MINIREVIEW

Genetic Elements Responsible for Erythromycin Resistance in Streptococci[†]

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During the last two decades, growing rates of erythromycin resistance have been reported in many countries among both *Streptococcus pyogenes* (29) and *Streptococcus pneumoniae* (63) clinical isolates. This trend was partly due to a further spread of the conventional methylase-mediated target site modification mechanism of resistance, but to an even greater extent it reflected the emergence of an active efflux-mediated mechanism of erythromycin resistance.

Besides less common mutations in 23S rRNA or ribosomal proteins, target site modification consists in posttranscriptional methylation of an adenine residue in 23S rRNA; this is caused by *erm* class gene-encoded methylases and usually results in coresistance to macrolide, lincosamide, and streptogramin B antibiotics (MLS phenotype) (62, 63). The traditional and widely predominant *erm* determinant in streptococci, *erm(B)*, can be expressed either constitutively or inducibly and is usually associated with high-level resistance (62). A more recently described methylase gene, *erm(TR)* (92), an *erm(A)* subclass (84), is normally inducible (46, 55) and is widely distributed in *S. pyogenes* isolates (55, 58), whose resistance level appears to depend on the contribution of a drug efflux pump (51). *erm(TR)* has been detected in other beta-hemolytic *Streptococcus* species (59, 67, 110), whereas it is quite uncommon in *S. pneumoniae* (12, 34, 41, 43, 97, 106). *erm(T)*, characterized by a very low G+C content (ca. 25%), was detected in inducibly erythromycin-resistant isolates of group D streptococci in Taiwan (99) and subsequently in the United States (40). Very recently, it has been detected in U.S. invasive isolates of inducibly resistant *S. pyogenes* that were negative for conventional erythromycin resistance determinants (111).

Efflux-mediated erythromycin resistance is associated, in streptococci, with a low-level resistance pattern affecting, among MLS antibiotics, only 14- and 15-membered macrolides (M phenotype) (96). Active efflux is encoded by *mef*-class genes, which include several variants. *mef(A)*, the first *mef* gene to be discovered, was originally described in *S. pyogenes* (22) and was subsequently found to be widespread in this species, but it is also common in *S. pneumoniae* and other streptococcal species (60). *mef(E)*, detected in *S. pneumoniae* shortly afterward (98), was found in a variety of other *Streptococcus* species (60), although it has only exceptionally been reported in *S. pyogenes* (4, 6, 87). Less common *mef* genes have

been detected in *S. pneumoniae* [*mef(I)* (28)] and *S. pyogenes* [*mef(O)* (87)], and *mef(B)* and *mef(G)* new alleles have recently been described in group B (15) and group G (5, 15) beta-hemolytic streptococci, respectively. An *msr* class gene with homology to *msr(A)*—an ATP-binding cassette gene associated with macrolide efflux in *Staphylococcus aureus* (85)—is located immediately downstream of the *mef* gene. This *msr* gene is usually designated *msr(D)*, even though different variants are associated with different *mef* genes. Studies carried out in pneumococci demonstrated that *mef* and *msr(D)* are cotranscribed, suggesting that the proteins encoded by the two genes may act as a dual efflux system (48), inducible by erythromycin (3). It has also been suggested that the *msr(D)*-encoded pump is capable of functioning independently of the one encoded by *mef* (3, 33).

Macrolide inactivation due to a phosphotransferase encoded by the *mph(B)* gene, formerly described only in gram-negative bacteria, has lately been detected in *Streptococcus uberis*, where the inactivation mechanism, however, conferred only resistance to spiramycin (1).

Until a decade ago, knowledge about the genetic elements responsible for erythromycin resistance in streptococci was virtually confined to a few plasmids or transposons carrying *erm(B)*, then called *ermAM* or simply *erm* (56, 65). Such transposons mainly included Tn917, detected in *Enterococcus faecalis* when it was still regarded as a *Streptococcus* species (94, 101, 102), and Tn1545, detected in *S. pneumoniae* and also encoding resistance, besides tetracycline, to erythromycin and kanamycin (31, 32). Remarkably, Tn1545 was related to Tn916 (47), the prototype of a family of broad-host-range conjugative transposons conferring tetracycline resistance via the *tet(M)* gene (24, 81). Other Tn916-related *erm(B)*-carrying transposons early described in *Streptococcus* species (81) ceased to be reported in later studies. During the last decade, the discovery of the above-mentioned variety of erythromycin resistance genes in streptococci has been closely followed by the identification and characterization of a variety of genetic elements responsible for the resistance and its possible spread via intra- and interspecific transfer. Different erythromycin resistance genes are carried by different elements: in the case of *mef* genes, such close gene-element association was a major argument for recommending that *mef(A)*, *mef(E)*, and any future *mef* variants continue to be discriminated and kept apart (60) as opposed to being collected in a single class, *mef(A)*, due to their high degree of similarity (84). This minireview is aimed at presenting such new knowledge about the genetic elements responsible for erythromycin resistance in streptococci. Elements and their essential characteristics are summarized in Table 1.

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TABLE 1. Essential characteristics of established genetic elements responsible for erythromycin resistance in streptococci

Element	Erythromycin resistance		Size ^b	Sequence accession no. ^c	<i>Streptococcus</i> or related species	Other resistance gene(s) ^d	Reference(s)
	Gene(s)	Phenotype ^a					
Tn917	<i>erm</i> (B)	iMLS, cMLS	5,614 bp	M11180	<i>E. faecalis</i> ^e , <i>S. pyogenes</i> , <i>S. pneumoniae</i>		13, 19, 94, 101, 102
Tn3872	<i>erm</i> (B)	cMLS	23.6 kb	AH007276, AM411624, AM411452	<i>S. pneumoniae</i> , <i>S. pyogenes</i> , <i>Abiotrophia defectiva</i> ^f , <i>S. agalactiae</i>	<i>tet</i> (M)	13, 70, 78, 80
Tn6002	<i>erm</i> (B)	cMLS	20,880 bp	AY898750	<i>S. oralis</i> , <i>S. cristatus</i> , <i>S. pneumoniae</i> , <i>S. pyogenes</i>	<i>tet</i> (M)	13, 19, 27, 35, 108
Tn6003	<i>erm</i> (B) ^g	cMLS	25,101 bp	AM410044	<i>S. pneumoniae</i>	<i>tet</i> (M), <i>aadE</i> , <i>sat4</i> , <i>aphA-3</i>	27
Tn1545	<i>erm</i> (B) ^g	cMLS	26.3 kb	X04388, X05577, X61025, X52632, AM889142, AM903082	<i>S. pneumoniae</i>	<i>tet</i> (M), <i>aadE</i> , <i>sat4</i> , <i>aphA-3</i>	16, 17, 18, 26, 31, 32, 68, 79, 103
Tn2010	<i>erm</i> (B), <i>mef</i> (E), <i>msr</i> (D)	cMLS	26,390 bp	DQ426906, DQ426907, EF592165, AB426620	<i>S. pneumoniae</i>	<i>tet</i> (M)	35, 37
Tn1116	<i>erm</i> (B)	iMLS	50 kb	AM411377	<i>S. pyogenes</i>		13
ICE 10750-RD.2	<i>erm</i> (TR)	iMLS	48.9 kb	CP000262	<i>S. pyogenes</i>	SpyM4_1701, SpyM4_1702, SpyM4_1705	11
Unnamed	<i>erm</i> (TR)	iMLS	40 kb	FM162351	<i>S. pyogenes</i>	<i>orf3</i> , <i>orf4</i> , <i>orf7</i>	54
Tn1806	<i>erm</i> (TR)	iMLS	56 kb	EF469826	<i>S. pneumoniae</i>	<i>orf3</i> , <i>orf4</i> , <i>orf6</i>	20
Unnamed	<i>erm</i> (T)	iMLS		AY894138	Group D streptococci		104
pRW35	<i>erm</i> (T)	iMLS	4,962 bp	EU192194	<i>S. pyogenes</i>		111
Tn1207.1	<i>mef</i> (A), <i>msr</i> (D)	M	7,244 bp	AF227520	<i>S. pneumoniae</i>		36, 89
Tn1207.3	<i>mef</i> (A), <i>msr</i> (D)	M	52,491 bp	AY657002	<i>S. pyogenes</i> , <i>S. agalactiae</i> , <i>S. dysgalactiae</i> subsp. <i>equisimilis</i>		7, 14, 42, 45, 53, 67, 88
Φ10394.4	<i>mef</i> (A), <i>msr</i> (D)	M	58,761 bp	AY445042	<i>S. pyogenes</i>		7, 9, 10, 14, 42
Φm46.1	<i>mef</i> (A), <i>msr</i> (D)	M	60 kb	AJ715499, AM492531	<i>S. pyogenes</i>	<i>tet</i> (O)	7, 14, 53
mega	<i>mef</i> (E), <i>msr</i> (D)	M	5,532 bp	AF274302	<i>S. pneumoniae</i>		35, 48, 109
mega variant	<i>mef</i> (E), <i>msr</i> (D)	M	5,511 bp	AJ318993	<i>S. salivarius</i>		95
Tn2009	<i>mef</i> (E), <i>msr</i> (D)	M	23.5 kb	AF376746, AY466609, AY466610, AY466395	<i>S. pneumoniae</i>	<i>tet</i> (M)	35, 38
5216IQ complex	<i>mef</i> (I), <i>msr</i> (D)	M	30,505 bp	AJ971089	<i>S. pneumoniae</i>	<i>catQ</i> , <i>tet</i> (M)	73

^a That is, the generally corresponding or expected phenotype. The resistance phenotype expressed by an erythromycin-resistant isolate may also be affected by factors other than the genetic element harbored, especially when an *erm* class gene is involved. In case of copresence of *erm* and *mef* genes carried by the same or by different genetic elements, the MLS phenotype usually overrides the M phenotype (52, 71, 106). cMLS, constitutive phenotype; iMLS, inducible phenotype; M, M phenotype (55, 63, 96).

^b The size of the genetic element is expressed as base pairs when it results from sequence analysis, otherwise it is expressed as kilobases.

^c Accession numbers refer to complete or partial sequencing of the genetic element.

^d Resistance genes carried on the same genetic element responsible for erythromycin resistance. Reported genes confer antibiotic resistances as follows: *tet*(M) and *tet*(O), tetracycline; *aadE*, streptomycin; *sat4*, streptothricin; *aphA-3*, kanamycin; SpyM4_1701 and SpyM4_1702 (ICE 10750-RD.2) or *orf3* and *orf4* (sequence accession no. FM162351) or *orf3* and *orf4* (Tn1806), tetracycline; SpyM4_1705 (ICE 10750-RD.2) or *orf7* (sequence accession no. FM162351) or *orf6* (Tn1806), spectinomycin; and *catQ*, chloramphenicol.

^e For some time after discovery and in early studies of Tn917, *E. faecalis* was regarded as a *Streptococcus* species (*S. faecalis*).

^f Formerly *S. defectivus*.

^g Two *erm*(B) genes, one lacking the stop codon.

erm(B)-CARRYING ELEMENTS

Tn917 and Tn3872. Tn917 was originally identified on a non-conjugative plasmid from *E. faecalis* (101, 102). Exposure of cells to low concentrations of erythromycin stimulated its transposition to conjugative coresident plasmids, making it transferable to plasmid-free recipients of the same species (25, 102). Tn917 was sequenced in the mid-1980s (94); its subsequently updated sequence (5,614 bp) revealed five open reading frames (ORFs), of which *orf2* is *erm*(B) and *orf4* and *orf5* are two specific transposition-related genes, *tnpR* (resolvase) and *tnpA* (transposase), respectively. After Tn917-like sequences had been demonstrated in

erythromycin-resistant *S. pneumoniae* isolates (61), McDougal et al. (70) described in the same species a new composite structure, designated Tn3872 (23.6 kb), resulting from the insertion of Tn917 into *orf9* of Tn916, at base 14525 of its published sequence (accession no. U09422), with the *erm*(B) gene of Tn917 thus linked to the *tet*(M) gene of Tn916. In recent studies of *S. pyogenes* (13), Tn3872-like elements were detected in several isolates, while in a few others Tn917 was probably carried by a plasmid. A composite element apparently corresponding to Tn3872 has been recently described in *Streptococcus agalactiae* (80). In mating experiments, Tn3872 could not be transferred from *S. pneumoniae*

donors to *S. pneumoniae* (27, 70), *S. pyogenes* and *E. faecalis* (27) recipients, or from *S. agalactiae* to *S. agalactiae* (80). However, conjugal transfer did occur from *S. pyogenes* to *S. pyogenes* (13) and from *Abiotrophia defectiva* (*Streptococcus defectivus*) to *E. faecalis* (78), albeit at low frequencies.

Tn6002, Tn6003, Tn1545, and Tn2010. An *erm*(B)/*tet*(M) linkage is also typical of a number of *erm*(B)-carrying elements (Tn6002, Tn6003, Tn1545, and Tn2010) that share with Tn3872 a Tn916-like structure, but where the *erm*(B)-carrying insertion is the so-called *erm*(B) element, a 2,847-bp fragment formed by five ORFs (*orfP0* to *orfP4*), of which *erm*(B) is the third (*orfP2*). This fragment is found between *orf20* (here detected in a larger form called *orf20F*, whose right end corresponds to *orfP0*) and *orf19* of Tn916 at base 3847 of its published sequence. Insertion of the *erm*(B) element alone distinguishes transposon Tn6002 (20,880 bp), detected in both *S. pyogenes* (13) and *S. pneumoniae* (19, 27, 35) but originally found in *Streptococcus cristatus* and designated Tn916Erm, whose complete sequence (later updated) was available in GenBank since 2005 (accession no. AY898750). Intraspecific conjugal transfer of Tn6002 has been demonstrated in *S. pyogenes* (13), whereas transconjugants were obtained from a *S. pneumoniae* donor only using a *S. pyogenes* recipient (27). As shown by Cochetti et al. (27), another pneumococcal transposon, Tn6003 (25,101 bp), is distinguished by the further insertion into Tn6002 of the so-called MAS (for macrolide-aminoglycoside-streptothricin) element (4,225 bp). The MAS element is found within the *erm*(B) element between *orfP0* and *orfP1*, at position 4110 of the Tn6002 sequence. A role in its insertion may have been played by a 222-bp sequence, absent in Tn916, detected both on the left end of the *erm*(B) element and on the right end of the MAS element (27). The MAS element appears to be a rearrangement of part of plasmid pRE25 (90) of *E. faecalis* and contains, from upstream to downstream, another *erm*(B) gene (lacking the stop codon) with its leader peptide; an aminoglycoside-streptothricin resistance cluster (*aadE-sat4-aphA-3*), with a 511-bp deletion in *aadE*; and an ORF identical to *orf47* of pRE25 (27). Conjugal transfer of Tn6003 failed to *S. pneumoniae* and *S. pyogenes* recipients, whereas it was obtained to an enterococcal recipient (27). Tn6003 shares its resistance gene combination [*erm*(B) (MLS antibiotics), *tet*(M) (tetracyclines), and *aphA-3* (kanamycin and structurally related aminoglycosides)] with Tn1545—originally detected in a multiply resistant clinical *S. pneumoniae* isolate—which was the first *erm*(B)-carrying element to be described in this species (31, 32). Since early studies, Tn1545 was shown to be transferable by conjugation to various gram-positive bacterial genera. In the years after its discovery, the genetic organization of Tn1545 has essentially been analyzed by restriction mapping (16); only specific portions have been sequenced, including its ends (17), the three above-mentioned resistance genes (18, 68, 103), and transposition-related genes such as *int* (integrase) and *xis* (excisase) (79), which are characteristically associated with the conjugative transposons of the Tn916 family (24, 81). The structural organization of Tn1545 has been elucidated quite recently, when two new regions of the transposon were sequenced and its genetic organization was compared with, and shown to be closely related to, that of Tn6003 (26). In particular, Tn1545 was found to contain a MAS element virtually identical (99.7% homology,

accession no. AM903082) to that of Tn6003. Remarkably, the second *erm*(B) in the MAS element of Tn1545 lacked the stop codon, exactly like the one of Tn6003. The sole dissimilarity detected between the two transposons was a 1,245-bp insertion between *orf13* and *orf12* (at base 18833 of the Tn6003 sequence) found in Tn1545 (accession no. AM889142) but not in Tn6003, containing a putative IS1239 insertion sequence (26). This being the sole difference between Tn6003 (25,101 bp, as resulting from its complete sequencing) and Tn1545 (never completely sequenced), the actual size of the latter should be ~1 kb greater than the 25.3 kb reported originally on the basis of restriction mapping (16, 31, 32). Another *erm*(B)-carrying Tn916-like transposon (described in *S. pneumoniae*) is Tn2010 (26,390 bp, accession no. AB426620), which results from the insertion of a variant of the *mef*(E)-containing mega element (see below) into Tn6002 at position 17014 of the Tn6002 sequence and does not appear to be transferable by conjugation (35, 37). Less-common structures are also occasionally observed, such as two nonmobile Tn916-related elements with intermediate genetic organizations (perhaps vestiges of previous recombination events) lacking *tet*(M) but bearing both Tn917 and a complete or an incomplete MAS element (27).

Tn1116. A special *erm*(B)-carrying transposon, Tn1116 (~50 kb), has been described in *S. pyogenes* by Brenciani et al. (13). It differs from the other Tn916-related elements by the involvement of Tn5397, a unique Tn916 family transposon originally found in *Clostridium difficile* (75, 83), where the *tndX* (resolvase) gene replaces *int* and *xis*. Tn1116 results from the insertion of *erm*(B)-containing DNA into the coding sequence of the *tet*(M) gene of Tn5397, at base 15019 of the published sequence of the transposon (accession no. AF333235); the whole portion of Tn5397 upstream of this insertion site is lacking. The *erm*(B)-containing insertion is largely unknown. However, the DNA region between the *erm*(B) and the *tndX* gene has been sequenced (accession no. AM411377), showing an insertion sequence, identical to the enterococcal IS1216 (64), between *erm*(B) and the truncated *tet*(M). Further sequencing in progress in our laboratory has shown homologies with the *S. pyogenes* plasmid pSM19035 (21). Tn1116 was easily transferred in intraspecific matings (13). A different Tn5397-related element, with a regularly expressed *tet*(M) instead of a truncated one, has been reported in *S. pneumoniae* isolates, but its actual features and organization have not yet been determined (19, 50).

Detection of and discrimination between *erm*(B)-carrying elements. The maps of Tn916 and Tn916-related *erm*(B)-carrying transposons Tn3872, Tn6002, Tn6003, Tn1545, and Tn1116 are shown and compared in Fig. 1. In order to detect and discriminate between these genetic elements, a PCR using the primer pair J12/J11 (the former targeting *orf20* and the latter the intergenic region between *orf18* and *orf19* of Tn916) (27) is sufficient to distinguish among Tn3872, Tn6002, and Tn6003/Tn1545, which yield amplicons of 0.8, 3.6, and 7.9 kb, respectively (26). Tn6003 and Tn1545 can be distinguished using the primer pair O₆/TETM2dg [the former targeting *orf13* and the latter *tet*(M) of Tn916], that yield amplicons of 2.4 and 3.6 kb, respectively (26). This scheme does not consider Tn2010, distinguished by the presence of the *mef*(E) gene. Transposon Tn1116 can be detected by pairing primers

FIG. 1. *erm(B)*-carrying elements. The *erm(B)* gene is indicated as a checked red arrow. Light-blue arrows indicate Tn916 and Tn917-related ORFs other than *tet(M)* (blue). Pink, green, and orange arrows indicate ORFs from Tn917, the *erm(B)* element, and the MAS element, respectively, other than *erm(B)*. A brown arrow indicates a putative IS1239 insertion sequence of Tn1545. Pink, green, orange, and brown areas between ORF maps denote insertions and homologies.

ERMB2 and *tndX*-2 (13), targeting *erm(B)* and *tndX*, respectively, and yielding a PCR product of 6.5 kb.

Spread and prevalence of *erm(B)*-carrying elements. Very recently, some data have become available about the spread and prevalence of the different *erm(B)*-carrying elements in streptococcal populations. In *S. pneumoniae* isolates, the *erm(B)* gene seems to be carried primarily by Tn917 or Tn917-containing elements in Japan (76), whereas Tn6002 ranks first (half to two-thirds) in Italy (26, 27) and Spain (19), followed by Tn3872 and, far distanced, by Tn6003/Tn1545. Interestingly, in the only study distinguishing between Tn6003 and Tn1545, only the former was detected (26). In fact, most epidemiological surveys of erythromycin-resistant pneumococci have neglected the phenotypic or genotypic assessment of kanamycin susceptibility; this may have resulted in some confusion not only between Tn1545 and Tn6003 but also between Tn1545 and Tn6002. However, the few studies addressing the question confirm that kanamycin resistance is associated with a tiny minority of *erm(B)*-positive pneumococci (19, 49, 57, 70, 93). It is noteworthy that Tn6003 and the MAS element have thus far been demonstrated only in *S. pneumoniae* (27), whereas there is as yet no evidence of their presence in *erm(B)*-carrying isolates of *S. pyogenes* (13). Conversely, Tn1116 has been detected only in the latter species: it was invariably associated with *erm(B)*-positive isolates with the inducible resistance phenotype, while a greater variability has been recorded in those with the constitutive phenotype, where Tn6002 accounted for almost half and Tn3872 for more than one-quarter of the isolates (13).

erm(TR)-CARRYING ELEMENTS

In *S. pyogenes*, the *erm(TR)* gene, formerly reported as prophage associated in a serotype M6 isolate (10), has subsequently been reported to be carried on an integrated conjugative element (ICE) in a serotype M4 strain whose genome was completely sequenced (accession no. CP000262) (11). ICEs are large segments of exogenous DNA with the characteristics of conjugative transposons or plasmids; they are regarded as putatively mobile even when their transferability has not been shown experimentally (11). In particular, *erm(TR)* was found in a 49-kb ICE, designated ICE 10750-RD.2, that is integrated into the *hsdM* chromosomal gene encoding host DNA restriction modification methyltransferase. Remarkably, other antibiotic resistance genes have been detected in the *erm(TR)*-flanking region, both upstream (tetracycline) and downstream (spectinomycin) of *erm(TR)*.

The *erm(TR)* gene is uncommon in *S. pneumoniae* but has been shown to be carried on a genetic element, designated Tn1806, possibly representing a transposon/prophage remnant chimera (20). Tn1806 (56 kb, i.e., ca. 7 kb larger than ICE 10750-RD.2 from *S. pyogenes*) is inserted into a chromosomal ORF homologous to *spr0790* of *S. pneumoniae* R6, corresponding to an *hsdM* gene coding for a type I restriction modification system. Sequencing of an ~11-kb *erm(TR)*-containing DNA fragment from Tn1806 and of its left and right junctions (accession no. EF469826) disclosed substantial homologies to

FIG. 2. Schematic and comparative representation of the *erm*(TR)-flanking regions from three *erm*(TR)-carrying elements (Tn1806 from *S. pneumoniae*, and an unnamed element [sequence accession no. FM162351] and ICE 10750-RD.2 from *S. pyogenes*). The *erm*(TR) gene is indicated as a checkered red arrow. White arrows indicate matching ORFs; arrows in other colors indicate other ORFs. Gray areas between ORF maps indicate areas of homology (with the approximate percentage reported).

ICE 10750-RD.2, despite the identification of ORFs unique to either element. Tn1806 did not appear to be conjugative but could be transferred by transformation to a pneumococcal recipient (20).

Before the detection and characterization of ICE 10750-RD.2 from *S. pyogenes* and of Tn1806 from *S. pneumoniae*, mating experiments had demonstrated the transfer of the *erm*(TR) gene, contained in putative elements of 30 to 40 kb, from *S. pyogenes* to the same and to other gram-positive species (54). Sequence analysis of the transposable element from one of these donors (accession no. FM162351) demonstrated extensive similarities to ICE 10750-RD.2, but to an even greater extent to Tn1806. The sequences of the *erm*(TR)-flanking regions from the three elements are compared in Fig. 2.

erm(T)-CARRYING ELEMENTS

The genetic organization of the *erm*(T) gene and its flanking regions was described in group D streptococci (*S. galloyticus* subsp. *pasteurianus*) (104). The *erm*(T)-carrying genetic element has a chromosomal location, but only a ca. 4.1-kb fragment has been sequenced and examined, revealing that *erm*(T) was flanked by two IS1216V-like insertion sequences with the same polarity.

A plasmid-borne *erm*(T) has lately been described in *S. pyogenes* (111). The plasmid, designated pRW35 (4,962 bp), contains three distinct ORFs, *erm*(T) and two ORFs highly similar to plasmid replication and transfer genes found in broad-host-range plasmids, a finding consistent with the hypothesis that pRW35 is capable of broad dissemination (111).

mef(A)-CARRYING ELEMENTS

The first *mef*(A)-carrying structure to be discovered was Tn1207.1 (7,244 bp), a chromosomal element described in *S. pneumoniae* by Santagati et al. (89) and considered as a defective transposon because the 5' end of *orf8* appears truncated. It contains eight ORFs, of which *mef*(A) and *msr*(D) are the fourth and the fifth; while *orf2* is homologous to site-specific recombinases of gram-positive bacteria, *orf6*, *orf7*, and *orf8* are homologous to three ORFs of the pneumococcal conjugative transposon Tn5252 (2). Tn1207.1 is integrated at a specific site of the pneumococcal chromosome into *celB*, a late competence gene (notably, Tn1207.1-carrying pneumococci are unable to become competent [36]). Tn1207.1 has been reported to be transferable by transformation but not by conjugation (89), even though variable results have been obtained in mating experiments with different pneumococcal donors (36).

Whereas Tn1207.1 is the sole recognized element carrying *mef*(A) in *S. pneumoniae*, in *S. pyogenes* it is not detected as such, but as part of larger composite elements, all shown to be chimeric, i.e., resulting from insertion of a transposon (identical or related to Tn1207.1) into a prophage (9, 53). In tetracycline-susceptible M-phenotype isolates of this species, a regular Tn1207.1 (with a nontruncated *orf8*) forms the left end of Tn1207.3 (52,491 bp) (88) or is part of Φ 10394.4 (58,761 bp) (9, 10). Tn1207.3 and Φ 10394.4 are closely related elements: they are integrated into the same chromosomal gene (*comEC*) and are inserted into the same prophage, as initially suggested by PCR assays (14) and subsequently confirmed by sequence

FIG. 3. *mef(A)*-carrying elements. Yellow arrows indicate Tn1207.1 and related ORFs other than *mef(A)* (oblique pink stripes) and *msr(D)* (horizontal orange stripes). A dark blue arrow indicates *tet(O)*. Gray areas between ORF maps indicate areas of homology.

analysis of the large fragments of phage origin located to the right of Tn1207.1 (accession no. AY657002). Φ10394.4 differs from Tn1207.3 by an additional left-hand region that has been reported to be ~6-kb long (with one ORF encoding an R28-like protein) but also to be quite variable in size (9), suggesting that Tn1207.3 could represent one extreme, completely lacking this region, of the variability range (53). Tn1207.3, which appears to be more common than Φ10394.4 in *mef(A)*-positive, tetracycline-susceptible *S. pyogenes* isolates (14, 39, 45), has also been occasionally detected in other beta-hemolytic *Streptococcus* species (45, 67). Tn1207.3 has been shown to be transferable in intra- and interspecific mating experiments (88). In tetracycline-resistant M-phenotype isolates of *S. pyogenes*, where tetracycline resistance is consistently mediated by the *tet(O)* determinant (52), *mef(A)* is linked to *tet(O)* in a mobile element, also chimeric, where, however, the prophage is different from the one shared by Tn1207.3 and Φ10394.4 (53). In fact, there is a variety of related *tet(O)*-*mef(A)* elements in which *mef(A)*, located 2.3 to 5.5 kb downstream of *tet(O)*, is harbored by a range of changeable and defective variants of Tn1207.1: of the eight typical ORFs of the latter, only *msr(D)* and a modified *orf6*, in addition to *mef(A)*, were detected by PCR in all isolates tested, while *orf1* and *orf2* were always undetectable, and *orf3*, *orf7*, and *orf8* were found in variable percentages (14). The one currently designated Φm46.1 (~60 kb), ostensibly the most common of such elements, has been shown to be transferable in mating experiments to *S. pyogenes*, but not to an enterococcal recipient (52), and is not integrated within the *comEC* gene (14). An ~12.1-kb region of Φm46.1, from upstream of the *tet(O)* gene through the end of a Tn1207.1-related transposon, has been sequenced (accession no. AJ715499), disclosing four new ORFs (*orfA* to *orfD*) downstream of the *tet(O)* gene; *tet(O)* was 5,717 bp upstream of *mef(A)* (14). It should be noted that tetracycline-susceptible M-phenotype isolates of *S. pyogenes* are usually SmaI nontypeable by pulsed-field gel electrophoresis (82, 105) because their DNA is made refractory to SmaI restriction by a DNA-modifying methyltransferase (45). This enzyme is encoded by a restriction modification cassette lo-

cated just downstream of Tn1207.1 in the conserved region of phage origin of Tn1207.3 and Φ10394.4 (42). The cassette is absent in Φm46.1, a finding consistent with the SmaI typeability of tetracycline-resistant M-phenotype isolates (7).

The maps of Tn1207.1, Tn1207.3, Φ10394.4, and Φm46.1 are represented and compared in Fig. 3.

mef(E)-CARRYING ELEMENTS

The genetic structure typically carrying the *mef(E)* gene in *S. pneumoniae*, first described by Gay and Stephens (48), has been designated as mega (macrolide efflux genetic assembly) element. It contains five ORFs having related sequences to the last five ORFs of Tn1207.1, of which *mef(E)* [91.6% homology to the *mef(A)* gene of Tn1207.1] is the first and *msr(D)*, originally named *mel*, is the second. The mega element has a variety of insertion sites in the pneumococcal chromosome, possibly associated with different variants. The deposited mega sequence (5,532 bp) refers to the first investigated variant (48); this is probably less common than a slightly smaller variant, with a 99-bp deletion in the intergenic region between *mef(E)* and *msr(D)*, detected in pneumococci isolated in North America (48, 109) and Italy (35). The mega element was shown to be transferable by transformation (36, 48) and not by conjugation (36). The *mef(E)* gene was detected in other streptococcal species, but the carrier element has been investigated only in *Streptococcus salivarius*. In this species, Stadler and Teuber (95) described a new mega variant (5,511 bp) whose sequence was almost identical to that of the pneumococcal element; an additional ORF (*orf6*) was detected between *orf4* and *orf5*, both partly modified compared to the corresponding ORFs in the pneumococcus. The mega element from *S. salivarius* was transformed into a pneumococcal recipient (95). A mega element 100% identical to the one of *S. salivarius*, but with an additional ORF (*orf7*) located upstream of *mef(E)*, was detected in *S. pneumoniae* in the composite transposons Tn2009 (23.5 kb) (38) and Tn2010 (26,390 bp) (35, 37). The former results from insertion of the mega variant in *orf6* of transposon Tn916, at position 14166 of its published sequence, the latter

FIG. 4. *mef(E)*-carrying elements. Yellow arrows indicate Tn1207.1-related ORFs other than *mef(E)* (oblique pink stripes) and *msr(D)* (horizontal orange stripes). A blue arrow indicates *tet(M)*. Green arrows indicate ORFs from the *erm(B)* element other than *erm(B)* (checkered red).

from the same insertion at the same position in Tn6002. In both Tn2009 and Tn2010 *mef(E)* is oriented opposite to *tet(M)* of Tn916. Tn2009 was shown to be transferable by transformation (38). Neither Tn2009 nor Tn2010 could be transferred by conjugation to pneumococcal or enterococcal recipients (35). The maps of the *mef(E)*-carrying elements are shown and compared in Fig. 4.

ELEMENTS CARRYING OTHER *mef* GENES

Of the *mef* genes other than *mef(A)* and *mef(E)* described thus far (5, 15, 28, 87), the gene-carrying element has been studied only for *mef(I)*, detected in *S. pneumoniae* and exhibiting 91.4 and 93.6% homologies to the *mef(A)* gene of Tn1207.1 and the *mef(E)* gene of the *mega* element, respectively (28). As shown by Mingoia et al. (73), *mef(I)* is carried by an apparently nonmobile composite structure, designated 5216IQ complex (30,505 bp), consisting of two halves of comparable size (Fig. 5). The left half (15,316 bp) is formed by parts of the known transposons Tn5252 (2) and Tn916, the latter containing a silent *tet(M)* gene; the Tn916 ORFs upstream of *tet(M)* are lacking, as is most of Tn5252. The right half (15,115 bp) of the 5216IQ complex is formed by a new fragment, designated the IQ element, that contains the *mef(I)*

gene with a new adjacent *msr(D)* gene variant, in the context of a genetic organization totally unlike those of the genetic elements carrying other *mef* genes. In the IQ element, *mef(I)* and *msr(D)* are the seventh and the eighth of 13 ORFs, of which the first and the last are two identical transposase genes and the twelfth is a *catQ* chloramphenicol acetyltransferase determinant. This was the first time this resistance gene, thus far detected only in *Clostridium perfringens* (91), was demonstrated in *S. pneumoniae* and shown to be linked with a *mef* gene. The IQ element should probably be considered as a novel transposon, although it does not appear to be mobile in the genetic context of the pneumococcus. The right junction of the 5216IQ complex was just upstream of the *spr0601* gene and the left junction just downstream of the *spr1199* gene of the *S. pneumoniae* R6 chromosome. However, further sequencing beyond the left junction disclosed a chromosomal organization more similar to that of the virulent pneumococcus TIGR4 (100) than to that of strain R6.

CONSIDERATIONS AND CONCLUSIONS

The streptococcal genetic elements considered here, chosen and reviewed as carriers of macrolide resistance determinants (*erm* or *mef/msr* class genes), also carry a number of other

FIG. 5. *mef(I)*-carrying element (5216IQ complex). Purple arrows indicate ORFs from transposon Tn5252. Light-blue arrows indicate ORFs from transposon Tn916 other than *tet(M)* (blue). White arrows indicate ORFs from the IQ element other than *mef(I)* (oblique pink stripes), *msr(D)* (horizontal orange stripes), and *catQ* (bright green).

genes conferring resistance to a variety of other antibiotics, including tetracyclines, aminoglycosides, spectinomycin, and chloramphenicol, but also minor drugs such as streptothricin and tetroneasin. On the other hand, recruitment of exogenous resistance genes and their concentration in particular genetic elements, as exemplified in gram-negative bacteria by the integrons and superintegrons (69), is the most rapid adaptation mechanism against antimicrobial compounds and is likely to have an important role in the horizontal gene transfer and the evolution of bacterial genomes (86). Del Grosso et al. (38) advocated that, in gram-positive bacteria such as the streptococci, a similar function can be exerted by conjugative transposons such as Tn916-like elements, which are highly evolved for broad-host-range transfer and highly capable of capturing other resistance elements to form composite structures, thus playing a major role in disseminating multiple drug resistances (24, 81). In some instances, particularly when the species *S. pyogenes* is involved, a major role can also be played by bacteriophages (8).

Most *erm*(B)-carrying elements detected in streptococci result from the insertion of *erm*(B)-containing DNA into conjugative transposons of the Tn916 family. The *tet*(M) gene is typically carried by these elements (24, 81), and until a short time ago this steady *tet*(M)-Tn916 association was held to be consistent with the fact that tetracycline-resistant *erm*(B)-positive streptococci are far more prevalent than tetracycline-susceptible ones. However, recent studies have unexpectedly demonstrated that tetracycline-susceptible *erm*(B)-positive isolates of both *S. pyogenes* (13, 74) and *S. pneumoniae* (27, 74) also most often carry Tn916-related elements, where, however, the *tet*(M) gene is present in a silent form. Retrospectively, it should be noted that tetracycline-susceptible *tet*(M)-positive isolates had been observed in some collections of pneumococci (4, 30, 66, 70, 77, 107).

Conjugal transfer of transposons or other genetic elements is often efficient from *S. pyogenes* but is lacking (or inefficient) from *S. pneumoniae*. Indeed, the latter has highly developed transformation systems, which are the basis for the unique recombination-mediated genetic plasticity that is a distinctive feature of the species (23). Conversely, natural transformation is not a common event in *S. pyogenes*, although the species has many of the genes essential for competence and transformation. It has been suggested that, in *S. pyogenes* strains, competence may have been lost due to the growing role assumed by phages (44); in fact, *S. pyogenes* is the only *Streptococcus* species containing complete bacteriophage genomes, indicating that this mechanism of horizontal gene transfer is an important factor in gene acquisition and/or loss, strain heterogeneity, and the overall evolution of the species (8, 44). In particular, a chimeric nature—i.e., a transposon inserted into a prophage—has been demonstrated for all recognized *mef*(A)-carrying elements of *S. pyogenes*, i.e., the closely related Φ 10394.4 (9, 10) and Tn1207.3 (88) (inserted into the same prophage) in tetracycline-susceptible isolates, and the *tet*(O)-*mef*(A) elements (e.g., Φ m46.1, inserted into a different prophage) in tetracycline-resistant isolates (53). Both in the case of *mef*(A), primarily carried by a nonconjugative transposon such as Tn1207.1 (89), and in the case of *tet*(O), believed to be unable to move from one chromosome to another prior to the discovery of the *tet*(O)-*mef*(A) elements (14, 52), a nontransferable

resistance determinant of *S. pneumoniae* (or of other streptococci from the upper respiratory tract) could have become easily transferable once transfer to *S. pyogenes* and integration into the prophage had successfully occurred. In the 1970s, several experimental studies provided evidence that prophages might have participated in the dissemination of erythromycin resistance among *S. pyogenes* isolates (8, 72). The subsequent hypothesis that the resistance determinant involved in those transduction experiments was *mef*(A) (8) appears far sounder now that specific prophage-associated *mef*(A) elements have been identified. Although the molecular mechanisms mediating the horizontal transfer of *mef*(A) have not yet been clearly defined, several lines of evidence suggest that transduction can play a role (9, 72). In any case, phage transfer is likely to play a critical role in the emergence of active efflux as the most widespread mechanism of macrolide resistance in this species.

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