ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2010, p. 1152–1159 Vol. 54, No. 3
0066-4804/10/$12.00 doi:10.1128/AAC.01268-09

Distribution of Serotypes, Genotypes, and Resistance Determinants among Macrolide-Resistant *Streptococcus pneumoniae* Isolates▽

Xiaoping Xu,[1,2]† Lin Cai,[2,3]† Meng Xiao,[2,4]† Fanrong Kong,[2] Shahin Oftadeh,[2] Fei Zhou,[2] and Gwendolyn L. Gilbert[2]*

Department of Laboratory Medicine, Shenzhen Second People's Hospital, Shenzhen 518035, People's Republic of China[1]; Centre for Infectious Diseases and Microbiology (CIDM), Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, New South Wales, Australia[2]; Department of Dermatology, Peking University People's Hospital, Beijing 100044, People's Republic of China[3]; and Life Science College, Peking University, Beijing, People's Republic of China[4]

Received 6 September 2009/Returned for modification 11 December 2009/Accepted 2 January 2010

Macrolide resistance in *Streptococcus pneumoniae* has emerged as an important clinical problem worldwide over the past decade. The aim of this study was to analyze the phenotypes (serotype and antibiotic susceptibility), genotypes (multilocus sequence type [MLST] and antibiotic resistance gene/transposon profiles) among the 31% (102/328) of invasive isolates from children in New South Wales, Australia, in 2005 that were resistant to erythromycin. Three serotypes—19F (47 isolates [46%]), 14 (27 isolates [26%]), and 6B (12 isolates [12%])—accounted for 86 (84%) of these 102 isolates. Seventy four (73%) isolates had the macrolide-lincosamide-streptogramin B (MLS_B) resistance phenotype and carried Tn*916* transposons (most commonly Tn*6002*); of these, 73 (99%) contained the erythromycin ribosomal methylase gene [*erm*(B)], 34 (47%) also carried the macrolide efflux gene [*mef*(E)], and 41 (55%) belonged to serotype 19F. Of 28 (27%) isolates with the M phenotype, 22 (79%) carried *mef*(A), including 16 (57%) belonging to serotype 14, and only six (19%) carried Tn*916* transposons. Most (84%) isolates which contained *mef* also contained one of the *msr*(A) homologues, *mel* or *msr*(D); 38 of 40 (95%) isolates with *mef*(E) (on *mega*) carried *mel*, and of 28 (39%) isolates with *mef*(A), 10 (39%) carried *mel* and another 11(39%) carried *msr*(D), on Tn*1207.1*. Two predominant macrolide-resistant *S. pneumoniae* clonal clusters (CCs) were identified in this population. CC-271 contained 44% of isolates, most of which belonged to serotype 19F, had the MLS_B phenotype, were multidrug resistant, and carried transposons of the Tn*916* family; CC-15 contained 23% of isolates, most of which were serotype 14, had the M phenotype, and carried *mef*(A) on Tn*1207.1*. Erythromycin resistance among *S. pneumoniae* isolates in New South Wales is mainly due to the dissemination of multidrug-resistant *S. pneumoniae* strains or horizontal spread of the Tn*916* family of transposons.

Streptococcus pneumoniae is an important cause of respiratory tract infections, bacteremia, and meningitis, for which antibiotic treatment is often difficult because of resistance to penicillin and other antibiotics, especially macrolides. During the last decade, macrolide resistance among *S. pneumoniae* isolates has increased, with considerable geographical variation among the genotypes and phenotypes involved (3, 14, 17, 34, 35).

Macrolide resistance in *S. pneumoniae* is mediated by two main mechanisms. Target modification due to a ribosomal methylase, encoded by *erm*(B) confers high-level resistance to macrolides, lincosamides, and streptogramin B (MLS_B phenotype). In *S. pneumoniae* and related *Streptococcus* spp., the frequent association of erythromycin and tetracycline resistance is often related to insertion of *erm*(B) into a conjugative transposon of the Tn*916* family that harbors *tet*(M) and carries integrase (*int*) and excisase (*xis*) genes. Members of this family, which carry *erm*(B), include Tn*6002*, Tn*1545* (which also carries the kanamycin resistance gene *aphA3*), and Tn*3872* (which also carries transposase genes *tnpA* and *tnpR*) (2, 8).

The second macrolide resistance mechanism is an efflux pump system encoded by *mef* which confers resistance to 14- and 15-member macrolides only (M phenotype) (22). The two main subclasses of *mef* in *S. pneumoniae*, *mef*(E) and *mef*(A), are carried on different, but related elements: *mef*(A) on the defective transposon Tn*1207.1* (32, 33) or the closely related Tn*1207.3* and *mef*(E) on an element named "macrolide efflux genetic assembly" (*mega*) (11, 19). Both of these elements carry an open reading frame downstream of *mef*, designated *msr*(D) (9, 10) or *mel* (1, 11, 19), which are homologues of *msr*(A), which codes for an ATP-dependent efflux pump in *Staphylococcus* spp. (31). *msr*(D) and *mel* are cotranscribed with *mef* and contribute to an erythromycin-inducible dual efflux system in *S. pneumoniae* (1, 9, 19). Different investigators have reported and named these *msr*(D) homologues separately, and it is not clear whether or not they are identical.

The prevalence of isolates carrying both *mef* and *erm*(B) has reportedly increased as a result of the worldwide spread of a limited number of multidrug-resistant clonal complexes (CCs), of which the most prevalent is Taiwan^{19F}-14 (CC-271) (15). Recently, two new composite elements of the Tn*916* family, containing *tet*(M) plus *mega* (Tn*2009*) and *tet*(M), *erm*(B), and *mega* (Tn*2010*), have been described (10, 12). The distribution of these transposons and the genes they carry also vary in

* Corresponding author. Mailing address: Centre for Infectious Diseases and Microbiology (CIDM), Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Darcy Road, Westmead, New South Wales, 2145 Australia. Phone: (612) 9845 6255. Fax: (612) 9893 8659. E-mail: l.gilbert@usyd.edu.au.

† X. Xu, L. Cai, and M. Xiao contributed equally to the work.

▽ Published ahead of print on 11 January 2010.

TABLE 1. Oligonucleotide primers used in this study

Primer[a]	GenBank accession no.	T_m (°C)[b]	Sequence (5′→3′)[c]	Source or reference
tetm-Sb	X90939	60.69	2668TCCGGTAAATCATTAGAAGCATT2690	This study
tetm-Ab	X90939	61.62	3101TGTGGCAATAGYTTTGTATCTCC3079	This study
teto-Sb	Y07780	61.34	121TCACATGAAAATAATTAACTTAGGCA146	This study
teto-Ab	Y07780	60.02	683CTAATAGTTCATCGTTTCCCATAAT659	This study
int-Sb	U09422	60.52	16854CATGATGGTATTGATGTTGTAGG16876	37
int-Ab	U09422	60.41	17386TGATGGTCTATATTGACAAGACG17364	37
tnpR-01-Upb	AM490850	64.94	6642CCAAGGAGCTAAAGAGGTCCC6662	2
tnpR-01-Dnb	AM490850	62.46	6937TACTCACTCGAGCATAGCCAA6917	This study
tnpR-02-Upb	AM490850	61.61	7936TTTCCATCTATAGCTACACTTGAAGA7962	This study
tnpR-02-Dnb	AM490850	70.18	8189GTCCCGAGTCCCATGGAAGC8170	2
tnpA-01-Upb	AM490850	61.67	8190AATTAATGTCTCCCATATTAATCGG8214	This study
tnpA-02-Dnb	AM490850	62.37	8476CATCAATTAAAGAAGCATAATGTTCC8451	This study
tnpA-02-Upb	AM490850	63.06	10031CAGATAGTGAGCTACGGCGA10050	This study
tnpA-02-Dnb	AM490850	63.87	10318CAAGAAAAGTGATATGCTCCCAA10296	This study
xis-forb	X61025	60.29	230ATGAAGCAGACTGACATTCCTA251	This study
xis-revb	X61025	61.03	433CTAGATTGCGTCCAATGTATCTATAA408	This study
ermb-Sb	M11180	59.84	828GGTAAAGGGCATTTAACGAC847	37
ermb-Ab	M11180	59.44	1321CGATATTCTCGATTGACCC1303	37
mefE/A-Sb	AF227521	63.41	3314GGCAGGGCAAGCAGTATC3331	37
mefE/A-Ab	AF227521	59.76	3674CTGTTCTTCTGGTACTAAAAGTGG3651	37
msrD-Sb	AF227520	62.98	5463CCATAATCCATACCCTATAGTCGG5486	This study
msrD-Ab	AF227520	60.65	5645GAAATAGAAATTCCTTCTTCATGG5622	This study
mel/mefI-Sb	AF376746	61.77	3306GAACAATTTATTGCGGAACG3325	This study
mel/mefI-Ab	AF376746	61.90	3661TGAAAAGATGCATTTTCAAACA3640	This study
aphA3-Sb	AF060241	63.77	854TGCCTGTTCCAAAGGTCC871	38
aphA3-Ab	AF060241	59.41	1407TTTTATTTTCTCCCAATCAGG1387	38
cat-Sb	V01277	61.16	1318ATTTGAACCAACAAACGACTTT1339	This study
cat-Ab	V01277	60.08	1657GGTGTTTTGGGAAACAATTT1638	This study

[a] Biotin-labeled primer.
[b] T_m, melting temperatures provided by manufacturer.
[c] Numbers relate to positions in GenBank sequences.

different geographic regions (4, 9) and provide a clue to the origins of antibiotic-resistant strains of *S. pneumoniae.*

This study is the first to analyze the distribution of antibiotic susceptibility patterns and phenotypic and genotypic characteristics of erythromycin-resistant invasive *S. pneumoniae* isolates in Australia, including the transposons on which resistance genes are carried. The isolates studied had been referred to the Pneumococcal Reference Laboratory at the Centre for Infectious Diseases and Microbiology, which receives all sterile-site isolates from patients with invasive pneumococcal disease, in New South Wales, for serotyping (30). The 7-valent pneumococcal conjugate vaccine (PCV7) first became available in Australia in 2001, with limited uptake. It was introduced into the routine infant immunization schedule in January 2005 as a 3-dose regimen given at 2, 4, and 6 months.

MATERIALS AND METHODS

Isolates, identification, and antibiotic susceptibility testing. As part of a detailed study of antibiotic resistance in invasive *S. pneumoniae*, before the widespread use of conjugate vaccine, all 328 invasive (mainly blood culture) isolates, from children less than 5 years old referred in 2005, were tested for susceptibility to 15 antibiotics.

S. pneumoniae isolates were identified by bile solubility and optochin susceptibility and were serotyped by the Quellung reaction, using antisera provided by the Statens Serum Institute (Copenhagen, Denmark). Antimicrobial susceptibility testing was performed by the broth microdilution method using Sensititre Microtiter Trays (Trek Diagnostics Systems, West Sussex, England) and cation-adjusted Mueller-Hinton broth supplemented with 3 to 5% lysed horse blood (28). The results were interpreted according the Clinical and Laboratory Standards Institute (CLSI) criteria, using penicillin breakpoints in place before they were changed in 2008 (susceptible, ≤0.06 mg/liter; intermediate, 0.12 to 1 mg/liter; and resistant, ≥2 mg/liter) (6). *S. pneumoniae* ATCC 49619 was used for quality control. Isolates for which the erythromycin MIC was ≥2 mg/liter were selected for further study.

Phenotypic characterization of macrolide resistance, for erythromycin-resistant/clindamycin-susceptible (MIC, <2 mg/liter) isolates was performed by the double-disk diffusion method. The clindamycin disk was placed approximately 22 mm from the edge of the erythromycin disk (7); after incubation, organisms that showed flattening of the clindamycin zone adjacent to the erythromycin disk ("D zone") were interpreted as having inducible resistance ($iMLS_B$ phenotype), whereas those with a conserved inhibition zone around the clindamycin disk were considered to have the M phenotype. Isolates resistant to both erythromycin and clindamycin by broth dilution had the constitutive ($cMLS_B$) phenotype.

Detection of resistance and transposon genes. We used a multiplex PCR-based reverse line blot (mPCR/RLB) assay (21) to identify the presence of antibiotic resistance and transposon genes [*tet*(M), *tet*(O), *int*, *xis*, *tnpR*, *tnpA*, *erm*(B), *mef*(A), *mef*(E), *mef*(I), *mel*, *msr*(D), *cat*, and *aphA3*], as shown in Table 1. DNA was prepared as described previously (21). Briefly, five individual *S. pneumoniae* colonies were sampled using a disposable loop and resuspended in 0.2 ml digestion buffer (10 mM Tris-HCl, pH 8.0, 0.45% Triton X-100, and 0.45% Tween 20) in 2-ml Eppendorf tubes. The tubes containing *S. pneumoniae* suspension were heated at 100°C (dry block heater) for 10 min and then cooled on ice and centrifuged for 2 min at 13,000 × *g* to pellet the cell debris. A 2-μl aliquot of each supernatant containing extracted DNA was used as a template for mPCR.

Some primers and probes from a previous study (37) were used, and new primers and probes were designed or modified based on published sequences in GenBank (2) so that they could be amplified, without interference, in a single mPCR reaction (21) (Tables 1 and 2). The mPCR system contained the following: 2 μl template DNA, 0.1 μl each forward (50 pmol/μl) and reverse (50 pmol/μl) primers, 2 μl deoxynucleoside triphosphates (dNTPs; 2.5 mM each dNTP), 2.5 μl 10× PCR buffer, 0.2 μl Qiagen HotStar *Taq* polymerase (5 U/μl), and water to 25 μl. PCR was performed according to the Qiagen Hotstar *Taq* polymerase kit instructions: 95°C for 15 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; 72°C for 10 min; and 22°C hold.

TABLE 2. Oligonucleotide probes used in this study

Probe[a]	GenBank accession no.	T_m (°C)[b]	Sequence (5′→3′)[c]	Source or reference
tetm-AP	X90939	61.70	2711CTTTCCTCTTGTTCGAGTTCC2691	This study
tetm-SP	X90939	61.46	3049AATGAGTTTTTGAAGTTAAATAGTGTTCTT3078	This study
teto-AP	Y07780	63.87	164CGTCAACGTGAGCCAGAA147	This study
teto-SP	Y07780	62.20	641GAACAGTGGGATGCGGTA658	This study
int-AP	U09422	60.64	16905CGTAAAGCTGGCAGAGTGT16887	37
int-SP	U09422	59.02	17321AGAGTTTGGTGGTTTGACAC17340	37
xis-AP	X61025	63.49	274GGTTAGGGTATAACGTTCCCAAA252	This study
xis-SP	X61025	63.14	368GGCAATCGTATTCAGATTAAACG390	This study
tnpR-AP	AM490850	67.79	6682CAAATTCCCCGTAGGCGCTA6663	This study
tnpR-SP	AM490850	61.75	8149TGTAAAAAAGTGGAAGTGATTGC8171	This study
tnpA-AP	AM490850	62.05	8239GTCTAGCTAGCTGAAGAAACCTGTT8215	This study
tnpA024-SP	AM490850	61.54	10261GTAACTAAGTATATGAAGCATGTATCTCCT10290	This study
ermb-AP	M11180	58.50	876TTACCTGTTTACTTATTTTAGCCAG852	37
ermb-SP	M11180	61.98	1236CTTACCCGCCATACCACA1253	37
mef(A/E)-AP	AF227521	60.04	3353CAAGATGGCACTAGTGATTAATG3331	37
mef(A)-SP	AF227521	60.04	3632GGCTCTCAATGCGGTTAC3649	37
msrD-AP	AF227520	61.99	5524ACAGTGCCTTATCCCCAAATA5504	This study
msrD-SP	AF227520	61.61	5580TAATGGAACCGGAAAAACAA5599	This study
mel-AP	AF376746	61.05	3493AGGGTTTTAGCAGCATTATACATC3470	This study
mel-SP	AF376746	60.58	3601TCGGTGCAGAAATTAATAAAGTATT3625	This study
mefI-AP	AJ971089	62.80	2397GGGATTTAACGGCATTATGC2378	This study
mefI-SP	AJ971089	60.64	2506TCGGTACGGAAATTACTAAAATATTT2531	This study
aphA3-SP	AF060241	59.63	891ATCATGCCGTTCAAAGTG874	38
aphA3-AP	AF060241	59.72	1341GGAAGAACAGTATGTCGAGC1360	38
cat-AP	V01277	60.72	1372AAAACACTAATATCAATTTCTGTGGTT1346	This study
cat-232-SP	V01277	65.55	1616GGTTATTGGGATAAGTTAGAGCCACTTTAT1637	This study

[a] Amine-labeled probe.
[b] T_m, melting temperatures provided by manufacturer.
[c] Numbers relate to positions in GenBank sequences.

Reverse line blot (RLB) hybridization. The RLB hybridization assay was based on a method described previously (21), except that the hybridization temperature was 60°C and the time of exposure to X-ray film (Hyperfilm; Amersham) was 15 min. RLB results were regarded as positive when both probes for a particular sequence gave positive signals. To optimize hybridization conditions, the probes were tested at several 2-fold dilutions, starting at a concentration of 1.2 pM and with final labeling concentrations of between 5.0 and 10 pM (21). Any discrepancies between phenotypic susceptibility to erythromycin or tetracycline and RLB results were confirmed by Etest (AB Biodisk, Solna, Sweden) and single-gene-specific PCR [for *erm*(B), *mef*(A), and *mef*(E) or *tet*(M) and *tet*(O), respectively] (37).

MLST analysis. Multilocus sequence typing (MLST) was performed as described previously (37). The seven genes targeted were *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*. Clusters of related sequence types (STs) were grouped into clonal complexes (CCs) using the eBURST program (http://www.mlst.net).

Statistical analysis. All statistical analyses were performed using SPSS version 11.0 (SPSS, Chicago, IL). The χ^2 test was used to compare dichotomous variables.

RESULTS

Susceptibility phenotypes of erythromycin-resistant *S. pneumoniae*. One hundred two of the 328 (31%) *S. pneumoniae* isolates tested were resistant to erythromycin (MIC, ≥2 mg/liter). A summary of susceptibilities of these isolates to the 15 antibiotics tested is shown in Table 3. All isolates were susceptible to the three fluoroquinolones levofloxacin, gatifloxacin, and moxifloxacin and to vancomycin and linezolid, but the majority were resistant (MIC, ≥2 mg/liter) or of intermediate susceptibility (MIC, 0.12 to 1 mg/liter) to penicillin (80 isolates [78%]), tetracycline (75 isolates [74%]), and trimethoprim-sulfamethoxazole (79 isolates [77%]). Eighty-four isolates (82%) were multidrug resistant (i.e., resistant to three or more classes of antibiotic), which is significantly higher than the proportion (50/226 [22%]) which was resistant to penicillin among erythromycin-susceptible isolates in this set ($P < 0.0001$) (data not shown).

Seventy-four (73%) isolates expressed the MLS_B phenotype, including 69 that were constitutively resistant to clindamycin and five that demonstrated inducible resistance in the D-test (Table 4). Of these 67 (91%) and 68 (92%) were resistant to penicillin and tetracycline, respectively. There were nine different resistance profiles among these 74 isolates, but two (Pen^r Ery^r Cli^r Chl^r SXT^r Tet^r [$n = 23$] and Pen^r Ery^r Cli^r Chl^s SXT^r Tet^r [$n = 31$]) were represented by 54 (73%) isolates; of these, 36 (67%) belonged to serotype 19F.

Twenty-eight (27%) isolates expressed the M phenotype. Of these, 10 (37%) were resistant to penicillin and nine (32%) to tetracycline. There were seven different antibiotic resistance profiles: one, Pen^s Ery^r Cli^s Chl^s SXT^s Tet^s, accounted for 15 (54%) isolates, 14 of which were serotype 14.

There were 9 serotypes and 28 sequence types (STs) among these 102 erythromycin-resistant isolates. Three serotypes, 19F ($n = 47$), 14 ($n = 27$), and 6B ($n = 12$), accounted for 84% of isolates. Among isolates with the MLS_B phenotype, serotypes 19F (41/74 [55%]) and 6B (12/74 [16%]) were predominant, whereas serotype 14 was the most common among those with the M phenotype (16/28; 57%). Three STs (ST-320 [$n = 28$], ST-352 [$n = 10$], and ST-9 [$n = 16$]) accounted for 53% of the erythromycin-resistant isolates (Fig. 1). Among 41 penicillin-resistant, serotype 19F isolates with the MLS_B phenotype, ST-320 (25 isolates [61%]) and ST-352 (10 isolates [24%]) were predominant. ST-9 was predominant (15/16 isolates [94%]) among serotype 14 isolates with the M phenotype. The distri-

TABLE 3. Antimicrobial susceptibilities of 102 erythromycin-resistant *S. pneumoniae* strains

Antibiotic	MIC (μg/ml)[a]			No. (%) of strains		
	50%	90%	Range	Susceptible	Intermediate	Resistant
Penicillin[b]	2	4	<0.03–>8	25 (24.5)	7 (6.9)	70 (68.6)
Amoxicillin-clavulanic acid	<2/1	>8/4	<2/1–8/4	68 (66.7)	5 (4.9)	29 (28.4)
Cefotaxime	1	2	<0.06–>2	64 (62.7)	32 (31.4)	6 (5.9)
Tetracycline	≥8	≥8	<0.5–>8	25 (24.5)	1 (1.0)	76 (74.5)
Chloramphenicol	4	8	<2–>16	68 (66.7)	0 (0)	34 (33.3)
Levofloxacin	1	1	<0.5–1	102 (100.0)	0 (0)	0 (0)
Gatifloxacin	<0.5	<0.5	<0.5	102 (100.0)	0 (0)	0 (0)
Moxifloxacin	<0.25	<0.25	<0.25–1	102 (100.0)	0 (0)	0 (0)
Erythromycin	>2	>2	>2	0 (0)	0 (0)	102 (100.0)
Azithromycin	>2	>2	>2	0 (0)	0 (0)	102 (100.0)
Linezolid	1	1	0.5–2	102 (100.0)	0 (0)	0 (0)
Clindamycin	>2	>2	<0.06–>2	31 (30.4)	2 (2.0)	69 (67.6)
Vancomycin	<0.5	<0.5	<0.5–1	102 (100.0)	0 (0)	0 (0)
SXT[c]	>4/76	>4/76	<0.5/9.5–4/76	23 (22.6)	18 (17.6)	61 (59.8)
Meropenem	0.5	1	<0.25–1	34 (33.3)	33 (32.4)	35 (34.3)

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] Susceptible, intermediate, and resistant MIC breakpoints for penicillin were ≤0.06, 0.12-1, and ≥2 mg/liter, respectively.
[c] SXT, trimethoprim-sulfamethoxazole.

bution of serotypes and sequence types between phenotypic categories is shown in Table 4.

Antibiotic resistance and transposon-related genes of erythromycin-resistant *S. pneumoniae*. Among 78 (76%) of 102 erythromycin-resistant isolates that were also tetracycline resistant, four strains contained neither *tet*(M) nor *tet*(O): all were of the MLS_B phenotype—two were serotype 14 (ST-15 and ST-143) and two serotype 6B (ST-322 and ST-1645). One phenotypically susceptible isolate (MLS_B, serotype 19F, ST-271) carried *tet*(M). None of the 102 isolates contained *mef*(I), *cat*, or *tet*(O); four isolates contained *apha3* (serotype 14, ST-143 [3 isolates]; and nontypeable, ST-344 [1 isolate]).

erm(B) was identified in 73 of 74 (99%) isolates with the MLS_B phenotype, and of these, 40 (55%) also carried *mef*—34 *mef*(E) and 6 *mef*(A). Neither *erm*(B) nor *mef* was detected in the other isolate with the MLS_B phenotype (serotype 19F,

TABLE 4. Distribution of serotypes and sequence types among different antibiotic resistance phenotypic categories of 102 erythromycin-resistant *S. pneumoniae* isolates

Phenotype (*n*)	Penicillin resistance/ susceptibility (*n*)[a]	Serotype (*n*)	ST (*n*)
Constitutive MLS_B (69)	R (65)	19F (39)	236 (1), 242 (1), 271 (1), 320 (24), 352 (10), 4234 (1), 4235 (1)
		14 (11)	15 (6), 143 (3), 230 (1), 1492 (1)
		6B (6)	315 (4), 322 (1), 4233 (1)
		19A (2)	320 (2)
		23F (3)	242 (1), 342 (1), 880 (1)
		6A (2)	81 (1), 320 (1)
		NT (1)	90 (1)
		9V (1)	156 (1)
	S (4)	6B (3)	90 (1), 315 (1), 4236 (1)
		NT (1)	344 (1)
Inducible MLS_B (5)	R (2)	19F (2)	271 (1), 320 (1)
	S (3)	6B (3)	1645 (3)
M (28)	R (10)	19F (5)	9 (1), 81 (1), 242 (1), 651 (1), 2477 (1)
		23F (3)	81 (2), 242 (1)
		14 (1)	242 (1)
		19A (1)	156 (1)
	S (18)	14 (15)	9 (15)
		15B (1)	411 (1)
		19F (1)	33 (1)
		4 (1)	205 (1)

[a] S, susceptible; R, resistant. MLS_B resistance is either constitutive (always produced) or inducible following exposure to a macrolide (as shown by D-test [see text]).

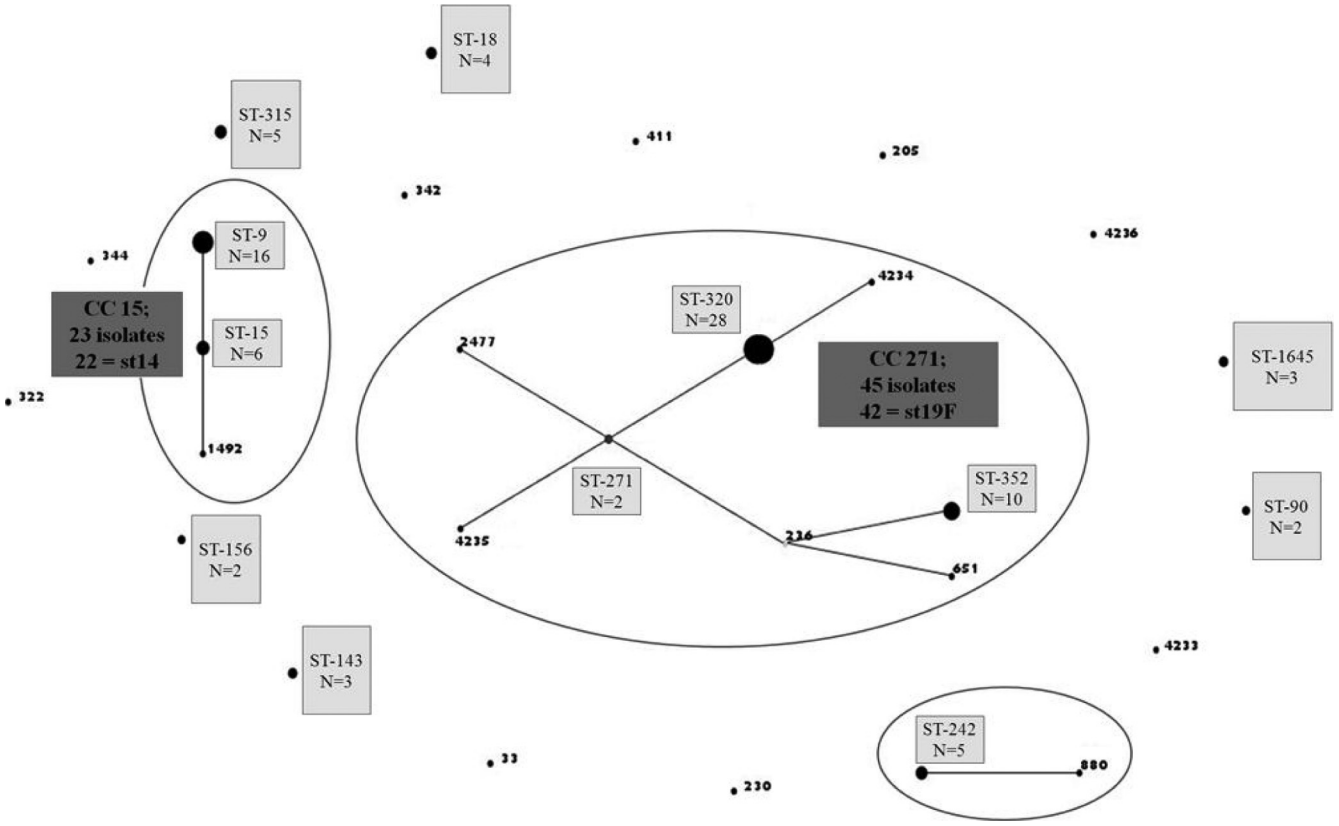

FIG. 1. Population structure of 102 erythromycin-resistant *S. pneumoniae* isolates. Relationships between sequence types (ST) of 102 erythromycin-resistant *S. pneumoniae* isolates were demonstrated using eBURST v3. Three groups were defined using stringent criteria (6/7 shared alleles). Two were identified as clonal complexes (CC): CC-271 and CC-15. The predicted clonal ancestor of CC-271 is shown in blue (ST-271), and a subgroup founder is shown in yellow (ST-236). Most of the isolates in each of the CCs belonged to single serotypes (st): CC-271, serotype 19F, and CC-15, serotype 14.

ST-352). All isolates with the M phenotype carried *mef*—22 *mef*(A) and 6 *mef*(E). Three also carried *erm*(B). Sixteen of 22 (79%) M phenotype isolates with *mef*(A) belonged to serotype 14. Overall, 36 of 102 (35%) isolates carried both *erm*(B) and *mef*(E). Of these isolates, 29 (81%) belonged to serotype 19F and 33 (92%) carried Tn*6002*.

All 74 isolates with the MLS_B phenotype and nine of 28 isolates with the M phenotype carried transposons belonging to the Tn*916* family: 49 carried Tn*6002* [characterized by *int*, *xis*, *tet*(M), and *erm*(B)], and four carried a variant without *tet*(M). Fifty-one of these 53 isolates had the MLS_B phenotype: 31 carried *mef*(E), and 30 belonged to serotype 19F. Three isolates carried Tn*1545* [*int*, *xis*, *tet*(M), and *erm*(B), plus *aphA*3], 15 carried Tn*3872* [*int*, *xis*, *tnpA*, *tnpR*, *tet*(M), and *erm*(B)], and another 5 carried a variant without *tet*(M)—all but one of these 20 isolates had the MLS_B phenotype.

Of 68 isolates with either *mef*(A) or *mef*(E), 59 (87%) carried one of the *msr*(A) homologues: 38/40 (95%) with *mef*(E) and 10/28 (36%) with *mef*(A) carried *mel*, and 11/28 (39%) with *mef*(A) carried *msr*(D). The distributions of antibiotic resistance genes and transposon markers among different serotypes and sequence types are shown in Table 5.

MLST of erythromycin-resistant *S. pneumoniae*. The distribution of STs among isolates with different antibiotic resistance phenotypes and genotypes is shown in Tables 4 and 5. Among 74 isolates with the MLS_B phenotype, which carried *erm*(B) and *mef*(E) on Tn*6002* or Tn*3872*, 37 (50%) belonged to ST-320 and ST-352, and of these, 34 belonged to serotype 19F (Table 5). Among the remaining 13 serotype 19F isolates, there were 11 STs, including two new ones, ST-4234 and ST-4235, which were single-nucleotide variants of ST-320 and ST-236, respectively.

Twelve serotype 6B isolates were distributed among six STs: five isolates for ST-315; three isolates for ST-1645; one isolate each for ST-322 and ST-90; and one isolate each for the two new STs, ST-4233 and ST-4236.

Among 28 M phenotype isolates, there were nine sequence types; one (ST-9), which carries *mef*(A)—and usually *msr*(D)—on Tn*1207.1* and belongs to serotype 14, accounted for 15 (57%) of these isolates. Among 27 serotype 14 isolates, 15 belonged to ST-9, six to ST-15, and three to ST-143, and the remaining three isolates each belonged to a different ST.

The 28 STs generated in this data set were separated by eBURST into two CCs, one doublet, and 15 singletons (Fig. 1). The largest clonal cluster was CC-271; 45 of 102 (44%) isolates belonged to this cluster, and of these, 42 belonged to serotype 19F, corresponding with the widely distributed antibiotic-resistant strain, Taiwan^{19F}-14 (CC-271). This CC was represented by eight STs (ST-271, ST-236, ST-320, ST-2477, ST-352, ST-651, ST-4234, and ST-4235, of which ST-271

TABLE 5. Antibiotic resistance and transposon genes identified among 102 erythromycin-resistant *S. pneumoniae* isolates

Isolate type	Antibiotic resistance and transposon gene profile[a]								Presumed transposon[b]	Serotype (*n*) and ST/CC (*n*)[c]
	tet(M)/*int*/*xis*	*tnpA*/*tnpR*	*erm*(B)	*mef*(E)	*mef*(A)	*msr*(*D*)	*mel*	*aphA3*		
MLS_B	−/+/+	+/+	+	−	−	−	−	−		Serotype 14 (5), ST-15 (5)
	+/+/+	−/−	+	−	−	−	−	−	Tn*6002*	Serotype 6B (8), ST-1645 (2), ST-4236 (1), and ST-315 (5); serotype 23F (1), ST-342 (1); serotype NT (1), ST-90 (1); serotype 14 (2), ST-230 (1) and ST-1492 (1)
	+/+/+	−/−	+	−	−	−	−	+	Tn*1545*	Serotype 14 (2), ST-143 (2); serotype NT (1), ST-344 (1)
	+/+/+	+/+	+	-	-	-	-	-	Tn*3872*	Serotype 19F (7), ST-352 (7); serotype 23F (1), ST-242 (1)
	+/+/+	+/+	+	−	−	+	−	−	Tn*3872*	Serotype 6B (1), ST-4233 (1)
	−/+/+	−/−	+	−	−	−	−	−		Serotype 6B (2), ST-1645 (1) and ST-322 (1); serotype 14 (1), ST-15 (1)
	−/+/+	−/−	+	−	−	−	−	+		Serotype 14 (1), ST-143 (1)
	+/+/+	−/−	−	−	−	−	−	−	Tn*916*	Serotype 19F (1), ST-352 (1)
	+/+/+	−/−	+	−	+	−	+	−	Tn*6002* + Tn*1207.1*	Serotype 19A (1), ST-320 (1); serotype 19F (3), ST-320 (2) and ST-4234 (1)
	+/+/+	+/+	+	−	+	−	−	−	Tn*3872* + Tn*1207.1*	Serotype 19F (2), ST-352 (2)
	+/+/+	−/−	+	+	−	−	+	−	Tn*6002* + *mega*	Serotype 6A (2), ST-320 (1) and ST-81 (1); serotype 19A (1), ST-320 (1); serotype 19F (27), CC-271 (27)
	+/+/+	−/−	+	+	−	−	−	−	Tn*6002* + *mega*	Serotype 6B (1), ST-90 (1)
	+/+/+	+/+	+	+	−	−	+	−	Tn*3872* + *mega*	Serotype 9V (1), ST-156 (1); serotype 19F (1), ST-242 (1)
	+/+/+	+/+	+	+	−	−	−	−	Tn*3872* + *mega*	Serotype 23F (1), ST-880 (1)
M	+/+/+	−/−	+	+	−	−	+	−	Tn*6002* + *mega*	Serotype 19F (1), ST-2477 (1); serotype 23F (1), ST-242 (1)
	+/+/+	−/−	−	+	−	−	+	−	Tn*916* + *mega* or Tn*2009*	Serotype 19F (1), ST-651 (1); serotype 23F (1), ST-81 (1)
	−/−/−	−/−	−	+	−	−	+	−	*mega*	Serotype 19F (2), ST-33 (1) and ST-9 (1)
	−/−/−	−/−	−	−	+	+	−	−	Tn*1207.1*	Serotype 14 (11), ST-9 (11)
	−/−/−	−/−	−	−	+	−	−	−	Tn*1207.1*	Serotype 14 (4), ST-9 (4)
	−/−/−	−/−	−	−	+	−	+	−	Tn*1207.1*	Serotype 19A (1), ST-156 (1); serotype 15B (1), ST-411 (1)
	+/+/+	+/+	+	−	+	−	+	−	Tn*3872* + Tn*1207. 1*	Serotype 4 (1), ST-205 (1)
	+/+/+	−/−	−	−	+	−	+	−	Tn*916* + Tn*1207.1*	Serotype 19F (2), ST-242(1) and ST-81 (1); serotype 23F (1), ST-81 (1)
	+/+/+	−/−	−	−	+	−	−	−	Tn*916* + Tn*1207.1*	Serotype 14 (1), ST-242 (1)

[a] +, positive; −, negative.
[b] Transposons were identified by the following markers: Tn*916*, *tet*(M), *int*, and *xis*; Tn*6002*, *tet*(M), *int*, *xis*, and *erm*(B); Tn*1545*, *tet*(M), *int*, *xis*, *erm*(B), and *aphA*3; Tn*3872*, *tet*(M), *int*, *xis*, *tnpA*, *tnpR*, and *erm*(B); Tn*1207.1*, *mef*(A); *mega*, *mef*(E); and Tn*2009*, *tet*(M), *int*, *xis*, and *mef*(E).
[c] NT, nontypeable; ST, sequence type; CC, clonal cluster.

was defined by eBURST as the founder of the CC). The next largest CC was CC-15, comprising 23 isolates, of which 22 belonged to serotype 14. This CC comprised three STs (ST-15, ST-9, and ST-1492), of which ST-15 was identified as the founder. There was one doublet containing five isolates belonging ST-242 and ST-880. All of the remaining isolates belonged to individual STs that were not closely related to others identified in this study.

Antibiotic resistance genes and transposons carried by erythromycin-susceptible *S. pneumoniae*. Of the original 328 *S. pneumoniae* isolates tested, 226 were phenotypically sensitive to erythromycin. Of these, four were tetracycline resistant and all carried *tet*(M), *int*, and *xis*. Their serotypes were (one each of) 6B, 19A, 23F, and 14; one isolate (serotype 14) was also clindamycin resistant and carried *erm*(B). Another four erythromycin- and clindamycin-sensitive isolates carried *mef*(E) or *mef*(A), and one carried *msr*(D); of these isolates, two belonged to serotype 14 and one each belonged to serotypes 6B and 6A.

DISCUSSION

Erythromycin-resistant *S. pneumoniae* strains are already distributed worldwide: their numbers appear to be increasing rapidly, and resistance is expanding to include multiple antimicrobial agents (20). There is considerable geographic variation in prevalence, from 30 to 55% in France, Spain, South Africa, the United States, and Asia to as low as 4 to 7% in parts of northern and western Europe (e.g., the Czech Republic, the Netherlands, and Sweden) (35). Differences in erythromycin resistance are believed to reflect variations in macrolide consumption (European Surveillance of Antibiotic Consumption; http://www.ua.ac.be/esac) and the spread of multidrug-resistant clones.

This is the first study of genotypes among invasive pneumococcal isolates from young children in Australia. In 2005, before the widespread use of pneumococcal conjugate vaccine, 31% of invasive isolates (mainly from blood cultures), were erythromycin resistant; 82% of erythromycin-resistant isolates were multidrug resistant, compared with only 22% of those that were erythromycin susceptible.

The MLS_B phenotype is predominant in most European countries, whereas the M phenotype predominates in North America, England, and Germany (13, 18, 24, 29). Although the MLS_B phenotype was the more common in New South Wales, a substantial minority of isolates had the M phenotype (27%). Serotypes 19F and 14 accounted for more than half of the MLS_B and M phenotype isolates, respectively.

Worldwide, the proportion of *S. pneumoniae* isolates harboring both *erm*(B) and *mef*(E) genes is increasing (15, 25) and is associated with the clonal dissemination of the Taiwan^{19F}-14 (ST-237) clone and, less frequently, the Taiwan^{23F}-15 and Spain^{23F}-1 clones (16). None of the isolates in our study belonged to ST-237, but two single-locus variants, ST-320 and ST-352, together represented 51% of isolates, and of these, most belonged to serotype 19F and carried *erm*(B) and *mef*(E) on Tn*6002* or Tn*3872*. eBURST analysis identified them as belonging to CC-271, which is closely related to ST-237. Two isolates carrying both *erm*(B) and *mef*(E) showed unusual serotype-sequence type combinations—6A and ST-81 and 6A and ST320. The serotype 6A–ST-81 combination has been described previously in Korea (http://www.mlst.net), but we have been unable to find any previous examples of the 6A–ST-320 combination. We assume this represents a "serotype switch" from 19F to 6A.

Another of the international clones identified by the Pneumococcal Molecular Epidemiology Network (26), which carries *mef*(A) and belongs to serotype 14 and ST-9 (13, 15), is a major contributor to the worldwide dissemination of M phenotype erythromycin resistance (http://www.mlst.net). Isolates with the same allelic profile and resistance pattern as England^{14}-9 were the most frequently identified among isolates with the M phenotype in this study. eBURST analysis showed that they were in CC-15. ST-9 is a single-locus variant of ST-15.

Most of the isolates that contained either *mef*(E) or *mef*(A) contained one of the *msr*(A) homologues *msr*(D) or *mel.* These elements appear to be closely related variants with about 98% homology, but based on sequence heterogeneity between available sequences in GenBank, we were able to design primers and probes specific for each. There was a difference in their distribution between isolates carrying *mef*(E) (carried on *mega*), most of which were associated with *mel*, and those carrying *mef*(A) (carried on Tn*1207.*1), which were associated with *msr*(D) (all serotype 14–ST-9 isolates) and *mel* (belonging to various serotypes and STs); *mel* and *msr*(D) were not identified together. Presumably this indicates that *mel*, at least, can be carried on either *mega*, with *mef*(E), or Tn*1207.1* with *mef*(A). However, the differences in distribution between genotypes and transposons suggest that they are distinct. Previous studies have shown that they are cotranscribed with *mef*, including the newly described variant *mef*(I) (27), which together act as an erythromycin-inducible dual-efflux system (19). However, it appears that the *msr*(D)/*mel*-encoded efflux system can also function independently (1, 9).

Most *S. pneumoniae* isolates with *erm*(B)-mediated erythromycin resistance are also tetracycline resistant, because they result from the insertion of *erm*(B) into conjugative transposons of the Tn*916* family, which typically carry *tet*(M) (5, 8, 23). For example, the composite transposon Tn*3872* is formed by the insertion of the *erm*(B) and *mef*(E) on transposable element Tn*917* (which also carries *tnpA* and *tnpR*) (10, 23). Among the 102 isolates studied, 66% harbored both *erm*(B) and *tet*(M). Most of these carried either Tn*6002* or Tn*3872*. In common with others (23), we found a low prevalence of Tn*1545*, a Tn*916* transposon that, in addition to *erm*(B), also carries *aphA3* (36).

The finding of one clindamycin-resistant and erythromycin-susceptible isolate is unusual. The isolate carried *erm*(B), and we assume that erythromycin resistance was not expressed, but there are other uncommon mechanisms of clindamycin resistance in *Streptococcus agalactiae* isolates (37), which, presumably, could also occur in *S. pneumoniae.*

In conclusion, the high prevalence of erythromycin-resistant *S. pneumoniae* strains among isolates from children in New South Wales, before the widespread use of pneumococcal conjugate vaccine, was mainly due to the dissemination of multiresistant *S. pneumoniae* strains and to the horizontal spread of the Tn*916* family of transposons. This study is the first of its kind in Australia and will provide a valuable baseline from which to monitor changes in the prevalence of vaccine serotypes and antibiotic resistance following introduction of the vaccine.

ACKNOWLEDGMENT

This study was funded, in part, by NHMRC grant 358351 (2005).

REFERENCES

1. **Ambrose, K. D., R. Nisbet, and D. S. Stephens.** 2005. Macrolide efflux in *Streptococcus pneumoniae* is mediated by a dual efflux pump (*mel* and *mef*) and is erythromycin inducible. Antimicrob. Agents Chemother. **49:**4203–4209.
2. **Brenciani, A., A. Bacciaglia, M. Vecchi, L. A. Vitali, P. E. Varaldo, and E. Giovanetti.** 2007. Genetic elements carrying *erm*(B) in *Streptococcus pyogenes* and association with *tet*(M) tetracycline resistance gene. Antimicrob. Agents Chemother. **51:**1209–1216.
3. **Brown, S. D., D. J. Farrell, and I. Morrissey.** 2004. Prevalence and molecular analysis of macrolide and fluoroquinolone resistance among isolates of *Streptococcus pneumoniae* collected during the 2000–2001 PROTEKT US Study. J. Clin. Microbiol. **42:**4980–4987.
4. **Calatayud, L., C. Ardanuy, E. Cercenado, A. Fenoll, E. Bouza, R. Pallares, R. Martin, and J. Linares.** 2007. Serotypes, clones, and mechanisms of resistance of erythromycin-resistant *Streptococcus pneumoniae* isolates collected in Spain. Antimicrob. Agents Chemother. **51:**3240–3246.
5. **Clewell, D. B., S. E. Flannagan, and D. D. Jaworski.** 1995. Unconstrained bacterial promiscuity: the Tn*916*-Tn*1545* family of conjugative transposons. Trends Microbiol. **3:**229–236.
6. **CLSI.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, document M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **CLSI.** 2005. Performance standards for antimicrobial susceptibility testing. Fifteenth international supplement M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Courvalin, P., and C. Carlier.** 1987. Tn1545: a conjugative shuttle transposon. Mol. Gen. Genet. **206:**259–264.
9. **Daly, M. M., S. Doktor, R. Flamm, and D. Shortridge.** 2004. Characterization and prevalence of MefA, MefE, and the associated *msr*(D) gene in *Streptococcus pneumoniae* clinical isolates. J. Clin. Microbiol. **42:**3570–3574.
10. **Del Grosso, M., R. Camilli, F. Iannelli, G. Pozzi, and A. Pantosti.** 2006. The *mef*(E)-carrying genetic element (*mega*) of *Streptococcus pneumoniae*: insertion sites and association with other genetic elements. Antimicrob. Agents Chemother. **50:**3361–3366.
11. **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.

12. **Del Grosso, M., J. G. Northwood, D. J. Farrell, and A. Pantosti.** 2007. The macrolide resistance genes *erm*(B) and *mef*(E) are carried by Tn*2010* in dual-gene *Streptococcus pneumoniae* isolates belonging to clonal complex CC271. Antimicrob. Agents Chemother. **51:**4184–4186.
13. **Dias, R., and M. Canica.** 2004. Emergence of invasive erythromycin-resistant *Streptococcus pneumoniae* strains in Portugal: contribution and phylogenetic relatedness of serotype 14. J. Antimicrob. Chemother. **54:**1035–1039.
14. **Doern, G. V., S. S. Richter, A. Miller, N. Miller, C. Rice, K. Heilmann, and S. Beekmann.** 2005. Antimicrobial resistance among *Streptococcus pneumoniae* in the United States: have we begun to turn the corner on resistance to certain antimicrobial classes? Clin. Infect. Dis. **41:**139–148.
15. **Farrell, D. J., S. G. Jenkins, S. D. Brown, M. Patel, B. S. Lavin, and K. P. Klugman.** 2005. Emergence and spread of *Streptococcus pneumoniae* with *erm*(B) and *mef*(A) resistance. Emerg. Infect. Dis. **11:**851–858.
16. **Farrell, D. J., I. Morrissey, S. Bakker, L. Morris, S. Buckridge, and D. Felmingham.** 2004. Molecular epidemiology of multiresistant *Streptococcus pneumoniae* with both *erm*(B)- and *mef*(A)-mediated macrolide resistance. J. Clin. Microbiol. **42:**764–768.
17. **Felmingham, D., R. Canton, and S. G. Jenkins.** 2007. Regional trends in beta-lactam, macrolide, fluoroquinolone and telithromycin resistance among *Streptococcus pneumoniae* isolates 2001–2004. J. Infect. **55:**111–118.
18. **Fotopoulou, N., P. T. Tassios, D. V. Beste, S. Ioannidou, A. Efstratiou, E. R. Lawrence, J. Papaparaskevas, R. C. George, and N. J. Legakis.** 2003. A common clone of erythromycin-resistant *Streptococcus pneumoniae* in Greece and the UK. Clin. Microbiol. Infect. **9:**924–929.
19. **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.
20. **Klugman, K. P., and J. R. Lonks.** 2005. Hidden epidemic of macrolide-resistant pneumococci. Emerg. Infect. Dis. **11:**802–807.
21. **Kong, F., and G. L. Gilbert.** 2006. Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB)—a practical epidemiological and diagnostic tool. Nat. Protoc. **1:**2668–2680.
22. **Leclercq, R., and P. Courvalin.** 2002. Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **46:**2727–2734.
23. **McDougal, L. K., F. C. Tenover, L. N. Lee, J. K. Rasheed, J. E. Patterson, J. H. Jorgensen, and D. J. LeBlanc.** 1998. Detection of Tn*917*-like sequences within a Tn*916*-like conjugative transposon (Tn*3872*) in erythromycin-resistant isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **42:**2312–2318.
24. **McEllistrem, M. C., J. M. Adams, K. Shutt, L. T. Sanza, R. R. Facklam, C. G. Whitney, J. H. Jorgensen, and L. H. Harrison.** 2005. Erythromycin-nonsusceptible *Streptococcus pneumoniae* in children, 1999–2001. Emerg. Infect. Dis. **11:**969–972.
25. **McGee, L., K. P. Klugman, A. Wasas, T. Capper, and A. Brink.** 2001. Serotype 19F multiresistant pneumococcal clone harboring two erythromycin resistance determinants [*erm*(B) and *mef*(A)] in South Africa. Antimicrob. Agents Chemother. **45:**1595–1598.
26. **McGee, L., L. McDougal, J. Zhou, B. G. Spratt, F. C. Tenover, R. George, R. Hakenbeck, W. Hryniewicz, J. C. Lefevre, A. Tomasz, and K. P. Klugman.** 2001. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the Pneumococcal Molecular Epidemiology Network. J. Clin. Microbiol. **39:**2565–2571.
27. **Mingoia, M., M. Vecchi, I. Cochetti, E. Tili, L. A. Vitali, A. Manzin, P. E. Varaldo, and M. P. Montanari.** 2007. Composite structure of *Streptococcus pneumoniae* containing the erythromycin efflux resistance gene mefI and the chloramphenicol resistance gene catQ. Antimicrob. Agents Chemother. **51:** 3983–3987.
28. **Perez-Trallero, E., J. M. Marimon, M. Ercibengoa, M. J. Gimenez, P. Coronel, and L. Aguilar.** 2007. Antimicrobial susceptibilities of amoxycillin-non-susceptible and susceptible isolates among penicillin-non-susceptible *Streptococcus pneumoniae*. Clin. Microbiol. Infect. **13:**937–940.
29. **Rikitomi, N., P. S. Sow, K. Watanabe, D. S. Nunez, G. Martinez, and T. Nagatake.** 1996. Rapid increase of pneumococcal resistance to beta-lactam and other antibiotics in isolates from the respiratory tract (Nagasaki, Japan: 1975–1994). Microbiol. Immunol. **40:**899–905.
30. **Roche, P. W., V. L. Krause, M. Bartlett, D. Coleman, H. Cook, C. Davis, J. E. Fielding, R. Holland, C. Giele, R. Gilmour, R. Kampen, M. Brown, L. Gilbert, G. Hogg, D. Murphy, and a Pneumococcal Working Party of the Communicable Diseases Network.** 2006. Invasive pneumococcal disease in Australia, 2004. Commun. Dis. Intell. **30:**80–92.
31. **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.
32. **Santagati, M., F. Iannelli, C. Cascone, F. Campanile, M. R. Oggioni, S. Stefani, and G. Pozzi.** 2003. The novel conjugative transposon tn*1207.3* carries the macrolide efflux gene *mef*(A) in *Streptococcus pyogenes*. Microb. Drug Resist. **9:**243–247.
33. **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.
34. **Schito, G. C.** 2004. Resistance trends in *Streptococcus pneumoniae* (PROTEKT years 1–3 [1999–2002]). J. Chemother. **16**(Suppl. 6)**:**19–33.
35. **Schito, G. C., and D. Felmingham.** 2005. Susceptibility of *Streptococcus pneumoniae* to penicillin, azithromycin and telithromycin (PROTEKT 1999–2003). Int. J. Antimicrob. Agents **26:**479–485.
36. **Seral, C., F. J. Castillo, M. C. Rubio-Calvo, A. Fenoll, C. Garcia, and R. Gomez-Lus.** 2001. Distribution of resistance genes *tet*(M), aph3′-III, *cat*pC194 and the integrase gene of Tn*1545* in clinical *Streptococcus pneumoniae* harbouring *erm*(B) and *mef*(A) genes in Spain. J. Antimicrob. Chemother. **47:**863–866.
37. **Zeng, X., F. Kong, H. Wang, A. Darbar, and G. L. Gilbert.** 2006. Simultaneous detection of nine antibiotic resistance-related genes in *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization assay. Antimicrob. Agents Chemother. **50:**204–209.