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Phenotypic and Genotypic Characterization of *Streptococcus pyogenes* Isolates Displaying the MLS_B Phenotype of Macrolide Resistance in Spain, 1999 to 2005▽

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The aim of this study was to describe the genetic characteristics of *Streptococcus pyogenes* showing the MLS_B phenotype of macrolide resistance from 1999 to 2005 in Spain and to highlight the substantial increase in these isolates in the last few years. The antimicrobial susceptibilities of 17,232 group A streptococci isolated from Madrid and Gipuzkoa from 1999 to 2005 were studied. The presence of the resistance genes *ermA*, *ermB*, *mef*, *tetM*, and *tetO* and the presence of the *intTn* and *xis* genes of the Tn*916*-Tn*1545* transposon family were studied in a sample of 739 MLS_B-resistant isolates. The epidemiological relationships among these isolates were analyzed by *emm* typing, T typing, and multilocus sequence typing. Erythromycin resistance was found in 21.3% of the isolates analyzed (annual variation of 14.3% to 28.9%). Until 2003, most erythromycin-resistant isolates showed the M phenotype, but in 2004 and 2005, about 50% of isolates showed the MLS_B phenotype. Among the MLS_B-resistant isolates studied, 16 clones were identified. The most prevalent clone was a strange *emm*11/T11/ST403 clone with a null *yqiL* allele. All but one of the 463 *emm*11/T11/ST403 isolates carried the *ermB*, *tetM*, *intTn*, and *xis* genes. The second most prevalent MLS_B-resistant clone was *emm*28/T28/ST52, which comprised two subclones: one bacitracin-resistant, tetracycline-susceptible subclone carrying the *ermB* gene ($n = 115$) and another bacitracin-susceptible, tetracycline-resistant subclone carrying the *ermB* and *tetM* genes ($n = 33$). The rapid diffusion of these two clones, and especially of *emm*11/T11/ST403, caused the large increase in MLS_B-resistant *S. pyogenes* isolates in Spain, suggesting a potential ability for international dissemination.

Streptococcus pyogenes is a pathogen with worldwide distribution that causes a broad spectrum of infections, from uncomplicated pharyngitis to severe life-threatening infections (6). In the absence of a β-lactam allergy, the treatment of choice is penicillin, while the first-line alternative treatments are macrolides or lincosamides. There are two main phenotypes of macrolide resistance: the M phenotype, mediated by the *mef* genes (8), which confer low-level resistance to 14- and 15-membered macrolides but not to 16-membered macrolides, lincosamides, or streptogramin B, and the MLS_B phenotype, mediated by the *erm* genes (20, 34), which confer resistance to macrolides, lincosamides, and streptogramin B antimicrobial agents. This latter phenotype can be constitutive, generally mediated by the *ermB* gene, or inducible, generally mediated by the *ermA* subclass TR (*ermA*) gene (31). Other mechanisms of resistance to macrolides or lincosamides in *S. pyogenes* (e.g., mutations in ribosomal proteins) are infrequently involved and currently have little clinical impact (4, 17).

The factor most directly associated with the increase in antimicrobial resistance is the high level of antibiotic consumption among the population (2, 15). Nonetheless, the final cause of a higher or lower prevalence of antimicrobial resistance depends on the circulating clones.

The aim of this study was to highlight the substantial increase in the MLS_B phenotype of macrolide resistance in *S. pyogenes* strains isolated in the last few years in Spain and to describe the genetic characteristics of these isolates.

MATERIALS AND METHODS

The province of Gipuzkoa (whose capital is Donostia-San Sebastián) is located in the northeastern area of the Basque Country in Spain, bordered by the Bay of Biscay and France to the north; Madrid is located in the center of Spain (415 km from San Sebastián).

All group A streptococci (GAS) isolated at the Microbiology Department of Hospital Donostia (Gipuzkoa) and in the Centro de Salud de Argüelles (Madrid) between 1999 and 2005 were included in this study. During the 7 years of the study, 17,232 GAS isolates (8,772 from Madrid and 8,460 from Gipuzkoa) were tested for antimicrobial susceptibility. More than 85% of the isolates were obtained from the pharyngeal tract.

Clinical isolates were characterized as being GAS according to their colony morphology, beta-hemolysis on blood agar, bacitracin susceptibility, and/or coagglutination with specific antisera (Slidex, Streptokit; bioMérieux, Marcy l'Etoile, France). GAS were confirmed as being *S. pyogenes* by the commercially available identification system Rapid ID 32 STREP (bioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing was performed by the broth microdilution method using Sensititre microtiter trays (Trek Diagnostics Systems, West Sussex, England) and cation-adjusted Mueller-Hinton broth supplemented with 3 to 5% lysed horse blood. The results were interpreted according to CLSI (formerly NCCLS) criteria (9). *Staphylococcus aureus* ATCC 29213 was used as the control. To expand the range of dilutions available in the broth microdilution trays, Etest strips (AB Biodisk, Solna, Sweden) were used. Inducible resistance was classified on the basis of a double-disk test with erythromycin (15 μg) and clindamycin (2 μg) disks (Bio-Rad, Marnes-la-Coquette, France).

Molecular detection of erythromycin resistance genes and typing methods

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TABLE 1. PCR primers

Target gene	Sequence primer (5′–3′)	Product size (bp)
Macrolide resistance genes		
ermB forward	ATTGGAACAGGTAAAGGGC	442
ermB reverse	GAACATCTGTGGTATGGCG	
ermA forward	AACTTGTGGAAATGAGTCAACGG	375
ermA reverse	CAGAATCTACATTAGGCTTAGGG	
mef forward	AGTATCATTAATCACTAGTGC	345
mef reverse	TTCTTCTGGTACTAAAAGTGG	
Tetracycline resistance genes		
tetM forward	AGTTTTAGCTCATGTTGATG	1,862
tetM reverse	TCCGACTATTTGGACGACGG	
tetO forward	GCGGAACATTGCATTTGAGGG	538
tetO reverse	CTCTATGGACAACCCGACAGAAG	
intTn and *xis* genes		
intTn forward	GGTCTTCGTATTTCAGAGTTTGG	473
intTn reverse	GTTGCATGTGCGTAATAGTTCAG	
xis forward	AAGCAGACTGACATTCCTA	193
xis reverse	GCGTCCAATGTATCTATAA	

were performed using a sample of 739 MLS_B-resistant randomly selected isolates (284 from Madrid and 455 from Gipuzkoa). This sample represented 55.7% of all MLS_B-resistant isolates obtained during the study period.

Detection of macrolide resistance genes (*mef*, *ermA*, and *ermB*), tetracycline resistance genes (*tetM* and *tetO*), and the *intTn* and *xis* genes of the Tn*916*-Tn*1545* transposon family was performed by PCR using the primers described in Table 1. Determinants of resistance from representative isolates (one to four) of each clone were sequenced and analyzed using BLAST software (available at http://www.ncbi.nlm.nih.gov/BLAST/).

T typing was performed by slide agglutination of trypsin-digested suspensions of bacteria with rabbit type-specific antiserum (pharmaSEVAC, Prague, Czech Republic) (18).

emm types were determined according to guidelines of the Division of Bacterial and Mycotic Diseases, Centers for Disease Control and Prevention (available at http://www.cdc.gov/ncidod/biotech/strep/doc.htm).

Multilocus sequence typing (MLST) was performed according to recommendations provided at http://spyogenes.mlst.net.

PCR-RFLP. To genetically characterize all the isolates and to obviate the need to sequence the *emm* gene and the seven alleles of the MLST of each isolate, the amplified products of the *emm* gene were subjected to digestion with the restriction endonucleases HaeIII and MboII. The amplified products of three of the seven housekeeping alleles of MLST (*gki*, *murI*, and *yqiL*) were digested with the enzymes AciI and BsrI, HphI, and NlaIV, respectively. The grouping obtained with the PCR-restriction fragment length polymorphism (RFLP) method was confirmed by sequencing of the *emm* gene, and the seven alleles of the MLST of each PCR-RFLP pattern were detected. The MLST was assigned by the RFLP method only to isolates previously well characterized by *emm* type, T type, and genotypic and phenotypic resistance patterns.

The *emm* gene and the seven alleles of MLST were sequenced in all the isolates of the 10 clones with less than 5 isolates each, in 50% of the isolates of the 2 clones with between 5 and 20 isolates each, and in more than 10% of the isolates of the clones with more than 40 isolates each. Overall, the seven alleles of the MLST were sequenced in 125 isolates.

Pulsed-field gel electrophoresis (PFGE) was performed as previously described (28).

In this work, a clone was defined as a group of isolates showing the MLS_B phenotype of resistance with the same *emm* type, the same sequence type (ST) complex, and a similar T type. In this study, an ST complex describes a group of STs that share six out of the seven MLST loci.

RESULTS

Erythromycin resistance among the GAS isolated between 1999 and 2005 was 21.3% (annual variation of 14.3% to 28.9%) (Fig. 1). The overall percentage of isolates with the MLS_B phenotype of resistance increased from 3.3% in 1999 to 10.2% between 2003 and 2005 (7.6 to 13.1%). The annual percentage of isolates with the M phenotype of resistance showed variations through the entire period (6.8 to 19.1%), although the tendency since 2003 was to decrease (Fig. 1).

Among the 739 *S. pyogenes* clinical isolates showing the MLS_B resistance phenotype studied in depth, 652 isolates carried the *ermB* gene, 80 carried *ermA*, three carried both *ermB* and *mef*, and one carried both *ermA* and *mef*; in the remaining three isolates, neither *ermA*, *ermB*, nor *mef* genes were detected. Overall, the isolates with the *ermB* gene showed a high level of erythromycin and clindamycin resistance (MIC_{50}, >64 μg/ml) as well as telithromycin resistance (MIC_{50}, >4 μg/ml). The isolates harboring the *ermA* gene showed moderate erythromycin resistance (MIC_{50}, 4 μg/ml) and, without induction, were susceptible to clindamycin (MIC_{50}, <0.5 μg/ml) and telithromycin (MIC_{50}, <0.25 μg/ml).

Sixteen *emm* types were detected. Thirteen *emm* types correlated with an ST, and three correlated with an ST complex. The three ST complexes were as follows: ST20, ST21, and ST403 in the same *emm*11 type; ST63 and ST369 in the same *emm*77 type; and ST38 and ST39 in the same *emm*4 type. In contrast, different *emm*/ST combinations shared the same T type, and frequently, more than one T type was detected in a single isolate. For the purposes of clarity, each clone was designed with the most prevalent T type found in that clone, although more detailed information is included as a footnote to Table 2.

When the STs were combined with the *emm* and T types, the 739 MLS_B-resistant isolates were grouped into 16 clones (Tables 2 and 3).

The most frequent clone was *emm*11/T11/ST403 (ST403-ST20-ST21 complex) whose ST could not initially be determined in most of the isolates because the housekeeping *yqiL* allele was absent (null *yqiL* allele). This ST is currently designated ST403. The first isolates of this clone were detected in August 2002, and their rapid dissemination since 2003 has made it the most prevalent clone, with 463 (62.7%) isolates. This clone included 448 ST403 isolates (which did not amplify the *yqiL* allele), 9 ST21 isolates, and 6 ST20 isolates. Of the seven alleles that define an ST, six are shared by STs 20, 21, and 403, with the noncommon allele being *yqiL*. The *yqiL* allele is absent in ST403 and has only two nucleotide differences between ST20 and ST21.

By PFGE, these three STs shared a common profile (pattern similarity of >80%). All these isolates except one carried the *ermB*, *tetM*, *intTn*, and *xis* genes (Table 2), suggesting the presence of a Tn*1545*-like transposon. The remaining isolate showed moderate erythromycin resistance (MIC, 8 μg/ml) and tetracycline susceptibility (MIC, <0.5 μg/ml) and carried the *ermA* gene.

The second most frequent clone between 1999 and 2005 was *emm*28/T28/ST52, which included 148 (20%) isolates. All isolates of this clone shared a single T type (T28) and *emm* type (*emm*28), but two subpopulations of isolates, designated as

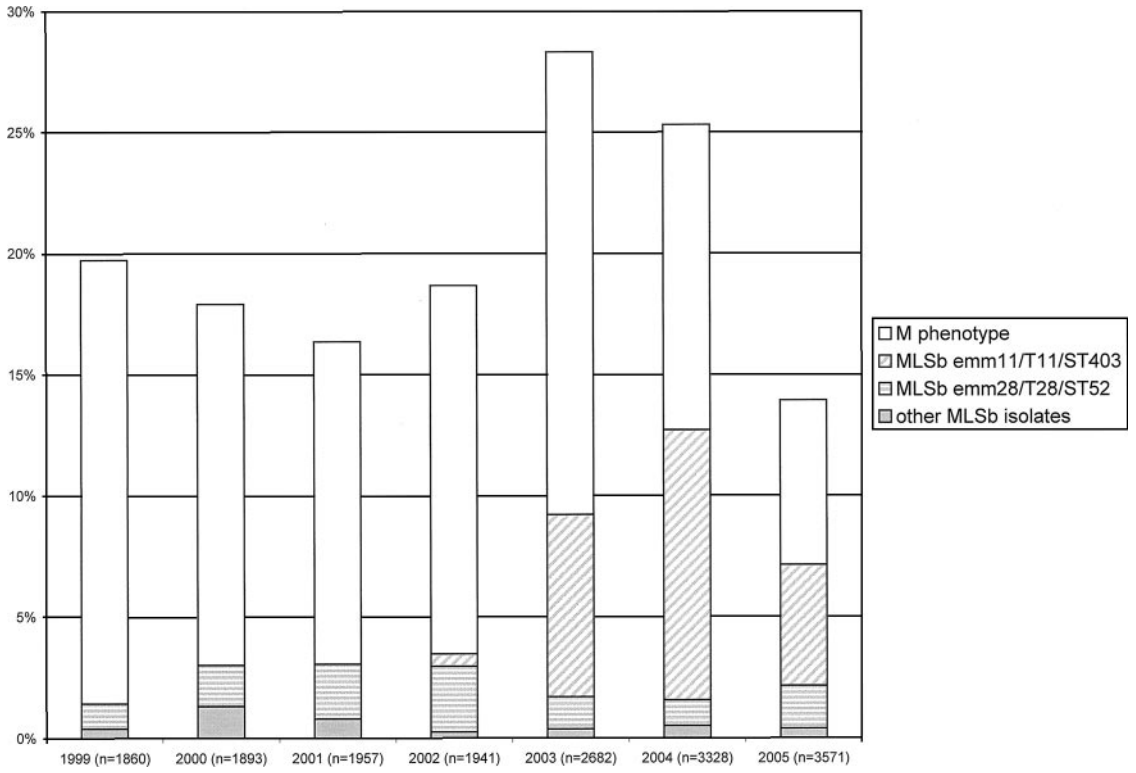

FIG. 1. Annual percentage of isolates (n = 17,232) showing the M (n = 2,356) and MLS_B (n = 1,326) resistance phenotypes. The proportional participation of *emm*11/T11/ST403 and *emm*28/T28/ST52 clones is illustrated by data extrapolated from a sample of 739 MLS_B-resistant isolates studied in depth.

subclones in this study, could be differentiated by their phenotypes of resistance and especially by their different bacitracin susceptibilities. One subclone comprised 115 bacitracin-resistant isolates (bacitracin MIC of ≥2 U/ml with no zone of inhibition around a 0.04-U bacitracin disk) that harbored the *ermB* gene and were tetracycline susceptible. The second subclone comprised 33 *emm*28/T28/ST52 bacitracin-susceptible isolates, 31 of which were tetracycline resistant and carried the *ermB* and *tetM* genes. One of the remaining isolates carried the *ermA* gene (erythromycin and tetracycline MICs of 2 and <0.25 μg/ml, respectively) and the other carried the *ermB* gene (erythromycin and tetracycline MICs of >64 and <0.25 μg/ml, respectively).

The third clone in order of frequency was the *emm*22/T12/ST46 clone, which included 62 isolates, 38 with the *ermA* gene and 24 with the *ermB* gene; all the isolates that had the *ermA* gene were tetracycline susceptible. Ten out of the 24 isolates with the *ermB* gene were tetracycline resistant and harbored the *tetM*, *intTn*, and *xis* genes.

The fourth most frequent clone was the *emm*77/T28/ST63 clone (ST63-ST369 complex) (n = 24). Most of the isolates of this clone had the *ermA* gene (Table 2). The *intTn* and *xis* genes were detected in the single isolate of this clone and carried both the *ermB* and *mef* genes. This clone included 23 ST63 isolates and 1 ST369 isolate. ST63 and ST369 have only one nucleotide difference at the *yqiL* allele, with the sequences of the other six alleles being identical (single-locus variant). The PFGE pattern of the ST369 isolate showed >80% similarity with the patterns of other ST63 isolates. A more detailed description of this clone has previously been described (24).

We also included two *emm*4/T4 isolates in the same clone (Tables 2 and 3), because ST38 and ST39 are differentiated by only one nucleotide at the *gtr* allele, and both PFGE patterns showed >80% similarity.

DISCUSSION

Macrolides and lincosamides are considered to be a suitable alternative to penicillin in patients who are allergic to penicillin. In animal models, clindamycin has proven to be more effective than penicillin in the treatment of some invasive infections (33) and is the treatment of choice, alone or in combination with penicillin, for sepsis of soft tissue origin (1, 5). When *S. pyogenes* shows resistance to clindamycin, it nearly always shows a high level of resistance to erythromycin and other macrolides. Consequently, although the M phenotype of resistance is important, the MLS_B phenotype of resistance has greater implications by limiting the therapeutic possibilities in cases of infection.

Several reports of the antimicrobial susceptibility of *S. pyogenes* isolates in Spain from 1984 to 1999 showed that until 1990, erythromycin resistance in *S. pyogenes* was low (less than 3%). After 1990, resistance increased, reaching 25 to 35% of all *S. pyogenes* strains isolated in 1995 (15, 27, 29). As in other countries, total macrolide consumption in Spain showed a good correlation with the erythromycin resistance curve (15).

TABLE 2. Annual distribution of 16 clones among a sample of 739 *Streptococcus pyogenes* isolates with the MLS_B-resistant phenotype[a]

Clone	No. of isolates							
	1999	2000	2001	2002	2003	2004	2005	Total
*emm*11/T11/ST403[b]	0	0	0	5	103	202	153	463
*emm*28/T28/ST52, bacitracin resistant	3	8	13	20	12	12	47	115
*emm*28/T28/ST52, bacitracin susceptible	10	1	1	1	6	7	7	33
*emm*22/T12/ST46[c]	12	9	10	6	8	6	11	62
*emm*77/T28/ST63[d]	12	5	4	1	0	1	1	24
*emm*73/T13/ST331[e]	0	4	3	0	4	1	1	13
*emm*94/TB3264/ST89	2	1	0	0	0	3	2	8
*emm*89/TB3264/ST101	0	0	0	0	0	0	4	4
*emm*58/T NT/ST19	0	0	0	0	0	0	3	3
*emm*12/T12/ST36	1	1	0	1	0	0	0	3
*emm*6/T6/ST37	0	0	1	0	0	1	1	3
*emm*2/T2/ST55	0	0	0	0	0	1	1	2
*emm*4/T4/ST39[f]	0	0	0	0	0	2	0	2
*emm*81/TB3264/ST117	1	0	0	0	0	0	0	1
*emm*68/T3,B3264/ST247	0	0	1	0	0	0	0	1
*emm*75/T8,25/ST150	0	1	0	0	0	0	0	1
*emm*102/T4/ST376	1	0	0	0	0	0	0	1
Total	42	30	33	34	133	236	231	739

[a] The two subclones of the *emm*28/T28/ST52 clone are represented separately. Each clone was designed with the most prevalent T type found in that clone.
[b] Two isolates (ST403/T11) were *emm* nontypeable(NT). T11 ($n = 413$), the T5,8,9,imp19,25,27 complex ($n = 28$), T28 ($n = 2$), and T nontypeable ($n = 20$) were also included. A total of 448 ST403 isolates (which did not amplify the *yqiL* allele), 9 ST21 isolates, and 6 ST20 isolates were included.
[c] TB3264 ($n = 3$); T3,5 ($n = 1$), and T nonetypeable ($n = 2$). The remaining isolates were of the T12 type and belonged to the T3,12,13,B3264 complex, which comprised isolates that were mainly T12 alone ($n = 40$).
[d] All isolates belonged to the T9,13,28 complex, and all but one (T13,9) agglutinated with the T28 type. A total of 23 ST63 isolates and 1 ST369 isolate were included.
[e] All isolates belonged to the T3,13,B3264 complex, and all agglutinated with the T13 type.
[f] One ST38 isolate and one ST39 isolate were included.

Before 1999, the M phenotype predominated among erythromycin-resistant *S. pyogenes* isolates, representing around 95% of these isolates (15, 27, 29). In the present study, we found that in 1999, the M phenotype was expressed by approximately 85% (340/402) of all erythromycin-resistant isolates. In 2004 and 2005, about 50% of erythromycin-resistant isolates (705/1,368) displayed the MLS_B phenotype. Four major clones grouped 94.3% of all *S. pyogenes* isolates displaying the MLS_B phenotype of resistance. In 2003, 2004, and 2005, the *emm*11/T11/ST403 clone represented 77.4%, 85.6%, and 66.2% of MLS_B-resistant isolates, respectively, with similar prevalences in Madrid and in Gipuzkoa. ST403 isolates of this clone are probably the only *S. pyogenes* strains that do not have one of the seven housekeeping alleles used for MLST.

Although the origin of this clone in Europe is not known, *emm*11 isolates carrying the *ermB* gene were frequently isolated in Belgium (22) and France (3) and occasionally in Italy (36). Because the isolates in these studies were characterized by *emm* typing only (the French isolates by *emm* and T typing), it cannot be ascertained whether they were of the same type as *emm*11/T11/ST403 found in Spain.

The *emm*28/T28/ST52 bacitracin-resistant subclone has persisted in Spain since its appearance in 1999. The characteristic of being bacitracin resistant will hamper its detection in laboratories that use bacitracin susceptibility as a presumptive identification test. *emm*28/T28/ST52 bacitracin-resistant isolates were first detected in Spain and Portugal in 1999 (28, 32), in France and Belgium between 2000 and 2002 (21, 23), and in the Czech Republic probably in the same period (35), proving its spread through Europe in the last few years.

Tetracycline resistance has been considered to be an important cofactor in the selection of erythromycin resistance (26). Tetracycline resistance in *S. pyogenes* has been associated with the *tetM* gene and, less frequently, with the *tetO* gene (14). In this study, the *tetM* gene was present in 70.6% of the MLS_B-resistant isolates

TABLE 3. Macrolide and tetracycline determinants of resistance in different clones of *Streptococcus pyogenes* with the MLS_B resistance phenotype

Clone	No. of isolates						Erythromycin		Tetracycline	
	Total	*ermB*	*ermA*	*tetM*	*tetO*	*int/xis*	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
*emm*11/T11/ST403	463	462[a]	1	462	0	462	>64	>64	>4	>4
*emm*28/T28/ST52, bacitracin resistant	115	115	0	0	0	0	>64	>64	<0.5	<0.5
*emm*28/T28/ST52, bacitracin susceptible	33	32	1	31[b]	0	22	>64	>64	>4	>4
*emm*22/T12/ST46	62	24	38[c]	10	0	10	4	>64	<0.5	>4
*emm*77/T28/ST63	24	1[d]	23	0	24	1	4	8	>4	>4
*emm*73/T13/ST331	13	3	10	2	0	2	8	>64	<0.5	>4
*emm*94/TB3264/ST89	8	1	7	8	0	8	8	>64	>4	>4
*emm*89/TB3264/ST101	4	4	0	0	0	0	>64	>64	<0.5	<0.5
*emm*58/TNT/ST19	3	3	0	0	0	0	>64	>64	<0.5	<0.5
*emm*12/T12/ST36	3	3	0	2	0	1	>64	>64	>4	>4
*emm*6/T6/ST37	3	3	0	1	0	1	>64	>64	<0.5	>4
*emm*2/T2/ST55	2[e]	1	0	1	0	1	>64	>64	>0.5	>4
*emm*4/T4/ST39	2	1	1	1	0	1	4	>64	<0.5	>4
*emm*81/TB3264/ST117	1	1	0	1	1	1	>64	>64	>4	>4
*emm*68/TB3264,3/ST247	1	0	0	1	0	1	2		>4	
*emm*75/T8,25/ST150	1	1	0	1	0	1	>64		>4	
*emm*102/T4/ST376	1	0	0	1	0	1	>64		2	

[a] Two isolates were *ermB*$^+$ and *mef*$^+$.
[b] One isolate was *ermA*$^+$, *tetM* negative, and *int/xis* negative, and another was *ermB*$^+$, *tetM* negative, and *int/xis* negative.
[c] One isolate was *ermA*$^+$ and *mef*$^+$.
[d] One isolate was *ermB*$^+$, *mef*$^+$, and *int/xis*$^+$.
[e] One isolate was *ermB* negative, *ermA* negative, *mef* negative, *tetM* negative, and *int/xis* negative.

(95.6% of tetracycline-resistant isolates), while the *tetO* gene was detected exclusively in MLS_B-resistant isolates of the *emm*77/T28/ST63 clone. This clone included 23 ST63 isolates and 1 ST369 isolate.

Most isolates with both the *tetM* and *ermB* genes carried the *intTn* and *xis* genes. The presence of the *intTn* and *xis* genes suggested the existence of a transposon related to the Tn*916*-Tn*1545* family of conjugative transposons. Conjugative transposons from the Tn*916*-Tn*1545* family are found in different species of the genus *Streptococcus* (7, 10, 30). These elements can carry several determinants of resistance and contribute to the dissemination of multidrug resistance.

Because of the inherent limitations of conventional phenotyping methods for detecting circulating strains, as well as their mechanisms of resistance, molecular techniques that complement the information provided by these methods have been developed. Molecular methods such as MLST, *emm* typing, and PFGE are highly discriminatory genotypic techniques that provide valuable information for cluster analysis. MLST is an unambiguous sequence-based method that allows the genetic relationship between the organisms of bacterial species to be identified (11). MLST is more highly discriminative than *emm* typing, and in this study, it was considered more appropriate to reduce its discriminative power and to introduce the concept of the ST complex in the definition of the clone. Since all STs assigned to the same ST complex shared alleles at six of the seven MLST loci and since their PFGE patterns, *emm* types, T types, and macrolide and tetracycline determinants of resistance showed strong similarity, the different STs assigned to a single ST complex were considered to be minor variants of the same clone.

emm genes encode M proteins that constitute an important virulence factor, and to date, more than 120 *emm* types have been well characterized (12, 13). Classic M protein serotyping has been replaced with the sequence-based methodology of *emm* typing. *emm* typing has enabled new types to be added to the Lancefield scheme. Only recently, the *emm*94-*emm*102 types were incorporated (13). In this study, *emm*94 and *emm*102 were detected and could not have been M-protein typed using all typing serum stocks available to international GAS reference laboratories (13). Ten out of the 16 *emm* types found in this study, which grouped more than 95% of all the total MLS_B-resistant isolates studied, were targeted by a 26-valent GAS vaccine currently undergoing clinical trials (16).

T serotyping, a phenotypic method, was the less discriminative of all the typing methods used in this study, but as in other studies, it added important information and validated data provided by genotypic methods (19). Moreover, as recently suggested, the induction of a protective immune response against T antigens may be another useful strategy for the development of vaccines against *S. pyogenes* (25).

The extensive and rapid diffusion of a clone in a specific region is indicative of its high infectious ability, and therefore, it becomes a threat for international dissemination. The advantage of using molecular methods, together with the T serotype, is that comparisons among *S. pyogenes* isolates from different countries and at different times can easily be made, increasing knowledge of the spread of resistant clones and facilitating future preventive measures.

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