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Molecular Epidemiology of Erythromycin Resistance in *Streptococcus pneumoniae* Isolates from Blood and Noninvasive Sites

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Erythromycin-resistant isolates of *Streptococcus pneumoniae* from blood cultures and noninvasive sites were studied over a 3-year period. The prevalence of erythromycin resistance was 11.9% (19 of 160) in blood culture isolates but 4.2% (60 of 1,435) in noninvasive-site isolates. Sixty-two of the 79 resistant isolates were available for study. The M phenotype was responsible for 76% (47 of 62) of resistance, largely due to a serotype 14 clone, characterized by multilocus sequence typing as ST9, which accounted for 79% (37 of 47) of M phenotype resistance. The ST9 clone was 4.8 times more common in blood than in noninvasive sites. All M phenotype isolates were PCR positive for *mef*(A), but sequencing revealed that the ST9 clone possessed the *mef*(A) sequence commonly associated with *Streptococcus pyogenes*. All M phenotype isolates with this *mef*(A) sequence also had sequences consistent with the presence of the Tn*1207.1* genetic element inserted in the *celB* gene. In contrast, isolates with the *mef*(E) sequence normally associated with *S. pneumoniae* contained sequences consistent with the presence of the mega insertion element. All MLS$_B$ isolates carried *erm*(B), and two isolates carried both *erm*(B) and *mef*(E). Fourteen of the 15 MLS$_B$ isolates were tetracycline resistant and contained *tet*(M). However, six M phenotype isolates of serotypes 19 (two isolates) and 23 (four isolates) were also tetracycline resistant and contained *tet*(M). MICs for isolates with the *mef*(A) sequence were significantly higher than MICs for isolates with the *mef*(E) sequence ($P < 0.001$). Thus, the ST9 clone of *S. pneumoniae* is a significant cause of invasive pneumococcal disease in northeast Scotland and is the single most important contributor to M phenotype erythromycin resistance.

There are two commonly described mechanisms of erythromycin resistance: active drug efflux and methylation of the antibiotic target site. In *Streptococcus pneumoniae* these result in two major phenotypes, M and MLS$_B$ (18, 30, 34). M phenotype isolates are resistant to macrolides via an active efflux mechanism that requires the presence of the *mef*(A) gene (30, 34, 35). This gene was first identified in *Streptococcus pyogenes* and originally designated *mef*(A), while a similar gene with 90% identity to *mef*(A) was later identified in *S. pneumoniae* and designated *mef*(E) (4, 35). More recently, it has been proposed that these two genes are members of the same family and should be referred to by the generic label of *mef*(A) (30). The product of the *mef*(A) gene has not been directly characterized, but its predicted amino acid sequence shows homology with other transporter proteins (4). Santagati et al. (31) described, in a clinical isolate of *S. pneumoniae,* a 7.244-kb chromosomal element, Tn*1207.1* that contained 8 open reading frames (ORFs), one of which (ORF4) was 100% identical to the original *mef*(A) sequence of *S. pyogenes*. Downstream from *mef*(A), ORF5 coded for a protein that showed homology to MsrA, an ATP-binding protein that mediates resistance to macrolides and streptogramin B in staphylococci. Upstream from *mef*(A), ORF2 was thought to represent an integrase or site-specific recombinase, although Tn*1207.1* was considered a defective transposon because it terminates at the 3′ end in a truncated ORF. In the isolate studied, Tn*1207.1* was inserted in the pneumococcal genome within the competence gene *celB*. A further 5.4- or 5.5-kb chromosomal insertion element has recently been described by Gay and Stephens (12) and has been designated the macrolide efflux genetic assembly (mega). mega contains 5 ORFs, of which ORF1 is identical to the original *mef*(E) sequence of *S. pneumoniae*. As with Tn*1207.1*, there is also a homologue of the *msr*(A) gene downstream from *mef*(E) designated *mel*, after the first three amino acids of the predicted protein (12). The sequences of the two *msr*(A) homologues found in Tn*1207.1* and mega are 98% identical. The five ORFs of mega show a high degree of identity with ORFs 4 to 8 of Tn*1207.1*, but mega does not contain ORFs with integrase or recombinase homology. PCR studies on 89 *mef*(E)-positive clinical isolates from Atlanta revealed that there are more than four different insertion sites for mega in the pneumococcal genome. None of the four insertion sites identified were in the *celB* gene (12).

In the MLS$_B$ phenotype, resistance to the structurally unrelated macrolide, lincosamide, and streptogramin B antibiotics is brought about by methylation of 23S rRNA, the common target of these agents (18). MLS$_B$ resistance is determined by members of the *erm* gene family, and in *S. pneumoniae* the *erm*(B) gene is usually carried on the 25.3-kb conjugative transposon Tn*1545* along with a separate gene, *tet*(M), that codes for tetracycline resistance (5, 30). Transfer of Tn*1545* between strains is mediated by the excisionase (*xis*) and integrase (*int*) genes (28). Other transposable elements such as Tn*917*-like elements and the composite transposon-like structure Tn*3872* can also carry *erm*(B) in *S. pneumoniae* (19).

The prevalence of erythromycin resistance in *S. pneumoniae* has increased in several countries over the past few years (13,

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17, 24). However, there are considerable differences between countries in the relative contributions of the M and MLS_B phenotypes to the overall prevalence of resistance. Surveys in the United States show a predominance of the M phenotype (8, 13), while in Italy (24), Belgium (7), and Germany (29) the MLS_B phenotype is more common. A survey of *S. pneumoniae* bacteremia carried out in our laboratory showed that the prevalence of erythromycin-resistant isolates was 12% and that 75% of these isolates had the M phenotype (21). All of the M phenotype isolates belonged to serotype 14 and had very similar profiles on pulsed-field gel electrophoresis (21). The aim of the present study was to compare the prevalence of the two major erythromycin resistance phenotypes in pneumococcal isolates from blood and from noninvasive sites. We serotyped all erythromycin-resistant isolates and identified the resistance genes and associated insertion elements by PCR and sequencing.

MATERIALS AND METHODS

Bacterial isolates. All bacterial isolates, unless otherwise stated, were cultured from clinical specimens submitted to the routine diagnostic laboratories of the Department of Medical Microbiology, University of Aberdeen, Aberdeen, United Kingdom. Over a 3-year period, from 1998 to 2000, all blood culture isolates of *S. pneumoniae* and all erythromycin-resistant isolates from other sites were collected. *S. pneumoniae* was identified by alpha-hemolysis on blood agar and sensitivity to optochin, and erythromycin resistance was detected in the first instance by disk diffusion (39). Isolates were serotyped by the Scottish Pneumococcal Reference Laboratory, Stobhill Hospital, Glasgow, United Kingdom, by coagglutination (33); selected isolates were also characterized by multilocus sequence typing (MLST) (10, 11) by the same laboratory. Isolates from sites other than blood, designated noninvasive, were obtained from the upper respiratory tract, sputum, eye swabs, and ear swabs. We obtained details on all pneumococci isolated during the study period from the computer database of our diagnostic laboratory and thus obtained baseline figures for the number of isolates from sites other than blood. A small number of isolates from invasive sites other than blood, e.g., cerebrospinal fluid, were excluded, and duplicate isolates from the same episode of infection in any one patient were counted only once.

Three *S. pneumoniae* M phenotype isolates (serotype 14) from the South of England (M44, M47, and M58), two from Australia (M231 and M238), and one from Belgium (M27) were kindly supplied by M. C. Enright and B. G. Spratt, Wellcome Trust Centre for the Epidemiology of Infectious Disease, Department of Zoology, Oxford University, Oxford, United Kingdom. Three susceptible isolates (serotype 14) from Australia (M222, M229, and M237) were also obtained from the same source. The isolates donated are all listed in the MLST database (http://www.mlst.net) as sequence type 9 (ST9).

Isolates were stored at −70°C in Protect (TSC Ltd., Heywood, United Kingdom) and recovered when required by culture on blood agar plates at 37°C in air with 5% CO_2. Resistance to erythromycin and the resistance phenotype were confirmed by disk diffusion assay with the disks adjacent to detect inducible resistance (34). Erythromycin MICs were determined by E-test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. *S. pneumoniae* strain ATCC 49619 was tested simultaneously as a quality control, and the MIC for this strain was within the manufacturer's recommended range. MICs were also determined by broth microdilution according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (23), except that additional antibiotic concentrations of 3, 6, 12, and 24 mg/liter were added to the recommended test range.

PCR. *S. pneumoniae* cells were harvested from one confluent blood agar plate. Chromosomal DNA was extracted from cell suspensions by the method of Pitcher et al. (27) or with a DNA extraction kit for gram-positive bacteria (Puregene; Gentra Systems, Minneapolis, Minn.). The *mef*(A) gene was amplified by using primers based on the published sequence of *S. pyogenes* (4) (5′ ATGGAAAAATACAACAATTG [forward] and 5′ TTATTTTAAATCTAATT TTCTAAC [reverse]). PCR conditions for amplification of the *mef*(A) gene comprised an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s. After the amplification cycles, a final elongation step at 72°C for 5 min was carried out. The primer set used to amplify the *erm*(B) gene was based on the erythromycin resistance gene carried in the conjugative transposon Tn*1545* from *S. pneumoniae* (36) and consisted of 5′ ATTGGAACAGGTAAAGGGC (forward) and 5′ GAACATCTGTGGTATGGCG (reverse). PCR conditions for amplification of the *erm*(B) gene comprised an initial denaturation step at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and elongation at 72°C for 1 min. After the amplification cycles, a final elongation step was performed at 72°C for 7 min. The homologue of *msr*(A) was amplified by using primers based on the sequence of this gene contained in the transposable element Tn*1207.1* of *S. pneumoniae* (GenBank accession number AF227520), 5′ TGCCTATATTCCCCAGTT (forward) and 5′ TTAATTTC CGCACCGACTA (reverse). PCR conditions for amplification of the *msr*(A) homologue comprised an initial denaturation step at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min, and elongation at 72°C for 1 min. After the amplification cycles, a final elongation step at 72°C for 10 min was carried out.

To establish whether the transposable element carrying *mef*(A) in our isolates had the same insertion site as Tn*1207.1*, specific PCR primers were designed. The forward primer, 5′ CTTTCCTTTCTCTATCCA, lies upstream of the known insertion site of Tn*1207.1* in the *celB* gene (31) (GenBank accession number AF052208). The reverse primer, 5′ TACATCAACATTACCATCTG, was based on the 5′-end sequence of Tn*1207.1* (GenBank accession number AF227520). Amplification conditions were the same as for the *msr*(A) homologue.

Primers for the *tet*(M) (5′ AGTTTTAGCTCATGTTGATG [forward] and 5′ TCCGACTATTTGGACGACGG [reverse]) and *int* (5′ GCGTGATTGTATCT CACT [forward] and 5′ GACCTCCTGTTGCTTCT [reverse]) genes were as described by Doherty et al. (9). Primers for the *xis* gene, 5′ AAGCAGACTGA CATTCCTA (forward) and 5′ GCGTCCAATGTATCTATAA (reverse), were based on the sequence of this gene available in the database (GenBank accession number X61025). PCR conditions for amplification of *tet*(M) comprised an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and elongation at 72°C for 1 min, 30 s. After the amplification cycles, a final elongation step at 72°C for 10 min was carried out. Conditions for amplification of *int* and *xis* were the same as those used for the *msr*(A) homologue.

All PCR amplification mixtures contained 100 ng of genomic DNA, forward and reverse primers (250 nM), $MgCl_2$ (1.5 mM), deoxynucleoside triphosphates (200 μM; Amersham Pharmacia Biotech UK Ltd., Little Chalfont, United Kingdom), and *Taq* polymerase (5 U) plus buffer (Bioline, London, United Kingdom). PCR was performed on a Perkin-Elmer (PE) Biosystems (Warrington, United Kingdom) 9700 Thermocycler. PCR products were detected by electrophoresis on agarose gels, followed by staining with ethidium bromide and UV transillumination.

DNA sequence analysis. PCR amplification products were purified by using Centricon C100 columns (Millipore UK Ltd., Watford, United Kingdom). Purified PCR products were sequenced by using the corresponding amplification primers. In addition, a specific region of interest within the *tet*(M) gene was sequenced in both directions by using internal sequencing primers 5′ CGAAC TTTACCGAATCTGAA (forward) and 5′ CAACGGAAGCGGTGATACAG (reverse); these were based on the published sequence of *tet*(M) (GenBank accession number X90939). Sequencing reactions were performed by using the BigDye Terminator cycle sequencing kit (PE Biosystems) according to the manufacturer's instructions. Sequencing products were run on an ABI 377 automated DNA sequencer, and sequencing analysis was carried out with the SeqEd 1.0.3 DNA analysis program (PE Biosystems).

Statistical analysis. The significance of differences in the distribution of isolates between blood and noninvasive sites was determined by the chi-square test, and the significance of differences between erythromycin MICs was determined by the Mann-Whitney test. Both tests were performed with the SPSS statistical package.

RESULTS

Erythromycin resistance in *S. pneumoniae* isolates from blood and noninvasive sites. Over the 3-year study period, there were 160 isolates of *S. pneumoniae* from blood and 1,435 from other sites. Nineteen blood isolates (11.9%) were erythromycin resistant, and 18 of these were available for confirmation of phenotype and further study. The prevalence of erythromycin resistance was much lower in isolates from noninvasive sites. Sixty of the 1,435 isolates (4.2%) were eryth-

TABLE 1. Distribution of resistance phenotypes and serotypes in erythromycin-resistant *S. pneumoniae* isolates collected from blood ($n = 160$) and noninvasive sites ($n = 1,435$)

Erythromycin resistance phenotype	Serotype	No. of isolates from:	
		Blood	Noninvasive sites
M	14	15	22
M	9		4
M	19		2
M	23	1	3
MLS_B	6		3
MLS_B	14		2
MLS_B	15		3
MLS_B	19	2	2
MLS_B	23		3
Total		18	44

romycin resistant, and 44 of these were available for confirmation of phenotype and further study. The distribution of resistance phenotypes and serotypes among available isolates from blood and noninvasive sites is shown in Table 1. The M phenotype was responsible for 76% (47 of 62) of erythromycin resistance overall, largely due to the contribution of M phenotype, serotype 14 (M14) isolates, which accounted for 60% (37 of 62) of all resistant isolates and 79% (37 of 47) of M phenotype resistance. In contrast, MLS_B isolates were responsible for 24% (15 of 62) of erythromycin resistance and were distributed across serotypes 6, 14, 15, 19, and 23 (Table 1).

The M14 clone was responsible for 83% (15 of 18) of erythromycin resistance in blood isolates but 50% (22 of 44) of resistance in isolates from other sites. All M14 isolates were penicillin susceptible (data not shown). If we assume that the distribution of resistance phenotypes was the same in isolates which were not available for examination as in those that were, then 10% of blood isolates were M14 (16 of 160) compared with 2.1% of isolates from noninvasive sites (30 of 1,435). Thus, the M14 clone was proportionately 4.8 times more common in blood than in noninvasive sites, and this difference is statistically significant ($P < 0.001$ by the chi-square test).

Resistance genes in erythromycin-resistant isolates. All M phenotype isolates and one erythromycin-susceptible isolate from blood were PCR positive for the *mef*(A) gene. Sequence analysis revealed that the *mef*(A) gene carried by all M14 isolates and by one isolate of serotype 9 was 100% identical to the *mef*(A) sequence originally described for *S. pyogenes* (GenBank accession number U70055), and all these isolates carried the sequence of the *msr*(A) homologue found in Tn*1207.1* (GenBank accession number AF227520) (Table 2). All isolates with this *mef*(A) sequence were positive by PCR with primers designed to amplify Tn*1207.1* when inserted into the *celB* gene (31). All remaining M phenotype isolates of serotypes 9, 19, and 23 carried the *mef*(E) sequence originally described for *S. pneumoniae* (GenBank accession number U83667). These isolates all carried *mel*, the *msr*(A) homologue found in the transposable element mega (GenBank accession number AF274302) (Table 2). Isolates with the mega sequence did not produce a PCR product with primers designed to amplify Tn*1207.1* when inserted into the *celB* gene. All MLS_B isolates were *erm*(B) positive by PCR. Two serotype 19 noninvasive isolates, included in Table 1 as MLS_B phenotype isolates, contained both the *mef*(E) and *erm*(B) genes (Table 2).

Tetracycline resistance. Tetracycline resistance is commonly associated with the MLS_B phenotype, since the *tet*(M) and *erm*(B) genes are both found on the Tn*1545* transposon. Fourteen of the 15 MLS_B isolates were resistant to tetracycline and were PCR positive for *tet*(M) (Table 2). Nine of these 14 isolates were also PCR positive for *int* and *xis,* the integrase and excisionase genes commonly associated with Tn*1545*; 4 isolates were negative for both *int* and *xis*, and 1 isolate was positive only for *xis*. One MLS_B isolate of serotype 23 was susceptible to tetracycline. This isolate was positive for *tet*(M) by PCR, but sequence analysis of the PCR product demonstrated a 10-bp deletion from base 619 to 628 (this deletion was also found in an isolate with the same phenotype collected in our laboratory in 1997). The tetracycline-susceptible isolate

TABLE 2. Distribution of resistance-related genes and transposable elements in erythromycin-resistant *S. pneumoniae* isolates

Erythromycin resistance phenotype	Serotype (no. of isolates)	Erythromycin resistance gene or insertion element[a]				Tetracycline resistance		Tn*1545*-associated gene	
		mef(A)[b]	*erm*(B)	Tn*1207.1*	mega	Phenotype[c]	*tet*(M)	*int*	*xis*
M	14 (37)	A	−	+	−	S	ND[d]	ND	ND
M	9 (1)	A	−	+	−	S	ND	ND	ND
M	9 (3)	E	−	−	+	S	ND	ND	ND
M	19 (2)	E	−	−	+	R	+	+	+
M	23 (4)	E	−	−	+	R	+	+	+
MLS_B	19 (2)	E	+	−	+	R	+	+	+
MLS_B	6 (3), 14 (1), 19 (2), 23 (1)	−	+	−	−	R	+	+	+
MLS_B	23 (1)	−	+	−	−	R	+	−	+
MLS_B	14 (1), 15 (3)	−	+	−	−	R	+	−	−
MLS_B	23 (1)	−	+	−	−	S	+[e]	+	+

[a] +, present; −, absent. The presence of the insertion elements Tn*1207.1* and mega was determined on the basis of the sequences of *mef*(A) and the *msr*(A) homologue. In addition, the insertion of Tn*1207.1* in the *celB* gene was confirmed by PCR.
[b] A and E represent the two different *mef*(A) gene sequences, *mef*(A) and *mef*(E), respectively.
[c] S, susceptible; R, resistant.
[d] ND, not determined.
[e] Ten-base-pair deletion in *tet*(M) (see the text).

was PCR positive for *int* and *xis*. Six M phenotype isolates of serotypes 19 (two isolates) and 23 (four isolates) were also tetracycline resistant and were PCR positive for *tet*(M), *int*, and *xis* (Table 2).

Erythromycin MICs for M phenotype isolates. Erythromycin MICs for all resistant isolates were determined by E-test and by broth microdilution (NCCLS). By broth microdilution, median erythromycin MICs were 12 mg/liter (range, 8 to 24 mg/liter) for 38 M phenotype isolates with the *mef*(A) sequence and 4 mg/liter (range, 2 to 8 mg/liter) for 9 isolates with the *mef*(E) sequence. By E-test, median MICs were 20 mg/liter (range, 12 to 32 mg/liter) for 38 M phenotype isolates with the *mef*(A) sequence and 3 mg/liter (range, 2 to 4 mg/liter) for 9 isolates with the *mef*(E) sequence. The difference in MICs between *mef*(A) and *mef*(E) isolates was statistically significant by both methods ($P < 0.001$ by the Mann-Whitney test).

Comparison with isolates from other geographical locations. To determine whether the *mef*(A) sequence found in our Scottish M14 isolates reflected a local phenomenon, we sequenced the gene in M14 isolates from other locations, three from the south of England, two from Australia, and one from Belgium. All six isolates also carried the *mef*(A) sequence. Erythromycin MICs for these isolates by E-test were in the range of 12 to 32 mg/liter, similar to that of the local M14 clone. It has previously been shown by pulsed-field gel electrophoresis analysis that the profiles of our Scottish M14 isolates are very similar to those of isolates from the south of England (21). The serotype 14 isolates from England, Australia, and Belgium have all been previously characterized by MLST as ST9 and belong to an M phenotype clone associated with meningitis in the United Kingdom (10, 11) (http://www.mlst.net). MLST analysis of nine representative isolates from our local M14 clone confirmed that they were also ST9.

DISCUSSION

This study demonstrates that the M phenotype is the commonest form of erythromycin resistance in northeast Scotland, largely due to the predominance of a serotype 14 clone that has been characterized by MLST as ST9. Thus, the phenotypic pattern of erythromycin resistance observed in the United Kingdom is closer to that of the United States than to that of other European countries, with M phenotype resistance at least three times more prevalent than MLS_B resistance overall. We have demonstrated that the ST9 clone is 4.8 times more common in blood than in other sites and is therefore more invasive than the average *S. pneumoniae* isolate. The ST9 clone has also been identified as an important cause of meningitis throughout the United Kingdom (10, 15, 37), and this clone is now recognized as a cause of invasive disease in other countries (http://www.mlst.net). There are many virulence factors other than the capsule involved in the pathogenesis of pneumococcal infection (14), and there is evidence that particular strains of pneumococci have a predilection for blood and cerebrospinal fluid (16). Further characterization of invasive clones such as ST9 will help to explain this process and may in the future offer targets for treatment or prevention of invasive pneumococcal disease. An increase in the expression of a variant form of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in our M14 clone, now known to be ST9, has been demonstrated previously (3). In other species such as *S. pyogenes* and *Staphylococcus aureus*, GAPDH is located in the cell wall and is associated with virulence (22, 25, 26, 38). The role of GAPDH in the pathogenicity of the ST9 clone is therefore worthy of further study.

The *mef*(A) genes of *S. pneumoniae* and *S. pyogenes* were found initially to be different and were designated *mef*(E) and *mef*(A), respectively. We have shown that an unusual characteristic of the ST9 clone is that it possesses the *mef*(A) sequence normally associated with *S. pyogenes*. The original *S. pyogenes* sequence [*mef*(A)] was reported for four isolates of *S. pneumoniae* from Italy (24), and more recently it has been reported for a further 17 isolates, also from Italy (6). Conversely, there is a report of the original pneumococcal sequence [*mef*(E)] in a single isolate of *S. pyogenes* (2), and we have also observed this (data not shown). A survey of erythromycin-resistant viridans streptococci found the *mef*(E) sequence in *S. mitis*, *S. oralis*, and *S. anginosus*, while the *mef*(A) sequence was found in only one isolate of *S. oralis* (1). Thus, the potential for the spread of M phenotype resistance in *S. pneumoniae* is increased by the widespread presence of the *mef*(A) gene in other, less pathogenic species. The original characterization of Tn*1207.1* showed that it was associated with the *mef*(A) sequence (31), and our data confirmed that all pneumococcal isolates with the *S. pyogenes mef*(A) sequence contained Tn*1207.1*. In contrast, the nine M phenotype isolates with the pneumococcal *mef*(E) sequence contained the mega insertion element. It seems likely, therefore, that the two *mef*(A) sequences are associated with different transposable elements. In support of this conclusion, Del Grosso et al. have also demonstrated an association between *mef*(A) sequence and genetic element in six Italian isolates (6).

Our results indicate that the erythromycin MIC for pneumococci possessing *mef*(A) together with the associated transposable element Tn*1207.1* is higher than that for pneumococci possessing *mef*(E) in association with mega. Gay and Stephens (12) demonstrated that in the transposable element mega, the *mef*(E) gene is cotranscribed with *mel*, the *msr*(A) homologue. In staphylococci, *msr*(A) encodes an ATP-binding cassette that provides energy for the efflux of macrolides and streptogramin B. Thus, the erythromycin MIC may be influenced not only by the Mef(A) protein but also by the actions of the MsrA homologue. Different sites of insertion in the genome may also result in different rates of transcription. However, relatively few of our isolates contained the *mef*(E) gene sequence, and therefore further studies are required to confirm the MIC difference.

Resistance to tetracycline is a common characteristic of the MLS_B phenotype because the *erm*(B) and *tet*(M) genes can be found on the same transposon, Tn*1545*. The presence of both resistance genes *erm*(B) and *mef*(A) in MLS_B isolates has been described previously (20), and these isolates are expected to be resistant to tetracycline. In contrast, *mef*(A) in M phenotype isolates is not known to be linked to tetracycline resistance. We have shown that M phenotype isolates of serotypes 19 and 23 can carry the *tet*(M) gene and other elements of Tn*1545*, such as *int* and *xis*, without possessing the *erm*(B) gene. Similar findings were reported for Spanish isolates (32). In this study we have identified a 10-bp deletion in the sequence of the *tet*(M) gene of an MLS_B isolate that was susceptible to tetra-

cycline, relative to the *tet*(M) sequence in tetracycline-resistant isolates. Susceptibility to tetracycline in MLS_B isolates has been reported for Spanish isolates, but these isolates did not possess the *tet*(M) gene (32). Our data show that tetracycline resistance is not a reliable guide to MLS_B phenotype erythromycin resistance, since we found both M phenotypes that were resistant to tetracycline and MLS_B phenotypes that were susceptible to tetracycline.

In conclusion, the present study has demonstrated the clinical importance in our region of an M phenotype, serotype 14 clone of *S. pneumoniae* that has invasive properties and for which MICs are higher than for other M phenotype isolates. The clone has been identified as ST9 and has been isolated in other parts of the world. Further characterization of this clone may yield further insights into both invasiveness and the detailed mechanisms of M phenotype resistance.

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