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Temporal Trends of Antimicrobial Resistance and Clonality of Invasive *Streptococcus pneumoniae* Isolates in Finland, 2002 to 2006▽

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The antimicrobial resistance of *Streptococcus pneumoniae*, or pneumococcus, is a growing global problem. In our study, 3,571 invasive pneumococcal isolates, recovered from blood and cerebrospinal fluid samples from patients in Finland between the years 2002 and 2006, showed an increase in erythromycin nonsusceptibility from 16% to 28% ($P < 0.0001$) over the 5-year study period, as well as a doubling of penicillin nonsusceptibility from 8% to 16% ($P < 0.0001$). Erythromycin nonsusceptibility increased especially in isolates derived from 0- to 2-year-old children and was 46% for this age group in 2006. Although multiresistance, defined as nonsusceptibility to penicillin, erythromycin, and tetracycline, was fairly rare (5.1% in 2006), 38% of the erythromycin-nonsusceptible isolates were also penicillin nonsusceptible, while 74% of the penicillin-nonsusceptible isolates were nonsusceptible to erythromycin. In contrast to the situation in continental Europe, but mirroring that in North America, the most frequent macrolide resistance determinant carried by 56% of the tested macrolide-resistant pneumococci was the *mef* gene. Serotypes 14, 9V, 19A, 6B, and 19F were most frequently nonsusceptible to erythromycin or penicillin. The penicillin-resistant invasive isolates ($n = 88$) were genotyped by multilocus sequence typing, which revealed the presence of 25 sequence types, 9 of which were novel. The majority of the isolates were related to one of several globally disseminated penicillin- or multiresistant clones, most importantly the *rlrA* adhesion pilus carrying clones Spain^{9V} ST156 and Taiwan^{19F} ST236. The penicillin-resistant pneumococcal population in Finland is therefore a combination of internationally recognized genotypes as well as novel ones.

Streptococcus pneumoniae, or pneumococcus, is a frequent cause of otitis media, respiratory infections, and community-acquired pneumonia. It can also cause life-threatening invasive infections like bacteremia and meningitis. The emerging antimicrobial resistance of pneumococci is a major problem worldwide and may lead to treatment failures (31). Several international penicillin (PEN)- or multiresistant pneumococcal clones have been identified that contribute to the global rise in drug resistance. In addition, the loss and acquisition of resistance determinants within the international drug-resistant clones, as well as in other lineages, affect resistance numbers (36). Some drug-resistant clones carry a pilus-encoding *rlrA* islet (40), which, based on a mouse model study, provides a competitive advantage over nonpiliated pneumococci in the nasopharynx, and may contribute to the spread of these piliated pneumococci (53). In the years 1999 to 2000, 7% of the invasive pneumococci isolated in Finland were resistant to macrolides and 4% were nonsusceptible to PEN, but multiresistance and resistance to fluoroquinolones or extended-spectrum cephalosporins were even rarer (43). Nevertheless, the 2002 study indicated that antimicrobial resistance among pneumococci in Finland was emerging (48).

A recent evaluation group recommended the introduction of the 7-valent pneumococcal conjugate vaccine (PCV-7) in the Finnish national vaccination program. In many countries, PCV-7 has effectively reduced the incidence of invasive pneumococcal disease not only in children but also in other age groups due to herd immunity (46). There are also some reports about decreasing antimicrobial resistance in pneumococci after introducing the vaccine (7, 35). However, the emergence of serotypes that are not covered by the vaccine threatens the positive effects of the vaccine (28). The pneumococcal population can be selected by the vaccine, and therefore, we need baseline data on antimicrobial resistance and pneumococcal population structure to monitor possible changes. In this study, we investigated antimicrobial resistance trends in invasive pneumococcal isolates from 2002 to 2006 and genetic mechanisms of macrolide resistance and studied the clonality of PEN-resistant (PEN R) isolates by genotype. The PEN R isolates were also studied for the presence of the adhesion pilus-encoding genomic *rlrA* islet.

MATERIALS AND METHODS

Isolates. The bacterial population in this study consisted of all Finnish blood and cerebrospinal fluid (CSF) pneumococcal isolates ($n = 3,571$) from the year 2002 through the year 2006 collected in the Strain Collection of the National Infectious Disease Register housed at the National Institute for Health and Welfare (THL; formerly the National Public Health Institute [KTL]). The number of isolates per year ranged from 593 to 752. Of the 3,571 isolates, 4.1% ($n = 148$) were from cerebrospinal fluid (CSF), and the rest were from blood. The clinical laboratories had identified pneumococci by conventional methods and sent them to THL for antimicrobial susceptibility and serotype surveillance. The following information was provided for each isolate: the laboratory identification, date of birth of the patient, date and place of isolation, and specimen type

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(blood or CSF). The number of bacterial isolates sent to the strain collection corresponded to 97.8% of the number of blood and CSF pneumococcal isolates reported to the National Infectious Disease Register during the same period.

Antimicrobial susceptibility. Susceptibility to PEN, erythromycin (ERY), tetracycline (TET), ceftriaxone, and levofloxacin was tested by the agar plate dilution method (44). Ceftriaxone susceptibility was tested from January 2005 and levofloxacin susceptibility from August 2004 onwards. In addition, 105 randomly selected ERY-resistant isolates [77 isolates carrying *erm*(B), 11 carrying *mef*, 2 carrying *erm*(B) and *mef*, and 15 isolates with unknown resistance determinants] and 24 susceptible isolates were tested for telithromycin susceptibility by the disk diffusion method with 15 μg telithromycin disks (Oxoid Ltd., Basingstoke, Hampshire, England) using the CLSI disk diffusion technique (11). *S. pneumoniae* ATCC 69419 was used as a quality-control strain.

The CLSI breakpoints referring to intermediate susceptibility were used for cutoffs when calculating the proportion of nonsusceptible bacteria, except for PEN, for which the proportion of fully resistant strains (≥2 mg/liter) was also calculated. For ceftriaxone, the meningitis breakpoint (intermediate) was used (11). An isolate that was nonsusceptible to ERY, PEN, and TET was defined as multiresistant. Resistance percentages were analyzed by age group (0 to 2 years, 3 to 15 years, 16 to 64 years, and ≥65 years) and by tertiary-care region in Finland (Helsinki, Turku, Tampere, Kuopio, and Oulu). Poisson regression was used for testing the statistical significance of the trends of antimicrobial resistance over the study time period. For statistical testing, the age groups 0 to 2 years and 3 to 15 years were combined due to the small size of the latter age group. Risk ratio (RR) estimates (representing the relative change of the risk for a strain being resistant to an antimicrobial within 1 year) and 95% confidence intervals (CI) were calculated; a P value of ≤0.05 was considered statistically significant.

Macrolide resistance determinants. A multiplex PCR method (24) was used to detect the macrolide resistance determinants [*mef*, *erm*(B), and *erm*(A)] of 223 randomly selected ERY-nonsusceptible isolates (MIC ≥ 0.5 mg/liter). For the multiplex PCR, primers from the following sources were used: *mef* (24, 48), *erm*(B) (57), and *erm*(A) [*erm*(TR) subclass] (44). Separate PCRs were performed to differentiate efflux gene subclasses *mef*(A) and *mef*(E) in the 60 randomly selected *mef*-positive isolates described previously (48) by using published primers (10, 14).

Serotyping. Isolates were serotyped by latex agglutination for the neutral serogroups/types 7 and 14, followed by counterimmunoelectrophoresis with pneumococcal Omni, pool, group/type, and factor sera (Statens Serum Institut, Copenhagen, Denmark). A quellung reaction was used as a confirmation method when needed (29, 34).

Genotyping by multilocus sequence typing (MLST). One PEN R pneumococcal isolate per case was typed by MLST (n = 88). If both blood and CSF isolates from the same patient were available (n = 7), the isolate from CSF was analyzed, since all pairs of isolates had the same serotypes and the resistance profiles were alike. The genomic DNA was isolated with the DNeasy tissue kit (Qiagen GmbH, Hilden, Germany), and the seven loci defined by the MLST scheme (http://spneumoniae.mlst.net/) were amplified by PCR using Ampli*Taq* Gold (Applied Biosystems, Foster City, CA). The primer sequences were taken from several sources: *aroE*, *recP*, and *xpt* (19); *gdh* and *gki* (20); *ddl* down (6); *spi* (45); and *ddl* up, 5′-TTGCCATGGATAAAATCACGAC-3′ (B. Pichon, personal communication). For *aroE* and *gki*, the thermal cycling conditions consisted of 35 cycles with a 60°C annealing temperature. The remaining loci were amplified using a 53°C annealing temperature. The PCR products were purified using the QiaQuick PCR purification kit (Qiagen) or the GeneClean Turbo kit (Q-Bio-Gene; MB Biomedicals, OH). Sequencing was performed using BigDye 1.3 chemistry (Applied Biosystems) as described by the manufacturer, and for analyzing, the Vector NTI Advance 10 software suite (Invitrogen Corporation, Carlsbad, CA) was used. The sequences were compared with the material in the MLST database (http://spneumoniae.mlst.net/), according to which sequence types (STs) were assigned. New allele sequence traces and STs were submitted to the MLST database. Lineage assignment to clonal complexes (CCs) for each ST was performed by eBURST analysis using default stringent parameters (http://eburst.mlst.net/). In this study, the CCs were named after the ST with the highest number of single-locus variants in November 2008.

Detection of the pilus-encoding *rlrA* islet. The first isolate (n = 27) of each serotype and ST as revealed by the MLST of the PEN R isolates was studied for the presence of *rlrA* (1) and *rrgC* (53) by PCR. The annealing temperature was 59°C.

RESULTS

Antimicrobial susceptibility. ERY nonsusceptibility increased from 16% to 28% (RR, 1.12; 95% CI, 1.06 to 1.18; $P <$ 0.0001) over the 5-year study period. The proportion of PEN-nonsusceptible isolates doubled from 8% to 16% (RR, 1.15; 95% CI, 1.07 to 1.23; $P < 0.0001$), and the proportion of PEN R isolates increased from 0.8% to 3.7% (RR, 1.18; 95% CI, 1.02 to 1.36; $P = 0.03$). TET resistance remained stable, ranging from 10 to 12%. The proportion of multiresistant isolates increased slightly from 3.7% (2002) to 5.1% (2006), but the change was not significant. On average, 38% (range by year, 31 to 46%) of the ERY-nonsusceptible isolates were also PEN nonsusceptible, while 74% (range by year, 65 to 78%) of the PEN-nonsusceptible isolates were nonsusceptible to ERY. By using the meningitis nonsusceptibility breakpoint, 2.9% and 3.7% of the isolates were nonsusceptible to ceftriaxone in 2005 and 2006, respectively. Only two isolates (0.3%) in 2006 had ceftriaxone MICs of ≥2 mg/liter, but none did in 2005. Nine levofloxacin-resistant isolates were observed from the years 2004 to 2006. There were no differences between the resistance proportions of blood and CSF isolates. The presence of heterogeneous telithromycin resistance, manifested by the presence of separate colonies inside the growth inhibition zone around the telithromycin disk (47), was detected in nine (7%) of the 128 tested isolates. All were *erm*(B) positive and belonged to serotypes 19A, 19F, 14, 6B, and 4.

A very high prevalence of ERY nonsusceptibility was observed in isolates derived from 0- to 2-year-olds, reaching 46% in 2006. ERY nonsusceptibility increased in all age groups during the study period (Fig. 1). The respective RR estimates were 1.12 ($P = 0.01$) for the 0- to 15-year-olds, 1.11 ($P = 0.003$) for the 16- to 64-year-olds, and 1.16 ($P < 0.001$) for the ≥65-year-olds, while PEN nonsusceptibility increased among the 16- to 64-year-olds (RR, 1.22; $P < 0.001$) and the ≥65-year-olds (RR, 1.15; $P = 0.02$) (Fig. 1). The proportion of PEN R isolates increased significantly only among the 16- to 64-year-olds (RR, 1.53; $P < 0.001$). At the tertiary-care-region level, the ERY and PEN nonsusceptibility ranges were 25 to 34% and 14 to 21%, respectively, and the PEN resistance range was 2 to 10% in 2006 (Fig. 2). A significant increase in ERY nonsusceptibility over the study time period was observed in two regions in Finland: Tampere, from 7% (2002) to 29% (2006) (RR, 1.35; 95% CI, 1.22 to 1.51; $P < 0.0001$), and Oulu, from 20% (2002) to 27% (2006) (RR, 1.12; 95% CI, 1.00 to 1.25; $P = 0.04$). PEN nonsusceptibility increased significantly in Tampere, from 5% (2002) to 15% (2006) (RR, 1.25; 95% CI, 1.07 to 1.45; $P = 0.004$), and Kuopio, from 5% (2002) to 21% (2006) (RR, 1.30; 95% CI, 1.04 to 1.61; $P = 0.02$). PEN resistance also increased significantly in Tampere, from 1% (2002) to 5% (2006) ($P = 0.002$), and in Kuopio, from 0% (2002) to 10% (2006) ($P = 0.006$).

Macrolide resistance genes. The *mef* gene was the most common macrolide resistance determinant and was detected in 56% (125/223) of the screened ERY-nonsusceptible isolates (Table 1). Of the subtyped *mef* isolates, 72% (43/60) were *mef*(E) and 28% (17/60) *mef*(A). All *mef*(A) isolates possessed serotype 14 and were susceptible to PEN. There was no significant difference in the ERY MICs between *mef*(A)-positive and *mef*(E) isolates, although the geometric mean of the ERY

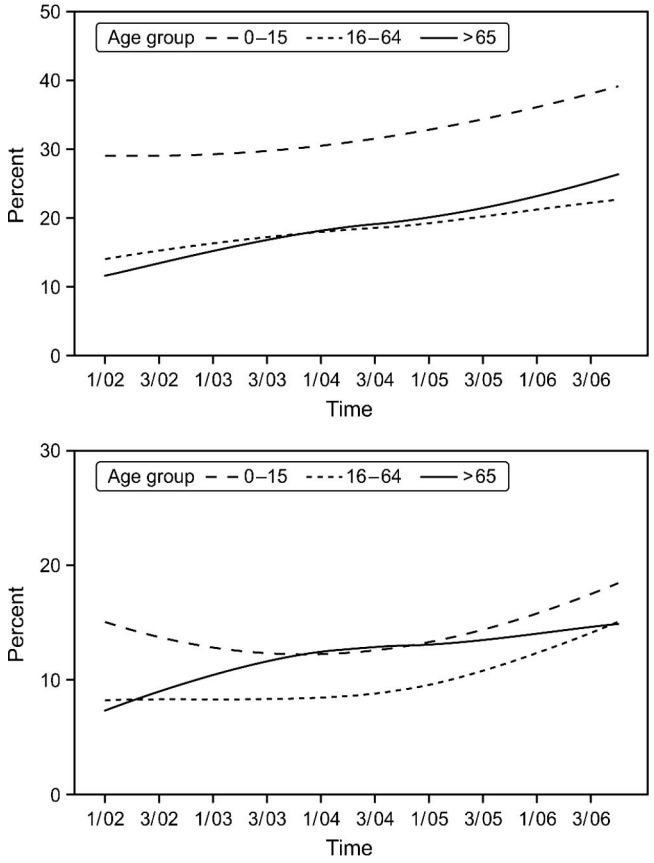

FIG. 1. Time trends of ERY and PEN nonsusceptibility by age group (in years). The curves have been smoothened by an interpolation method to facilitate the viewing of trends. Top panel, ERY nonsusceptibility; bottom panel, PEN nonsusceptibility.

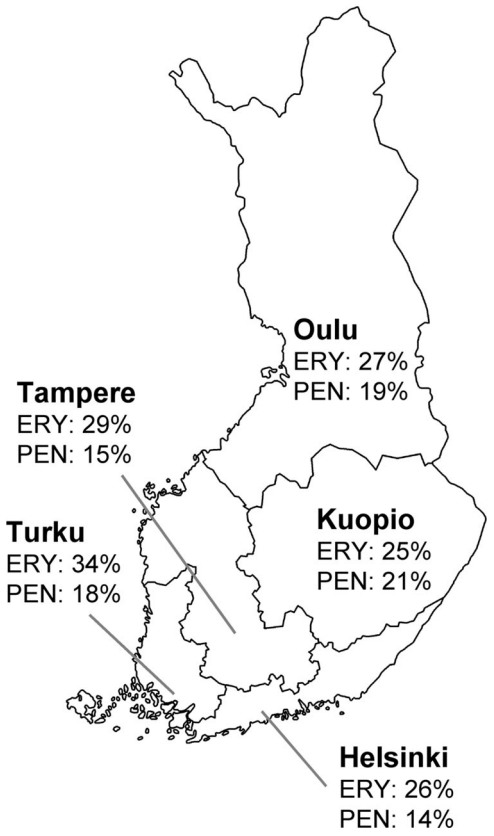

FIG. 2. The nonsusceptibility to ERY and PEN by tertiary-care region (Helsinki, Kuopio, Oulu, Tampere, or Turku) among invasive pneumococci in Finland in the year 2006.

MICs was slightly higher among the former (36.2 mg/liter versus 28.1 mg/liter). *erm*(B) was present in 30% ($n = 68$) of the isolates, and both the *mef*(E) and *erm*(B) genes were carried by two isolates (0.9%). No *erm*(A)-positive isolates were found. In 28 isolates, the macrolide resistance mechanism remained unknown.

Serotype distribution. In 2006, the most common serotypes were 14 (20%), 4 (11%), 6B (9%), 23F (8%), 3 (6%), 7F (6%), 19F (5%), and 9V (5%). The most prominent change was detected in serotype 14, the proportion of which increased from 14% in 2002 to 20% in 2006. The hypothetical PCV-7 serotype coverage varied between age groups, being highest (72%) among the isolates derived from the 0- to 2-year-olds and lowest (54%) among those derived from 16- to 64-year-olds. The overall hypothetical serotype coverage for all age groups and isolates was 58% for the whole study period (Table 2). The vaccine would cover 80% of the ERY-resistant isolates and 87% of the PEN-nonsusceptible isolates.

Genotype distribution. Twenty-five STs were detected (Table 3). Nine of the STs were novel, and to date, another two (ST961 and ST2306) of the previously described STs have not been found outside Finland. The detected STs could be assigned to 10 genetic lineages or CCs, and two STs (ST3248 and ST3249) were singletons. CC156 was the most frequent CC among the PEN R isolates. Only two of the serotype 9V ST156 isolates were multiresistant, and the first of two ERY-resistant isolates in the serotype 14 ST156 population was detected in 2005. However, all isolates of the other STs within CC156 were consistently either multiresistant or resistant to both macrolides and PEN. The macrolide resistance determinants of 12 macrolide-nonsusceptible CC156 isolates were detected, and the most common resistance gene was *erm*(B), although *mef*(E) was present in one isolate each of ST156 serotype 14 and serotype 9V. ST271 and ST2917 of CC271 were isolated from children aged 2 years or less. Two out of the three CC90 isolates were multiresistant, whereas one was susceptible to TET. Both patients with CC63 isolates were infants under the

TABLE 1. Macrolide resistance gene distribution by PEN susceptibility among macrolide-nonsusceptible invasive pneumococci ($n = 223$) from 2002 to 2006 in Finland

Macrolide resistance gene	No. (%) of isolates with indicated PEN susceptibility			Overall macrolide resistance gene distribution [no. (%) of isolates]
	Susceptible	Intermediate	Resistant	
mef[a]	91 (68)	26 (39)	8 (35)	125 (56)
erm(B)	24 (18)	33 (50)	11 (48)	68 (30)
erm(B) + *mef*(E)	0 (0)	0 (0)	2 (9)	2 (1)
erm(A)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	19 (14)	7 (11)	2 (9)	28 (13)

[a] Of the subtyped *mef* isolates, 72% (43/60) carried *mef*(E) and 28% (17/60) *mef*(A).

TABLE 2. The frequency of different serotypes and the proportion of antimicrobial nonsusceptibility by serotype among invasive pneumococci (n = 3,571) in Finland from 2002 to 2006

Serotype	Serotype frequency [no. (%) of isolates]	% of isolates with nonsusceptibility to:	
		ERY	PEN
14[a]	551 (15.4)	57.8	36.3
4[a]	405 (11.3)	4.7	3.2
23F[a]	272 (7.6)	8.5	6.6
9V[a]	268 (7.5)	41.8	13.4
3	243 (6.8)	2.9	3.3
6B[a]	250 (7.0)	40.2	20.8
7F	228 (6.4)	2.6	1.3
19A	163 (4.6)	41.7	11.0
18C[a]	156 (4.4)	3.2	1.9
19F[a]	155 (4.3)	26.5	20.6
9N	125 (3.5)	12.8	4.0
22F	119 (3.3)	5.9	2.5
6A	108 (3.0)	10.2	3.7
12F	97 (2.7)	2.1	0
11A	51 (1.4)	5.9	0
Others	380 (10.6)	8.4	1.6

[a] Included in PCV-7.

age of 1 year in 2005 in one tertiary-care region (Kuopio). These isolates are multiresistant, and one was tested for macrolide-resistant determinants and carried *erm*(B).

Distribution of the pilus-encoding *rlrA* islet. The *rrgC* and *rlrA* genes were present in 19 of the 27 tested PEN R isolates (Table 3). If assumed that all members of an ST carry the *rlrA* islet when the analyzed representative does so, 79.5% (n = 70) of the PEN R isolates carry the islet.

DISCUSSION

A continuous increase in ERY nonsusceptibility from 16 to 28% was observed during the 5-year study period from 2002 to 2006 among invasive pneumococci in Finland. Compared to that for the years 1999 to 2000, there was a fivefold increase in ERY nonsusceptibility (43). In Europe, ERY resistance prevalences similar to that for Finland (20 to 30%) have been reported from Germany, the United Kingdom, and Switzerland (21), while countries with low macrolide resistance prevalences (<11%) include Austria, the Czech Republic, Denmark, Norway, Portugal, Sweden, and The Netherlands (2, 21, 49, 54). Globally, the highest ERY resistance prevalences are found in the Far East (~80%) and the lowest in Latin America (~15%) (23).

The *mef* gene was the most frequent macrolide resistance determinant in this study carried by 56% of the macrolide-resistant pneumococci, while 30% of the isolates had *erm*(B). Our results are in accordance with previous studies concerning Finland (21, 43, 48). The macrolide resistance gene distribution in Finland is more comparable with that of North America and Scotland than with that of continental Europe (21, 60). Of the *mef* subtypes, *mef*(E) was the prevailing one in Finland. It also dominates in Canada, the United States, South Africa, and eastern Europe, while both *mef*(A) and *mef*(E) occur in Mediterranean and western European countries (14). All *mef*(A) isolates in our study were serotype 14 and had an identical antibiogram, suggesting that they are clonally related. In Scotland, Italy, and Norway, the spread of *mef*(A)-carrying isolates was related to the emergence of the ERY-resistant but PEN-susceptible England[14] ST9 clone (3, 12, 51). However, ST13, a single-locus variant of this international clone, was present in our PEN R material, and the macrolide resistance determinant of a representative of this genotype was *mef*(E). Previously, 10% of the invasive pneumococci in Finland were shown to have a mutation in the ribosome or ribosomal protein that confers macrolide resistance (43). Although the presence of ribosomal mutations was not investigated in this study, our results indicate that the proportion of such isolates is similar, as 13% of the macrolide-resistant pneumococci did not carry an efflux or methylase gene.

Apart from ERY nonsusceptibility, PEN nonsusceptibility doubled from 8 to 16% during the study period. This is a fourfold increase compared to the level from year 1999 to 2000 (43). The global situation of PEN nonsusceptibility among pneumococci is similar to that of ERY, although PEN nonsusceptibility figures are usually somewhat lower (23). Worldwide PEN nonsusceptibility prevalence has reached 36 to 37%, while the proportion of fully resistant strains is 23% (23). Of the European countries, Denmark, Sweden, Norway, Iceland, and The Netherlands have so far managed to keep the PEN-nonsusceptibility prevalence between 0 and 10% (8, 49), most likely because of conservative antimicrobial policy and low total antimicrobial consumption in these countries (5, 38, 49).

Several internationally recognized PEN R or multiresistant genotypes are present in the invasive PEN R pneumococcal population in Finland. Spain[9V] ST156, the predicted founder of our largest CC, CC156, has been described on all continents and displays a variety of serotypes, indicating that it is capable of capsular gene switching (36). Similar expansion, diversification, and development of further resistance of the CC156 genotypes as seen in our study have been described elsewhere. In Poland, the dissemination of the ST143 clone together with the ST156 clone has been implicated in the rise in PEN R cases (50), and in Portugal, ST143 has contributed to the rise in ERY-resistant isolates (16). One example of possible capsular switching by genotypes related to ST156 is provided by ST671, which is represented in our material by a serotype 19F isolate. In the United States, multiresistant serotype 14 ST671 isolates have caused invasive disease (26).

CC271, which includes the international Taiwan[19F] ST236 clone, merits attention not only for the dual macrolide resistance of two of the isolates but also because all isolates in this CC are multiresistant. All five ST271 and ST2917 isolates were derived from children aged 2 years or less. Dual macrolide resistance is reportedly significantly more frequent among isolates derived from 0- to 2-year-olds than from the other age groups (21).

In our study, the *rlrA* islet-positive genotypes were concentrated in five CCs, including CC156 and CC271, and one singleton. This is in accordance with previous reports indicating that carriage of the *rlrA* islet correlates with the genotype and is high among drug-resistant isolates (1, 40). In isolates from Portugal, Spain[9V] ST156 is associated with the presence of *rlrA*, regardless of serotype, and 61% of the PEN-nonsusceptible isolates carried *rlrA*, while the corresponding figure for susceptible isolates was 16% (1). The high proportion of *rlrA*-positive PEN R isolates described in our study may be ex-

TABLE 3. CCs, STs, serotypes, and antimicrobial susceptibilities of the PEN R isolates (n = 88)

CC[a]	ST[b]	Allelic profile[b,c]	Serotype	Antimicrobial susceptibility (mg/liter)			ERY resistance gene(s)[d]	*rlrA* islet[e]	No. of patients	Year(s) of isolation	Related international clone or degree of relatedness[f]
				PEN	ERY	TET					
156	156	7-11-10–1-6-8-1	9V	2	0.063–>128	0.25–64	*erm*(B) or *mef*(E)	+	13	2003–2006	Spain^{9V} ST156
	156	7-11-10-1-6-8-1	14	2–4	0.125–16	0.25–0.5	*mef*(E)	+	15	2003–2006	Spain^{9V} ST156
	156	7-11-10-1-6-8-1	19F	2	0.125	0.25	Not determined	+	1	2002	Spain^{9V} ST156
	2918	7-11-10-1-6-8-267	14	2	>128	0.5	Not determined	+	1	2006	SLV of ST156
	2306	7-11-10-1-6-8-119	14	2	>128	32–64	*erm*(B)	+	11	2002–2006	SLV of ST156
	3247	7-8-10-1-6-8-119	14	2	>128	32	Not determined	+	1	2003	DLV of ST156
	143	7-5-10-18-6-8-1	14	2	>128	0.25–32	*erm*(B)	+	7	2004–2006	DLV of ST156
	2916	7-5-10-18-6-5-1	14	2	>128	32	*erm*(B)	+	4	2005–2006	TLV of ST156
	671	7-11-10-1-5-76-98	19F	2	32	0.5	Not determined	+	1	2006	TLV of ST156
271	236	15-16-19-15-6-20-26	19F	2–4	8–16	32	Not determined	+	2	2003, 2006	Taiwan^{19F} ST236
	271	4-16-19-15-6-20-26	19F	2	129	16–32	*erm*(B) and *mef*(E)	+	2	2003, 2005	SLV of ST236
	2694	15-16-19-1-6-20-26	19F	2	8	32	*mef*(E)	+	1	2004	SLV of ST236
	2917	4-16-19-15-6-20-266	19F	2–4	>128	32	*erm*(B) and *mef*(E)	+	3	2005–2006	DLV of ST236
	3245	4-16-19-15-6-20-286	19F	4	>128	32	Not determined	+	1	2002	DLV of ST236
81	81	4-4-2-4-4-1-1	23F	2–4	0.125–2	1–8	*mef*(E)	−	2	2003, 2005	Spain^{23F} ST81
	961	4-4-2-4-10-1-1	23F	2	>128	0.25–4	*erm*(B)	−	5	2003–2004, 2006	SLV of ST81
	3250	4-4-180-4-4-1-1	23F	2	>128	16	Not determined	−	1	2004	SLV of ST81
90	90	5-6-1-2-6-3-4	6B	2	>128	0.5–64	*erm*(B)	+	3	2005–2006	Spain^{6B} ST90
	3246	5-6-33-16-6-3-4	6B	2	>128	32	*erm*(B)	+	1	2003	DLV of ST90
15	13	1-5-4-5-5-27-8	14	2–4	16–32	0.25	*mef*(E)	−	4	2003, 2005	SLV of England^{14} ST9
63	2678	2-60-36-12-17-21-14	14	2–4	>128	32–64	*erm*(B)	−	2	2005	SLV of Sweden^{15A} ST63
496	496	42-35-29-36-9-39-18	18C	2	129	0.25	*erm*(B)	−	2	2003–2004	None
205	205	10-5-4-5-13-10-18	4	2	32	1	Not determined	+	1	2004	Sweden^{4} ST205
138	138	7-5-8-5-10-6-14	6B	4	16	0.25	Not determined	+	1	2006	None
460	461	5-40-4-1-10-1-27	10	2	>128	32	Not determined	−	1	2003	None
S	3248	115-12-2–16-6-19-245	17	2	>128	32	Not determined	−	1	2004	None
S	3249	10-6-1-2-49-26-14	6B	2	>128	32	Not determined	+	1	2004	None

[a] CC named after the ST with the highest number of single-locus variants in the MLST database (November 2008). S, singleton.
[b] New STs and alleles are underlined.
[c] In the following order: *aroE-gdh-gki-recP-spi-xpt-ddl*.
[d] ERY resistance determinant results shown here are based on 223 randomly chosen ERY-nonsusceptible isolates, which were not selected on the basis of PEN susceptibility (see text).
[e] Based on the detection of *rlrA* and *rrgC* by PCR in one isolate per ST and serotype. +, presence; −, absence.
[f] SLV, single-locus variant; DLV, double-locus variant; TLV, triple-locus variant.

plained by the clonal association, because a majority of our PEN R isolates were clonally related to ST156. Additionally, in our study, only fully PEN R isolates were investigated for the presence of *rlrA*, which could further affect the proportion of *rlrA*-carrying isolates. In a mouse model, the pilus provided a competitive advantage over nonpiliated pneumococci in the nasopharynx, and it has been suggested that this may contribute to the spread of the piliated clones (53).

A third of the PEN R isolates had STs that have not been described elsewhere, a situation similar to that in Norway, where 42% of the genotyped PEN-nonsusceptible isolates had a previously unknown genotype (55). Interestingly, the isolates with novel genotypes described in this study are, with one exception, multiresistant, and many are related to successful international clones, with the potential to spread and diversify. Four novel genotypes consisted of new combinations of known alleles, whereas five contained previously unknown alleles. Although 11 of the detected STs have not been described previously internationally, we do not know the time and place of their origins. The MLST database relies on the voluntary submission of typed isolates and is thus unlikely to include all analyzed isolates. Furthermore, a possible bias toward studying drug-resistant and invasive pneumococci may be reflected in the database material. This combined with the limitations of the eBURST algorithm may also affect the assignment of both subgroup founders and the predicted primary founder of a CC.

Ceftriaxone-resistant pneumococci are still rare in Finland, most likely because of the low proportion of fully PEN R pneumococci (<5%). Nevertheless, reduced ceftriaxone susceptibility (the meningitis breakpoint) was detected in 3 to 4% of the isolates, which warrants careful continuous surveillance in the future. In countries with high PEN R prevalence, such as Taiwan, the United States, and Spain, 6 to 9% of the pneumococci are nonsusceptible to ceftriaxone (9, 33, 58), while in countries with low PEN R prevalences, such as Italy, Hungary, and Portugal, the respective proportion is below 1% (18, 27, 39). However, comparing the studies is sometimes difficult because of differences in the representativeness of the isolates and the breakpoints used. Levofloxacin-resistant isolates were rare (≤0.5%) in this study. According to several studies, the fluoroquinolone resistance prevalence is ≤3% in most countries (18, 23, 49), although studies, for example, from Canada and Portugal indicate the increasing prevalence of fluoroquinolone resistance (1, 24).

In our previous study, we described the presence of heterogeneous telithromycin resistance that was associated with the *erm*(B) gene in a Finnish pneumococcal collection in 2002 (47). That type of telithromycin resistance was also observed in nine *erm*(B) isolates in this study. According to an international longitudinal study, started in 1999, globally less than 1% of pneumococci are nonsusceptible to this agent, and no increasing trend was detected by 2005 (23). However, that study used the CLSI broth microdilution method, which, in our experience, does not favor the detection of this resistance type (47). The significance of this type of resistance is not clear, but it can offer an advantage in the presence of antimicrobial pressure favoring the selection of telithromycin-resistant pneumococci. Previous laboratory experiments have shown that *erm*(B)-positive pneumococci require only two to three passages in telithromycin in order to achieve telithromycin resistance that is stable and maintained without continuing selective pressure from the antimicrobial agent (15, 59).

The proportion of multiresistant isolates remained near 5% among invasive pneumococci in Finland. In general, the higher the resistance to ERY or PEN among pneumococci, the higher the prevalence of multiresistance; for example, in the United States, multiresistance percentages range from 9 to 25% by region and correspond with the proportions of PEN and ERY resistance (13, 32). The definition of pneumococcal multiresistance varies between studies, making a comparison of proportions difficult. The highest antimicrobial nonsusceptibility prevalences were seen among pneumococci isolated from children less than 15 years old, particularly those less than 2 years of age. This may be explained by the high consumption of antimicrobials among young children, compared to other age groups (4), and is in accordance with studies from other countries (18, 21).

Our results showed that the hypothetical serotype coverage by PCV-7 varies by age group but includes approximately 60% of all invasive isolates in our study. The vaccine hypothetical serotype coverage exceeded 80% among both the ERY-resistant and the PEN-nonsusceptible isolates, which suggests that the introduction of PCV-7 into the national vaccination program could reduce the proportion of antimicrobial resistance in Finland. Nevertheless, postvaccine follow-up studies imply that the longer-term impact of the vaccine on antimicrobial resistance is still unclear. Some investigators have observed a reduction in antimicrobial resistance (7, 35), while others did not (17, 25, 37). The prevalence of antimicrobial resistance can also rapidly increase in nonvaccine- and vaccine-related pneumococci (22, 30). Vaccine serotypes are most likely replaced by nonvaccine- or vaccine-related serotypes, while the genotypes remain unchanged (28, 41, 52, 56). One such example may be provided by isolates in CC271. The CC271 isolates in this study were serotype 19F, but several genotypes in this CC also express serotype 19A, according to the MLST database. Serotype 19A is the most important replacement serotype which caused invasive pneumococcal disease in the United States following the introduction of PCV-7 (42). At present, the prevalence of serotype 19A isolates in Finland is around 5% and has remained stable between the years 1995 and 2006 as described previously (H. Nohynek et al., presented at the 18th European Congress of Clinical Microbiology and Infectious Diseases [ECCMID], Barcelona, Spain, 19 to 22 April 2008). Continuing surveillance is important, not least after PCV-7 is widely introduced. In the future, perhaps a multivalent protein vaccine including one or several pilus protein components may help to control the piliated international drug-resistant clones.

In conclusion, the emergence of antimicrobial resistance among invasive pneumococci is a public health concern in Finland. The high proportion of macrolide resistance, especially in children, should be taken into account when recommendations of first-line drugs are issued. When macrolides are used, the response should be carefully monitored to ensure that treatment is successful. The introduction of PCV-7 into the national vaccination program will cause changes in pneumococcal epidemiology, but whether this has an impact on antimicrobial resistance remains to be seen. Nevertheless, close surveillance of antimicrobial resistance and changes in

the clonality of the pneumococcal population is of utmost importance in the near future.

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