Vol. 42, No. 8

Characterization and Prevalence of MefA, MefE, and the Associated *msr*(D) Gene in *Streptococcus pneumoniae* Clinical Isolates

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Recent work has shown that the efflux genes in *Streptococcus pneumoniae* that are responsible for acquired macrolide resistance can be distinguished as either *mef*(E) or *mef*(A). The genetic elements on which *mef*(A) and *mef*(E) are found also carry an open reading frame (ORF) that is 56% homologous to *msr*(A) in *Staphylococcus*. The prevalence of *mef*(A/E) and of the *msr*-like ORF [*msr*(D)] was evaluated in 153 *mef*$^+$ *S. pneumoniae* clinical isolates collected in North America, Europe, Africa, and Asia from 1997 to 2002. Clinical isolates were screened with PCR primers specific for either *mef*(A) or *mef*(E) and for *msr*(D). *mef*(A), *mef*(E), and *msr*(D) were cloned from *mef*$^+$ strains and transformed into a susceptible, competent strain of *S. pneumoniae*. The transformants were tested for antimicrobial susceptibilities and efflux pump induction. The results of this work demonstrated that *mef*(A) is more often isolated in parts of Europe, with some incidence in Canada, and that the *msr*-like gene alone can confer the efflux phenotype.

Recent work has shown that the efflux genes in *Streptococcus pneumoniae* responsible for acquired macrolide resistance can be distinguished as either *mef*(E) or *mef*(A) (6). Originally, *mef* in *S. pneumoniae* had been labeled *mef*(E), while *mef*(A) had been reserved for *Streptococcus pyogenes*. The two *mef* genes show a 90% sequence homology between the start and stop codons, but they can be distinguished with specific primer sets. Due to sequence similarity, these genes were merged under *mef*(A) by Roberts et al. (16). However, for clarity in the present discussion, the genes will be referred to as *mef*(A) and *mef*(E). Whether there are sufficient differences in the epidemiology and/or function of the genes to return to separate designations has not been determined.

The *mef* genes are carried on transposons comprised of additional open reading frames (ORFs). Both of these genetic elements also carry an ORF downstream from *mef* that is 56% homologous to the coding region of *msr*(A) in *Staphylococcus*. The upstream region of the *msr*-like gene in *Streptococcus* lacks the leader peptide found in the *Staphylococcus msr*(A) gene (17). The *msr*-like homologs found associated with either *mef*(A) or *mef*(E) have 98% sequence homology. Although the *msr*-like homolog is believed to be a part of the efflux system, it has not been previously studied independently in *Streptococcus*.

mef(A) in *S. pneumoniae* has been previously described in Italy (6). Given our worldwide clinical isolate collection, we studied the prevalence rates of *mef*(A) versus that of *mef*(E) in *S. pneumoniae* isolates collected in Europe, Asia, and North and South America. The prevalence and geographic distributions of *mef*(A) versus *mef*(E) in 153 clinical isolates of *mef*$^+$ *S. pneumoniae* from six regions of the world were evaluated in this study. The prevalence and function of the *msr* homolog were also evaluated. This gene has been given the designation *msr*(D) (M. Roberts, personal communication) and was shown

to be capable of independent function when cloned and expressed individually.

MATERIALS AND METHODS

Bacteria strains. One hundred fifty-three strains of *S. pneumoniae* exhibiting the *mef* phenotype were screened for this study. Bacterial strains were from worldwide clinical trials or surveillance studies from 1997 to 2002. Strains were subcultured from frozen stocks onto Trypticase soy agar plus 5% sheep blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and grown in 5% CO_2 at 37°C. Crude lysates were made by suspending a loopful of bacteria in 100 µl of water and boiling at 95°C for 15 min. Lysates were centrifuged, and the supernatant was used in PCRs.

Serotypes were determined using the slide agglutination method as previously described (5). Briefly, serotypes were determined by mixing 40 µl of a cell suspension (turbidity equal to 2 to 3 McFarland standard) in saline with 10 µl of pneumococcal antiserum purchased from Statens Serum Institut (Copenhagen, Denmark) on a hanging drop slide. Positive agglutination reactions were usually visible within 2 min. Strains with known serotypes were used as a positive control.

MIC testing was performed using the broth microdilution method according to NCCLS standards (13). Due to growth requirements of some strains, the modification of Todd-Hewitt broth supplemented with 0.5% yeast extract (THYE; Becton Dickinson Microbiology Systems) was also used when necessary for growth. In addition, the MIC testing for the *msr*(D) transformants was performed in the presence of CO_2 in order to facilitate growth when required. Cethromycin, telithromycin, streptogramin A (dalfopristin), and streptogramin B (quinupristin) were prepared at Abbott Labs (Abbott Park, Ill.). All other antibiotics were purchased from Sigma (St. Louis, Mo.). Susceptibility testing of the parent strain was performed under both growth conditions for comparison. *S. pneumoniae* ATCC 49619 was also tested for quality control.

PCR amplification and gene cloning. The presence of *mef*(E), *mef*(A), and *msr*(D) was determined by PCR amplification. Primers and genes used in this study are listed in Table 1. Primers were picked from sequences deposited in GenBank using Oligo 6 (MBI, Inc., Cascade, Colo.) One microliter of each lysate was used in a 25-µl reaction mixture at the annealing temperature indicated in Table 1 (Readymix *Taq*; Sigma). Products were run on a 1.5% agarose gel and visualized with ethidium bromide stain. Products were sequenced using the Big Dye sequencing kit (Applied Biosystems Inc., Foster City, Calif.). Sequencing reactions were purified by using an Auto-Seq G-50 column (Amersham Pharmacia Biotech, Piscataway, N.J.) and run on an ABI 377 automated sequencer. Clinical isolate DNA sequences of genes to be cloned were compared with published sequences.

A representative *mef*(E) isolate (5645) and a *mef*(A) isolate (2511) were selected for further genetic study. Strain 5645 was also used for cloning of *msr*(D). Chromosomal DNA was extracted using a detergent lysis and ethanol precipitation method as previously described (8). The entire *mef* and *msr* coding regions along with their respective upstream regions were individually PCR

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TABLE 1. Primers used in this study

Gene	Primer sequence (5′ to 3′)	Annealing temp (°C)	Region amplified	Sequence reference[b]
mef (E) (screening)[a]	Upper: GGGAGATGAAAAGAAGGAGT	52	616–979	Tait-Kamradt et al.
	Lower: TAAAATGGCACCGAAAG			
mef (A) (screening)	Upper: TGGTTCGGTGCTTACTATTGT	52	574–1127	Clancy et al.
	Lower: CCCCTATCAACATTCCAGA			
msr (D) (screening)	Upper: TTGGACGAAGTAACTCTG	50	1814–2184	Del Grosso et al.
	Lower: GCTTGGCTCTTACGTTC			
msr (D) (cloning)	Upper: TTGCCAAATGATAACTGA	45	1436–3081	Del Grosso et al.
	Lower: GACCAGCGACTACCTT			
mef (A&E) (cloning)	Upper: TGTTGTGCTTATTTATACG	45	40–1538	Del Grosso et al.
	Lower: GCGATTTTAGCAGGAAGAG			
ami locus	Upper: AGAAATTTCCTTCGGTGAA	45	5843–6132	Alloing et al.
	Lower: AATCAACAGTCGCACGTTC			

[a] Screening primers were used to characterize all isolates in this study, while cloning primers were used to isolate the genes from the donor strains.
[b] Tait-Kamradt et al. (19); Clancy et al. (3); Del Grosso et al. (6); Alloing et al. (1).

amplified. The ends were treated with T4 polymerase and ligated individually into a shuttle vector (pRKH1) at the EcoRV site in *amiF*. pRKH1 is a hybrid construct of the *ami* locus and chloramphenicol acetyltransferase gene from pR327 (4) and the multiple cloning sites of pFW6 (15). This plasmid construct was used to transform *Escherichia coli* DH5α cells. Transformants were selected on Luria-Bertani agar with 10 μg of chloramphenicol/ml and were screened using the aforementioned primers. The orientation of the genes was determined by PCR with primers from the *ami* locus. For each gene, two clones were selected, one in each orientation, for further transformation into *S. pneumoniae*. Plasmid DNA was extracted using the plasmid mini-prep kit (Bio-Rad, Hercules, Calif.).

Transformation. Transforming DNA was PCR amplified from the plasmid construct using primers specific for the *ami* locus on either side of the inserted gene. A transformation-competent, macrolide-susceptible strain of *S. pneumoniae* (CP1250) (14) was used as the recipient strain. Transforming PCR product (0.1 to 1 μg) was added to culture aliquots as described previously (20). The transformants were selected on THYE agar plates containing erythromycin. The *msr*(D) gene was selected for with 1 μg of erythromycin/ml, while either the *mef*(E) or *mef*(A) gene was selected for at 0.5 μg of erythromycin/ml. Plates were incubated in 5% CO_2 at 37°C for up to 72 h. Colonies were screened using the respective gene-specific primers. Orientation was also verified using the flanking *ami* primers, and transformants of each orientation were picked for each gene.

Induction of the *msr* efflux pump has been demonstrated in *Staphylococcus* (12) and was detected in this study by placing cethromycin, telithromycin, and clindamycin disks 15 mm apart from erythromycin disks on blood agar plates using a similar technique as used in methylase induction (6). Induction was present when the zone on the erythromycin side of the test drug disk was blunted, forming a D-zone diffusion pattern. Induction was also detected by broth microdilution in the presence of 0.05 μg of erythromycin/ml. An inducible *erm*(A) methylase-containing *S. pyogenes* strain was used as a positive control.

RESULTS

The presence of *mef*(A) or *mef*(E) was determined in 153 *S. pneumoniae* clinical isolates. We identified *mef*(A) in one-third (10 of 30) of the European *mef*⁺ isolates tested as well as 1 isolate each from Canada and South America (Table 2). All of the *mef*⁺ isolates tested from the United States, Asia, and South Africa were identified as *mef*(E).

Eleven of the 12 *mef*(A) isolates were serotype 14 (91.7%) (Table 3). The majority of *mef*(E) isolates were grouped into four serotypes: serotype 19 (48 strains [35.8%]), serotype 6 (24 strains [17.9%]), serotype 14 (23 strains [17.2%]), and serotype 23 (19 strains [14.2%]). The remaining 20 typeable isolates fell into five serotypes: serotype 9 (5.2%), serotype 12 (6%), serotype 15 (2.2%), serotype 16 (0.75%), and serotype 18 (0.75%). Seven isolates could not be serotyped. All isolates tested also contained *msr*(D). This gene was not found alone or in 50 other non-*mef*, macrolide-resistant strains (data not shown).

In order to study the function of *msr*(D) in the absence of Mef, *msr*(D) was cloned and inserted into the *ami* locus of macrolide-susceptible *S. pneumoniae* strain CP1250. Transformants carrying *msr*(D) exhibited the efflux phenotype (Table 4). The *msr*(D) transformant MICs of erythromycin and clarithromycin increased 64-fold over the those of the parent strain (0.015 and 0.03 versus 2 μg/ml). The cethromycin MIC increased twofold (0.002 versus 0.004 μg/ml), while the telithromycin MIC increased 16-fold (0.004 versus 0.06 μg/ml). The MICs of clindamycin, streptogramin A, streptogramin B, and other drug classes remained the same or increased by one twofold dilution (Table 4). This phenotypic profile remained consistent and stable through multiple passages on agar and regardless of the gene orientation.

To compare phenotypes among the three efflux determinants, *mef*(A) and *mef*(E) were also cloned and individually inserted into CP1250. Transformants carrying *mef*(E) or *mef*(A) exhibited the typical efflux phenotype (Table 4), with MICs similar to those for donor strains. No meaningful difference was observed in MICs between the *mef*(A) and *mef*(E) transformants for the drugs tested. MICs of erythromycin and clarithromycin for transformant versus parent were 0.015 and 0.03

TABLE 2. Distribution of *mef*(A) and *mef*(E)

Genotype	Country of origin[a]	No. of isolates
mef (A)	Canada	1
	Mediterranean	4
	South America	1
	Western Europe	6
mef (E)	Asia	10
	Canada	25
	Eastern Europe	3
	Mediterranean	10
	South Africa	31
	South America	5
	United States	49
	Western Europe	8
Total		153

[a] E. Europe (Czech Republic, Estonia, and Russia) Mediterranean (France, Greece, Italy, and Spain); W. Europe (Austria, Belgium, England, Germany, Scandinavia, Switzerland, and United Kingdom); S. America (Argentina, Chile, Dominican Republic, and Panama).

TABLE 3. Serotype distribution

Geographic origin (no. of isolates)	mef(A)		mef(E)	
	Serotype	No. of isolates (%)	Serotype	No. of isolates (%)
Asia (10)			19	10 (100)
Canada (26)	14	1 (3.8)	19	7 (26.9)
			12	7 (26.9)
			14	4 (15.4)
			6	3 (11.5)
			23	2 (7.7)
			9	1 (3.8)
			Untypeable	1 (3.8)
Eastern Europe (3)			6	2 (66.7)
			15	1 (33.3)
Mediterranean (14)	14	4 (28.6)	14	5 (35.7)
			23	2 (14.3)
			19	1 (7.1)
			6	1 (7.1)
			Untypeable	1 (7.1)
South Africa (31)			19	11 (35.5)
			6	8 (25.8)
			23	6 (19.4)
			9	3 (9.7)
			15	2 (6.5)
			Untypeable	1 (3.2)
South America (6)	14	1 (16.7)	14	2 (33.3)
			19	1 (16.7)
			6	1 (16.7)
			18	1 (16.7)
United States (49)			19	15 (30.6)
			14	11 (22.4)
			23	9 (18.4)
			6	7 (14.3)
			9	3 (6.1)
			12	1 (2)
			Untypeable	3 (6.1)
Western Europe (14)	14	5 (35.7)	19	3 (21.4)
	15	1 (7.1)	6	2 (14.3)
			14	1 (7.1)
			16	1 (7.1)
			Untypeable	1 (7.1)

µg/ml versus 4 and 8 µg/ml. The cethromycin and telithromycin MICs did not increase, while the clindamycin MIC increased eightfold (0.015 versus 0.125 µg/ml). The MICs for other drug classes remained the same or increased by one twofold dilution. As with *msr*(D), the gene orientation did not impact the observed phenotype for *mef*(A) or *mef*(E).

Since MsrA is inducibly regulated in staphylococci, we investigated the presence of induction in MsrD (8). Disk diffu-

sion induction testing revealed a slight blunting of the zone around the cethromycin and telithromycin disks in the *mef*[+] *msr*[+] donor strains and a more pronounced blunting of the zones with the *msr* transformants. This D-shaped inhibition zone was absent with the *mef* transformants.

Efflux pump induction was confirmed with broth microdilution susceptibility testing in the presence of 0.05 µg of erythromycin/ml. The MICs of both cethromycin and telithromycin for the donor *mef* strain increased by twofold (0.015 versus 0.03 µg of cethromycin/ml; 0.06 versus 0.125 µg of telithromycin/ml). The MIC of telithromycin increased fourfold (0.03 versus 0.125 µg/ml) for the transformant with *msr*(D), while the cethromycin MIC increased eightfold (0.004 versus 0.03 µg/ml) in the presence of erythromycin.

DISCUSSION

The majority of the 153 isolates screened were *mef*(E). The highest incidence of *mef*(A) was in Europe, while only *mef*(E) was found in the United States, South Africa, and Asia. This supports findings by other researchers that *mef*(A) is found more in Europe than in other parts of the world although, unlike other studies (2, 9), we found *mef*(E) to be more common, with two-thirds of the European strains containing *mef*(E). *msr*(D) was always associated with *mef*(A) or *mef*(E) in the strains examined in this study and was genetically identical in both *mef*(A)- and *mef*(E)-containing elements. The serotyping data suggest that the *mef*(A)[+] strains in this study are clonal, as 11 of 12 strains were serotype 14. Similar results were reported in a study of Italian *S. pneumoniae* isolates (6). Ribotyping done on these strains (7) showed them all to be in the same EcoRI ribogroup, with one isolate differing in the HindIII group (data not shown).

The most common serotypes observed in this study (6, 14, 19, and 23) are also the most common serotypes associated with infection. *mef*(E) was associated with multiple serotypes in each of the geographic regions studied, with the exception of Asia. The 10 Asian *mef*(E) strains were all serotype 19.

msr(D) expression alone is sufficient to confer the efflux phenotype, although the erythromycin MIC was lower than the MIC for the donor strain as well as the *mef*(A/E) transformants, suggesting that it was not the sole gene responsible for macrolide efflux. *msr*-containing transformants also appeared to have slightly increased ketolide MICs, which *mef*-containing transformants did not. The increase in the telithromycin MIC

TABLE 4. MIC profiles for transformants and associated strains

S. pneumoniae isolate	MIC (µg/ml)[a]									
	ERY	CLR	CLI	CETH	TEL	CIP	TET	PEN	SGRA	SGRB
CP1250	0.03	0.015	0.015	0.002	0.004	1	0.25	0.125	4	1
CP1250 + *msr*(D)	2	2	0.03	0.004	0.06	2	0.5	0.125	4	1
CP1250 + *mef*(E)	4	4	0.125	0.002	0.004	1	0.25	0.125	ND[b]	ND
CP1250 + *mef*(A)	8	4	0.125	0.002	0.004	1	0.25	0.125	ND	ND
Clinical isolate 5645 [*mef*(E) and *msr*(D) donor DNA]	4	4	0.03	0.004	0.06	2	1	8	16	2
Clinical isolate 2511 [*mef*(A) donor DNA]	8	8	0.06	0.008	0.06	0.5	0.5	0.125	16	2
ATCC 49619	0.015	0.03	0.03	0.0005	0.004	1	0.5	2	8	1

[a] ERY, erythromycin; CLR, clarithromycin; CLI, clindamycin; CETH, cethromycin; TEL, telithromycin; CIP, ciprofloxacin; TET, tetracycline; PEN, penicillin; SGRA, streptogramin A; SGRB, streptogramin B.
[b] ND, not done.

for the *msr* transformant was similar to the telithromycin MIC for the parent strain, suggesting that the slight increase in the telithromycin MIC reported in this study and by others may be due to MsrD rather than MefA/E (10, 21). The cethromycin MICs for the *msr*(D) transformants had a greater increase when induced by erythromycin than those induced by telithromycin. The *msr*(D) transformants did not show resistance to streptogramin B, as has been reported for MsrA in staphylococci (11). This may reflect a difference in the specificity of the MsrA and MsrD proteins.

We observed no substantial differences in the phenotypes of isolates with *mef*(A) versus *mef*(E) in this study, nor did we see a noteworthy phenotypic difference between the *mef*(A) and *mef*(E) transformants. The *mef* transformants did show resistance to erythromycin at the same level as the donor strains. The *mef*(A) and *mef*(E) transformants had an increase of three twofold dilutions in the clindamycin MIC relative to that of the susceptible recipient strain (0.015 versus 0.12 μg/ml), while the MIC for the *msr*(D) transformant increased one twofold dilution (0.015 to 0.03 μg/ml). The wild-type Mef/Msr donor strains had clindamycin MICs of 0.03 to 0.06 μg/ml. While the slight increase in the clindamycin MIC for the *mef*(A) and *mef*(E) transformants was reproducible, it is not known if this represents a slight affinity of the Mef(A/E) pump for clindamycin or if it is an experimental artifact due to the insertion and expression of *mef*(A) and *mef*(E) in the *ami* locus. The clindamycin MIC ranges previously reported for Mef-positive *S. pneumoniae* strains are 0.015 to 0.25 μg/ml and 0.12 to 0.5 μg/ml, which represent a slight shift in the MIC at which 90% of isolates are inhibited, compared to that for macrolide-susceptible strains (≤0.12 μg/ml), but Mef-containing strains remain clindamycin susceptible (9, 18).

The *mef* and *msr*(D) genes appeared to be expressed from their own promoters, as the phenotypes were the same with both gene orientations; however, we did not perform specific experiments to confirm expression.

Only *msr*(D) transformants were inducible with erythromycin. This efflux pump induction did not occur with the *mef* transformants but was observed with the Mef/Msr+ donor strains. The inducible expression that was described previously for *msr*(A) in *Staphylococcus* (12) was reported to require the leader peptide sequence in the upstream region. No similar structure was identified in the *Streptococcus* isolates examined here, suggesting that this induction is under different regulation in *Streptococcus*.

In summary, we have confirmed and expanded reports of others that *mef*(A) is found predominantly in Europe and rarely in Asia and North and South America, while *mef*(E) is the predominant efflux mechanism in North and South America, Europe, and Asia (2, 9). The *mef*(A)-containing strains in Europe appeared to be associated with serotype 14, although they were isolated in different countries. These isolates were also members of the same ribogroup, suggesting that *mef*(A) is more likely to be clonal than *mef*(E); however, the small number of *mef*(A) isolates here does not allow a definitive answer. The greater prevalence of MefE suggests that this is the primary efflux mechanism in *S. pneumoniae*, while the occurrence of MefA may have resulted in horizontal gene transfer from *S. pyogenes* to specific clones of *S. pneumoniae*. The alternative explanation that the difference in prevalence is due to a difference in the transmissibility of MEGA and TN1207.1 elements cannot be ruled out.

We have also described here the cloning and expression of a second macrolide efflux pump in *S. pneumoniae*. *msr*(D) was found to always be associated with the *mef* genes, yet it was shown to be capable of functioning independently of Mef. The Msr pump of *S. pneumoniae* appears to differ in regulation and specificity from Mef, with both potentially contributing to the efflux phenotype. Further studies on its role in macrolide resistance are under way.

REFERENCES

1. **Alloing, G., M. C. Trombe, and J. P. Claverys.** 1990. The *ami* locus of the gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operons of gram-negative bacteria. Mol. Microbiol. **4:**633–644.
2. **Amezaga, M. R., P. E. Carter, P. Cash, and H. McKenzie.** 2002. Molecular epidemiology of erythromycin resistance in *Streptococcus pneumoniae* isolates from blood and noninvasive sites. J. Clin. Microbiol. **40:**3313–3318.
3. **Clancy, J., J. Petitpas, F. Dib-Hajj, W. Yuan, M. Cronan, A. V. Kamath, J. Bergeron, and J. A. Retsema.** 1996. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes.* Mol. Microbiol. **22:**867–879.
4. **Claverys, J. P., A. Dintilhac, E. V. Pestova, B. Martin, and D. A. Morrison.** 1995. Construction and evaluation of new drug-resistance cassettes for gene disruption mutagenesis in *Streptococcus pneumoniae*, using an ami test platform. Gene **164:**123–128.
5. **Colman, G., E. M. Cooke, B. D. Cookson, P. G. Cooper, A. Efstratiou, and R. C. George.** 1998. Pneumococci causing invasive disease in Britain 1982–1990. J. Med. Microbiol. **47:**17–27.
6. **Del Grosso, M., F. Iannelli, C. Messina, M. Santagati, N. Petrosillo, S. Stefani, G. Pozzi, and A. Pantosti.** 2002. Macrolide efflux genes *mef*(A) and *mef*(E) are carried by different genetic elements in *Streptococcus pneumoniae.* J. Clin. Microbiol. **40:**774–778.
7. **Doktor, S. Z., V. D. Shortridge, J. M. Beyer, and R. K. Flamm.** 2004. Epidemiology of macrolide and/or lincosamide resistant *Streptococcus pneumoniae* clinical isolates with ribosomal mutations. Diagn. Microbiol. Infect. Dis. **49:**47–52.
8. **Gusaffero, C.** 1993. Chemiluminescent ribotyping, p. 584–594. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
9. **Hoban, D. J., A. K. Wierzbowski, K. Nichol, and G. G. Zhanel.** 2001. Macrolide-resistant *Streptococcus pneumoniae* in Canada during 1998–1999: prevalence of *mef*(A) and *erm*(B) and susceptibilities to ketolides. Antimicrob. Agents Chemother. **45:**2147–2150.
10. **Jorgensen, J. H., S. A. Crawford, M. L. McElmeel, and C. G. Whitney.** 2004. Activities of cethromycin and telithromycin against recent North American isolates of *Streptococcus pneumoniae.* Antimicrob. Agents Chemother. **48:**605–607.
11. **Leclercq, R., and P. Courvalin.** 1991. Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. Antimicrob. Agents Chemother. **35:**1273–1276.
12. **Matsuoka, M., L. Janosi, K. Endou, and Y. Nakajima.** 1999. Cloning and sequences of inducible and constitutive macrolide resistance genes in *Staphylococcus aureus* that correspond to an ABC transporter. FEMS Microbiol. Lett. **181:**91–100.
13. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility testing; 12th informational supplement (aerobic dilution). Supplemental tables M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14. **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.
15. **Podbielski, A., B. Spellerberg, M. Woischnik, B. Pohl, and R. Lutticken.** 1996. Novel series of plasmid vectors for gene inactivation and expression analysis in group A streptococci (GAS). Gene **177:**137–147.
16. **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.
17. **Ross, J. I., E. A. Eady, J. H. Cove, W. J. Cunliffe, S. Baumberg, and J. C. Wootton.** 1990. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. Mol. Microbiol. **4:**1207–1214.
18. **Shortridge, V. D., R. K. Flamm, N. Ramer, J. Beyer, and S. K. Tanaka.** 1996.

Novel mechanism of macrolide resistance in *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. **26:**73–78.

19. **Tait-Kamradt, A., J. Clancy, M. Cronan, F. Dib-Hajj, L. Wondrack, W. Yuan, and J. Sutcliffe.** 1997. *mef*E is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **41:**2251–2255.

20. **Yu, L., A. H. Gunasekera, J. Mack, E. T. Olejniczak, L. E. Chovan, X. Ruan,** **D. L. Towne, C. G. Lerner, and S. W. Fesik.** 2001. Solution structure and function of a conserved protein SP14.3 encoded by an essential *Streptococcus pneumoniae* gene. J. Mol. Biol. **311:**593–604.

21. **Zhanel, G. G., T. Hisanaga, K. Nichol, A. Wierzbowski, and D. J. Hoban.** 2003. Ketolides: an emerging treatment for macrolide-resistant respiratory infections, focusing on *S. pneumoniae*. Expert Opin. Emerg. Drugs **8:**297–321.