ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2005, p. 1257–1261 Vol. 49, No. 3
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.3.1257–1261.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Molecular Epidemiology and Prevalence of Macrolide Efflux Genes *mef*(A) and *mef*(E) in *Streptococcus pneumoniae* Obtained in Canada from 1997 to 2002

Aleksandra K. Wierzbowski,[1,2] Dean Swedlo,[3] Dave Boyd,[4] Michael Mulvey,[4] Kim A. Nichol,[1,2] Daryl J. Hoban,[1,2] and George G. Zhanel[1,2,3]*

Department of Medical Microbiology, Faculty of Medicine, University of Manitoba,[3] Departments of Medicine[2] and Clinical Microbiology,[1] Health Sciences Centre, and Health Canada,[4] Winnipeg, Manitoba, Canada

Received 30 March 2004/Returned for modification 26 June 2004/Accepted 28 October 2004

One hundred forty M phenotype *Streptococcus pneumoniae* isolates were evaluated by PCR-restriction fragment length polymorphism, serotyping, and pulsed-field gel electrophoresis. Molecular genotyping revealed that the predominant macrolide resistance mechanism in *S. pneumoniae* in Canada is *mef*(E) and resistance dissemination is due to both spread of the genetic element MEGA as well as clonal dissemination of penicillin- and/or macrolide-resistant strains.

Low-level resistance to 14- and 15-member macrolides and susceptibility to lincosamides and streptogramin B (M phenotype) in *Streptococcus pneumoniae* (16) are conferred by the presence of a membrane-bound efflux protein, encoded by the *mef* gene *mef*(A) or *mef*(E) (1, 4, 5, 10, 13). The genetic elements carrying the *mef* genes, MEGA and Tn*1207.1*, have recently been described and are well described in the literature (5, 14). Clinical isolates of *S. pneumoniae* with reduced susceptibility to macrolides may arise through the horizontal acquisition of the genetic element carrying the *mef* gene or through clonal expansion of resistant strains. Unlike penicillin-resistant *S. pneumoniae*, the molecular epidemiology of macrolide-resistant *S. pneumoniae* in Canada has not been examined extensively (9, 12). The aim of this study was to identify the prevalence of *mef* genes in a large collection of *S. pneumoniae* strains isolated in Canada from 1997 to 2002 and to determine the genetic relatedness between *mef*(E)- and *mef*(A)-carrying isolates.

One hundred forty macrolide-resistant (erythromycin MIC, ≥1 μg/ml) and clindamycin-susceptible (MIC, ≤0.25 μg/ml) (M phenotype) *S. pneumoniae* clinical isolates were selected from among 6,991 isolates collected between 1997 and 2002 as part of an ongoing annual national surveillance study, the Canadian Respiratory Organism Susceptibility Study (7). Study isolates were collected from medical centers in 9 out of 10 Canadian provinces. Isolates for this study were collected from respiratory tract specimens only and were limited to one isolate per patient.

Erythromycin, clindamycin, and penicillin susceptibilities were determined by the NCCLS M7-A4 broth microdilution method (11). MIC interpretive standards for erythromycin, clindamycin, and penicillin were defined according to the NCCLS breakpoints for 2000 (11). The presence of the *mef* gene was determined by a previously described PCR assay that did not distinguish between the two variants (15). Discrimination between *mef*(A) and *mef*(E) was performed by PCR-restriction fragment length polymorphism analysis according to a previously described protocol (5). The relatedness among *mef*(A)- and *mef*(E)-carrying isolates was examined by pulsed-field gel electrophoresis (PFGE) by published methods (9, 12). Genomic DNAs were digested with SmaI prior to electrophoresis with a contour-clamped homogenous electric field apparatus (CHEF DRIII; Bio-Rad Laboratories, Hercules, Calif.). Isolates that differed by one to three bands were considered clonally related (12). DNA patterns were digitized for analysis with Molecular Analyst (Fingerprinting Plus, version 1.12) software. A dendrogram was calculated by the unweighted pair group method with arithmetic averages. Isolates were serotyped by the capsular swelling in antisera (Quellung reaction) from the Statens Serum Institut (Copenhagen, Denmark) according to the manufacturer's instructions.

The distribution of the *mef*(A) and *mef*(E) variants of the *mef* gene among pneumococcal isolates is summarized in Table 1. Among a sample of 140 M phenotype *S. pneumoniae* isolates, 133 (95%) isolates carried the *mef*(E) gene and 7 (5%) isolates carried the *mef*(A) gene. Both *mef*(E)- and *mef*(A)-carrying isolates were resistant to erythromycin (MIC, ≥1 μg/ml); however, all *mef*(A)-carrying isolates were susceptible to other antibiotics, including penicillin (penicillin-susceptible MIC, ≤0.06 μg/ml), while 66% (92 of 140) of *mef*(E)-carrying

TABLE 1. Prevalence of *mef*(E) and *mef*(A) *S. pneumoniae* genotypes in Canada between 1997 and 2002

Yr of isolation	No. (%) of isolates		
	Total	With *mef*(E)	With *mef*(A)
1997–1998	29	28	1
1998–1999	29	28	1
1999–2000	27	24	3
2000–2001	29	29	0
2001–2002	26	24	2
Total	140	133 (95)	7 (5)

* Corresponding author. Mailing address: Clinical Microbiology, Health Sciences Centre, MS673-820 Sherbrook St., Winnipeg, Manitoba R3A 1R9, Canada. Phone: (204) 787-4902. Fax: (204) 787-4699. E-mail: ggzhanel@pcs.mb.ca.

TABLE 2. Dendrogram depicting the genetic relatedness of 140 clinical *S. pneumoniae* isolates on the basis of PFGE results

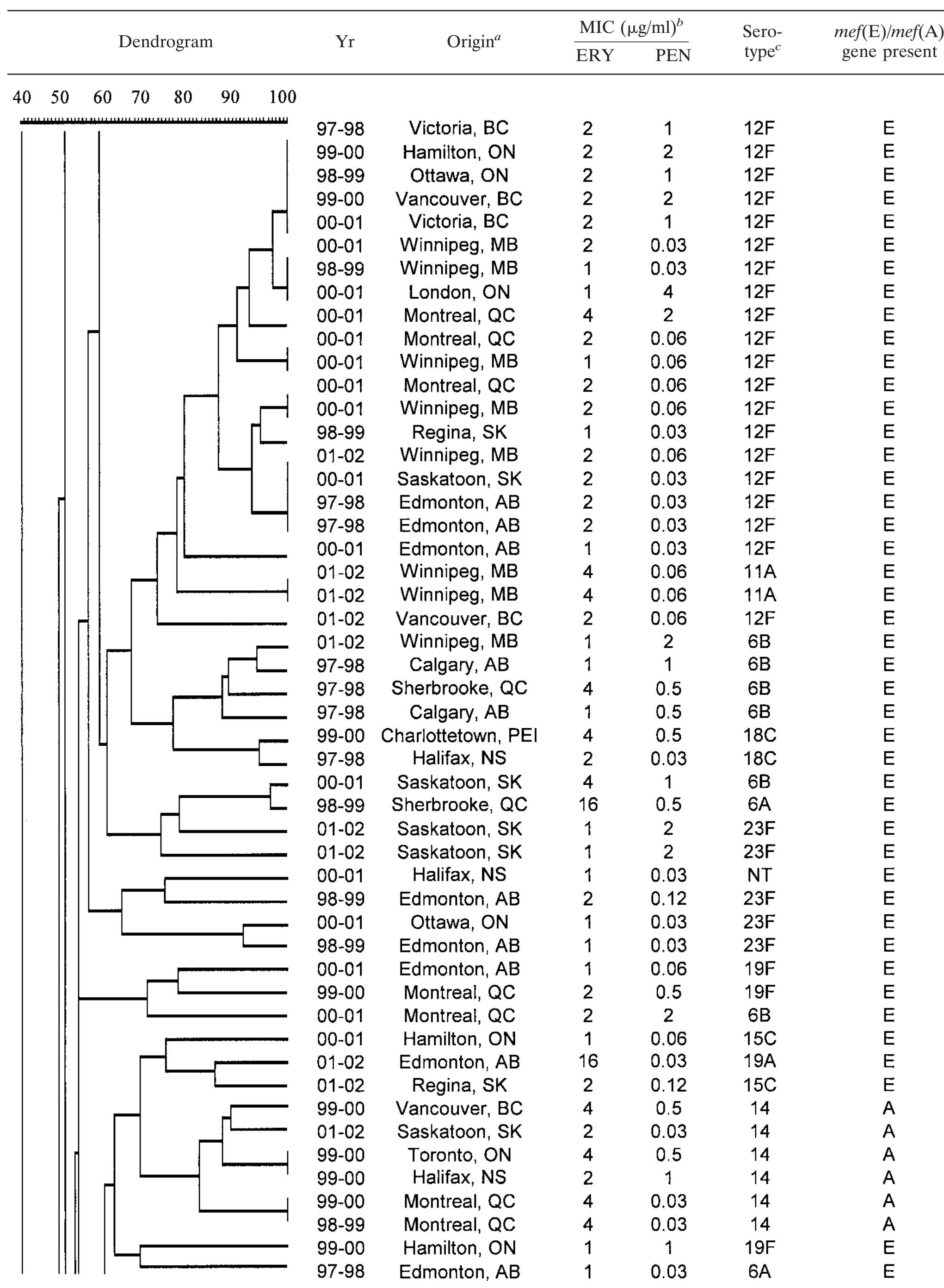
Dendrogram	Yr	Origin[a]	MIC (μg/ml)[b]		Serotype[c]	*mef*(E)/*mef*(A) gene present
			ERY	PEN		
40 50 60 70 80 90 100						
	01-02	Moncton, NB	1	0.06	NT	E
	01-02	Montreal, QC	2	0.06	NT	E
	99-00	Edmonton, AB	2	2	NT	E
	97-98	Charlottetown, PEI		1	NT	E
	99-00	Vancouver, BC	4	1	NT	E
	97-98	Edmonton, AB		0.12	NT	E
	97-98	London, ON	1	4	NT	E
	97-98	Montreal, QC	4	2	6B	E
	01-02	Halifax, NS	1	4	19F	E
	01-02	Halifax, NS	1	4	19F	E
	01-02	Montreal, QC	1	8	19F	E
	98-99	Victoria, BC	1	0.03	19B	E
	97-98	Hamilton, ON	1	1	19F	E
	99-00	Halifax, NS	1	0.5	19F	E
	99-00	Victoria, BC	4	0.03	6A	E
	98-99	Victoria, BC	2	0.03	6A	E
	97-98	Edmonton, AB	1	1	NT	E
	97-98	Sherbrooke, QC	1	0.03	19F	E
	97-98	Edmonton, AB	2	2	19F	E
	98-99	Saskatoon, SK	16	0.06	NT	E
	99-00	Halifax, NS	2	2	NT	E
	00-01	Edmonton, AB	2	0.03	NT	E
	01-02	London, ON	1	0.03	NT	E
	00-01	Montreal, QC	2	0.06	NT	E
	01-02	Charlottetown, PEI	1	2	NT	E
	00-01	Hamilton, ON	1	0.12	NT	E
	97-98	Vancouver, BC	4	4	9V	E
	98-99	Halifax, NS	2	0.5	19B	E
	00-01	Ottawa, ON	4	4	9V	E
	00-01	Ottawa, ON	2	4	9V	E
	99-00	Winnipeg, MB	1	1	9V	E
	99-00	Regina, SK	1	1	9V	E
	98-99	Montreal, QC	32	0.06	6B	E
	01-02	Ottawa, ON	4	0.06	34	E
	97-98	London, ON	2	0.25	6A	E
	98-99	London, ON	2	0.25	6A	E
	97-98	Regina, SK	1	1	9V	E
	99-00	Ottawa, ON	1	0.25	23F	E
	99-00	Hamilton, ON	2	1	NT	E
	00-01	Calgary, AB	8	1	6A	E
	99-00	Calgary, AB	1	1	6A	E
	98-99	Winnipeg, MB	4	0.03	11A	E
	98-99	Sherbrooke, QC	2	0.12	19F	E
	97-98	Montreal, QC	4	1	11A	E
	98-99	Winnipeg, MB	4	0.03	11A	E
	99-00	London, ON	4	0.5	14	E
	99-00	Montreal, QC	4	1	NT	E
	98-99	Victoria, BC	2	0.03	6B	E
	97-98	London, ON	2	0.03	6B	E
	98-99	Montreal, QC	1	0.03	6B	E
	98-99	Victoria, BC	2	0.03	6B	E

Continued on following page

isolates demonstrated reduced susceptibility to penicillin (MIC, ≥0.12 μg/ml).

Fourteen unique capsular serotypes were identified among the 140 isolates. Predominant serotypes included 12F (14%), 19F (13%), 23F (12%), and 14 (12%). Seventy-four of 140 (53%) *mef*-positive *S. pneumoniae* isolates belonged to serotypes 6B, 9V, 18C, 19F, 14, and 23F. One hundred seven of the 140 (76%) *mef*-positive *S. pneumoniae* isolates studied belonged to serotypes 8, 14, 11A, 12F, 18C, 19F, 23F, 6B, and 9V. Nontypeable strains accounted for 18% (25 of 140). Based on

TABLE 2—*Continued*



Dendrogram	Yr	Origin[a]	MIC (μg/ml)[b] ERY	MIC (μg/ml)[b] PEN	Serotype[c]	mef(E)/mef(A) gene present
	97-98	Victoria, BC	2	1	12F	E
	99-00	Hamilton, ON	2	2	12F	E
	98-99	Ottawa, ON	2	1	12F	E
	99-00	Vancouver, BC	2	2	12F	E
	00-01	Victoria, BC	2	1	12F	E
	00-01	Winnipeg, MB	2	0.03	12F	E
	98-99	Winnipeg, MB	1	0.03	12F	E
	00-01	London, ON	1	4	12F	E
	00-01	Montreal, QC	4	2	12F	E
	00-01	Montreal, QC	2	0.06	12F	E
	00-01	Winnipeg, MB	1	0.06	12F	E
	00-01	Montreal, QC	2	0.06	12F	E
	00-01	Winnipeg, MB	2	0.06	12F	E
	98-99	Regina, SK	1	0.03	12F	E
	01-02	Winnipeg, MB	2	0.06	12F	E
	00-01	Saskatoon, SK	2	0.03	12F	E
	97-98	Edmonton, AB	2	0.03	12F	E
	97-98	Edmonton, AB	2	0.03	12F	E
	00-01	Edmonton, AB	1	0.03	12F	E
	01-02	Winnipeg, MB	4	0.06	11A	E
	01-02	Winnipeg, MB	4	0.06	11A	E
	01-02	Vancouver, BC	2	0.06	12F	E
	01-02	Winnipeg, MB	1	2	6B	E
	97-98	Calgary, AB	1	1	6B	E
	97-98	Sherbrooke, QC	4	0.5	6B	E
	97-98	Calgary, AB	1	0.5	6B	E
	99-00	Charlottetown, PEI	4	0.5	18C	E
	97-98	Halifax, NS	2	0.03	18C	E
	00-01	Saskatoon, SK	4	1	6B	E
	98-99	Sherbrooke, QC	16	0.5	6A	E
	01-02	Saskatoon, SK	1	2	23F	E
	01-02	Saskatoon, SK	1	2	23F	E
	00-01	Halifax, NS	1	0.03	NT	E
	98-99	Edmonton, AB	2	0.12	23F	E
	00-01	Ottawa, ON	1	0.03	23F	E
	98-99	Edmonton, AB	1	0.03	23F	E
	00-01	Edmonton, AB	1	0.06	19F	E
	99-00	Montreal, QC	2	0.5	19F	E
	00-01	Montreal, QC	2	2	6B	E
	00-01	Hamilton, ON	1	0.06	15C	E
	01-02	Edmonton, AB	16	0.03	19A	E
	01-02	Regina, SK	2	0.12	15C	E
	99-00	Vancouver, BC	4	0.5	14	A
	01-02	Saskatoon, SK	2	0.03	14	A
	99-00	Toronto, ON	4	0.5	14	A
	99-00	Halifax, NS	2	1	14	A
	99-00	Montreal, QC	4	0.03	14	A
	98-99	Montreal, QC	4	0.03	14	A
	99-00	Hamilton, ON	1	1	19F	E
	97-98	Edmonton, AB	1	0.03	6A	E

Continued on following page

the incidence of the particular serotypes among our *S. pneumoniae* population, we found that the currently available heptavalent and 23-valent pneumococcal vaccinations would provide potential coverage for 53 and 76% of the isolates, respectively. This vaccine coverage was greater for penicillin-resistant isolates, increasing to 77 and 86%, respectively.

All *S. pneumoniae* strains were typeable by PFGE; the results are summarized in Table 2. Molecular analysis by PFGE with SmaI-restricted chromosomal DNA revealed 127 distinct DNA profiles among 140 macrolide-resistant *S. pneumoniae* isolates. One hundred twenty-two unique genotypes were found among the 133 *mef*(E)-carrying isolates. Dendrogram analysis of the *mef*(E)-carrying isolates identified 19 clusters (≥85% genetic relatedness), each containing between 2 and 11 isolates, which accounted for 47% (63 of 133) of the *mef*(E) *S. pneumoniae* isolates. The majority of the *mef*(E) isolates within clusters were coresistant to penicillin. Among the 19 clusters, 13 (68%) demonstrated cluster-specific serotypes. Isolates

TABLE 2—*Continued*

Dendrogram	Yr	Origin[a]	MIC (μg/ml)[b]		Serotype[c]	*mef*(E)/*mef*(A) gene present
			ERY	PEN		
40 50 60 70 80 90 100						
	00-01	Montreal, QC	2	2	23F	E
	98-99	Regina, SK	1	2	23F	E
	00-01	Saskatoon, SK	1	2	23F	E
	01-02	Edmonton, AB	1	2	14	E
	00-01	Edmonton, AB	1	2	14	E
	99-00	Edmonton, AB	1	1	14	E
	97-98	Montreal, QC		1	23F	E
	99-00	Calgary, AB	1	0.5	19F	E
	98-99	Edmonton, AB	1	2	14	E
	01-02	Edmonton, AB	1	4	14	E
	97-98	Calgary, AB	1	2	NT	E
	97-98	Edmonton, AB	1	4	14	E
	01-02	Saskatoon, SK	2	4	23F	E
	97-98	Saskatoon, SK	1	8	14	E
	97-98	Victoria, BC	1	4	19F	E
	00-01	Montreal, QC	1	4	23F	E
	98-99	Montreal, QC	1	2	23F	E
	98-99	Calgary, AB	4	0.5	19F	E
	98-99	Sherbrooke, QC	1	0.5	8	E
	00-01	Sherbrooke, QC	4	2	19F	E
	01-02	Regina, SK	1	4	23F	E
	99-00	Saskatoon, SK	2	2	23F	E
	99-00	Calgary, AB	1	1	14	E
	97-98	Sherbrooke, QC	4	2	23F	E
	97-98	Victoria, BC	1	4	19F	E
	00-01	Edmonton, AB	1	8	14	E
	01-02	Saskatoon, SK	2	0.12	23F	E
	98-99	London, ON	1	1	19F	E
	01-02	Halifax, NS	1	2	19F	E
	97-98	Edmonton, AB	1	0.06	14	A
	00-01	Montreal, QC	1	0.12	NT	E
	99-00	Winnipeg, MB	2	1	NT	E
	98-99	Moncton, NB	1	0.03	NT	E
	01-02	London, ON	16	2	6B	E
	98-99	Calgary, AB	16	2	6B	E
	98-99	Winnipeg, MB	1	1	NT	E
	98-99	Halifax, NS	1	0.03	6A	E
	99-00	Saskatoon, SK	1	1	NT	E
	01-02	Halifax, NS	1	0.03	NT	E

[a] Province: AB, Alberta; BC, British Columbia; MB, Manitoba; NB, New Brunswick; NS, Nova Scotia; ON, Ontario; QC, Quebec; SK, Saskatchewan.
[b] ERY, erythromycin; PEN, penicillin.
[c] NT, nontypeable.

within these 13 clusters belonged to serotype 6B (3 clusters), 6A (2 clusters), 12F (2 clusters), 23F (2 clusters), 11A (1 cluster), 9V (1 cluster), 18C (1 cluster), or 14 (1 cluster).

Among the seven *mef*(A)-carrying isolates, five unique genotypes were found. Dendrogram analysis identified one cluster (≥80% genetic relatedness) which accounted for 86% (six of seven) of the *mef*(A) *S. pneumoniae* isolates. All seven *mef*(A)-carrying isolates belonged to serotype 14, and all were susceptible to penicillin.

The higher prevalence of the *mef*(E) variant found among the macrolide-resistant *S. pneumoniae* population in Canada adds to the conclusion that the *mef*(E) gene is more prevalent in North America than in Europe (1, 4–6, 10, 13). It has been proposed that the incidence of the two variants occurs as a result of the carriage rates of *Streptococcus pyogenes* and viridans group streptococci, which carry *mef*(A) and *mef*(E), respectively (6). The low incidence of *mef*(A)-positive *S. pyogenes* isolates might explain the low incidence of *mef*(A) among *mef*-positive *S. pneumoniae* isolates in Canada (8); however, as the incidence of macrolide-resistant *mef*(A)-carrying *S. pyogenes* isolates appears to be increasing, it might affect the incidence of the *mef*(A) gene in the *S. pneumoniae* population (8). A low incidence of the *mef*(A) gene in the *S. pneumoniae* population might also be due to greater ability of the MEGA *mef*(E)-containing element to spread horizontally in *S. pneumoniae* compared to the Tn*1207.1* *mef*(A)-containing element, which has been referred to as "defective" (5, 14).

Genotyping of 133 *mef*(E)-carrying *S. pneumoniae* isolates showed that approximately half of the isolates were genetically related and the other half remained genetically unrelated (Ta-

ble 2). This indicates that macrolide resistance associated with the genetic element MEGA is a result of both clonal dissemination (vertical) as well as spread of the genetic element (horizontal). Further analysis showed that the majority of the isolates that are genetically related (cluster) were also resistant to penicillin, while the majority of the genetically unrelated *mef*(E)-carrying isolates remained susceptible to penicillin, suggesting that penicillin resistance is driving the clonal spread of the MEGA element. Genotyping of seven *mef*(A)-carrying *S. pneumoniae* isolates demonstrated genetic relatedness among these isolates. As the isolates are not related in terms of date and location of isolation, the presence of a single cluster containing six of the seven *mef*(A) strains indicates that resistance due to the genetic element Tn*1207.1* is occurring through the expansion of a single penicillin-susceptible serotype 14 clone that has acquired the *mef*(A) gene. These PFGE patterns are similar to those of other investigators who found that *mef*(E) strains did not appear to be related by PFGE, while *mef*(A) strains were genetically indistinguishable (5).

In conclusion, although both *mef*(E) and *mef*(A) genes were present in Canadian isolates of *S. pneumoniae*, the majority of isolates screened were *mef*(E). This is in contrast to the European studies that reported *mef*(A) as the major efflux gene among their *S. pneumoniae* isolates (1, 4, 5, 10, 13). Similar to the findings of others (5), all *mef*(A) isolates found in our study belonged to serotype 14, and unlike some studies (1, 10) that identified *mef*(A) isolates scattered over seven different serotypes (23F,19A,3,6B,15B,33A, and 9), no other serotypes were found in our *mef*(A) *S. pneumoniae* isolates. The *mef*(E)-carrying isolates, in concordance with other studies (1, 5, 10), were more scattered (over 14 serotypes).

Because *mef*(A) and *mef*(E) in pneumococci appear to originate from different essentially invariant elements, acquired from group A and viridans group streptococci, respectively, and because acquisition of either gene may have implications regarding streptococcal physiology and antibiotic resistance, particularly penicillin, it remains important for *mef*(E) and *mef*(A) to be considered independently and to continue to document their horizontal and vertical spread within *S. pneumoniae* as this may lead to a better understanding of the spread of macrolide-resistant *S. pneumoniae*. In addition, since both *mef*(E) and *mef*(A) have been found in *S. pyogenes* and *Streptococcus agalactiae* in at least one study (2, 3), it would be interesting to see whether a similar mixed occurrence of *mef*(A) and *mef*(E) is also present in Canadian M phenotype strains of *S. pyogenes* and viridans group streptococci and this may lead to a better understanding of the dissemination of macrolide resistance in Canada.

REFERENCES

1. **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.
2. **Arpin, C., M. H. Canron, P. Noury, and C. Quentin.** 1999. Emergence of mefA and mefE genes in beta-haemolytic streptococci and pneumococci in France. J. Antimicrob. Chemother. **44:**133–134.
3. **Arpin, C., H. Daube, F. Tessier, and C. Quentin.** 1999. Presence of *mefA* and *mefE* genes in *Streptococcus agalactiae*. Antimicrob. Agents Chemother. **43:** 944–946.
4. **Bogaert, D., P. W. M. Hermans, I. N. Grivea, G. S. Katopodis, T. J. Mitchell, M. Sluijter, R. De Groot, N. G. Beratis, and G. A. Syrogiannopoulos.** 2003. Molecular epidemiology of penicillin-susceptible non-β-lactam-resistant *Streptococcus pneumoniae* isolates from Greek children. J. Clin. Microbiol. **41:**5633–5639.
5. **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.
6. **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.
7. **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.
8. **Katz, K. C., A. J. McGeer, C. L. Duncan, A. Ashi-Sulaiman, B. M. Willey, A. Sarabia, J. McCann, S. Pong-Porter, Y. Rzayev, J. S. de Azavedo, and D. E. Low.** 2003. Emergence of macrolide resistance in throat culture isolates of group A streptococci in Ontario, Canada, in 2001. Antimicrob. Agents Chemother. **47:**2370–2372.
9. **Louie, M., L. Louie, G. Papia, J. Talbot, M. Lovgren, and A. E. Simor.** 1999. Molecular analysis of the genetic variation among penicillin-susceptible and penicillin-resistant Streptococcus pneumoniae serotypes in Canada. J. Infect. Dis. **179:**892–900.
10. **Montanari, M. P., M. Mingoia, I. Cochetti, and P. E. Varaldo.** 2003. Phenotypes and genotypes of erythromycin-resistant pneumococci in Italy. J. Clin. Microbiol. **41:**428–431.
11. **NCCLS.** 2000. Methods for antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5, 5th ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
12. **Nichol, K. A., G. G. Zhanel, and D. J. Hoban.** 2003. Molecular epidemiology of penicillin-resistant and ciprofloxacin-resistant *Streptococcus pneumoniae* in Canada. Antimicrob. Agents Chemother. **47:**804–808.
13. **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.
14. **Santagati, M., F. Iannelli, M. R. 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.
15. **Sutcliffe, J., T. Grebe, A. Tait-Kamradt, and L. Wondrack.** 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother. **40:**2562–2566.
16. **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.