal genotypes from pediatric throat isolates in Rome, Italy. J. Clin. Microbiol. **39:**1687–1690.
10. **Facklam, R., B. Beall, A. Efstratiou, V. Fischetti, D. Johnson, E. Kaplan, P. Kriz, M. Lovgren, D. Martin, B. Schwartz, A. Totolian, D. Bessen, S. Hollingshead, F. Rubin, J. Scott, and G. Tyrrell.** 1999. *emm* typing and validation of provisional M types for group A streptococci. Emerg. Infect. Dis. **5:**247–253.
11. **Facklam, R. F., D. R. Martin, M. Lovgren, D. R. Johnson, A. Efstratiou, T. A. Thompson, S. Gowan, P. Kriz, G. J. Tyrrell, E. Kaplan, and B. Beall.** 2002. Extension of the Lancefield classification for group A sreptococci by addtion of 22 new M protein gene sequence types from clinical isolates: *emm*103 to *emm*124. Clin. Infect. Dis. **34:**28–38.
12. **Granizo, J. J., L. Aguilar, J. Casal, R. Dal-Ré, and F. Baquero.** 2000. *Streptococcus pyogenes* resistance to erythromycin in relation to macrolide consumption in Spain (1986–1997). J. Antimicrob. Chemother. **46:**959–964.
13. **Johnson, D. R., D. L. Stevens, and E. L. Kaplan.** 1992. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J. Infect. Dis. **166:**374–382.
14. **Kaplan, E. L.** 1997. Recent evaluation of antimicrobial resistance in beta-hemolytic streptococci. Clin. Infect. Dis. **24**(Suppl. 1)**:**S89–S92.
15. **Martin, J. M., M. Green, K. A. Barbadora, and E. R. Wald.** 2002. Erythromycin-resistant group A streptococci in schoolchildren in Pittsburgh. N. Engl. J. Med. **346:**1200–1206.
16. **Maslow, J. N., A. M. Slutsky, and R. D. Arbeit.** 1993. The application of pulsed field gel electrophoresis to molecular epidemiology, p. 563–571. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
17. **Musser, J. M., V. Kapur, J. Szeto, X. Pan, D. S. Swanson, and D. R. Martin.** 1995. Genetic diversity and relationships among *Streptococcus pyogenes* strains expressing serotype M1 protein: recent intercontinental spread of a subclone causing episodes of invasive disease. Infect. Immun. **6:**994–1003.
18. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard. NCCLS document M7–45. National Committee for Clinical Laboratory Standards, Wayne, Pa.
19. **Pérez-Trallero, E., C. Fernández-Mazarrasa, C. García-Rey, E. Bouza, L. Aguilar, J. García de Lomas, F. Baquero, and the Spanish Surveillance Group for Respiratory Pathogens.** 2001. Antimicrobial susceptibilities of 1,684 *Streptococcus pneumoniae* and 2,039 *Streptococcus pyogenes* isolates and their ecological relationships: results of a 1-year (1998–1999) multicenter surveillance study in Spain. Antimicrob. Agents Chemother. **45:**3334–3340.

20. **Pérez-Trallero, E., M. J. Marimón, M. Montes, B. Orden, and M. de Pablos.** 1999. Clonal differences among erythromycin-resistant *Streptococcus pyogenes* in Spain. Emerg. Infect. Dis. **5:**235–240.
21. **Pérez-Trallero, E., M. Urbieta, M. Montes, I. Ayestarán, and J. M. Marimón.** 1998. Emergence of *Streptococcus pyogenes* strains resistant to erythromycin in Gipuzkoa, Spain. Eur. J. Clin. Microbiol. Infect. Dis. **17:**25–31.
22. **Scott, R. J. D., J. Naidoo, N. F. Lightfoot, and R. C. George.** 1989. A community outbreak of group A beta-haemolytic streptococci with transferable resistance to erythromycin. Epidemiol. Infect. **102:**85–91.
23. **Seppala, H., A. Nissinen, H. Jarvinen, S. Houvinen, T. Henriksson, and E. Herva.** 1992. Resistance to erythromycin in group A streptococci. N. Engl. J. Med. **326:**292–297.
24. **Seppala, H., Q. Y. Nissinen, and P. Huovinen.** 1993. Three different phenotypes of erythromycin resistance in group A streptococci in Finland. J. Antimicrob. Chemother. **32:**885–891.
25. **Seppala, H., M. Skurnik, H. Soini, M. C. Roberts, and P. Huovinen.** 1998. A novel erythromycin resistance methylase gene (*ermTR*) in *Streptococcus pyogenes.* Antimicrob. Agents Chemother. **42:**257–262.
26. **Stanley, J., D. Linton, M. Desai, A. Efstratiou, and R. George.** 1995. Molecular subtyping of prevalent M serotypes of *Streptococcus pyogenes* causing invasive disease. J. Clin. Microbiol. **33:**2850–2855.
27. **Tanaka, D., Y. Gyobu, H. Kodama, J. Isobe, S. Hosorogi, Y. Hiramoto, T. Karasawa, and S. Nakamura.** 2002. *Emm* typing of group A streptococcus clinical isolates: identification of dominant types for throat and skin isolates. Microbiol. Immunol. **46:**419–423.
28. **Tenover, R. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
29. **Whatmore, A. M., V. Kapur, D. J. Sullivan, J. M. Musser, and M. A. Kehoe.** 1994. Non-congruent relationships between variation in *emm* gene sequences and the population genetic structure of group A streptococci. Mol. Microbiol. **14:**619–631.