

## Distribution of Subclasses *mefA* and *mefE* of the *mefA* Gene among Clinical Isolates of Macrolide-Resistant (M-Phenotype) *Streptococcus pneumoniae*, Viridans Group Streptococci, and *Streptococcus pyogenes*

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**The distribution of subclasses *mefA* and *mefE* of the *mefA* gene among 116 M-phenotype streptococci was as follows: pneumococci (38 strains had *mefE* and 4 *mefA*), viridans streptococci (49 *mefE* and 1 *mefA*), and *Streptococcus pyogenes* (24 *mefA*). Spain<sup>9V</sup>-3-14 and England<sup>14</sup>-9 clones of serotype 14 were dominant among pneumococci.**

The emergence of macrolide resistance in streptococci in the past decade in Spain has been associated with an increased consumption of these drugs (10). In two world surveillance studies carried out throughout the period from 1997 to 2000 (9, 12), the regional rates of erythromycin resistance (Ery<sup>r</sup>) ranged from 7 to 67.3% among *Streptococcus pneumoniae* strains, from 26.3 to 42.2% among viridans group streptococci (VGS), and from 2.7 to 18.6% among beta-hemolytic streptococci. In the European countries, the MLS<sub>B</sub> phenotype (due to *ermB* or *ermTR* genes) is prevalent among Ery<sup>r</sup> pneumococci, whereas the M phenotype (due to the *mefA* gene) predominates among VGS and beta-hemolytic streptococci (9, 13, 19). The macrolide resistance genes are usually located in mobile elements such as transposons, suggesting a putative transmission of macrolide-resistant genes between different bacteria, with special emphasis in *S. pneumoniae* or *Streptococcus pyogenes* (19).

Although two *mef* genes have been described (the *mefA* gene in *S. pyogenes* and the *mefE* gene in *S. pneumoniae* strains), they have been considered a single class of *mefA* gene and MefA protein due to their high homology (19, 22). However, a recent study (5) found that the *mefA* and the *mefE* elements had important genetic differences and proposed to refer them as *mefA* subclass *mefA* or subclass *mefE*.

During the period 1998 to 2003, 24.2% (51 of 211) of *S. pyogenes* strains, 32.9% (754 of 2,292) of *S. pneumoniae* strains, and 39.7% (209 of 527) of VGS strains isolated in our laboratory from adult patients were Ery<sup>r</sup>. Among these Ery<sup>r</sup> strains, 24 (47.1%) *S. pyogenes*, 42 (5.6%) *S. pneumoniae*, and 124 (59.3%) VGS isolates had the M phenotype (22). All 24 M-

phenotype *S. pyogenes* strains and 42 *S. pneumoniae* strains of this period and 50 selected M-phenotype VGS strains were studied.

The origins of the pneumococci were as follows: 30 strains from sputum, 7 from blood, 1 from cerebrospinal fluid, and 4 from other sites. Six *S. pyogenes* strains were isolated from blood, 5 from skin, 5 from respiratory tract, 6 from wounds, and 2 from other sites. All 50 VGS strains were isolated from blood (35 were *Streptococcus mitis* group, 12 were *Streptococcus sanguinis* group, 2 were *Streptococcus anginosus* group, and 1 was *Streptococcus salivarius* group) (7).

The in vitro activities of 10 antimicrobials against M-phenotype Ery<sup>r</sup> streptococci are shown in Table 1. All strains showed cross-resistance to clarithromycin and azithromycin. A high rate of penicillin and cotrimoxazole resistance was found among pneumococci and VGS strains, whereas all *S. pyogenes* strains were susceptible to these antibiotics. Among the streptococci studied, only pneumococci showed resistance to chloramphenicol (4.8%). Tetracycline resistance was observed in 14.3% of M-phenotype pneumococci, and this association was more frequent among nontypeable pneumococci (27.8%) than among capsulated pneumococci (4.2%). In addition, 31.5% of M-phenotype VGS were tetracycline resistant, suggesting a spread of the Tn916-like transposon harboring *mefE-tetM* (1, 6, 20) among streptococci isolated in our area. Clindamycin, quinupristin-dalfopristin, and telithromycin showed good in vitro activity against all M-phenotype streptococci, and no resistant strains were found.

The *mefA/E* gene was detected by PCR (23) in all 116 streptococcal strains, and none had the *ermB* gene. After digestion with BamHI (16), the distribution of *mefA* gene subclasses was as follows: 38 *S. pneumoniae* strains had *mefE* and 4 strains had *mefA*, 49 VGS isolates had *mefE*, and 1 *S. salivarius* group strain had *mefA*, whereas all 24 *S. pyogenes* isolates had *mefA*.

The majority (40.5%) of pneumococci were nontypeable, being serotype 14, the most frequent type found (33.3%) (Table 2). Forty-two *S. pneumoniae* strains were studied by pulsed-

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TABLE 1. In vitro activity of 10 antimicrobials against M-phenotype streptococci

Antimicrobial	Breakpoint (μg/ml) <sup>a</sup>	<i>S. pneumoniae</i> (n = 42)				VGS (n = 50)				<i>S. pyogenes</i> (n = 24)			
		MIC (μg/ml) <sup>b</sup>			% Resistant	MIC (μg/ml) <sup>b</sup>			% Resistant	MIC (μg/ml) <sup>b</sup>			% Resistant
		50%	90%	Range		50%	90%	Range		50%	90%	Range	
Penicillin	≥0.12 <sup>c</sup>	0.06	2	0.01–8	50	0.25	4	≤0.03–8	59.3	≤0.03	≤0.03	≤0.03	0
Azithromycin	≥1	16	32	4–64	100	8	32	2–>64	100	16	32	2–32	100
Erythromycin	≥0.5	8	32	1–32	100	4	32	1–≥64	100	8	32	1–64	100
Clarithromycin	≥0.5	2	4	0.5–8	100	1	2	0.5–4	100	4	8	4–16	100
Clindamycin	≥0.5	≤0.12	≤0.12	≤0.12	0	≤0.12	≤0.12	≤0.12	0	≤0.12	≤0.12	≤0.12–0.25	0
Q/dalfopristin <sup>d</sup>	≥2	0.5	1	0.5–1	0	0.5	1	0.5–1	0	0.5	0.5	0.25–0.5	0
Telithromycin	≥2	0.25	0.5	≤0.03–1	0	0.25	0.5	0.12–1	0	0.5	1	0.25–1	0
Tetracycline	≥4	0.5	≥8	≤0.25–≥8	14.3	≤2	≥8	≤2–≥8	31.5	≤2	≤2	≤2	0
Chloramphenicol	≥8	≤2	4	≤2–16	4.8	2	4	≤2–4	0	≤2	≤2	≤2	0
Cotrimoxazole	≥1	≤0.5	≥4	≤0.5–≥4	40.5	≤0.5	≥4	≤0.5–≥4	42.6	≤0.5	≤0.5	≤0.5	0

<sup>a</sup> Breakpoints according NCCLS criteria (resistant + intermediate) (17).

<sup>b</sup> 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

<sup>c</sup> The penicillin breakpoint for VGS and *S. pyogenes* is 0.25 μg/ml (resistant + intermediate) (17).

<sup>d</sup> Q/dalfopristin, quinupristin-dalfopristin.

field gel electrophoresis (PFGE) as previously described (15, 21, 24). Although 33 PFGE-patterns were found, two international clones (England<sup>14-9</sup> and Spain<sup>9V-3-14</sup>) accounted for 26.2% of strains. Among the eight invasive pneumococci studied, one strain belonged to serotype 6B and seven belonged to serotype 14 (four of the Spain<sup>9V-3</sup> clone, one of the England<sup>14-9</sup> clone, and two of unrelated clones). All nontypeable M-phenotype pneumococci were isolated from sputum.

The increase in the M phenotype among pneumococci reported in some European countries has been associated with the England<sup>14-9</sup> clone of serotype 14, which harbors the *mefA* subclass of the *mefA* gene (1, 5, 8, 16). In our study, serotype 14 was also the most frequently serotype found, but the Spain<sup>9V-3</sup> clone of serotype 14 with *mefE* was more prevalent than the England<sup>14-9</sup> clone with *mefA*. The subclass *mefE* had been previously detected in the Spain<sup>9V-3-14</sup> pneumococcal clone (14), but the high frequency of this clone among M-phenotype pneumococci in Spain had not been known so far.

The Spain<sup>9V-3</sup> clone, first identified in 1987 in Spain and France, has become prevalent in many European countries and in other parts of the world (4, 15). In Spain, the majority of serotype 9V strains and nearly half of serotype 14 strains belonged to the Spain<sup>9V-3</sup> clone (13).

The England<sup>14-9</sup> clone, first described in the United Kingdom (11), is characterized by the presence of the *mefA* subclass *mefA* gene, which is part of a genetic element (Tn1207.1) that has been found in strains from Italy and the United Kingdom (1, 5). This clone is an important cause of meningitis throughout the United Kingdom (1, 11), and it has been recently described in Italy (16), Greece (8), and now in Spain, suggesting the spread of this clone among Mediterranean countries.

The subclass *mefA* of *mefA* gene was originally reported in *S. pyogenes* and is prevalent among strains of this species (3, 19, 22). According to this fact, we found the subclass *mefA* gene among all *S. pyogenes* strains tested. However, the original pneumococcal subclass *mefE* of this gene has been found among *S. pyogenes* isolates from France and the United Kingdom (1, 3). Our study shows that subclass *mefE* is prevalent among VGS, the only exception being one *S. salivarius* strain with the subclass *mefA*. Other studies have found a similar rate

of *mefA/mefE* subclass distribution among VGS in Spain (18) and in France (2).

In conclusion, in our geographical area, the majority of pneumococci and VGS had *mefA* subclass *mefE* gene, whereas

TABLE 2. Distribution of *mefA* subclass *mefA* and subclass *mefE* genes among 42 *S. pneumoniae* isolates by serotypes or groups and clones

Serotype [%] (no. of strains)	PFGE pattern	No. of strains of each subclass of <i>mefA</i> gene		Resistance pattern <sup>a</sup>
		<i>mefE</i>	<i>mefA</i>	
Nontypeable (18 [40.5])	Unrelated <sup>b</sup>	2		E
		1		EC
		2		ESxT
		5		ET
		3		PE
5			PESxT	
14 (14 [33.3])	Spain <sup>9V-3c</sup>	7		PESxT
	England <sup>14-9</sup>		4	E
	Unrelated	2		PESxT
1	Unrelated	1		E
23F (4 [9.5])	Unrelated	1		PESxT
		1		PE
		1		E
		1		PETSxT
3 (1)	Unrelated	1		EC
6A (1)	Unrelated	1		E
6B (1)	Unrelated	1		E
10 (1)	Unrelated	1		E
19A (1)	Unrelated	1		PE
23A (1)	Unrelated	1		E

<sup>a</sup> P, penicillin resistant; E, erythromycin resistant; SxT, cotrimoxazole resistant; T, tetracycline resistant; C, chloramphenicol resistant.

<sup>b</sup> PFGE patterns unrelated to international PMEN clones (15) represented by a single isolate.

<sup>c</sup> Capsular switching.

all *S. pyogenes* strains had *mefA* subclass *mefA*. The *S. pneumoniae* strains with the M phenotype were frequently nontypeable and isolated from respiratory tract specimens, whereas among invasive pneumococci, this phenotype was mainly found in serotype 14 strains related to Spain<sup>9V</sup>-3-14 and England<sup>14</sup>-9 clones.

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