ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2004, p. 3559–3562 Vol. 48, No. 9
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.9.3559–3562.2004

Emergence of Macrolide-Resistant *Streptococcus pyogenes* Strains in French Children

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Received 29 January 2004/Returned for modification 3 April 2004/Accepted 11 May 2004

We studied the antimicrobial susceptibility of 322 *Streptococcus pyogenes* throat isolates from French children and their serotype and genomic diversity. A total of 22.4% were erythromycin resistant, and 69.4, 4.2, and 26.4% of these isolates harbored *ermB*, *ermA*, and *mefA*, respectively. Increasing resistance in France is mainly associated with a few *emm* type 28 clones.

Streptococcus pyogenes pharyngitis is one of the most common bacterial upper respiratory tract infections in children. Group A streptococci (GAS) are uniformly susceptible to penicillin. Macrolides are recommended alternatives for penicillin-hypersensitive patients. However, resistance to erythromycin has become widespread among GAS (4, 6, 10, 18, 19; L. Mihaila-Amrouche, J. Loubinoux, and A. Bouvet, Abstr. 13th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. S6, 2003.). Here, we studied the prevalence of macrolide resistance among recent throat isolates of GAS collected from children throughout France. We also determined their mechanisms of resistance and their clonality by means of *emm* genotyping and pulsed-field gel electrophoresis (PFGE).

A total of 322 consecutive *S. pyogenes* isolates were collected between 2002 and 2003 throughout France. They were isolated from throat cultures of children 2 to 16 years of age (mean, 6 years) with pharyngitis. The isolates were identified as *S. pyogenes* by colony morphology, Gram staining, beta-hemolysis on blood agar, and the presence of group A antigen as determined by an agglutination test (Oxoid, Basingstoke, United Kingdom).

Antimicrobial susceptibility was tested as recommended previously (15). Erythromycin-resistant strains were identified by the disk diffusion method on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Bio-Rad, Marnes la Coquette, France) using 15-IU erythromycin disks (Bio-Rad) (15). Bacitracin susceptibility was studied by the disk diffusion method with 10-IU disks (Bio-Rad).

The MICs of amoxicillin, penicillin G, erythromycin, azithromycin, clarithromycin, telithromycin, clindamycin, and streptogramin B were determined for all isolates with erythromycin inhibition zone diameters of less than 21 mm (14). Telithromycin interpretative criteria for susceptibility and resistance were $\leq$0.5 and $\geq$4 μg/ml, respectively (NCCLS approved breakpoints are not available) (7).

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The *mefA*, *ermB*, and *ermA* genes were detected by PCR amplification as previously described (3).

Biotypes were determined as previously described (5).

T serotypes were determined on trypsinated bacteria by slide agglutination with anti-T sera obtained from the Institute of Sera and Vaccines, Prague, Czech Republic (9).

emm-specific PCR products were obtained with the primers MF2 and MR1 described by Podbielski et al. (17). The 5′ end of the *emm* genes was sequenced as described by Beall et al. (2) (see the Centers for Disease Control and Prevention website [http://www.cdc.gov/ncidod/biotech/strep/protocols.htm]). DNA sequences were subjected to homology searches (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm).

PFGE with SmaI was applied to all 72 erythromycin-resistant isolates, as previously described (4). The DNA of 19 isolates, all carrying *mefA*, could not be restricted by SmaI despite repeated attempts. We thus used SfiI for these isolates. Cluster analysis (unweighted pair group method with arithmetic mean) with whole-band analyzer software (Biogene, Vilber-Lourmat, Marne la Vallée, France) was used to calculate similarity or dissimilarity among GAS isolates. Clonally related PFGE patterns were defined by a similarity coefficient higher than 80% (usually corresponding to a difference of no more than four bands in our study) (20).

All isolates were susceptible to penicillin G and amoxicillin. Of the 322 isolates, 72 (22.4%) were resistant to erythromycin by the disk diffusion method. The MICs of the different antimicrobial agents are given in Table 1. Thirty-five isolates (10.9%) were resistant to bacitracin (disk diffusion zone diameters of 0 mm), and all these isolates were resistant to erythromycin.

Among the 72 erythromycin-resistant isolates, 69.4, 4.2, and 26.4% harbored the *ermB*, *ermA*, and *mefA* genes, respectively. Table 1 shows the MICs for erythromycin-resistant isolates according to the underlying mechanism. Isolates carrying *ermB* were resistant to 14-, 15- and 16-membered-ring macrolides and showed cross-resistance to clindamycin and streptogramin B; 47% of *ermB*-positive isolates were resistant to telithromycin. All but one of the 35 bacitracin-erythromycin-resistant isolates carried the *ermB* gene. Isolates carrying *mefA* were

TABLE 1. In vitro activities of eight antimicrobials against erythromycin-resistant *S. pyogenes* isolates according to known mechanisms of resistance

Group (*n*)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
All isolates (72)	Erythromycin	1–>128	>128	>128
	Azithromycin	4–>128	>128	>128
	Clindamycin	0.064–>128	>128	>128
	Streptogramin B	0.5–>128	8	128
	Clarithromycin	0.5–>128	>128	>128
	Telithromycin	0.064–128	2	4
	Penicillin G	≤0.008	≤0.008	≤0.008
	Amoxicillin	≤0.008–0.016	0.016	0.016
ermB (50)	Erythromycin	1–>128	>128	>128
	Azithromycin	4–>128	>128	>128
	Clindamycin	128–>128	>128	>128
	Streptogramin B	2–>128	8	128
	Clarithromycin	0.5–>128	>128	>128
	Telithromycin	0.064–128	4	8
	Penicillin G	≤0.008	≤0.008	≤0.008
	Amoxicillin	≤0.008–0.016	0.016	0.016
ermA (3)	Erythromycin	1–>128	ND	ND
	Azithromycin	4–>128	ND	ND
	Clindamycin	0.125–>128	ND	ND
	Streptogramin B	1–32	ND	ND
	Clarithromycin	0.5–>128	ND	ND
	Telithromycin	0.064–0.25	ND	ND
	Penicillin G	≤0.008	ND	ND
	Amoxicillin	≤0.008	ND	ND
mefA (19)	Erythromycin	8–16	8	16
	Azithromycin	8–16	16	16
	Clindamycin	0.064–0.125	0.064	0.125
	Streptogramin B	0.5–2	1	2
	Clarithromycin	2–8	4	8
	Telithromycin	0.25–0.5	0.5	0.5
	Penicillin G	≤0.008	≤0.008	≤0.008
	Amoxicillin	≤0.008–0.016	≤0.008	0.016

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively. ND, not determined.

resistant to erythromycin, clarithromycin, and azithromycin but susceptible to clindamycin and telithromycin. Isolates carrying *ermA* ($n = 3$) were susceptible to telithromycin.

Clindamycin and telithromycin remained active against 29 and 32%, respectively, of the 72 erythromycin-resistant isolates.

Molecular analysis of SmaI-digested DNA was possible for 53 of the 72 erythromycin-resistant isolates. The remaining 19 isolates could not be typed because SmaI did not restrict their DNA. These 53 GAS isolates were genetically diverse, as they yielded 35 PFGE patterns. However, 57% of isolates belonged to two clones (Fig. 1). The bacitracin-resistant isolates carrying *ermB* belonged to four clones, of which 82% belonged to the main two clones. The remaining 19 *mef* isolates yielded nine PFGE patterns after digestion with SfiI, but 68% of these isolates belonged to two clones (Fig. 2).

Various *emm* types were observed among the 72 erythromycin-resistant *S. pyogenes* isolates (Table 2). However, most of them were of the genotype *emm28*. There was only one *emm* type 1 resistant isolate. The *emm* type 28 isolates which contain either the *ermB* or the *ermA* gene exhibited two highly related PFGE patterns (Fig. 1).

The prevalence of erythromycin resistance among GAS isolates from French children was only 6.2% two years ago (3). Our study shows a threefold increase in the prevalence of erythromycin resistance among GAS in France compared to the results of a study carried out in 2000 (6.2% in 2000 and 22.4% in 2003; $P < 0.001$) (3).

The gene *ermB* was the main resistance mechanism in our isolates. In contrast, the M phenotype and/or the *mefA* gene predominates in other European countries such as Finland

TABLE 2. T serotypes and *emm* types of the 72 erythromycin-resistant *S. pyogenes* isolates with various resistance mechanisms

T serotype and *emm* type[a]	Total no. of isolates	No. of isolates with resistance gene		
		ermB	*ermA*	*mefA*
emm22 (T nontypeable)	2	2		
emm28 (T nontypeable)	4	4		
T1 *emm1*	1			1
T11 *emm11*	12	11	1	
T12 *emm12*	6	2		4
T28 *emm28*	33	31	2	
T4 *emm4*	9			9
T8/T25/lmp19 *emm75*	5			5

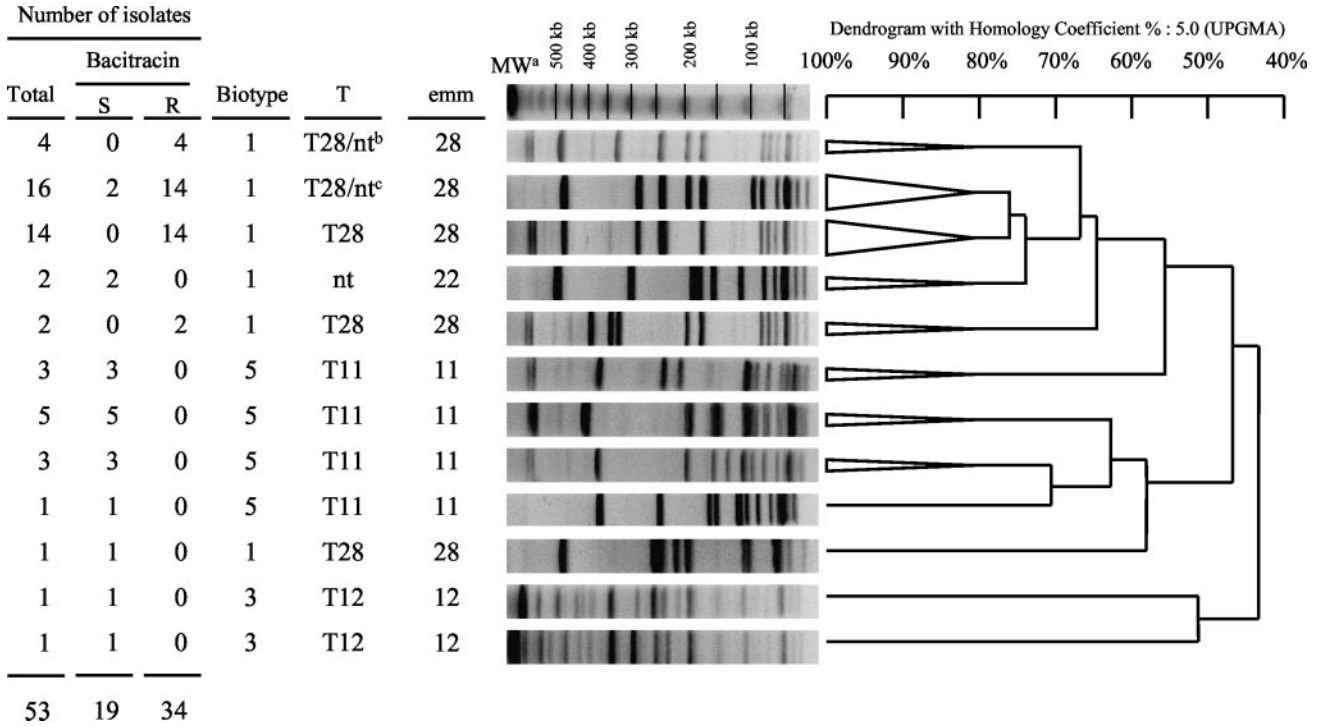

FIG. 1. Representative SmaI PFGE patterns of the 53 *erm*-positive erythromycin-resistant *S. pyogenes* isolates according to biotype, T serotype, and *emm* type. The triangles are collapsed branches gathering isolates with 80% similarity of banding patterns. MW[a], molecular weight marker (lambda ladder; Bio-Rad); T28/nt[b], one isolate nontypeable for T antigen. T28/nt[c], three isolates nontypeable for T antigen; UPGMA, unweighted pair group method with arithmetic mean.

(11), Spain (16), and Italy (1) and also in the United States (13) and Canada (12). The increase in erythromycin resistance among GAS isolates has been linked to the spread of serotype T28M28 in Quebec (21) and T28 *emm28* in Spain (16). In our study, 46% of the strains resistant to macrolides were associated with the type T28 *emm28*, with 94% carrying *ermB*. The major type encountered among our isolates carrying *mefA* was T4 *emm4* (47%). This type was also related to the increase in erythromycin resistance found in Finland (11).

Interestingly, in our study, all isolates whose DNA was not

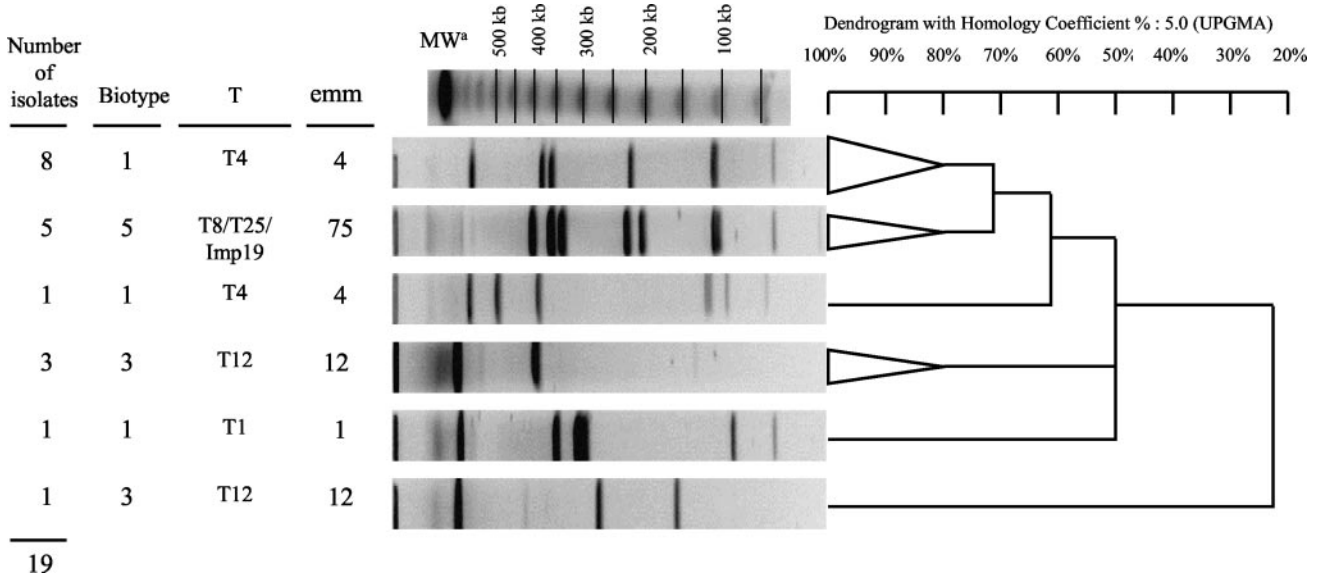

FIG. 2. Representative SfiI PFGE patterns of the 19 *mefA* erythromycin-resistant *S. pyogenes* isolates according to biotype, T serotype, and *emm* type. The triangles are collapsed branches gathering isolates with 80% similarity of banding patterns. MW[a], molecular weight marker (lambda ladder; Bio-Rad); UPGMA, unweighted pair group method with arithmetic mean.

restricted by SmaI carried the *mefA* gene, in keeping with a previous report (8). The diversity of the erythromycin-resistant isolates was shown by the fact that 35 different profiles were obtained with SmaI and 9 were obtained with SfiI, although a small number of PFGE patterns accounted for most of the isolates. Indeed, two highly related SmaI PFGE patterns included 57% of the *erm* isolates, and two different SfiI PFGE patterns included 68% of the *mefA* isolates. Thus, our results suggest that the emergence of macrolide resistance among GAS in France is due to the dissemination of a limited number of clones as described in Europe and North America (11–13; Mihaila-Amrouche et al., Abstr. 13th Eur. Congr. Clin. Microbiol. Infect. Dis.). The increase of prevalence of macrolide resistance in GAS in our study may be due to both the epidemic spread of resistant clones and the dissemination of resistance determinants among isolates of different genetic background.

Contrary to the situation in the United States, Spain, and Italy, where clindamycin remains active against M phenotype strains, the main mechanism of erythromycin resistance in our isolates involved the *erm* genes, leading to high-level resistance to both macrolides and clindamycin (16% of our isolates were resistant to clindamycin). Moreover, 15% of our isolates were intermediately resistant or resistant to telithromycin. Current data indicate that macrolide resistance is clinically relevant in GAS pharyngitis (13). Indeed, erythromycin-resistant GAS eradication failed in 47% of children in Finland (19) and 40% of children in Italy (1).

Macrolide GAS resistance must therefore be closely monitored in each country. Furthermore, the use of rapid group A antigen test must be followed by sensitivity testing when the patient is allergic to β-lactam agents.

Financial support was provided in part by Pfizer (France).

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