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Prevalence and Molecular Analysis of Macrolide and Fluoroquinolone Resistance among Isolates of *Streptococcus pneumoniae* Collected during the 2000-2001 PROTEKT US Study

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The PROTEKT US (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin in the United States) surveillance program was established to determine the prevalence and mechanisms of antibacterial resistance among bacterial pathogens from patients with community-acquired respiratory tract infections. In year 1 of the PROTEKT US study, 10,103 isolates of *Streptococcus pneumoniae*, including 3,133 erythromycin-resistant strains and 81 levofloxacin-resistant strains, were collected from 206 centers. We report on the molecular analyses of these resistant strains. The resistance genotypes among the 3,044 typed macrolide-resistant isolates overall were *mef*(A) ($n = 2,157$; 70.9%), *erm*(B) ($n = 530$; 17.4%), *mef*(A) *erm*(B) ($n = 304$; 10.0%), and *erm*(A) subclass *erm*(TR) ($n = 5$; 0.2%). Fifty (1.6%) macrolide-resistant isolates were negative for the *mef* and the *erm* resistance genes. Seventy-eight (96.3%) of the 81 levofloxacin-resistant isolates analyzed possessed multiple mutations in the *gyrA*, *gyrB*, *parC*, and/or *parE* quinolone resistance-determining regions. A total of 43 known multilocus sequence typing (MLST) profiles (or single- or double-locus variants) accounted for 75 of 81 isolates. There was no evidence of dissemination of fluoroquinolone-resistant clones within the United States; however, 12 isolates with the same MLST profile were located in one center in Massachusetts. Almost 90% of the erythromycin-resistant isolates and approximately one-third of the levofloxacin-resistant isolates were multidrug resistant.

Macrolides and fluoroquinolones are antibacterial agents used for the empirical treatment of community-acquired respiratory tract infections (CARTIs). The rate of erythromycin resistance among *Streptococcus pneumoniae* isolates in the United States was about 0.2% in 1987-1988 (23), but this rate increased to 17% by 1998 and 23% by 1999 (17). The rate of fluoroquinolone resistance among *S. pneumoniae* isolates remains low worldwide (1%) (14), but reports of increasing rates of resistance are emerging from several countries, such as Hong Kong (16) and the United States (31).

Macrolide resistance in *S. pneumoniae* is typically achieved through two mechanisms: efflux and target modification. Efflux resistance is mediated by the *mef*(A) gene, which encodes an efflux pump specific to 14- and 15-membered-ring macrolides. In the case of target modification, mediated by the *erm*(B) gene, an adenine residue in the 23S rRNA is methylated, leading to reduced binding of 14-, 15-, and 16-membered-ring macrolides, lincosamides, and streptogramin B to their shared target site on the 50S ribosomal subunit (32).

Fluoroquinolone resistance occurs primarily through mutations in the quinolone resistance-determining regions (QRDRs) of the *parC* and *gyrA* genes (involved in encoding the A subunits of DNA gyrase and topoisomerase IV) or the *gyrB* and *parE* genes (which encode the B subunits of DNA gyrase and topoisomerase IV). Resistance is generally imparted in two steps: a primary mutation reduces fluoroquinolone binding affinity, while a subsequent mutation in a secondary target results in a further reduction in in vitro activity (8, 15, 33).

In 2000, the PROTEKT US (Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin in the United States) surveillance program was initiated to longitudinally monitor antibacterial resistance in pathogens across the United States. The geographical distributions of fluoroquinolone- and macrolide-resistant isolates of *S. pneumoniae* across the United States have been reported in detail by Doern and Brown (7) and Waites and Brown (37). In this paper, we describe the prevalence of resistance genotypes responsible for macrolide and fluoroquinolone resistance among these isolates of *S. pneumoniae*.

MATERIALS AND METHODS

During 2000 and 2001, 206 centers from 154 cities or metropolitan areas took part in the PROTEKT US study. The participating centers were each requested to collect 50 clinical isolates of *S. pneumoniae*. Isolates were collected from patients with community-acquired pneumonia, acute bacterial sinusitis, acute otitis media, and acute exacerbation of chronic bronchitis or acute exacerbation of chronic obstructive airway disease. Suitable sources for isolates included blood, sputum, bronchoalveolar lavage fluid, middle ear fluid, nasopharyngeal swab or aspirates, and sinus aspirates. The centers were free to include isolates from patients of any age. Specimens were considered acceptable if they were the first positive specimen from each patient and had been obtained from outpatients with CARTIs or hospitalized patients within 48 h of admission. Isolates from patients hospitalized for 48 h or more or patients with nosocomial lower respiratory tract infections or cystic fibrosis were excluded, as were isolates from existing collections and duplicate strains or isolates from sputum samples with a poor Gram staining result.

All isolates were stored in Amies transport medium and were subsequently shipped to a central laboratory (Clinical Microbiology Institute, Wilsonville, Oreg.) for microbiological investigation. Molecular analysis was carried out at GR Micro Ltd. (London, United Kingdom).

MICs were determined by the NCCLS broth microdilution methodology (27). NCCLS breakpoints were used to define susceptibility (28). Tentative NCCLS 2004 breakpoints were applied for telithromycin: susceptible, MIC $\leq$ 1 mg/liter; intermediate, MIC = 2 mg/liter; and resistant, MIC $\geq$ 4 mg/liter (29).

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Amplification and detection of *erm*(A), *erm*(A) subclass *erm*(TR), *erm*(B), *erm*(C), and *mef*(A) macrolide resistance genes were carried out by a duplex PCR (12).

Extraction and amplification of the gene mutations in *gyrA*, *gyrB*, *parC*, and *parE* were carried out with a High Pure PCR template kit (Roche, Lewis, United Kingdom). Sequencing was conducted with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Warrington, United Kingdom) (26). Multilocus sequence typing (MLST), as described by Enright and Spratt (9), was conducted with all levofloxacin-resistant isolates to identify the existence of clonality among them. Data available from the Multi Locus Sequence Typing website (http://www.mlst.net) and the Pneumococcal Molecular Epidemiological Network website (http://www.sph.emory.edu/PMEN) were used to analyze and describe these isolates.

RESULTS

A total of 10,103 isolates of *S. pneumoniae* were collected from 206 centers in 42 states, the District of Columbia, and Puerto Rico during 2000 and 2001. Of these, 3,133 (31.0%) were erythromycin resistant and 81 (0.8%) were levofloxacin resistant (7, 37).

Macrolide-resistant isolates. Of the 3,133 erythromycin-resistant *S. pneumoniae* isolates collected during year 1 of the PROTEKT US study, genotyping data were available for 3,044. Five resistance genotypes were identified: *erm*(B), *mef*(A), *erm*(B) *mef*(A), *erm*(A) subclass *erm*(TR), and "other" (erythromycin-resistant isolates that were negative for the mechanisms tested for). All but 4 centers collected at least 1 resistant isolate, and of the remaining 202 centers, 51 collected 20 or more isolates (the range data presented below are only for these 51 centers). The *mef*(A) genotype was the most common resistance mechanism identified and accounted for 70.8% (n = 2,157) of the erythromycin-resistant isolates (median, 71.4% per center; range, 41.7 to 95.0% per center). The *erm*(B) gene was identified in 17.4% (n = 530) of isolates (median, 16.7% per center) and was the second most prevalent genotype in the majority (36 of 51) of centers (range, 0.0 to 45.5% per center). The third most common genotype overall was *erm*(B) *mef*(A), which was detected among a further 10.0% (n = 304) of isolates (median, 7.1% per center) and whose prevalence ranged from 0.0 to 35.0% per center. Five isolates were positive for *erm*(A) subclass *erm*(TR) and were located in five separate centers. Fifty (1.6%) erythromycin-resistant isolates were negative for the mechanisms tested for and have been described elsewhere (10).

Fluoroquinolone-resistant isolates. In contrast to erythromycin-resistant isolates, the majority of centers (74.3%; 153 of 206 centers) did not isolate any levofloxacin-resistant *S. pneumoniae* isolates. Of the remaining 53 centers, 40 collected only one levofloxacin-resistant isolate, 9 centers collected two levofloxacin-resistant isolates, and 2 centers collected three levofloxacin-resistant isolates. Such small sample sizes cannot be relied upon to accurately measure the prevalence of fluoroquinolone resistance within regions or states within the United States, but nevertheless, these results indicate that the rate of fluoroquinolone resistance among pneumococci is low. The two centers with the highest prevalence of levofloxacin-resistant isolates were in New York State and Massachusetts, with 4 and 12 resistant isolates, respectively (resistance rates, 3.1 and 21.8%, respectively).

MLST data for all 81 fluoroquinolone-resistant isolates are presented in Table 1. The most common MLST found was a single-locus variant (SLV) of Tennesee^{23F}-4 clone ST37. Interestingly, this clone represented all 12 levofloxacin-resistant isolates from the center in Massachusetts mentioned above. This indicates that the very high rate of fluoroquinolone resistance in this center was due to the dominance of a single clone. A double-locus variant (DLV) of ST37 was also isolated in Massachusetts, but from a different center, and ST37 itself was found in centers in Washington State and Tennessee (Table 1).

All 12 isolates of the ST37 SLV clone were from different patients, and 10 were recovered during outpatient visits. The period over which this clone was isolated (3 November 2000 to 10 January 2001) spanned most of the cold season associated with the majority of community-acquired lower respiratory tract infections. The age range of the patients was from 5 to 91 years, with eight elderly patients (>64 years old), three adult patients (15, 51, and 63 years old, respectively), and one child patient (5 years old). The amino acid changes responsible for resistance in all 12 isolates of the ST37 SLV clone were GyrA E85K plus ParC S79F; 7 isolates had a further ParE E474K change, and 1 had a ParE D435N change (data not shown). Therefore, it is possible that multiple separate mutational events may have occurred in the original fluoroquinolone-susceptible ST37 clone rather than the spread of resistance from a single source. The ST37 DLV is even more likely to be from a separate source because it contained resistance mutations (GyrA S81F and ParC S79F) distinct from those seen in the ST37 SLV (data not shown).

Spain^{23F}-1 clone ST81 was the second most prevalent isolate, but it accounted for only 7 of 81 (8.6%) of all fluoroquinolone-resistant pneumococci in the United States. This clone was distributed across the United States and was not concentrated in any one center. An SLV of ST81 was also observed in one center (Table 1). Third in order of prevalence, but found on only four occasions in four separate centers, was Spain^{9V}-3 clone ST156 (Table 1). Other clones observed in year 1 of the PROTEKT US study that have been highlighted by the Pneumococcal Molecular Epidemiology Network were Taiwan^{19F}-14 clone ST236 (two occurrences), Sweden^{15A}-25 clone ST63 (two occurrences), North Carolina^{6A}-23 clone ST376 (one occurrence), Taiwan^{23F}-15 clone ST242 (one occurrence), Spain^{6B}-2 clone ST90 (one occurrence), and an SLV of England^{14}-9 clone ST9 (ST13; one occurrence). The remaining isolates did not possess any distinguishing characteristics. However, three clones plus one SLV were typed and found to be unrelated to known pneumococcal sequence types (Table 1). Interestingly, these isolates had QRDR mutations indicative of the viridans group streptococci (see below), which suggests that either these isolates have undergone considerable genetic transfer with nonpneumococci or they are, in fact, not *S. pneumoniae*.

The topoisomerase amino acid subunit alterations in the QRDRs for all 81 levofloxacin-resistant isolates are listed in Table 2. The majority of isolates (58 of 81; 71.6%) possessed two amino acid changes. Of these, the GyrA S81F and ParC S79F/T modifications were the most common. One isolate had an unusual ParC S79A change, and two isolates had an S79R change in ParC. A novel Q90H change was found in ParC of one isolate, and a novel S80P change was found in another; both changes appeared to play a role in fluoroquinolone resistance. Some isolates (15 of 81; 18.5%) possessed three QRDR

TABLE 1. MLST profiles of levofloxacin-resistant isolates of *S. pneumoniae* collected during year 1 of the PROTEKT US study (2000–2001)[a]

MLST	No. (%) of isolates with the following MLST allele:							% Occurrence	State(s)	Levofloxacin MIC range (mg/liter)	Comment
	aroE	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*				
SLV of ST37	1	8	6	2	6	4	26	12	Massachusetts	16–>16	SLV of Tennessee^{23F}-4 clone
DLV of ST37	1	8	1	2	6	4	14	1	Massachusetts	16	DLV of Tennessee^{23F}-4 clone
37	1	8	6	2	6	4	6	2	Washington, Tennessee	16–>16	Tennessee^{23F}-4 clone
81	4	4	2	4	4	1	1	7	Pennsylvania, Iowa, North Carolina, New Jersey, Missouri, Texas, Michigan	8–>16	Spain^{23F}-1 clone
SLV of ST81	4	4	2	4	68 (96)	1	1	1	Kansas	8	SLV of Spain^{23F}-1 clone
156	7	11	10	1	6	8	1	4	Connecticut (2), Michigan, Nebraska	8–16	Spain^{9V}-3 clone
236	15	16	19	15	6	20	26	2	Iowa, Alaska	8–>16	Major multiresistant Taiwan 19F strain
63	2	5	36	12	17	21	14	2	New York	>16	Sweden^{15A}-25
376	6	11	1	1	15	72	77	1	North Carolina	>16	North Carolina^{6A}-23 clone
242	15	29	4	21	30	1	14	1	Massachusetts	16	Taiwan^{23F}-15 clone
90	5	6	1	2	6	3	4	1	Ohio	>16	Spain^{6B}-2 clone
13	1	5	4	5	5	27	8	3	Colorado, Oregon, Tennessee	16	SLV of England^{14}-9 clone
Unknown A	4 (95)	20 (96)	3 (96)	50 (96)	82 (96)	51 (98)	102 (98)	1	California	16	May have undergone extensive genetic transformation
SLV of Unknown A	4 (95)	40 (96)	3 (96)	50 (96)	82 (96)	51 (98)	102 (98)	1	Florida	16	May have undergone extensive genetic transformation
Unknown B	4 (95)	57 (98)	66 (97)	50 (96)	82 (96)	122 (95)	3 (96)	1	Illinois	16	May have undergone extensive genetic transformation
Unknown C	4 (96)	57 (96)	49 (95)	50 (98)	67 (95)	46 (96)	98 (94)	1	Missouri	8	May have undergone extensive genetic transformation
42	1	8	9	9	6	4	6	2	Massachusetts	8	Serotype 23A strain from Spain and Brazil
43	1	10	4	1	9	3	8	1	Kansas	8	Previously found in UK
53	2	5	1	11	16	3	14	1	New York	16	Previously found in UK, Holland, Spain, and Brazil
62	2	5	29	12	16	3	14	1	Ohio	16	Previously found in Spain
100	5	12	29	12	9	39	18	1	Nebraska	8	Previously found in Spain
138	7	5	8	5	10	6	14	1	Ohio	16	Previously found in Sweden, UK, Denmark, and USA
146	7	6	1	2	6	15	14	1	Arizona	8	Previously found in UK and USA
180	7	15	2	10	6	1	22	3	North Dakota, Ohio (2)	8–>16	Serotype 3 strain
199	8	13	14	4	17	4	14	1	Indiana	8	Previously found in UK, Holland, and Ireland
205	10	5	4	5	13	10	18	1	Michigan	16	Previously found in Sweden, Denmark, and Canada
220	10	20	14	1	9	1	29	1	Pennsylvania	16	Previously found in Denmark
244	16	2	4	1	6	10	18	1	Utah	8	Previously found in Spain
395	1	5	7	12	17	1	14	1	Texas	8	Previously found in UK
460	5	7	4	10	10	1	27	3	Alabama, California, Tennessee	8–16	Serotype 6A strain from England
473	7	25	4	4	15	20	28	2	New York, New Jersey	16–>16	Serotype 6A/B strain from UK, Austria, and Greece
574	2	1	1	1	6	31	14	1	Alabama	8	Previously found in UK
638	7	2	1	1	10	4	21	1	Pennsylvania	8	USA invasive strain
651	15	5	19	15	6	20	26	1	Kansas	16	USA invasive strain
659	1	5	4	4	6	58	19	2	New York, Michigan	8–16	USA invasive strain
695	16	13	4	4	6	113	18	1	Pennsylvania	>16	USA invasive strain

Continued on following page

TABLE 1—*Continued*

MLST	No. (%) of isolates with the following MLST allele:							% Occurrence	State(s)	Levofloxacin MIC range (mg/liter)	Comment
	aroE	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*				
816	5	40	4	19	10	1	27	1	Massachusetts	16	Previously found in UK
1257	15	13	8	18	15	1	31	1	Oregon	16	USA invasive strain
1269	7	11	10	1	6	76	14	1	California	8	USA invasive strain
DLV of ST26, ST327, and ST395	1	5	7	4	15	1	14	1	Ohio	16	Multiple possible origins
DLV of ST394 and ST414	8	5	27	5	1	48	1	1	Ohio	8	DLV of Serotype 16F strain from UK
DLV of ST662	5	35	29	12	42 (99.8)	39	25	1	Pennsylvania	>16	DLV of USA invasive strain
SLV of ST110, ST638, and ST1073	7	2	1	5	10	4	21	1	New York	16	SLV of USA invasive strain
SLV of ST271, ST328, and ST635	4	16	19	15	55	20	26	2	New York	16	SLV of a multiresistant serotype 19F strain
SLV of ST433	1	1	4	1	18	58 (99.8)	17	1	Michigan	16	SLV of a serotype 22F strain from the UK
SLV of ST916 and ST1190	7	6	4	1	15	20	14	1	Illinois	16	SLV of isolate previously found in Gambia and Mexico
TLV of ST384	7	6	4	15	6	1	50	2	Colorado	8–16	TLV of Maryland^{6B}-17 clone

[a] Abbreviations: USA, United States; UK, United Kingdom; TLV, triple-locus variant.

changes, and this additional QRDR change shifted the levofloxacin MICs from 8 to 16 mg/liter for most isolates with two modifications to >16 mg/liter for most isolates with three modifications. A minority of GyrB changes were implicated in resistance, as has been found previously (5, 21, 38). Surprisingly, 13 instances of ParE alterations (D435N and E474K in the main and one novel A325V change) occurred, which is greater than the number found in previous studies (3, 5, 21, 38). One isolate relied only on alterations in GyrB and ParE for resistance (Table 2). Only three levofloxacin-resistant isolates carried just one QRDR alteration, and the alteration was in the DNA gyrase in two isolates (Table 2).

Some other less common amino acid substitutions were discovered and are indicated in boldface in Table 2. These changes were detected in those isolates that presented numerous QRDR amino acid substitutions and did not have any major effect on the fluoroquinolone MICs. The GyrA S114G and the ParC N91D changes have previously been found in *S. pneumoniae* and are a consequence of the uptake of DNA from viridans group streptococci (2). These isolates also produced unusual MLST results, which are mentioned above.

Of the 81 levofloxacin-resistant isolates, 75 (92.6%) were gatifloxacin resistant, indicating almost complete cross-resistance between these two fluoroquinolones.

Antibacterial susceptibility among erythromycin- and levofloxacin-resistant isolates. The antibacterial susceptibilities of all *S. pneumoniae* isolates from year 1 of the PROTEKT US study are compared to the susceptibilities of the erythromycin-resistant and levofloxacin-resistant isolates in Table 3. The majority of the erythromycin-resistant pneumococci (irrespective of their mechanism of resistance) were also resistant to cefuroxime, co-trimoxazole, and penicillin. As would be expected, coresistance did not occur with clindamycin among isolates carrying *mef*(A) and almost complete cross-resistance occurred with those isolates carrying *erm*(B). Fewer *mef*(A) isolates than *erm*(B) isolates were coresistant to tetracycline (tetracycline resistance rates, 28.1 and 93.6%, respectively). In contrast, among the erythromycin-resistant isolates, no coresistance between erythromycin and levofloxacin or telithromycin was detected. Among the levofloxacin-resistant isolates, approximately 40 to 50% were also resistant to erythromycin, cefuroxime, penicillin, and co-trimoxazole. Resistance to tetracycline or clindamycin was not as prevalent among the levofloxacin-resistant isolates than among the erythromycin-resistant population.

An analysis of multidrug resistance is given in Table 4. Overall, 34.4% of the *S. pneumoniae* isolates were multidrug resistant, which is defined by the U.S. Food and Drug Administration to be resistance to two or more of the five classes of antibacterials represented by erythromycin, cefuroxime, co-trimoxazole, penicillin, and tetracycline (http://www.fda.gov/cder/foi/label/2004/21144_ketek_lbl.pdf). Among the levofloxacin-resistant population, a total of 53.1% of the isolates were multidrug resistant. In contrast, almost 90% of the erythromycin-resistant isolates were multidrug resistant, with virtually all *erm*(B) and *erm*(B) *mef*(A) isolates resistant to two or more agents. An alternative analysis of multidrug resistance was also made by replacing cefuroxime susceptibility with levofloxacin susceptibility, on the basis of the fact that β-lactam agents are represented twice by the inclusion of both penicillin and cefuroxime in the U.S. Food and Drug Administration definition. The results of this analysis showed little difference in the prevalence of isolates resistant to two or more or three or more agents, but fewer isolates with resistance to four or more or five or more agents were observed (results not shown). Isolates with double mechanisms of macrolide resistance are of

TABLE 2. Topoisomerase amino acid alterations and MICs of levofloxacin and gatifloxacin for levofloxacin-resistant *S. pneumoniae* isolates collected during year 1 of the PROTEKT US study (2000–2001)

No. of isolates	Amino acid alteration in the following QRDR:				Total no. of amino acid changes	MIC range (mg/liter)	
	gyrA	*gyrB*	*parC*	*parE*		Levofloxacin	Gatifloxacin
1	S81F				1	8	2
1			S79F			16	>4
1		D435N				>16	4
22	S81F		S79F		2	8–>16	4–>4
8	S81F		S79Y			8–16	4–>4
8	S81F			D435N		8–16	4
7	E85K		S79F			32	>4
3	S81Y		S79Y			8–16	4–>4
2	E85K			D435N		8–16	2–4
2	S81F		D83Y			8	2
1	E85G		S79F			8	2
1	S81F		D83N			8	2
1	S81F		S79A			8	4
1	S81Y		S79F			16	4
1			Q90H	E474K		8	4
1			S79F	D435N		>16	>4
7	E85K		S79F	E474K	3	>16	>4
1	E85K		**K136T**[a]	D435N		8	2
1	E85K		S79F	D435N		>16	>4
1	S81F		S79Y	A325V		16	>4
1	S81F		S79Y, D83G			16	4
1	S81F			D435N, E474K		16	4
1	S81Y		S79F, D83N			>16	>4
1		D435N		D435N, E474K		16	4
1	S81F		S52G	D435N		8	4
1	S81F, E85Q		S80P	D435N	4	>16	>4
1	S81F, **S114G**		**S52G**, S79Y, **N91D**		5	16	>4
1	S81F, **S114G**		**S52G**, D83N, **N91D**	**V144I, T186I, I241T**	8	8	2
1	S81F, **S114G**		**S52G**, S79R, **N91D**	**K214E, T2161, I241T, I493L**	9	16	>4
1	S81F, **S114G**		**S52G**, S79R, **N91D, E135D**	**T157M, G163A, 1241T, A425S**	10	16	4

[a] Uncommon amino acid substitutions that probably play no role in fluoroquinolone resistance are shown in boldface.

particular concern, as more than 80% were resistant to four or more agents according to either multidrug resistance definition. No isolate was found to be resistant to all eight antibacterials tested overall.

DISCUSSION

Traditional treatment options for CARTIs have been threatened in recent years by increasing levels of antibacterial resistance among *S. pneumoniae* isolates (6, 14, 22), an observation supported by the results of the PROTEKT US study for 2000-2001 (7). An understanding of local resistance patterns is essential to ensure that appropriate antibiotics are used for the treatment of CARTIs. The PROTEKT US study was undertaken not only to monitor national and local resistance patterns as a guide to the prescription of antibacterials but also to determine the prevalence of resistance mechanisms. Although there is often no proven direct link between in vitro resistance and clinical treatment failure, we have followed the principles laid out by the NCCLS and others who provide MIC breakpoints to help clinicians make informed decisions about appropriate patient therapy.

The prevalence of macrolide resistance mechanisms varies widely geographically. As this report shows, the *mef*(A) gene clearly predominates in the United States; other research has suggested that the same is true in Canada (20). In contrast, for Europe overall, *erm*(B) prevails among macrolide-resistant *S. pneumoniae* isolates (although this varies widely from country to country) (13). In the Far East, the prevalence of macrolide resistance mechanisms among isolates of *S. pneumoniae* is more diverse. In Hong Kong, *mef*(A) positive isolates are prevalent (72.8%) (19). In South Korea, *mef*(A) is the least common genotype (18.4%), with isolates positive for *erm*(B) and for both *erm*(B) and *mef*(A) occurring at similar levels (43.3 and 38.3%, respectively) (13). In Japan, isolates with the *mef*(A) (40.3%) and the *erm*(B) (43.5%) genes are equally prevalent, but isolates with both *erm*(B) and *mef*(A) are relatively common (16.1%) (30). Interestingly, most *erm*(B) *mef*(A) isolates appear to belong to a multidrug-resistant clonal complex (11). Telithromycin is one of the few antibacterials to have good activity against this clone (11). In this study, resistance to multiple antibacterial agents was shown to be highly prevalent among all erythromycin resistance genotypes, with the number of antibacterial agents to which isolates were resistant being higher for those that possessed the *erm*(B) gene or the *erm*(B) and the *mef*(A) genes.

Of the 3,044 erythromycin-resistant isolates discussed here for which genotyping data were available, 50 isolates were

TABLE 3. Percent susceptibility among all *S. pneumoniae* isolates, erythromycin-resistant isolates by genotype, and levofloxacin-resistant isolates of *S. pneumoniae* collected during year 1 of the PROTEKT US study (2000-2001)

Drug and breakpoint (MIC [mg/liter])	% Prevalence					
	Total isolates (n = 10,103)	Erythromycin-resistant isolates (n = 3,133)	*mef*(A) (n = 2,157)	*erm*(B) (n = 530)	*erm*(B) + *mef*(A) (n = 304)	Levofloxacin-resistant isolates (n = 81)
Erythromycin						
Susceptible (≤0.25)	68.8	0.0	0.0	0.0	0.0	56.8
Intermediate (0.5)	0.2	0.0	0.0	0.0	0.0	1.2
Resistant (≥1)	31.0	100	100	100	100	42.0
Cefuroxime[a]						
Susceptible (≤1)	68.7	24.4	23.4	31.1	5.6	54.3
Intermediate (2)	2.5	4.5	4.6	4.3	3.9	3.7
Resistant (≥4)	28.8	71.1	72.0	64.5	90.5	42.0
Co-trimoxazole						
Susceptible (≤0.5)	58.8	13.7	12.4	17.7	3.3	37.0
Intermediate (1–2)	7.3	8.4	8.5	10.6	1.0	7.4
Resistant (≥4)	33.9	77.9	79.1	71.7	95.7	55.6
Penicillin						
Susceptible (≤0.06)	61.2	13.4	12.3	11.5	2.0	39.5
Intermediate (0.12–1)	12.5	20.8	21.0	29.4	8.6	19.8
Resistant (≥2)	26.3	65.8	66.8	59.1	89.5	40.7
Clindamycin						
Susceptible (≤0.25)	90.9	71.5	98.4	1.5	5.6	85.2
Intermediate (0.5)	0.2	0.5	0.4	0.0	1.0	0.0
Resistant (≥1)	8.8	27.9	1.3	98.5	93.4	14.8
Tetracycline						
Susceptible (≤2)	83.8	54.0	71.7	4.9	3.6	72.8
Intermediate (4)	0.3	0.5	0.2	1.5	1.3	0.0
Resistant (≥8)	15.9	45.5	28.1	93.6	95.1	27.2
Levofloxacin						
Susceptible (≤2)	99.1	98.8	99.0	97.7	99.0	0.0
Intermediate (4)	0.1	0.2	0.1	0.6	0.0	0.0
Resistant (≥8)	0.8	1.1	0.9	1.7	1.0	100
Telithromycin						
Susceptible (≤1)	99.6	98.8	99.1	97.4	98.7	100
Intermediate (2)	0.3	1.1	0.9	1.9	1.3	0.0
Resistant (≥4)	0.04	0.1	0.0	0.8	0.0	0.0

[a] Breakpoints are for cefuroxime axetil (oral).

negative for the mechanisms of resistance for which tests were performed. Subsequent testing of these isolates has shown that resistance is imparted through ribosomal mutations (10).

The majority of *S. pneumoniae* isolates collected during year 1 of the PROTEKT US study were susceptible to fluoroquinolones: 10,014 (99.1%) were susceptible to levofloxacin at ≤2 mg/liter. Of the resistant isolates identified, 64.2% were resistant to one or more other antibacterials. Typically, resistance was mediated through mutations at three of the QRDRs: *gyrA*, *parC*, and *parE*. Only 2 of the 81 fluoroquinolone-resistant isolates identified in this study possessed mutations in the *gyrB* QRDR. Low incidences of mutation in the *gyrB* QRDR among fluoroquinolone-resistant isolates of *S. pneumoniae* have been reported previously (5). However, this study has highlighted the possibility that ParE may play a greater role in fluoroquinolone resistance than was previously thought. Four isolates possessed a large number of amino acid substitutions (5 to 10) within the QRDRs. The nature of these changes is evidence of transfer of genetic material from viridans group streptococci to pneumococci, as described previously (2). It would appear that this does not have any effect on fluoroquinolone susceptibility.

Of the levofloxacin-resistant isolates collected in this study, most possessed MLST profiles that have been identified previously, and some were recognized international clones. The profiles of 7 of the 51 isolates with previously identified MLST profiles matched the profile of clone Spain^{23F}-1, which has been highlighted as an important clone causing high-level fluoroquinolone resistance in Hong Kong (16, 18, 26). However, unlike in Hong Kong, the fluoroquinolone-resistant Spain^{23F}-1 clone did not dominate at any U.S. center. Overall, the MLST data provided in this study indicate that the rare occurrences of fluoroquinolone resistance in the United States have occurred as independent mutational events in a diverse number of clones. The exception to this occurred in one center in Massachusetts, where relatively high rates of fluoroquinolone resistance can be directly attributed to the dominance of the SLV of the Tennessee^{23F}-4 clone. Although all 12 isolates were the same clone, resistance may still have evolved independently.

TABLE 4. Multidrug resistance among all *S. pneumoniae* isolates, erythromycin-resistant isolates by genotype, and levofloxacin-resistant isolates of *S. pneumoniae* collected during year 1 of the PROTEKT US study (2000–2001)

Isolate and genotype	No. of isolates	% Resistant to the following no. of agents[a]:			
		≥2	≥3	≥4	≥5
Total	10,103	34.4	27.2	20.9	9.3
Levofloxacin resistant	81	53.1	50.6	25.9	13.6
Erythromycin resistant	3,133	89.1	74.6	66.4	30.1
mef(A)	2,157	87.3	73.0	65.7	19.2
erm(B)	530	97.0	75.7	63.1	49.2
erm(B) *mef*(A)	304	99.7	95.4	92.4	83.2

[a] Analysis includes erythromycin, cefuroxime, co-trimoxazole, penicillin, and tetracycline.

Interestingly, the four isolates with viridans group streptococci-like QRDR sequences also possessed unusual MLST profiles. This suggests that these isolates had undergone considerable DNA transformation or were not true *S. pneumoniae* strains (even though they were identified as such by three separate laboratories). The study of the MLST profiles of levofloxacin-resistant isolates will continue in the coming years as part of the PROTEKT US study to monitor the epidemiology of fluoroquinolone resistance in the United States.

The results of this study have highlighted the extent of coresistance among macrolide-resistant pneumococci. This resistance was greatest with the *erm*(B)-carrying subpopulation, which showed coresistance to clindamycin, β-lactams, tetracycline, and co-trimoxazole. The *mef*(A) isolates retained susceptibility to clindamycin (as would be expected), and considerably fewer isolates were resistant to tetracycline. This phenomenon may occur because *erm*(B) and *mef*(A) are associated with different conjugative transposons (Tn*1545* and Tn*916*, respectively) (25, 34, 36). It is possible that the insertion of *erm*(B) into transposon Tn*1545* already associated with *tet*(M) and other resistance genes has evolved over a long time period, whereas the association of *mef*(A) and *tet*(M) on Tn*916* has occurred more recently (1, 25, 35). It will be interesting to see if any further evolutionary changes are observed as the PROTEKT US surveillance study continues.

Isolates carrying both *mef*(A) and *erm*(B) have been reported previously (4, 11, 24) and are highly multidrug resistant (11), as the results of the present study have confirmed. This high-level resistance is most likely due to the presence of more than one mobile genetic element (11), but this hypothesis was not tested in the present study. The prevalence of these isolates in the United States may have increased slightly from 7% in 1996-1997 (4) to 10% in 2000-2001 (this study). However, as different centers were used for these two studies, it is difficult to form any firm conclusions. As stated above, we await the results of future PROTEKT US surveillance studies to see if this upward trend continues. It is noteworthy that only telithromycin and levofloxacin showed activity against these antibiotic-resistant *S. pneumoniae* isolates.

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