JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2002, p. 774–778 Vol. 40, No. 3
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.3.774–778.2002

Macrolide Efflux Genes *mef*(A) and *mef*(E) Are Carried by Different Genetic Elements in *Streptococcus pneumoniae*

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Received 9 August 2001/Returned for modification 14 October 2001/Accepted 14 December 2001

Susceptibilities to macrolides were evaluated in 267 *Streptococcus pneumoniae* isolates, of which 182 were from patients with invasive diseases and 85 were from healthy carriers. Of the 98 resistant isolates, 20 strains showed an M phenotype and carried *mef*. Strains that carried both *mef*(A) and *mef*(E) were found: 17 strains carried *mef*(A) and 3 carried *mef*(E). The characteristics of the strains carrying the *mef* genes and the properties of the *mef*-containing elements were studied. Strains carrying *mef*(A) belonged to serotype 14, were susceptible to all the antibiotics tested except erythromycin, and appeared to be clonally related by pulsed-field gel electrophoresis (PFGE). The three *mef*(E) strains belonged to different serotypes, showed different susceptibility profiles, and did not appear to be related by PFGE. The sequences of a fragment of the *mef*-containing element, which encompassed *mef* and the *msr*(A) homolog, were identical among the three *mef*(E)-positive strains and among the three *mef*(A)-positive strains, although there were differences between the sequences for the two variants at 168 positions. In all *mef*(A)-positive strains, the *mef* element was inserted in *celB*, which led to impairment of the competence of the strains. In line with insertion of the *mef*(E) element at a different site, the competence of the *mef*(E)-positive strains was maintained. Transfer of erythromycin resistance by conjugation was obtained from two of three *mef*(A) strains but from none of three *mef*(E) strains. Due to the important different characteristics of the strains carrying *mef*(A) or *mef*(E), we suggest that the distinction between the two genes be maintained.

Macrolide resistance in *Streptococcus pneumoniae* is typically due to acquisition of the *erm*(B) gene, which mediates ribosomal modification (10), or the *mef* gene, which encodes a drug efflux pump (28). Recently, mutations in the 23S rRNA or ribosomal proteins of *S. pneumoniae* have been found to confer erythromycin (ERY) resistance in some clinical isolates (30).

The Mef pump confers a low to moderate level of resistance to 14- and 15-membered macrolides but not to lincosamide or streptogramin B antibiotics (M phenotype). Of the two variants of the *mef* gene, *mef*(A) was originally found in *Streptococcus pyogenes* (3) and *mef*(E) was originally found in *S. pneumoniae* (29). *mef*(A) and *mef*(E) are 90% identical at the nucleotide level and were assigned to the same class of macrolide resistance determinants (22). In most subsequent studies, *mef* was detected by a PCR assay that did not distinguish between the two variants (27). However, the two variants were considered species specific; therefore, if a *mef* gene was found in *S. pneumoniae*, it was generally assumed to be *mef*(E) (9, 16, 26). However, *mef*(A) was shown to be present in macrolide-resistant Italian isolates of *S. pneumoniae* (18).

Genetic elements carrying *mef* genes in *S. pneumoniae* were recently detected and characterized. The *mef*(A)-carrying element is a 7.2-kb defective transposon (Tn*1207.1*) that contains eight open reading frames (ORFs), one of which is a putative site-specific recombinase (23). The element that contains *mef*(E) (macrolide efflux genetic assembly [the mega element]) is approximately 5.5 kb and contains five ORFs but no putative transposase or recombinase (7). Interestingly, both elements contain an ORF adjacent to *mef*, designated ORF5 in Tn*1207.1* and *mel* in the mega element, which has homology with the *msr*(A) gene of *Staphylococcus aureus*, which coded for a protein of the ABC transporter superfamily involved in macrolide efflux (7, 23).

The aim of this study was to identify the *mef* genes in a large collection of *S. pneumoniae* strains from Italy and to characterize the properties of the strains carrying them.

MATERIALS AND METHODS

Bacterial strains. Two-hundred sixty-seven *S. pneumoniae* clinical isolates were examined: 182 strains were from patients with invasive diseases (blood or cerebrospinal fluid) isolated in different areas of Italy over 4 years [19]; unpublished data) and 85 strains from the nasopharynges of healthy children attending day-care centers in Rome (20a).

Susceptibilities to ERY, penicillin (PEN), clindamycin, tetracycline (TET), and chloramphenicol (CHL) were assayed by the Etest (AB Biodisk). The breakpoints for resistance were those suggested by NCCLS (17). Isolates were serotyped by capsular swelling in antisera prepared at the Statens Seruminstitut, Copenhagen, Denmark.

Detection of *mef* genes and distinction between *mef*(A) and *mef*(E). The presence of the *mef* gene was detected by PCR with the primer pair designed by Sutcliffe et al. (27). In order to discriminate between *mef*(A) and *mef*(E), a PCR-restriction fragment length polymorphism analysis was performed, as suggested by Oster et al. (18). The primers pair used were MEF3 (5′-GCGTTTA AGATAAGCTGGCA-3′) and MEF4 (5′-CCTGCACCATTTGCTCCTAC-3′), both of which were derived from the work of Tait-Kamradt et al. (29), to generate a 1,743-bp PCR product. The amplicon was digested with the *Bam*HI or the *Dra*I restriction enzyme. In *mef*(A) there is one *Bam*HI site, so restriction generates two fragments of 1,340 and 403 bp, respectively, while in *mef*(E) there

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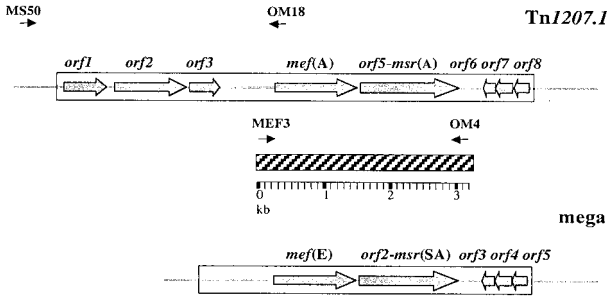

FIG. 1. Schematic representation of *mef*(A)-carrying element Tn*1207.1* (22) and the *mef*(E)-carrying mega element (7). The hatched bar represents the fragment amplified and sequenced with primers MEF3 and OM4. Primers MS50 and OM18, used to amplify the chromosomal insertion of the *mef*(A)-carrying element, are indicated above Tn*1207.1*. The positions of the primers are indicated by arrows.

are no *Bam*HI restriction sites. Restriction of *mef*(A) with *Dra*I yields two fragments of 1,493 and 250 bp, respectively, while restriction of *mef*(E) yields three fragments of 782, 711, and 250 bp, respectively.

PFGE. The relatedness among *mef*(A)- and *mef*(E)-carrying strains was examined by pulsed-field gel electrophoresis (PFGE) by published methods (19). Genomic DNAs were digested with *Sma*I prior to electrophoresis with a CHEF-Mapper system (Bio-Rad Laboratories, Milan, Italy). Strains that differed by one to six bands were considered clonally related (31).

Sequencing of *mef* and *orf5* genes. A 3,201-bp fragment that encompassed the *mef* gene and *orf5-msr*(A) homolog was obtained by amplification with primers MEF3 (29) and OM4 (5′-AGGAGCAGTTCGATTTACTG-3′), designed on the basis of the Tn*1207.1* sequence (23) (Fig. 1). Sequencing was performed with a Perkin-Elmer ABI 377 DNA sequencer and an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems).

As the *mef*(A) element of *S. pneumoniae* is inserted into chromosomal gene *celB* (23), a PCR was performed to amplify a segment spanning a portion of *celB* (upstream of the putative insertion) and a portion of the inserted *mef* element. The primers used were MS50 (5′-GCTTATGCTTTTATCCTGACCATG-3′), which anneals upstream of the Tn*1207.1* integration site in *celB* (20, 23), and OM18 (5′-TGCTTGCCCTGCCCATATT-3′), which is designed on the basis of a consensus sequence internal to the *mef* genes (Fig. 1). In order to detect the chromosomal insertion of the *mef*(E) element, PCRs were performed with primers specific for the sequences flanking the insertion sites found by Gay and Stephens (7).

Transformation assay. The assay used to determine the competence of the strains was carried out as described previously (21). The clinical isolates were tested along with a noncompetent control strain, strain MF4 (23), and a competent control strain, strain Rx1 (21). A competence curve was obtained for *mef*(E)-carrying strain 713. Briefly, transforming DNA and competence-stimulating peptide were added to pneumococcal cells harvested at 15-min intervals during the exponential phase of growth in competence medium (21). Since the transforming DNA contained a novobiocin resistance marker, the number of transformants was determined by plating the cells on selective plates containing novobiocin at 10 μg/ml. The transformation frequency was expressed as the number of CFU of the transformants divided by the number of CFU of the recipients.

Mating experiments. Transfer of *mef* by conjugation was tested. The recipient strain was FP10, a streptomycin-resistant derivative of strain Rx1 (rough type 2) (21) in which the *comC* gene that encodes the competence-stimulating peptide was deleted and replaced by a CHL resistance cassette, making the strain not spontaneously transformable (F. Iannelli et al., unpublished data). Donor and recipient bacteria, grown separately at 37°C in tryptic soy broth until the end of the log phase, were mixed at a 1:10 ratio, plated onto tryptic soy agar plates with 5% horse blood, and incubated in 5% CO_2 at 37°C for 4 h. Matings were performed in the presence of DNase at 10 μg/ml. After incubation, the cells were harvested, diluted, and plated. Scoring of transconjugants on multilayer plates was performed as described by Shoemaker et al. (25). In the overlay of the selection plates, ERY was added at 1 μg/ml, streptomycin was added at 500 μg/ml, and CHL was added at 3 μg/ml. The presence of *mef* genes in the transconjugants was confirmed by PCR.

Nucleotide sequence accession number. The nucleotide sequence of the 3,152-bp fragment containing *mef*(E) and the *msr*(A)-like gene of strain PN150 was assigned GenBank accession no. AF376746.

RESULTS

Characteristics of *mef*(A)- and *mef*(E)-positive isolates. Among a sample of 267 isolates of *S. pneumoniae*, 98 pneumococcal strains (36.7%) were resistant to macrolides. Twenty of these strains displayed the M phenotype and carried a *mef* gene. Of the *mef*-positive strains, 17 carried *mef*(A) and 3 carried *mef*(E). The 17 *mef*(A)-carrying isolates belonged to serotype 14. By PFGE their profiles appeared to be very similar (Fig. 2A). Six isolates shared an identical profile (indicated profile A1); the other isolates showed six different patterns (profiles A2 to A7) that differed from the principal profile by two to six bands. These differences are compatible with a clonal origin of the isolates (31), although they were not related in terms of times of isolation, geographical area of isolation in Italy, or the characteristics of the patients from which they were isolated (Table 1).

The *mef*(E)-carrying strains belonged to two different serotypes, and their macrorestriction profiles were different (Fig. 2B). All *mef*(A)-carrying strains were resistant to ERY and sensitive to other antibiotics, including PEN, TET, and CHL, while the *mef*(E)-carrying strains were resistant to ERY and to other drugs (Table 1).

***mef* and *msr*(A)-like genes.** The 3,201-bp DNA fragment containing the *mef* and *msr*(A)-like genes was obtained by PCR with primers designed on the basis of the sequence of *mef*(A)-carrying genetic element Tn*1207.1* (Fig. 1). PCR products of identical sizes were obtained from all 20 *mef* strains, and sequencing was performed for the 3 *mef*(E)-carrying strains and for 3 randomly chosen *mef*(A)-carrying strains (strains PN83, PN92, and PN137; Table 1). The nucleotide sequences of the *mef*(A)-carrying strains were identical to that of Tn*1207.1*, while the nucleotide sequences of the *mef*(E)-carrying strains were identical to each other but differed from that of Tn*1207.1* at 168 positions. In the *mef*(E)-carrying strains, the sequences encoding *mef*(E) and the *msr*(A) homolog were identical to the corresponding sequences published by Gay and Stephens (7). However, a 16-bp deletion and six single-base mismatches were found in the noncoding region upstream of *mef*(E), and one mismatch was found in the noncoding region downstream of the *msr*(A) homolog.

Chromosomal insertion site of the *mef* element. Since *mef*(A)-carrying genetic element Tn*1207.1* was found to be integrated into the pneumococcal *celB* gene (23), PCR primer pair MS50-OM18 was designed on the basis of *celB* and *mef* sequences (Fig. 1) to investigate the insertion sites of the *mef*-carrying genetic elements. Identical 3.9-kb fragments could be amplified from all the 17 *mef*(A) strains examined in this study, indicating integration in *celB*. Sequencing of the insertion site in one isolate showed that it was coincident at the nucleotide level with the insertion site previously described in transformant MF4 (23). No PCR products were obtained from the three *mef*(E) strains, suggesting integration elsewhere. In strain 713, the insertion site was found to correspond to class I of Gay and Stephens (7). In the other two *mef*(E) strains the

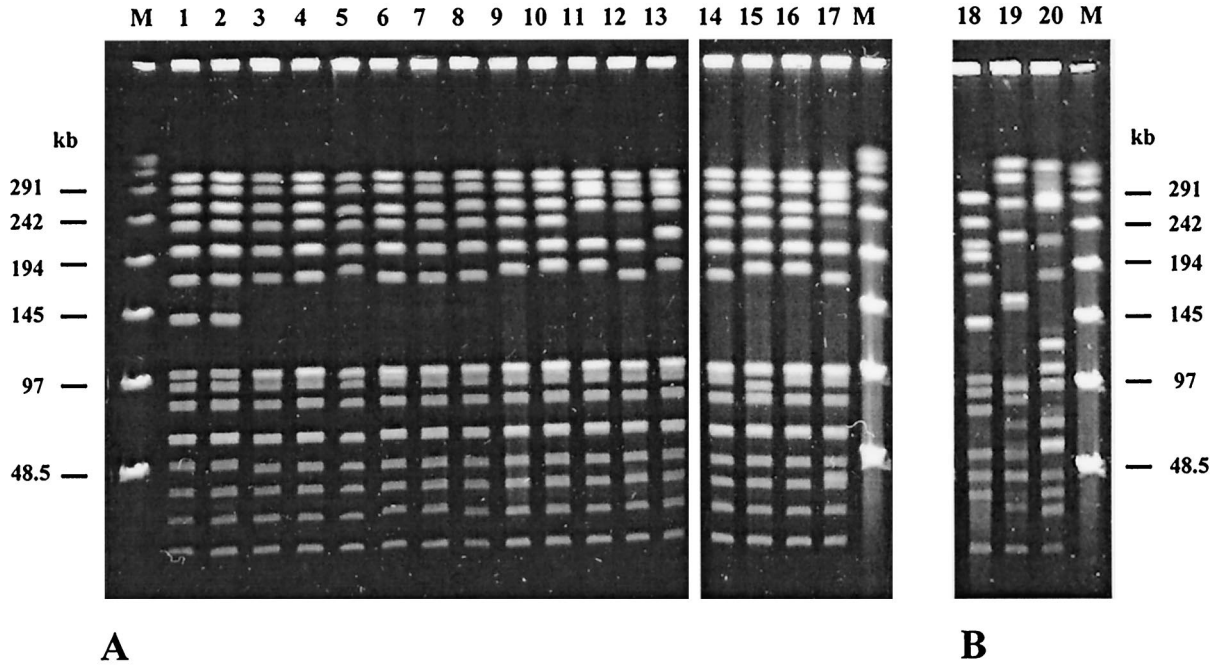

FIG. 2. PFGE of *Sma*I-digested chromosomal DNA of *S. pneumoniae*. (A) *mef*(A)-carrying serotype 14 strains; (B) *mef*(E)-carrying strains. Lane 1, PNS06; lane 2, PNS07; lane 3, 1514; lane 4, 1711; lane 5, PN67; lane 6, PN151; lane 7, PN83; lane 8, PN98; lane 9, PN88; lane 10, PN137; lane 11, PN165; lane 12, PN17; lane 13, 1044; lane 14, PN138; lane 15, PN139; lane 16, PN92; lane 17, PNS11; lane 18, PN150; lane 19, PN34; lane 20, 713; lanes M, bacteriophage lambda ladder molecular mass marker. See Table 1 for details about the strains.

insertion sites were different from those described by Gay and Stephens (7).

Transformability of *mef*-carrying pneumococci. Since insertional inactivation of *celB* should lead to impairment of natural transformation (20), our strains were tested for competence, as was noncompetent control strain MF4 (23) and competent control strain Rx1 (21). As expected, the three *mef*(A)-carrying strains (strains PN83, PN92, and PN137) did not become competent, while the *mef*(E) strains were transformed, confirming that the *mef*(E) elements are not integrated in *celB*. A competence curve was obtained for *mef*(E)-carrying *S. pneumoniae* strain 713 by using as a positive control competent strain Rx1. The transformation frequency was calculated at each time point. The maximum activity was at 45 min for both strains, with a value of 4.2×10^{-4} transformants per recipient in strain 713 and 3×10^{-2} transformants per recipient in strain Rx1.

Conjugal transfer of *mef*. The three *mef*(E)-carrying strains and the three *mef*(A)-carrying strains were used as donors in

TABLE 1. Characteristics of 20 clinical isolates carrying the *mef* gene

Strain	Place (yr) of isolation	Source	Patient	Serotype	Resistance pattern	*mef* gene	PFGE profile
PN17	Cuneo (1997)	CSF[a]	Adult	14	ERY	*mef*(A)	A5
PN67	Rome (1997)	CSF	Child	14	ERY	*mef*(A)	A3
PN83	Verona (1998)	CSF	Child	14	ERY	*mef*(A)	A1
PN88	Reggio Calabria (1998)	CSF	Child	14	ERY	*mef*(A)	A3
PN92	Monza (1998)	CSF	Child	14	ERY	*mef*(A)	A3
PN98	Rome (1998)	CSF	Child	14	ERY	*mef*(A)	A1
PN137	Arezzo (1999)	CSF	Adult	14	ERY	*mef*(A)	A3
PN138	Parma (1999)	CSF	Adult	14	ERY	*mef*(A)	A1
PN139	Arezzo (1999)	CSF	Adult	14	ERY	*mef*(A)	A7
PN151	Mantova (1999)	CSF	Adult	14	ERY	*mef*(A)	A1
PN165	Palermo (1999)	CSF	Child	14	ERY	*mef*(A)	A4
PNS06	Rome (1998)	Blood	Adult	14	ERY	*mef*(A)	A2
PNS07	Rome (1998)	Blood	Adult	14	ERY	*mef*(A)	A2
PNS11	Rome (1998)	Blood	Adult	14	ERY	*mef*(A)	A5
1514	Rome (1999)	Nasopharynx	Child	14	ERY	*mef*(A)	A1
1711	Rome (1999)	Nasopharynx	Child	14	ERY	*mef*(A)	A1
1044	Rome (1999)	Nasopharynx	Child	14	ERY	*mef*(A)	A6
PN34	Trento (1997)	CSF	Adult	6A	ERY, TET, CHL	*mef*(E)	B
PN150	Bologna (1999)	CSF	Adult	19F	ERY, PEN, TET	*mef*(E)	C
713	Rome (1999)	Nasopharynx	Child	6A	ERY, TET	*mef*(E)	D

[a] CSF, cerebrospinal fluid.

conjugation experiments in which rough strain FP10 was the recipient. *mef*(A) was transferred at a frequency of 1.5×10^{-3} transconjugants per donor from PN92 and at a frequency of 1.8×10^{-4} transconjugants per donor from PN137, while no transfer ($<5 \times 10^{-8}$ transconjugants per donor) was detected when PN83 was the donor. In the two transconjugants, the *mef*(A) element was found to be inserted in *celB*. No *mef*(E)-carrying strain was able to transfer macrolide resistance by conjugation.

DISCUSSION

The high rate of macrolide resistance observed in this study is not unprecedented among isolates from Italy. In the last decade a steep increase in the rate of resistance to macrolides has been observed, from 5% in 1993 (12) to over 30% in 1998 to 1999 (14, 19). In our study, ERY resistance was mediated by the drug efflux mechanism in only 20 of 98 isolates (20%). This confirms that in Italy, as in other European countries, the *mef* gene is relatively uncommon among macrolide-resistant *S. pneumoniae* isolates (2, 5, 13, 19), while in North America it is more frequent than the *erm*(B) gene (4, 6, 9). Although carriage of *mef*(E) has been considered typical of *S. pneumoniae*, we have found 17 strains that carry *mef*(A) and only 3 strains that carry *mef*(E).

As both the element carrying *mef*(A) and the element carrying *mef*(E) contain another putative efflux gene, a homolog to *msr*(A), we amplified and sequenced a fragment that included *mef* and the *msr*(A) homolog in six Italian isolates. The sequences were identical for the three *mef*(A) strains and the sequences were identical for the three *mef*(E) strains, while the sequences of the two variants were divergent at a number of positions. This suggests that the elements have recently emerged from a common ancestor and evolved in different hosts. It is possible that the original host of the *mef*(A) element is *S. pyogenes* (M. Santagati, F. Iannelli, C. Messina, M. R. Oggioni, S. Stefani, and G. Pozzi, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2014, 2001), while *mef*(E) is carried by viridans group streptococci (1). In the nasopharynx there are opportunities for these elements to be transferred from both species to *S. pneumoniae*. The presence of *mef*(A) in Italian isolates of *S. pneumoniae* might be the consequence of the high prevalence of macrolide-resistant *mef*-carrying *S. pyogenes* isolates in Italy (8).

An important characteristic that is different between the two elements is the insertion site: while the *mef*(A) element was found to be integrated at a single specific chromosomal site (*celB*) in all the strains examined, including transformant MF4 and the two transconjugants, the *mef*(E) element was found to be inserted at different chromosomal locations (7). Site-specific integration of the *mef*(A) element is a property shared by other mobile DNA elements, including transposons (32) and the pathogenicity islands of enteric pathogens (24).

As the target gene for *mef*(A) integration (*celB*) is involved in competence, *mef*(A)-carrying strains are defective in transformability, whereas *mef*(E) strains can be transformed.

We found that two of three *mef*(A)-carrying isolates were able to transfer the *mef*(A) element by conjugation. Previously described transposon Tn*1207.1* appeared to be a defective element and was not transferable (23). Although we did not examine the size of the *mef*(A) element in the clinical isolates, it is conceivable that the strains able to transfer macrolide resistance possess an element that is larger than Tn*1207.1*, similar to that described recently in *S. pyogenes* (Santagati et al., 41st ICAAC), and that represents a complete conjugative transposon. Conversely, the *mef*(E) element was not transferable by conjugation from our three isolates. Although *mef*(E) has been found to be transferable from *S. pneumoniae* in some instances (11), the mega element described by Gay and Stephens (7) has features of a defective transposon that lacks the enzymes required for DNA transposition.

Interestingly, the resistance phenotypes of the strains are different. *mef*(A)-positive strains are susceptible to all the antibiotics tested other than ERY, while *mef*(E)-positive strains display resistance to various antibiotics, including PEN. The uniform susceptibility *of mef*(A) strains might be due to the fact that all the *mef*(A) isolates belong to a single serotype 14 clone. This finding is intriguing and has also been confirmed in a subsequent large set of *mef*(A)-carrying invasive isolates (data not shown). As the isolates are not related in terms of times and areas of isolation, it appears that the spread of the *mef*(A) element in Italy occurred through the expansion of a single PEN-susceptible serotype 14 clone that has acquired *mef*(A). An alternate explanation is that different strains belonging to a well-established clone have acquired the element independently. On the basis of *Sma*I PFGE fingerprinting and multilocus sequence typing (data not shown), the *mef*(A)-positive clone appears to belong to the England^{14}-9 clone, one of the major antibiotic-resistant pneumococcal clones (15). It is noteworthy that clonal expansion has also been noted in ERY-resistant serotype 14 isolates carrying *mef*(E) (7) or *erm*(B) (19).

We do not know whether the prevalence of *mef*(A) in *S. pneumoniae* is a peculiarity of the Italian situation, as the identity of the *mef* genes has been investigated in only a few geographical areas. On the other hand, our data and those of Gay and Stephens (7) indicate that the *mef*(E) element is disseminated by horizontal transfer to different strains and therefore seems to be more adapted for *S. pneumoniae*.

In conclusion, as the *mef*(A) and the *mef*(E) elements are endowed with important genetic differences and confer distinctive characteristics to the strains, it might be appropriate to distinguish between them by referring to them as *mef*(A), subclass *mef*(A), or subclass *mef*(E). When describing the epidemiology of macrolide resistance in *S. pneumoniae* in different parts of the world, it might be important to distinguish these two genes, as this could contribute to an understanding of the spread of ERY resistance in *S. pneumoniae*.

ACKNOWLEDGMENTS

We are indebted to Kathryn Gay and David S. Stephens for making the sequence of the mega element available to us before publication. We thank Fabio D'Ambrosio for experienced technical assistance.

This work was supported in part by grants from Ministero della Sanità (Programmi per la Ricerca Finalizzata 1999 and Progetti di ricerca finalizzata IRCCS, ICS 120.5/RF 97.99) and from MURST (COFIN 2000, 22010709/16010190).

REFERENCES

1. **Arpin, C., M. H. Canron, J. Maugein, and C. Quentin.** 1999. Incidence of *mefA* and *mefE* genes in viridans group streptococci. Antimicrob. Agents Chemother. **43:**2335–2336.
2. **Baquero, F., J. A. Garcia-Rodriguez, J. G. De Lomas, L. Aguilar, and the**

Spanish Surveillance Group for Respiratory Pathogens. 1999. Antimicrobial resistance of 1,113 *Streptococcus pneumoniae* isolates from patients with respiratory tract infections in Spain: results of a 1-year (1996–1997) multicenter surveillance study. Antimicrob. Agents Chemother. **43:**357–359.

3. **Clancy, J., J. Pepitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes*. Mol. Microbiol. **22:**867–879.
4. **Doern, G. V., A. B. Brueggemann, H. Huynh, E. Wingert, and P. Rhomberg.** 1999. Antimicrobial resistance with *Streptococcus pneumoniae* in the United States, 1997–98. Emerg. Infect. Dis. **5:**757–765.
5. **Fitoussi, F., C. Doit, P. Geslin, N. Brahimi, and E. Bingen.** 2001. Mechanism of macrolide resistance in clinical pneumococcal isolates in France. Antimicrob. Agents Chemother. **45:**636–638.
6. **Gay, K., W. Baughman, Y. Miller, D. Jackson, C. G. Whitney, A. Schuchat, M. M. Farley, F. Tenover, and D. S. Stephens.** 2000. The emergence of *Streptococcus pneumoniae* resistant to macrolide antimicrobial agents: a 6-year population-based assessment. J. Infect. Dis. **182:**1417–1424.
7. **Gay, K., and D. S. Stephens.** 2001. Structure and dissemination of a chromosomal insertion element encoding macrolide efflux in *Streptococcus pneumoniae*. J. Infect. Dis. **184:**56–65.
8. **Giovanetti, E., M. P. Montanari, M. Mingoia, and P. E. Varaldo.** 1999. Phenotypes and genotypes of erythromycin-resistant *Streptococcus pyogenes* strains in Italy and heterogeneity of inducibly resistant strains. Antimicrob. Agents Chemother. **43:**1935–1940.
9. **Johnston, N. J., J. C. De Azavedo, J. D. Kellner, and D. E. Low.** 1998. Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2425–2426.
10. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
11. **Luna, V. A., P. Coates, E. A. Eady, J. H. Cove, T. T. H. Nguyen, and M. C. Roberts.** 1999. A variety of gram-positive bacteria carry mobile *mef* genes. J. Antimicrob. Chemother. **44:**19–25.
12. **Marchese, A., E. A. Debbia, A. Arvigo, A. Pesce, and G. C. Schito.** 1995. Susceptibility of *Streptococcus pneumoniae* strains isolated in Italy to penicillin and ten other antibiotics. J. Antimicrob. Chemother. **36:**833–837.
13. **Marchese, A., E. Tonoli, E. A. Debbia, and G. C. Schito.** 1999. Macrolide resistance mechanisms and expression of phenotypes among *Streptococcus pneumoniae* circulating in Italy. J. Antimicrob. Chemother. **44:**461–464.
14. **Marchese, M., E. Tonoli, G. Balistreri, E. Debbia, and G. C. Schito.** 2000. Antibiotic susceptibility patterns and serotypes of antibiotic resistant and/or invasive *Streptococcus pneumoniae* strains circulating in Italy. Microb. Drug Resist. **6:**163–170.
15. **McGee, L., L. McDougal, J. Zhou, B. G. Spratt, F. C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J. C. Lefevre, A. Tomasz, and K. P. Klugman.** 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J. Clin. Microbiol. **39:**2565–2571.
16. **Montanari, M. P., M. Mingoia, E. Giovannetti, and P. E. Varaldo.** 2001. Differentiation of resistance phenotypes among erythromycin-resistant pneumococci. J. Clin. Microbiol. **39:**1311–1315.
17. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 5th ed. NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
18. **Oster, P., A. Zanchi, S. Cresti, M. Lattanzi, F. Montagnani, C. Cellesi, and G. M. Rossolini.** 1999. Patterns of macrolide resistance determinants among community-acquired *Streptococcus pneumoniae* isolates over a 5-year period of decreased macrolide susceptibility rates. Antimicrob. Agents Chemother. **43:**2510–2512.
19. **Pantosti, A., F. D'Ambrosio, A. Tarasi, S. Recchia, G. Orefici, and P. Mastrantonio.** 2000. Antibiotic susceptibility and serotype distribution of *Streptococcus pneumoniae* causing meningitis in Italy, 1997–1999. Clin. Infect. Dis. **31:**1373–1379.
20. **Pestova, E. V., and D. A. Morrison.** 1998. Isolation and characterization of three *Streptococcus pneumoniae* transformation-specific loci by use of a *lacZ* reporter insertion vector. J. Bacteriol. **180:**2701–2710.

20a. **Petrosillo, N., A. Pantosti, E. Boroli, A. Spanò, M. Del Grosso, B. Tallaride, and G. Ippolito.** Prevalence and determinants of *Streptococcus pneumoniae* nasopharyngeal colonization and molecular epidemiology of the isolates from healthy children in Rome. Eur. J. Clin. Microbiol. Infect. Dis., in press.

21. **Pozzi, G., L. Masala, F. Iannelli, R. Manganelli, L. S. Havarstein, L. Piccoli, D. Simon, and D. A. Morrison.** 1996. Competence for genetic transformation in encapsulated strains of *Streptococcus pneumoniae*: two allelic variants of the peptide pheromone. J. Bacteriol. **178:**6087–6090.
22. **Roberts, M. C., J. Sutcliffe, P. Courvalin, L. B. Jensen, J. Rood, and H. Seppala.** 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob. Agents Chemother. **43:**2823–2830.
23. **Santagati, M., F. Iannelli, M. Oggioni, S. Stefani, and G. Pozzi.** 2000. Characterization of a genetic element carrying the macrolide efflux gene *mef*(A) in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **44:** 2585–2587.
24. **Schmidt, H., W.-L. Zhang, U. Hemmrich, S. Jelacic, W. Brunder, P. I. Tarr, U. Dobrindt, J. Hacker, and H. Karch.** 2001. Identification and characterization of a novel genomic island integrated at *selC* in locus of enterocyte effacement-negative, Shiga toxin-producing *Escherichia coli*. Infect. Immun. **69:**6863–6873.
25. **Shoemaker, N., M. Smith, and W. R. Guild.** 1980. DNase-resistant transfer of chromosomal cat and tet insertions by filter mating in pneumococcus. Plasmid **3:**80–87.
26. **Shortridge, V. D., G. V. Doern, A. B. Brueggemann, J. M. Beyer, and R. K. Flamm.** 1999. Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic resistance surveillance study conducted in the United States in 1994–1995. Clin. Infect. Dis. **29:** 1186–1188.
27. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
28. **Sutcliffe, J., A. Tait-Kamradt, and L. Wondrack.** 1996. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolide but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob. Agents Chemother. **40:**1817–1824.
29. **Tait-Kamradt, A., J. Clancy, M. Cronan, F. Dib-Hajj, L. Wondrack, W. Yuan, and J. Sutcliffe.** 1997. *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **41:**2251–2255.
30. **Tait-Kamradt, A., T. Davies, P. C. Appelbaum, F. Depardieu, P. Courvalin, J. Petitpas, L. Wondrack, A. Walker, M. R. Jacobs, and J. Sutcliffe.** 2000. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. Antimicrob. Agents Chemother. **44:**3395–3401.
31. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
32. **Vijayakumar, M. N., and S. Ayalew.** 1993. Nucleotide sequence analysis of the termini and chromosomal locus involved in site-specific integration of the streptococcal conjugative transposon Tn*5252*. J. Bacteriol. **175:**2713–2719.