Rapid Inversion of the Prevalences of Macrolide Resistance Phenotypes Paralleled by a Diversification of T and *emm* Types among *Streptococcus pyogenes* in Portugal

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In Portugal erythromycin resistance of 26.6% ($n = 352$) remained constant during 1998 to 2003, however in 1998 the MLS_B phenotype dominated (85%), whereas in 2003 the M phenotype prevailed (77%). A decline in T12/*emm*22 MLS_B isolates could partially explain the drop in this phenotype, but the rise of the M phenotype was not due to clonal expansion.

Although penicillin remains the antibiotic of choice in the treatment of Lancefield group A streptococci (GAS) infections, macrolides and lincosamides are recommended as suitable alternatives for patients allergic to penicillin (5). Newer macrolides, such as azithromycin, may be given once a day, making this an attractive option for the treatment of pharyngitis due to *Streptococcus pyogenes*. In Portugal, where penicillin V is not available, macrolides and lincosamides have the additional advantage of being a therapeutic option with an oral route of administration. High macrolide resistance in GAS was previously identified in Portugal (13) in line with other European countries (1, 4, 6) but in contrast to others (14, 16). The aims of this study were to determine the prevalence of macrolide resistance phenotypes and its temporal trends and to evaluate the correlation with T and *emm*-types.

A total of 1,321 GAS from clinical infections were collected from 30 laboratories, geographically distributed throughout Portugal, from January 1998 to December 2003. The isolates were distributed in the study period as follows: 153 in 1998, 240 in 1999, 213 in 2000, 216 in 2001, 270 in 2002, and 229 in 2003. The laboratories were asked to submit all nonduplicate GAS isolated from outpatients during the study period. Antimicrobial susceptibility testing, T-typing, and macrolide resistance phenotype and genotype were determined as previously described (7, 13). Strains were *emm* typed according to the recommendations of the Centers for Disease Control and Prevention (CDC) (<http://www.cdc.gov/ncidod/biotech/strep/protocols.htm>). The sequences of representatives of each restriction profile were searched against GenBank as well as the *emm* CDC database (<http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm>). An isolate was considered to be of a given *emm* type if it had >95% identity over the 160 bases considered (3).

Among this collection, 352 isolates (26.6%) were erythromycin resistant. Although there was a higher prevalence of

resistant isolates recovered in 1998 (34.6%), the variation of the overall prevalence in the following years (19.6% in 1999, 27.7% in 2000, 25.5% in 2001, 23.7% in 2002, and 20.5% in 2003) was not significant (χ^2 test, $P = 0.22$) (Fig. 1). Only the 325 erythromycin-resistant isolates recovered from throat swabs associated with a diagnosis of pharyngitis were characterized further.

Resistance to tetracycline was expressed by 38.7% ($n = 126$) of the isolates (MIC₉₀ = 96 μ g/ml, MIC range, 12 to 512 μ g/ml). The distribution of tetracycline-resistant isolates among the study years was as follows: 84.9% of the isolates recovered in 1998, 70.2% in 1999, 28.8% in 2000, 30.9% in 2001, 12.5% in 2002, and 12.8% in 2003.

The cMLS_B phenotype was expressed by 170 isolates (52.3%), 151 (46.4%) presented the M phenotype, and only 4 isolates (1.2%) the iMLS_B phenotype. Erythromycin resistance determinants were detected in all isolates by multiplex PCR. All isolates presenting the M phenotype carried the *mefA* gene, including one which carried both *mefA* and *ermB* genes. Nine of the isolates presenting the MLS_B phenotype (4.9%) carried both the *mefA* and *ermB* resistance determinants whereas all other isolates presenting this phenotype yielded a single PCR product consistent with the presence of the *ermB* gene. Three isolates presenting the iMLS_B phenotype carried the *erm(A)* gene, and one carried the *ermB* gene.

A summary of the number of T/*emm*-type associations with the two macrolide resistance phenotypes and their distribution over the different years of the study period is presented in Table 1. The majority (90%) of the tetracycline-resistant isolates were included in the T12 *emm*22 group.

The situation found in 1998 and 1999, where strains expressing the MLS_B phenotype accounted for approximately 80% of all macrolide-resistant isolates (Fig. 1), was similar to that found among a group of erythromycin-resistant isolates recovered in Italy in 2000 (6) and in France (2002 to 2003) (4). However, the dominance of the MLS_B phenotype was in sharp contrast to the situation found in neighboring Spain where isolates collected around the same years (1996 to 1999) expressed overwhelmingly (95%) the M phenotype (1). The tendency for the strains expressing the MLS_B phenotype to

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FIG. 1. Erythromycin resistance and prevalence of macrolide resistance phenotypes in Portugal during 1998 to 2003.

present a limited number of T/*emm*-type associations and for at most two associations (T12 *emm*22 and T28 *emm*28) to account for more than 80% of isolates recovered in each year was a constant during the study period. The only exception was 2001 when, not only was an unusually high number of associations identified ($n = 9$) but three different associations were necessary to account for 80% of the isolates (compare to 2000

TABLE 1. Distribution of T and *emm* types and macrolide resistance phenotypes among 325 pharyngitis-associated erythromycin-resistant GAS in Portugal during 1998 to 2003

T(<i>emm</i>) ^a	Result ^b for yr:						Total ^c
	1998	1999	2000	2001	2002	2003	
12(12)	1/1	0/5	0/4	1/5	0/7	0/2	2 (1)/24
12(22)	42/0	31/0	20/1	15/0	9/0	8/0	125 (113)/1
28(28)		2/0	8/0	7/0	10/0	1/1	28 (2)/1
1(1)			0/3	0/11	0/17	0/4	0/35
25(75)				0/3	0/5	0/3	0/11
4(4)	0/5	0/1	0/6	0/2	0/6	0/12	0/32
12(11)				2/0			2 (2)/0
B3264(4)						0/2	0/2
5/27/44(4)						0/2	0/2
1(12)			0/2				0/2
NT(12) ^d		0/2	0/4			0/1	0/7
NT(28) ^d					2/0		2/0
13(22)		3/0					3/0
2(2)	0/2						0/2
2(4)						0/2	0/2
9(9)			0/6		0/1		0/7
Other ^e	2/0	1/2	1/4	5/4	2/5	1/8	12 (10)/23
Total	45/8	37/10	29/30	30/25	23/41	10/37	174/151

^a All T/*emm* type combinations presenting ≥ 2 isolates in any year of the study period are discriminated.

^b Number of isolates presenting each of the erythromycin resistance phenotypes (MLSB/M).

^c Numbers in parenthesis indicate tetracycline-resistant isolates among those presenting the MLSB phenotype. None of the isolates presenting the M phenotype was resistant to tetracycline.

^d NT, nontypeable by T serotyping.

^e Single isolates presenting unique T/*emm*-type combinations in each year. Most (16/24) of these T/*emm* combinations were represented by a single isolate during the entire study period. The remaining 8 were represented by more than one isolate each recovered in different years: T13 *emm*4, T13 *emm*77, and T12 *emm*28 were expressed by 3 isolates; T12 *emm*4, T(B3264) *emm*89, T1 *emm*4, T13 *emm*1, and T(nontypeable) *emm*1 were expressed by 2 isolates.

when, for the same number of isolates, only three associations were identified) (Table 1). This indicates that other T/*emm*-type associations expressing the MLSB phenotype existed that could replace the T12 *emm*22 group; however none rose to significant numbers.

In the following years there was an increase in prevalence of isolates expressing the M phenotype, that accounted for 76.6% of erythromycin resistant isolates in 2003 (Fig. 1). In sharp contrast to the data presented here, the increases in occurrence of strains expressing the M phenotype documented elsewhere were also paralleled by an increase in the overall macrolide resistance rate (8, 9, 11). Contrary to isolates expressing the MLSB phenotype, those expressing the M phenotype were found among a diverse group of T/*emm*-type associations and usually at least one-half of the total number of associations found each year was necessary to account for 80% of the isolates. This increased diversity is better illustrated by the strains exhibiting the M phenotype recovered in 2003, when 17 different T/*emm*-type associations were found among the 36 isolates (Table 1). Unlike these findings studies from elsewhere in Europe have documented similar numbers of T/*emm*-type associations among isolates expressing the two phenotypes (4, 6). The contrast to the situation in Spain, where only four *emm* types accounted for 85% of the isolates expressing the M phenotype (1), indicates that, despite the geographic proximity, macrolide-resistant GAS isolated in the two countries constitute two different populations. Moreover, it suggests that the rise in isolates of the M phenotype was not due to invasion of the few *emm* groups prevalent in Spain.

The decrease observed in tetracycline resistance paralleled the decline of isolates presenting the MLSB phenotype, namely, those associated with the T12 *emm*22 types. This could be explained by the presence of *erm* and tetracycline resistance genes in the same transposon (12) in this group of isolates.

Although a situation where either phenotype dominates is not unusual, such a rapid shift in prevalence of the different phenotypes, while maintaining the same overall erythromycin resistance rate (Fig. 1), was not previously described in GAS to the best of our knowledge. This shift could not be attributed simply to the reduction in the number of isolates defined by T12 *emm*22 and the absence of other T/*emm*-type associations expressing the MLSB phenotype that could take its place, or by the emergence of a limited number of highly successful strains expressing the M phenotype and specific T/*emm*-type associations as documented in North America (8, 11). Since the data presented do not offer firm clues, we can only speculate as to the reasons behind this shift. Although Portugal is among the largest antibiotic consumers in Europe (www.ua.ac.be/ESAC), it is not remarkable for its macrolide consumption. Decreases in macrolide use were associated to a reduction of macrolide-resistant GAS (16). However, there was only a slight decrease in consumption for human use in the ambulatory setting during the study period in Portugal (www.ua.ac.be/ESAC). If, as suggested by Nielsen et al. (15), tetracycline use together with macrolide use could be correlated to macrolide resistance, especially the MLSB phenotype, then the slight reduction in tetracycline use (www.ua.ac.be/ESAC) could have an additive effect. These factors, as well as a possible immunity developed by the human population against the limited number of T antigens and M proteins associated with the MLSB phenotype,

could explain the observed reduction in prevalence. The diversity among the strains expressing the M phenotype could be explained by a higher mobility of the genetic element containing the *mefA* gene (10). Interestingly, an unusual chimeric genetic element combining a transposon and a bacteriophage, that could mediate the transfer of the *mefA* gene by a process similar to transduction, was recently described (2).

The data presented documents a major shift in the prevalence of macrolide resistance phenotypes in an unusual short time period, not paralleled elsewhere in Europe and without a concomitant change in the frequency of macrolide resistance. Continued surveillance is needed to establish if this shift will stabilize and if the major groups of isolates sharing the same T/*emm*-types are also found elsewhere.

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