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Mechanisms of Macrolide Resistance in Clinical Pneumococcal Isolates in France

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The genetic basis of macrolide resistance was investigated in a collection of 48 genotypically unrelated clinical isolates of *Streptococcus pneumoniae* obtained between 1987 and 1997 in France. All strains were resistant to erythromycin, clindamycin, and streptogramin B, exhibiting a macrolide-lincosamide-streptogramin B resistance phenotype, and harbored the *erm*(B) gene. None of the strains carried the *mef*(A) or *erm*(A) subclass *erm*(TR) gene.

Antibiotic resistance in *Streptococcus pneumoniae*, especially to β-lactam antibiotics, has been a matter of growing concern in the last two decades. Resistance in this species has also been noted with tetracyclines, chloramphenicol, cotrimoxazole, and macrolides. In the United States, 19 to 34% of pneumococcal isolates are currently resistant to macrolides (8; M. R. Jacobs, D. Felminghan, P. C. Appelbaum, and The Alexander Project Group, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1044, p. 151, 1999). In Western Europe, the low prevalence of macrolide resistance in Germany, Austria, Portugal, The Netherlands, and Switzerland (1.5 to 4.6%) contrasts with the high rates observed in Spain, Italy, and Belgium (33, 24, and 31%, respectively) (1, 17; D. Felmingham and R. N. Gruneberg, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1790, p. 108, 2000). In France, the prevalence of pneumococcal resistance to erythromycin was 53% in 1997 (10). Four mechanisms of macrolide resistance have been described in *S. pneumoniae*. The first is a target modification involving a ribosomal methylase, associated with the *erm*(B) gene (15, 25). A macrolide-specific efflux mechanism encoded by the *mef*(A) gene was described in 1996 (21, 23). The *erm*(B) gene is associated with high-level resistance to macrolides, lincosamides, and streptogramin B (MLS_B phenotype), while the *mef*(A) gene is associated with low-level resistance to 14- and 15-membered-ring macrolides (M phenotype). More recently, erythromycin resistance in clinical isolates of *S. pneumoniae* harboring the *erm*(A) subclass *erm*(TR) gene has been described (3; G. A. Syrogiannopoulos, I. N. Grivea, A. Tait-Kamradt, G. D. Katopodis, N. G. Beratis, J. Sutcliffe, P. C. Appelbaum, and T. A. Davies, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 139, p. 65, 2000). Finally, macrolide-resistant pneumococcal strains with mutations in the 23S rRNA and ribosomal protein L4 or L22 have been selected by macrolide passage in vitro (22; A. Canu, B. Malbruny, M. Coquemont, T. A. Davies, P. C. Appelbaum, and R. Leclercq, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1927, p. 118, 2000; J. Sutcliffe, A. Tait-Kamradt, A. Walker, and J. Petitpas, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1925, p. 117, 2000) or have been found in clinical isolates (T. A. Davies, P. C. Appelbaum, W. Hryniewicz, L. Drukalska, H. Hupkova, J. Kolman, J. Mieivleviciene, M. Pana, L. Setchanova, A. Tambic, M. K. Thege, J. Trupl, and P. Urbaskova, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 138, p. 65, 2000; A. Tait-Kamradt, T. Davies, L. Brennan, F. Depardieu, P. Courvalin, J. Duignan, J. Petitpas, L. Wondrack, M. Jacobs, P. Appelbaum, and J. Sutcliffe, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. LB 8, p. 15, 2000).

The mechanisms of pneumococcal resistance to macrolides have been investigated in several countries, but very few data deal with the French situation (C. Arpin, M. H. Canron, P. Noury, and C. Quentin, Letter, J. Antimicrob. Chemother. **44:**133–134, 1999; P. Angot, M. Vergnaud, and R. Leclercq, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1221, p. 158, 1999). The aim of this study was to determine the genetic mechanisms of *S. pneumoniae* macrolide resistance in a collection of genotypically unrelated clinical isolates obtained between 1987 and 1997 in French hospitals.

Forty-eight invasive strains of *S. pneumoniae* resistant to erythromycin by the disk diffusion method were collected throughout France between 1987 and 1997. The 48 isolates were previously shown to be of different clonal origins by means of restriction fragment length polymorphism analysis of the rRNA gene regions and of the *pbp2b* and *2x* genes (9). The serotypes were 6B ($n = 10$), 6A ($n = 4$), 19A ($n = 1$), 14 ($n = 9$), 15A ($n = 3$), 24 ($n = 2$), 23F ($n = 9$), and 19F ($n = 9$); one strain was nontypeable. The MICs of erythromycin, azithromycin, josamycin, clindamycin, streptogramin B, and penicillin G were determined by the agar dilution method, using Mueller-Hinton medium supplemented with 5% sheep blood and incubation in room air (7).

The *mef* and *erm* genes were detected after PCR amplification, as previously described (4, 12, 18, 20). *Streptococcus pyogenes* 02C 1061, *S. pyogenes* 02C 1110, and *S. pyogenes* 02C 1064 were used as positive PCR controls for the *erm*(B), *erm*(A) subclass *erm*(TR), and *mef*(A) genes, respectively (4–6).

The 48 pneumococcal isolates displayed a high level of re-

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TABLE 1. MICs of macrolides and related agents and of penicillin G for 48 erythromycin-resistant *S. pneumoniae* isolates

Antimicrobial agent	MIC (μg/ml)[a]		
	50%	90%	Range
Erythromycin	>128	>128	>128
Azithromycin	>128	>128	>128
Josamycin	>128	>128	>128
Clindamycin	>128	>128	64–>128
Streptogramin B	128	>128	2–>128
Penicillin G	0.5	4	0.016–8

[a] 50 and 90%, MICs at which the growth of 50 and 90% of isolates, respectively, was inhibited.

sistance to erythromycin and related agents (Table 1), which is typical of the MLS_B resistance phenotype. Seventy-six percent of the strains were intermediately susceptible or resistant to penicillin. PCR amplification with primers specific for the *erm* and *mef* genes yielded results in keeping with the high level of resistance to MLS_B. Indeed, all the strains harbored the *erm*(B) gene, and none bore the *mef* or *erm*(A) subclass *erm* (TR) gene.

The prevalence of erythromycin resistance in North American pneumococcal isolates ranges from 2.9% in Canada to 34% in the United States (8, 11, 24). This erythromycin resistance is associated with the M phenotype and the *mef*(A) gene in 56 to 63% of cases (11, 19, 24). In South Africa the prevalence of erythromycin-resistant pneumococci with the M phenotype has increased from 1 to 20% in the last 10 years (26). In Western Europe the prevalence of pneumococcal macrolide resistance varies geographically (1, 17; Felmingham et al., 40th ICAAC). Mechanisms of resistance have been investigated in countries with high prevalence rates. In Spain, 98% of erythromycin-resistant strains were found to have the MLS_B phenotype (2). In Italy and Belgium, more than 90% of strains carried the *erm*(B) gene (13, 16). The spread of multiresistant clonal epidemic pneumococcal strains has been recently reported in France (9). To avoid biasing our results, we determined the genetic mechanisms of macrolide resistance only in genotypically unrelated isolates, which were representative of the strains usually recovered in France during that period (9). All strains possessed the *erm*(B) gene. The location of the *erm* (B) gene on a transposon may explain the interstrain spread of erythromycin resistance. None of our isolates harbored the *mef*(A) gene, which is rare in France (Arpin et al., letter; Angot et al., 39th ICAAC). Macrolide resistance due to mutations in ribosomal protein L4, as recently reported for clinical isolates (Davies et al., 40th ICAAC), was not investigated for our strains. Our results contrast with the epidemiology of the mechanisms of resistance described for North American isolates; this may result from the use of 16-membered-ring macrolides in France, which tends to select strains with the MLS_B phenotype, conferring resistance to 14-, 15-, and 16-membered-ring macrolides (14; D. Guillemot, C. Carbon, N. Thibult, H. Lecoeur, P. Weber, and E. Eschewege, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1863, p. 463, 2000).

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