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Antimicrobial Susceptibility of *Streptococcus pneumoniae* in Eight European Countries from 2001 to 2003

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Susceptibility testing results for *Streptococcus pneumoniae* isolates ($n = 2,279$) from eight European countries, examined in the PneumoWorld Study from 2001 to 2003, are presented. Overall, 24.6% of *S. pneumoniae* isolates were nonsusceptible to penicillin G and 28.0% were resistant to macrolides. The prevalence of resistance varied widely between European countries, with the highest rates of penicillin G and macrolide resistance reported from Spain and France. Serotype 14 was the leading serotype among penicillin G- and macrolide-resistant *S. pneumoniae* isolates. One strain (PW 158) showed a combination of an efflux type of resistance with a 23S rRNA mutation (A2061G, pneumococcal numbering; A2059G, *Escherichia coli* numbering). Six strains which showed negative results for *mef*(A) and *erm*(B) in repeated PCR assays had mutations in 23S rRNA or alterations in the L4 ribosomal protein (two strains). Fluoroquinolone resistance rates (levofloxacin MIC ≥ 4 μg/ml) were low (Austria, 0%; Belgium, 0.7%; France, 0.9%; Germany, 0.4%; Italy, 1.3%; Portugal, 1.2%; Spain, 1.0%; and Switzerland, 0%). Analysis of quinolone resistance-determining regions showed eight strains with a Ser81 alteration in *gyrA*; 13 of 18 strains showed a Ser79 alteration in *parC*. The clonal profile, as analyzed by multilocus sequence typing (MLST), showed that the 18 fluoroquinolone-resistant strains were genetically heterogeneous. Seven of the 18 strains belonged to new sequence types not hitherto described in the MLST database. Europe-wide surveillance for monitoring of the further spread of these antibiotic-resistant *S. pneumoniae* clones is warranted.

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in humans (35). The worldwide increase in the rates of antibiotic resistance in this species has become a serious problem within the last 20 years (1). Macrolide resistance in *S. pneumoniae* is usually caused by the presence of the *erm*(B) or the *mefA* resistance determinant. The *erm*(B) protein encodes a 23S rRNA methylase, and most pneumococcal strains that harbor this gene are resistant to 14-, 15-, and 16-membered-ring macrolides, lincosamides, and streptogramin B (MLS_B phenotype). The *mef*(A) protein encodes an efflux pump that leads to resistance only to 14- and 15-membered-ring macrolides (45, 52). Other mechanisms of macrolide resistance include changes clustered in a highly conserved region of domain V of 23S rRNA, which plays a key role in macrolide binding (6, 10, 49, 55), and in ribosomal proteins L4 and L22. In addition, *erm*(TR) mutations (53) have also been described in a few clinical pneumococcal isolates (6, 10, 49, 55).

Newer fluoroquinolones with greater potencies against *S. pneumoniae* licensed in Europe include levofloxacin and moxifloxacin. Because of the emergence of antimicrobial resistance in pneumococci (1), newer fluoroquinolones are now recommended for the empirical treatment of pneumonia in adults when antimicrobial resistance is suspected (28). Fluoroquinolones are also recommended for initial empirical therapy of selected outpatients with community-acquired respiratory tract infections (e.g., patients with acute exacerbations of chronic bronchitis) in several countries (28, 59). Other therapeutic options (macrolides and doxycycline) are generally preferred for the treatment of uncomplicated infections in outpatients, but there is increasing concern about the misuse and overuse of fluoroquinolones, and it is believed that if abuse of this class of drugs continues unabated, we may see the demise of fluoroquinolones as useful antibiotics within the next 5 to 10 years (28).

Pneumococcal resistance to quinolones is usually due to mutations in either *parC* or *gyrA*, or both (40). Strains usually become fully fluoroquinolone resistant with the addition of a mutation in the other target gene (either *gyr*A or *par*C). Mutations in *parE* and *gyrB* may also contribute to resistance (37). In addition, an efflux mechanism has been described (16).

The PneumoWorld surveillance study was established in 2001 to study the susceptibilities of *Streptococcus pneumoniae* isolates from patients with noninvasive and invasive pneumococcal disease. This paper presents the results of the 2001 to 2003 PneumoWorld surveillance study of isolates submitted by 31 participating centers in eight European countries and focuses on the prevalence of macrolide and fluoroquinolone resistance geno- or phenotypes in these countries.

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MATERIALS AND METHODS

Study design. The PneumoWorld Study was established to study the antimicrobial susceptibilities of *S. pneumoniae* isolates from Latin America and Europe. In the European part of the study, 31 centers in eight European countries took part in the study: Austria (three centers), Belgium (two centers), France (5 centers), Germany (6 centers), Italy (6 centers), Portugal (3 centers), Spain (4 centers), and Switzerland (2 centers).

Bacterial isolates. Only pneumococcal isolates of probable clinical significance from adults ≥16 years of age were included. Strains were isolated and preliminarily identified (optochin sensitivity and bile solubility testing) in each center and were stored frozen at −70°C in porous beads (MICROBANK; Mast Diagnostica GmbH, Rheinfeld, Germany) for up to 3 months. Strains were then sent by courier in batches of up to 100 strains in transport medium (Port-A-Cul; Difco, Germany) to the German National Reference Centre for Streptococci for confirmation of species identification, susceptibility testing, and further investigation. Confirmation of the identities of the *S. pneumoniae* strains was performed by optochin sensitivity and bile solubility testing (2, 15). Demographic data collected during the study included the age and sex of the patient, infection type, culture source, inpatient or outpatient status, and the date of sample collection.

Susceptibility testing. MIC testing was performed by the broth microdilution method recommended by CLSI (formerly the National Committee for Clinical Laboratory Standards) (38). Microtiter plates (Sensititre susceptibility plates; TREK Diagnostic Systems Ltd., East Grinstead, England) containing penicillin G, amoxicillin, cefotaxime, cefuroxime, cefpodoxime, clarithromycin, clindamycin, gatifloxacin, levofloxacin, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol with cation-adjusted Mueller-Hinton broth (Oxoid, Wesel, Germany) plus 5% lysed horse blood (Oxoid) were used. The final inoculum was 5×10^5 CFU/ml. MICs were determined following incubation at 35°C for 20 to 24 h in ambient air. *S. pneumoniae* ATCC 49619 was included as a control strain. Current CLSI interpretive criteria were used to define antimicrobial resistance (38). The isolates were stored at −70°C as described above (MICROBANK). For determination of macrolide-resistant phenotypes, disks (Oxoid Ltd., Basingstoke, United Kingdom) of erythromycin (15 μg) and clindamycin (2 μg) were placed 15 to 20 mm apart on Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, MD) with 5% sheep blood (Oxoid, Wesel, Germany). The plates had previously been inoculated with a swab dipped into a 0.5 McFarland standard bacterial suspension (32). MICs of fluoroquinolone-resistant *S. pneumoniae* were additionally determined for clinafloxacin (Pfizer, Karlsruhe, Germany), grepafloxacin (Glaxo Wellcome, Hamburg, Germany), sparfloxacin (Aventis, Bad Soden, Germany), moxifloxacin (Bayer, Leverkusen, Germany), and ciprofloxacin (Bayer).

Determination of resistance genes. For the detection of *erm*(B) and *mef*(A), the primers described by Trieu-Cuot et al. (57) and by Tait-Kamradt et al. were chosen (54). Preparation of DNA and reverse transcription-PCR were performed as described previously (48). For seven isolates (six *mef*(A)- and *erm*(B)-negative isolates and one *mef*(A)-positive isolate with a constitutive MLS_B [$cMLS_B$] resistance phenotype), sequencing of the 23S rRNA genes and the ribosomal protein L4 and L22 genes was performed with an ABI Prism Big Dye terminator kit (Applied Biosystems, Foster City, CA). The sequencing primers were as reported earlier by Canu et al. (6) and Tait-Kamradt et al. (55). The nucleotide sequences of the 23S rRNA and the L4 and L22 ribosomal proteins in *Escherichia coli*, *S. pneumoniae* R6, and *S. pneumoniae* serotype 4 (56) were obtained from the Institute for Genomic Research website (http://www.tigr.org). For fluoroquinolone-resistant strains, prepared chromosomal DNA was used as a template for PCR amplification of the genes encoding the quinolone resistance-determining region (QRDR). The primers and PCR conditions were those defined previously (24, 47). Some strains with unusual resistance phenotypes (strains PW 555, PW 169, PW 1905, PW 2216, and PW 1571) were screened for resistance determinants *ermA*, *ermC*, *msrA*, *msrB*, and *msrD* as described by Shortridge et al. (51) and Daly et al. (7).

Serotyping. Pneumococcal strains with clarithromycin resistance, penicillin G resistance, or reduced susceptibility to fluoroquinolones were serotyped by Neufeld's Quellung reaction (39) by using type- and factor-specific sera provided by the Statens Serum Institut, Copenhagen, Denmark.

MLST. Multilocus sequence typing (MLST) of seven strains [six *mef*(A)- and *erm*(B)-negative isolates and one *mef*(A)-positive isolate with a $cMLS_B$ phenotype] was carried out as described previously (11). Briefly, internal fragments of the *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl* genes were amplified by PCR from chromosomal DNA with the primer pairs described by Enright and Spratt (11). The alleles at each of the seven loci provide the allelic profile of each isolate and also define their sequence type (ST). The allelic profiles are shown as the alleles at each of the seven loci, in the order *aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*.

Data on antibiotic consumption was obtained from the European Surveillance of Antimicrobial Consumption, University of Antwerp (http://www.ESAC-.UA.AC.be). The correlation between antibiotic consumption and fluoroquinolone resistance was analyzed by linear regression by using the Winstat statistical module (R. Fitch Software, Staufen, Germany).

RESULTS

A total of 2,279 isolates of *S. pneumoniae* from 31 European centers in eight countries were examined. Strains were isolated from the following sources: sputum (n = 1,055 [46.3%]), blood cultures (n = 421 [18.5%]), bronchoalveolar lavage fluid (n = 416 [18.2%]), sinus punctures (n = 150 [6.6%]), cerebrospinal fluid (CSF; n = 57 [2.5%]), other sterile body sites (n = 81 [3.6%]), pus from the middle ear (n = 54 [2.4%]), fluid obtained by tympanocentesis (n = 29 [1.2%]), and other sources (n = 16 [0.7%]). Pneumococci were isolated from individuals in the following age groups: ≥16 to 30 years (n = 261 [11.5% of cases]), >30 to 40 years (n = 275 [12.0% of cases]), >40 to 50 years (n = 276 [12.1% of cases]), >50 to 60 years (n = 421 [18.5% of cases]), >60 to 70 years (n = 494 [21.7% of cases]), and > 70 (n = 506 [22.2% of cases]). Among all patients, 63.6% were males and 35.1% were females (no data on the sex of the patients were available for 1.3% of the patients). The following clinical diagnoses were recorded: pneumonia (n = 1,056 [46.3%]), acute bacterial exacerbation of chronic bronchitis (n = 392 [17.2%]), chronic obstructive lung disease (n = 194 [8.5%]), acute sinusitis (n = 150 [6.6%]), acute otitis media (n = 85 [3.7%]), and other or unknown diagnosis (n = 402 [17.7%]).

Overall, 12.5% of the isolates were penicillin G intermediate (MIC = 0.12 to 1 μg/ml) and 12.1% were penicillin G resistant (MIC ≥ 2 μg/ml). However, there was considerable intercountry variation in these overall resistance rates (Table 1).

A total of 853 pneumococcal strains showed resistance to either macrolides or penicillin G, or both (n = 27), and were chosen for pneumococcal serotyping. Serotypes 14 (18.4%), 23F (13.7%), 6B (13.7%), and 19F (12.5%) were the leading serotypes among the antibiotic-resistant strains (Table 2). The rates of antibiotic resistance varied within countries (Table 3).

In total, 618 pneumococcal strains showed macrolide resistance. The prevalence of macrolide resistance geno- and phenotypes varied greatly between countries. In Spain and France, where among the eight countries in this study the highest rates of macrolide resistance were reported, *erm*(B) was the leading genotype (Spain, 119 of 129 strains [92.3%]); France, 199 of 202 strains [98.5%]) (Table 4). By contrast, in Germany and Austria, where relatively low rates of macrolide resistance were found, the proportion of macrolide-resistant strains with efflux was relatively high (Germany, 26 of 56 [46.2%]; Austria, 5 of 12 [41.7%]). In addition, in Italy a relatively high rate of *mef*(A)-positive strains was also observed (25.0% of all macrolide-resistant strains). Six strains from Spain (n = 2), Germany (n = 2), Italy (n = 1), and Belgium (n = 1) which showed no PCR product in repeated assays for *mef*(A) or *erm*(B) were further analyzed for mutations in 23S rRNA and alterations in ribosomal proteins L4 and L22 (Table 5). One strain from Spain (strain PW 160), which exhibited a $cMLS_B$ phenotype, showed a 23S rRNA mutation at A2060 (pneumococcal numbering; A2058 by *E. coli* numbering). Interestingly, this strain was ST 156 and serotype 13, indicating a serotype switch from serotype 14 to serotype 13. Two isolates, a serotype 3 strain from

TABLE 1. MIC_{50}s, MIC_{90}s, MIC ranges, and antibiotic resistance of 2,279 isolates of *S. pneumoniae* in eight European countries[a]

Country (no. of strains)	Antibiotic	MIC (μg/ml)			% Resistance (I + R)
		50%	90%	Range	
Austria (*n* = 160)	Penicillin G	0.016	0.03	0.008–2	4.4
	Amoxicillin	0.016	0.03	0.008–2	0
	Cefotaxime	0.03	0.03	0.03–1	0
	Cefuroxime	0.03	0.06	0.03–4	0.6
	Cefpodoxime	0.03	0.06	0.03–2	0.6
	Clarithromycin	0.125	0.25	0.125–≥32	10.0
	Clindamycin	0.125	0.125	0.125–≥32	4.4
	Gatifloxacin	0.25	0.25	0.125–0.5	0
	Levofloxacin	0.5	1	0.25–1	0
	COT	0.25/4.75	1/19	0.125/2.37–8/152	11.3
	Tetracycline	0.5	4	0.125–≥32	10.6
	Chloramphenicol	2	2	2–8	1.9
Belgium (*n* = 148)	Penicillin G	0.016	0.125	0.008–2	11.5
	Amoxicillin	0.016	0.03	0.008–2	0
	Cefotaxime	0.03	0.06	0.03–2	3.4
	Cefuroxime	0.03	0.25	0.03–8	8.8
	Cefpodoxime	0.03	0.06	0.03–4	6.8
	Clarithromycin	0.125	≥32	0.125–≥32	23.7
	Clindamycin	0.125	≥32	0.125–≥32	18.2
	Gatifloxacin	0.25	0.25	0.06–4	0.7
	Levofloxacin	0.5	1	0.125–≥32	0.7
	COT	0.25/4.75	4/76	0.125/2.37–16/304	18.9
	Tetracycline	0.5	≥32	0.125–≥32	23.7
	Chloramphenicol	2	2	2–16	2.7
France (*n* = 443)	Penicillin G	0.06	2	0.008–4	47.6
	Amoxicillin	0.03	2	0.008–8	3.6
	Cefotaxime[b]	0.06	2	0.016–4	11.1
	Cefuroxime	0.25	8	0.03–8	39.1
	Cefpodoxime	0.125	4	0.03–4	38.4
	Clarithromycin	0.125	≥32	0.125–≥32	46.1
	Clindamycin	0.125	≥32	0.125–≥32	44.2
	Gatifloxacin	0.25	0.25	0.125–4	0.9
	Levofloxacin	1	1	0.25–≥32	0.9
	COT	0.5/9.5	8/152	0.125/2.37–16/304	42.0
	Tetracycline	0.5	≥32	0.125–≥32	40.4
	Chloramphenicol	2	8	2–16	14.7
Germany (*n* = 530)	Penicillin G	0.016	0.03	0.008–2	6.0
	Amoxicillin	0.016	0.03	0.008–4	0.2
	Cefotaxime	0.03	0.03	0.016–2	0.6
	Cefuroxime	0.03	0.06	0.03–8	1.5
	Cefpodoxime	0.03	0.06	0.03–4	1.3
	Clarithromycin	0.125	1	0.125–≥32	10.6
	Clindamycin	0.125	0.125	0.06–≥32	5.1
	Gatifloxacin	0.25	0.25	0.06–4	0.2
	Levofloxacin	1	1	0.06–≥32	0.4
	COT	0.25/4.75	1/19	0.125/2.37–≥32/≥1,216	14.5
	Tetracycline	0.25	4	0.125–≥32	11.3
	Chloramphenicol	2	2	2–≥32	1.9
Italy (*n* = 462)	Penicillin	0.016	0.25	0.008–4	13.0
	Amoxicillin	0.016	0.06	0.008–4	0.2
	Cefotaxime	0.03	0.125	0.016–2	2.8
	Cefuroxime	0.03	0.5	0.03–8	7.4
	Cefpodoxime	0.03	0.25	0.03–4	8.0
	Clarithromycin	0.125	≥32	0.06–≥32	35.5
	Clindamycin	0.125	≥32	0.06–≥32	24.7
	Gatifloxacin	0.25	0.5	0.125–4	1.1
	Levofloxacin	1	1	0.125–64	1.3
	COT	0.5/9.5	4/76	0.125/2.37–≥32/≥1,216	41.1
	Tetracycline	0.5	≥32	0.125–≥32	23.8
	Chloramphenicol	2	2	2–≥32	6.7
Portugal (*n* = 174)	Penicillin G	0.016	2	0.008–4	19.0
	Amoxicillin	0.016	2	0.008–8	2.3
	Cefotaxime	0.03	1	0.016–4	6.9
	Cefuroxime	0.06	8	0.03–8	14.4
	Cefpodoxime	0.06	2	0.03–8	13.8

Continued on following page

TABLE 1—*Continued*

Country (no. of strains)	Antibiotic	MIC (μg/ml)			% Resistance (I + R)
		50%	90%	Range	
	Clarithromycin	0.125	1	0.125–≥32	10.3
	Clindamycin	0.125	0.125	0.125–≥32	8.6
	Gatifloxacin	0.25	0.25	0.06–8	1.1
	Levofloxacin	1	1	0.06–≥32	1.2
	COT	0.25/4.75	4/76	0.125/2.37–8/152	24.1
	Tetracycline	0.5	8	0.125–≥32	12.1
	Chloramphenicol	2	2	2–16	2.3
Spain (n = 310)	Penicillin G	0.25	2	0.008–4	61.9
	Amoxicillin	0.25	2	0.008–8	9.0
	Cefotaxime[c]	0.25	2	0.016–4	11.0
	Cefuroxime	1	8	0.03–8	43.9
	Cefpodoxime	0.5	4	0.03–8	44.8
	Clarithromycin	0.125	≥32	0.125–≥32	43.6
	Clindamycin	0.125	≥32	0.125–≥32	39.4
	Gatifloxacin	0.25	0.5	0.125–4	0.7
	Levofloxacin	1	1	0.25–≥32	1
	COT	2/38	8/152	0.125/2.37–8/152	66.5
	Tetracycline	1	≥32	0.125–≥32	47.1
	Chloramphenicol	2	16	2–≥32	25.8
Switzerland (n = 52)	Penicillin G	0.016	0.5	0.008–2	17.3
	Amoxicillin	0.016	0.25	0.008–2	0
	Cefotaxime	0.03	0.5	0.03–2	3.9
	Cefuroxime	0.03	2	0.03–8	11.5
	Cefpodoxime	0.03	0.5	0.03–4	9.6
	Clarithromycin	0.125	0.25	0.125–≥32	17.3
	Clindamycin	0.125	0.125	0.125–≥32	11.5
	Gatifloxacin	0.25	0.25	0.125–0.5	0
	Levofloxacin	1	1	0.125–1	0
	COT	0.25/4.75	1/19	0.125/2.37–8/152	28.9
	Tetracycline	0.5	4	0.125–≥32	13.5
	Chloramphenicol	2	2	2	0
All strains (n = 2,279)	Penicillin G	0.016	2	0.008–4	24.6
	Amoxicillin	0.016	1	0.008–8	2.2
	Cefotaxime[d]	0.03	1	0.016–4	5.1
	Cefuroxime	0.06	4	0.03–8	17.7
	Cefpodoxime	0.03	2	0.03–8	17.5
	Clarithromycin	0.125	≥32	0.125–≥32	28.0
	Clindamycin	0.125	≥32	0.06–≥32	22.6
	Gatifloxacin	0.25	0.25	0.06–8	0.7
	Levofloxacin	1	1	0.06–≥32	0.8
	COT	0.25/4.75	4/76	0.125/2.37–≥32/≥1,216	33.4
	Tetracycline	0.5	≥32	0.125–≥32	25.2
	Chloramphenicol	2	2	2–≥32	8.6

[a] Abbreviations: I, intermediate; R, resistant; COT, trimethoprim-sulfamethoxazole.

[b] Ninety-seven strains showed an MIC of 1 μg/ml. Of the 97 strains, 1 was a CSF isolate and was classified as cefotaxime intermediate. Of 46 isolates with an MIC of 2 μg/ml, all were nonmeningitis strains and were classified as cefotaxime intermediate. Two strains exhibited an MIC of 4 μg/ml and were classified as cefotaxime resistant, so that in total, 49 strains (11.1%) were cefotaxime intermediate or cefotaxime resistant.

[c] Seventy-six strains (all nonmeningitis isolates) showed an MIC of 1 μg/ml and were classified as cefotaxime susceptible. Of 28 isolates with an MIC of 2 μg/ml, all were nonmeningitis strains and were classified as cefotaxime intermediate. Six strains exhibited an MIC of 4 μg/ml and were classified as cefotaxime resistant, so that in total, 34 strains (11.0%) were cefotaxime intermediate or cefotaxime resistant.

[d] Two hundred seven strains showed an MIC of 1 μg/ml. Of the 207 strains, 1 was a CSF isolate and was classified as cefotaxime intermediate. Of the 108 isolates with an MIC of 2 μg/ml, all were nonmeningitis strains and were classified as cefotaxime intermediate. Nine strains exhibited an MIC of 4 μg/ml and were classified as cefotaxime resistant, so that in total, 118 strains (5.1%) were cefotaxime intermediate or cefotaxime resistant.

Belgium (PW 1571) and a serotype 6A strain from Italy (PW 1905), showed alterations in the L4 ribosomal protein (Ser20Asn). A German strain (PW 555) with an inducible MLS_B ($iMLS_B$) resistance phenotype and a clarithromycin MIC of 2 μg/ml showed no alterations in ribosomal proteins L4 and L22 and only A2937G and G2939T mutations located outside the 3′end of 23S rRNA (internal transcribed spacer). Interestingly, strain PW 158 from Spain showed a combination of an efflux mechanism and a 23S rRNA mutation, explaining the discrepancy between the preliminary genotype [*mef*(A) positive] and phenotype ($cMLS_B$).

Eighteen strains showed resistance to fluoroquinolones (defined as a levofloxacin MIC ≥ 4 μg/ml). Fluoroquinolone resistance rates (mean rate, 0.8%; 18 of 2,279 pneumococcal strains) in the eight participating countries were as follows: Austria, 0% (n = 160); Belgium, 0.7% (n = 148); France, 0.9% (n = 443); Germany, 0.4% (n = 530); Italy, 1.3% (n = 462); Portugal, 1.2% (n = 174); Spain, 1.0% (n = 310); and Swit-

TABLE 2. Serotype distributions of 853 antibiotic-resistant[a] pneumococcal strains isolated in eight European countries

Serotype	No. of isolates	% of isolates
14	157	18.4
23F	117	13.7
6B	117	13.7
19F	107	12.5
19A	68	8.0
9V	66	7.7
Rough	36	4.2
6A	31	3.6
15A	23	2.7
11A	20	2.3
3	13	1.5
15B	11	1.3
35B	10	1.2
9A	10	1.2
9N	7	0.8
1	5	0.6
10F	5	0.6
31	5	0.6
33F	5	0.6
20	4	0.5
4	4	0.5
Others	32	3.8
Total	853	100.0

[a] The selection of strains included all penicillin G- or macrolide-resistant isolates. Serotype data for strains showing reduced sensitivities to fluoroquinolones are presented in Table 7.

zerland, 0% ($n = 52$). The MICs and the fluoroquinolone resistance genotypes are presented in Table 6. Sixteen of the 18 strains showed high-level ciprofloxacin resistance (MICs ≥ 32 μg/ml). Among the fluoroquinolones tested, clinafloxacin (MIC_{90}, 0.5 μg/ml; MIC range, 0.125 to 0.5 μg/ml) showed the greatest potency against the levofloxacin-nonsusceptible strains. Gatifloxacin (MIC_{90}, 4 μg/ml; MIC range, 0.5 to 8 μg/ml) and moxifloxacin (MIC_{90}, 4 μg/ml; MIC range, 0.25 to 4 μg/ml) were also potent against levofloxacin-nonsusceptible isolates. Infections caused by fluoroquinolone-resistant pneumococci occurred more often in older patients (mean age, 66 years versus a mean age of 55.5 years for patients from whom fluoroquinolone-susceptible isolates were recovered; $P = 0.05$). Ten of the 18 strains additionally showed reduced sensitivity to penicillin G; and 10 strains also showed MLS_B resistance (multiply resistant isolates). Nine of the strains showed additional tetracycline resistance; and five strains had a combination of fluoroquinolone, penicillin G, MLS_B, and tetracycline resistance. Two clonally related strains exhibited MLS_B resistance in combination with tetracycline resistance (strains PW 2304 and PW 2305) (Table 6). Eight strains showed the classical Ser81 mutations (either Ser81Phe or Ser81Tyr) in *gyrA*. One strain (PW 904) showed a combination of Ser81Tyr and Ser114Gly alterations. A Glu85 alteration was found in two strains (PW 1698 and PW 1752). With the exception of one strain (PW 1601; Gly486Glu alteration) all isolates showed the wild type for *gyrB*. Thirteen of 18 strains showed the classical Ser79 alteration (either Ser79Phe or Ser79Tyr) in *parC*. The second most widespread *parC* alteration was Lys137Asn, found in 8 of the 18 isolates. This alteration was also seen in combination with Ser79Phe ($n = 5$). Nine of the 18 strains showed an Ile460Val *parE* alteration.

Serotype, MLST, and demographic data are presented together in Table 7. Fluoroquinolone resistance was associated with pneumococcal serogroup 19 (19A, $n = 5$; 19F, $n = 2$). The clonal profile of fluoroquinolone-resistant *S. pneumoniae* strains in Europe was heterogeneous, and only a very few clones belonged to identical sequence types (ST 66, ST 156, and a new ST, 1-5variant-6-5-6-20-1). Of note, 7 of the 18 strains belonged to new sequence types not described in the MLST database before.

Fluoroquinolone resistance has now reached some of the multiply antibiotic resistant pneumococcal clones described by the pneumococcal epidemiology network (31), such as the Poland^{6B}-20, the serotype 14 variant of the penicillin resistant Spain^{9V}-3, the multiply resistant Spain^{23F}-1 clone, the 19F variant, and the multiply resistant Spain^{23F}-1. Interestingly, *S. pneumoniae* PW 904 (sequence type 90) showed a serotype exchange from serotype 6B (Spain^{6B}-2) to serotype 23F, and the present study is the first to describe a serotype 23F variant of this clone (Table 8).

Analysis of a correlation of antibiotic consumption and fluoroquinolone resistance by linear regression showed that, with the exception of Belgium, all data were in the 95% confidence interval ($r^2 = 0.76$) (Fig. 1). These findings confirm that the consumption of fluoroquinolones may drive the development of fluoroquinolone resistance in Europe.

DISCUSSION

Large-scale surveillance programs are required to look for trends in antimicrobial resistance and are essential in the establishment of evidence-based guidelines for the treatment of infections, such as community-acquired respiratory tract infections, where pneumococci are regularly found as the key pathogen. Overall, 24.6% of the isolates of *S. pneumoniae* from European centers examined in the present study exhibited reduced sensitivity to penicillin G, with a generally low prevalence in countries of Central and Western Europe (Germany, 6.0%; Austria, 4.4%) and particularly high rates in France (47.6%) and Spain (61.9%). Moderate levels of resistance were found in Belgium (11.5%), Portugal (19.0%), Switzerland (17.2%), and Italy (13.0%). This intercountry variability was also found in the PROTEKT 1999 to 2000 study (12), the Alexander Project (22, 50), and the SENTRY Antimicrobial Surveillance Program (13, 18). The prevalence of strains with reduced sensitivity to penicillin G in Italy and Switzerland has significantly increased compared to the prevalence in previous studies from these countries (29, 34, 60). It remains to be seen whether this accurately represents evolution to higher rates of resistance in these countries.

Macrolides are important alternatives to β-lactams for the treatment of lower respiratory tract infections involving *S. pneumoniae*. The level of macrolide resistance in *S. pneumoniae* is increasing worldwide but varies widely between countries, as confirmed by the present study. In *S. pneumoniae* macrolide resistance rates are highest in Asian countries, such as Korea and Japan (>80%). In Europe, Italy, and France, >40% of pneumococcal isolates are reported to be macrolide resistant (12). These large differences in resistance profiles

TABLE 3. Antibiotic resistance of 2,279 isolates of *S. pneumoniae* in eight European countries, by center

Country and city	% Resistance						
	Penicillin G	Clarithromycin	Clindamycin	Levofloxacin	Trimethoprim-sulfamethoxazole	Tetracycline	Chloramphenicol
Austria							
Vienna	9.6	5.8	1.9	0	7.7	7.7	3.9
Linz	0	12.5	0	0	50.0	25	12.5
Innsbruck	2	12	6	0	10	11	0
Belgium							
Leuven	8.1	22.2	16.2	0	18.2	26.3	2.0
Brussels	18.4	26.5	22.5	2.0	20.4	18.4	4.1
France							
Le Chemay	74.3	54.3	54.3	0	65.7	54.3	25.7
Strasburg	39.2	49.5	45.4	1.0	28.9	41.2	9.3
Caen	47.0	45.2	44.4	0	43.5	35.7	13.9
Lyon	31.6	28.6	27.6	0	25.5	27.6	7.1
Creteil	63.3	58.2	56.1	3.1	61.2	53.1	24.5
Germany							
Aachen	6.7	13.3	6.7	0	8.3	13.3	3.3
Leipzig	2.3	18.6	4.7	0	23.3	4.7	0
Kaiserslautern	6.9	10.3	6.9	0	17.2	13.8	0
Berlin	8.7	11.6	8	0.7	17.4	15.2	2.9
Jena	5.7	6.7	2.9	0	17.2	15.2	1.9
Weingarten	4.5	9.0	3.2	0.7	9.7	5.2	1.3
Italy							
Genoa	9.1	32.3	18.2	0	37.4	20.2	2.0
Rome	22.5	33.8	26.8	1.4	40.9	26.8	9.9
Bari	10.6	48.5	43.9	0	40.9	34.9	9.1
Mailand	13.6	28.4	24.7	1.2	29.6	27.2	9.9
Padua	16.3	20.4	18.4	4.1	28.6	20.4	4.1
Catania (Sicily)	9.4	44.8	19.8	2.1	61.5	16.7	6.3
Portugal							
Lisbon	19	10.3	8.6	1.2	24.1	12.1	2.3
Porto	18.8	12.9	9.9	1.0	27.7	14.9	3.0
Coimbra	12.8	10.3	10.3	2.6	12.8	7.7	2.6
Spain							
Salamanca	71.4	46.2	40.7	0	70.3	50.6	23.1
Valencia	41.2	21.6	17.7	2.0	41.2	27.5	9.8
Madrid-1	60.6	49.5	45.5	2.0	70.7	51.5	26.3
Madrid-2	66.7	47.8	44.9	0	73.9	50.7	40.6
Switzerland							
Basel	18.4	18.4	13.2	0	29.0	15.8	0
La Chaux-de Fonds	14.3	14.3	7.1	0	28.6	7.1	0

may be explained in part by the strong association between increasing rates of macrolide usage, especially the longer-acting compounds, such as clarithromycin and azithromycin, and the development of resistance to this class of antimicrobials, as reported previously from studies in Spain and Germany (19, 46). The importance of ribosomal mutations in the development of macrolide resistance has only recently been recognized in pneumococci (6, 9, 10). As observed in the present study, the incidence of these strains is low (6 of 2,279) in Europe. Most information available on these resistance mechanisms today is based on in vitro selection studies showing that certain structures participate in the binding of macrolides involving domains V and II of 23S rRNA (6, 9, 10). In clinical isolates, most point mutations were identical to those found in in vitro selection studies, but new mutations were also observed. Point mutations at positions A2058, A2059, and A2062 (*E. coli* numbering; by pneumococcal numbering, positions A2060, A2061, and A2063) were associated with the development of macrolide resistance (27). The A2058G and A2058U (*E. coli* numbering; A2060 by pneumococcal numbering) substitutions confer the highest level of MLS_B resistance, with MICs of erythromycin and related macrolides of between 32 and >200 μg/ml (6, 26, 27, 36, 43, 55, 58). The relevance of some mutations in the 23S rRNA for macrolide resistance development in *S. pneumoniae*, such as A138G, A373T, A260G, T389C, A449C, and A1745T, as described in the present study, needs further investigation. Alterations in the L22 and L4 proteins also play an increasing role in macrolide resistance in pneumococci.

TABLE 4. Overview of macrolide resistance geno- and phenotypes of 618 pneumococcal strains isolated in eight European countries

Country	No. of strains	% of strains	No. of isolates with the following resistance phenotype:			No. of isolates with the following resistance genotype		
			$cMLS_B$	$iMLS_B$	M	*erm*(B)	*mef*(A)	*erm*(B) *mef*(A) negative
Spain	129	20.9	120	1	8	119	8	2
Switzerland	8	1.3	6	0	2	6	2	0
Portugal	18	2.9	15	1	2	16	2	0
Austria	12	1.9	7	0	5	7	5	0
Italy	160	25.9	112	7	41	119	40	1
France	202	32.7	188	11	3	199	3	0
Germany	56	9.1	27	1	28	28	26	2
Belgium	33	5.3	26	2	5	27	5	1
Total	618	100	501	23	94	521	91	6

Mutations in the L4 protein occur in a region of 32 amino acids and interfere with the binding of the protein to rRNA. Nagai and coworkers (36) studied the rates of macrolide resistance among 992 isolates of *S. pneumoniae* from clinical specimens collected in 1999 and 2000 in Central and Eastern European countries. Among 180 erythromycin-resistant *S. pneumoniae* isolates, L4 protein mutations were seen in 28 strains (15.6%). Similar pulsed-field gel electrophoresis patterns suggested that some strains from the Slovak Republic, Bulgaria, and Latvia that contained L4 mutations were clonally related (36). Identical amino acid alterations were seen in one isolate from Finland and two strains from Russia (26, 43).

Fluoroquinolone-resistant *S. pneumoniae* strains are currently relatively rare in the eight European countries participating in the study, and the present surveillance reports resistance levels between ranging from 0% in Austria to 1.2% in Portugal. By contrast, in Hong Kong, Ireland, and some areas of Spain, fluoroquinolone resistance has reached levels of 17.8%, 15.2%, and 5%, respectively (14, 17, 21). The newer fluoroquinolones are now recommended for the treatment of community-acquired respiratory tract infections in many European countries (59), with a corresponding increase in drug use. In 2001, the highest levels of consumption of quinolones were observed in Portugal (4.1 defined daily doses [DDD]/1,000 inhabitants/day, Italy (3.8 DDD/1,000 inhabitants/day), Belgium (3.3 DDD/1,000 inhabitants/day), Spain (2.5 DDD/1,000 inhabitants/day), and France (2.3 DDD/1,000 inhabitants/day). Significantly lower levels of consumption were reported from Austria (1.3 DDD/1,000 inhabitants/day) and Germany (1.1 DDD/1,000 inhabitants/day) (data for Switzerland were not available). Despite generally low resistance levels, linear regression analysis showed a strong correlation between the rates of fluoroquinolone consumption and the rates of resistance ($r^2 = 0.76$) (Fig. 1).

The clinical relevance of fluoroquinolone resistance has been proven by numerous studies (8, 42). Clearly, the spread of fluoroquinolone resistance determinants to international clones possessing the potential for worldwide spread is worrisome, and the present study documented that fluoroquinolone resistance was associated with these international clones (Poland^{6B}-20, serotype 14 variant of Spain^{9V}-3, serotype 19F variant of Spain^{23F}-1, the newly described 23F variant of the

TABLE 5. Mutations in 23S rRNA and changes in ribosomal proteins L4 and L22 in macrolide-resistant pneumococcal strains isolated in eight European countries[g]

PW strain no.	Country	Age of patient (yr)	Source	CLA MIC (μg/ml)	Genotype	Phenotype	23S rRNA mutation(s)[c]	L4 mutation	L22 mutation	Serotype	ST[a]
158	Spain	45	Sputum	≥32	*mef*(A)	$cMLS_B$	A138G, A260G, A2061G,[d] A1745T	WT	WT	14	156
160	Spain	39	Blood	≥32	Negative[b]	$cMLS_B$	A2060G[d]	WT	WT	13	156
169	Spain	47	Sputum	1	Negative[b,e]	M	T389C	WT	WT	35B	558
555	Germany	37	Sputum	2	Negative[b,e]	$iMLS_B$	A2937G,[f] G2939T[f]	WT	WT	11A	62
1571	Belgium	88	Sputum	16	Negative[b,e]	M	T389C, A138G	Ser20Asn	WT	3	180
1905	Italy	70	RT	2	Negative[b,e]	M	T449C, T389C	Ser20Asn	WT	6A	1547
2216	Germany	47	BAL	8	Negative[b,e]	M	A138G, A373T, T389C, A1745T	WT	WT	19F	1585

[a] The strains exhibited the following alleles and sequence types (*aroE, gdh, gki, recP, spi, xpt, ddl*, ST): PW 158, 7, 11, 10, 1, 6, 8, 1, ST 156; PW 160, 7, 11, 10, 1, 6, 8, 1, ST 156; PW 169, 18, 12, 4, 44, 14, 77, 97, ST 558; PW 555, 2, 5, 29, 12, 16, 3, 14, ST 62; PW 1571, 7, 15, 2, 10, 6, 1, 22, ST 180; PW 1905, 2, 13, 9, 1, 6, 19, 5, ST 1547 ST; PW 2216, 18, 5, 1, 1, 6, 4, 21, ST 1585.

[b] In these strains, neither *mef*(A) nor *erm*(B) was detected in repeated PCRs.

[c] Pneumococcal numbering.

[d] Mutations in the erythromycin binding site of the 23S rRNA: A2060 (pneumococcal numbering; A2058, *E. coli* numbering) and A2061 (pneumococcal numbering; A2059, *E. coli* numbering).

[e] Strains were negative for *ermA, ermC, msrA, msrB*, and *msrD*.

[f] Mutations outside the 23S rRNA (see text).

[g] Abbreviations: WT, wild type; CLA, clarithromycin; RT, respiratory tract; BAL bronchoalveolar lavage fluid.

TABLE 6. Characteristics of 18 pneumococcal strains with reduced susceptibility to fluoroquinolones isolated in Europe, 2001 and 2002[b]

Strain no.	ST	Fluoroquinolone resistance genotype by alteration in:				MIC (μg/ml)							Antibiotic(s) to which additional resistance was detected[a]
		gyrA	*gyrB*	*parC*	*parE*	GAT	CLI	GRE	SPA	MOX	CIP	LEV	
PW 735	1558	Ser81Phe	WT	Ser79Phe, Lys137Asn	Ile460Val	4	0.5	≥32	32	2	≥32	≥32	PEN, MLS_B, T
PW 1698	315	Glu85Lys	WT	Ser79Phe	Ile460Val	4	0.5	≥32	>32	4	≥32	≥32	PEN, MLS_B, T
PW 2304	66	WT	WT	Ser79Phe, Lys137Asn	Ile460Val	4	0.5	≥32	>32	4	>32	≥32	MLS_B, T
PW 2305	66	WT	WT	Ser79Phe, Lys137Asn	Ile460Val	4	0.5	≥32	≥32	4	≥32	≥32	MLS_B, T
PW 1443	513	WT	WT	Ser79Phe	WT	0.5	0.125	1	1	0.25	8	4	
PW 239	156	Ser81Phe	WT	Asp83Tyr, Lys137Asn	Ile460Val	2	0.25	16	32	1	≥32	8	PEN
PW 1752	156	Glu85Gly	WT	Lys137Asn	Asp435Asn	2	0.25	16	32	2	≥32	≥32	PEN, MLS_B
PW 603	416	WT	WT	Ser79Phe	WT	4	0.5	>32	>32	4	≥32	≥32	
PW 802	1596	WT	WT	Asp83Gly	Ile460Val	1	0.25	4	8	0.5	≥32	4	
PW 836	1596	Ser81Tyr	WT	Asp83Gly	Ile460Val	2	0.25	8	32	1	≥32	8	
PW 1601	81	WT	Gly486Glu	Ser79Phe, Lys137Asn	WT	1	0.25	2	2	0.25	≥32	4	PEN, T
PW 1891	1574	Ser81Phe	WT	Ser79Tyr	WT	4	0.5	≥32	≥32	4	≥32	≥32	
PW 786	1570	Ser81Phe	WT	Ser79Phe, Lys137Asn	Ile460Val	4	0.5	≥32	≥32	4	≥32	≥32	PEN, MLS_B, T
PW 904	90	Ser81Tyr Ser114Gly	WT	Ser79Phe Asn91Gly	Ile460Val	4	0.25	≥32	≥32	2	≥32	16	PEN, MLS_B
PW 931	1559	WT	WT	Ser79Tyr	Ile460Val	4	0.5	≥32	≥32	2	≥32	≥32	PEN, MLS_B, T
PW 1026	81	WT	WT	Ser79Phe	WT	8	0.5	≥32	≥32	4	≥32	≥32	PEN, MLS_B, T
PW 1872	1598	Ser81Tyr	WT	Lys137Asn	Asp435Asn	2	0.25	1	2	1	16	8	PEN, MLS_B, T
PW 2243	355	Ser81Tyr	WT	Ser79Phe	WT	4	0.5	≥32	≥32	2	≥32	≥32	

[a] Resistance to the following additional antibiotics was observed: PEN, penicillin G nonsusceptibility (MIC ≥ 0.1 μg/ml); MLS_B, resistance to macrolides, lincosamides, and streptogramin B; T, tetracycline resistance.
[b] Abbreviations: GAT, gatifloxacin; CLI, clinafloxacin; GRE, grepafloxacin; SPA, sparfloxacin; MOX, moxifloxacin; CIP, ciprofloxacin; LEV, levofloxacin; WT, wild type.

$Spain^{6B}$-2, and the $Spain^{23F}$-1 clone) in one-third of cases. Other investigators have shown this association of fluoroquinolone resistance with international clones, such as the $Spain^{23F}$-1 (20), $Spain^{9V}$-3 (30), $Tennessee^{23F}$-4 (5), $Spain^{14}$-5 (41), and $England^{14}$-9, $Taiwan^{19F}$-14, $Taiwan^{23F}$-15, and $Maryland^{6B}$-17 (5) clones. Furthermore, 6 of the 18 fluoroquinolone-resistant strains showed multiple resistances (additional reduced sensitivity to penicillin G, MLS_B resistance, and resistance to tetracycline). Therefore, further spread of these clones may be driven not only by an increase in the rate of fluoroquinolone consumption but also by an increased rate of use of β-lactams, macrolides, and tetracycline. In contrast to the close genetic relatedness observed in macrolide- and penicillin G-resistant pneumococcal clones throughout Europe (44), the fluoroquinolone-resistant clones observed in the present study were characterized by a relatively high genetic diversity.

In the present investigations, QRDR analysis showed that a combination of *gyrA* (Ser81) and *parC* (Ser81 and Ser79) mutations was the most widespread and responsible for high-level

TABLE 7. MLSTs and demographic data for 18 pneumococcal strains with reduced susceptibility to fluoroquinolones isolated in Europe, 2001 and 2002[b]

Strain	Yr[a]	Country	Specimen	Serotype	Age (yr)	Sex	MLST allele							ST
							aroE	*gdh*	*gki*	*recP*	*spi*	*xpt*	*ddl*	
PW 735	2001	Spain	Sputum	19A	75	M	7	13	8	12	9	1	1	1558
PW 1698	2002	Spain	Sputum	6B	72	M	20	28	1	1	15	14	14	315
PW 2304	2002	Italy	Sputum	9N	62	M	2	8	2	4	6	1	1	66
PW 2305	2002	Italy	Sputum	9N	67	M	2	8	2	4	6	1	1	66
PW 1443	2002	Germany	Sputum	11A	73	F	2	5	29	12	6	3	14	513
PW 239	2001	Portugal	Blood	14	68	M	7	11	10	1	6	8	1	156
PW 1752	2001	France	Sputum	14	67	M	7	11	10	1	6	8	1	156
PW 603	2001	Italy	Blood	19A	65	M	1	13	14	4	17	51	14	416
PW 802	2001	Italy	BAL	19A	35	F	1	100	6	5	6	20	1	1596
PW 836	2001	Italy	Sputum	19A	37	F	1	100	6	5	6	20	1	1596
PW 1601	2002	Spain	Others	19F	95	M	4	4	2	4	4	1	1	81
PW 1891	2002	Italy	Sputum	19F	42	F	8	14	4	12	9	1	14	1574
PW 786	2001	Belgium	Sputum	1	70	M	8	11	10	1	9	8	1	1570
PW 904	2001	France	Sputum	23F	70	M	5	6	1	2	6	3	4	90
PW 931	2001	France	Sputum	19A	62	M	7	19	2	17	9	22	14	1559
PW 1026	2001	Portugal	Sputum	23F	72	F	4	4	2	4	4	1	1	81
PW 1872	2002	France	SA	23F	55	F	11	4	2	4	4	1	169	1598
PW 2243	2003	Germany	Sputum	23F	91	F	1	8	4	2	6	4	6	355

[a] Year of isolation.
[b] Abbreviations: SA, sinus aspirate; V, variant; BAL, bronchoalveolar lavage fluid; M, male; F, female.

TABLE 8. Genetic relatedness of fluoroquinolone-resistant *S. pneumoniae* strains isolated in Europe

Strain	ST	Countries where strains were found	Serotype(s) described[a]	Other countries where strain was also reported[d]	Time	Resistance[f]	Clone[c]
PW 1698	315	Spain	**6B**	Poland, Bulgaria, Italy, Sri Lanka	1992–2001	P, E, T	Poland^{6B}-20
PW 2304, PW 2305	66	Italy	**9N**	Sweden, United Kingdom, Australia, Brazil	1993–2001	E, T	
PW 1443	513	Germany	**11A**	Finland	1995	ND[g]	
PW 239, PW 1752	156	Portugal, France	**14**, 9V, 19F, 11A	Spain, United Kingdom, Denmark, Uruguay, Poland, France, The Netherlands, Czech Republic, Brazil, Israel	1993–2003	P, E	Serotype 14 variant of Spain^{9V}-3
PW 603	416	Italy	**19A**	England	1999–2000	T	
PW 1601	81	Spain	**19F**	Spain, Poland, throughout Asia	1990–2001	P, E, T	Multiresistant Spanish serotype 23F clone (Spain^{23F}-1), serotype 19F variant
PW 904	90	Portugal	6B, **23F**[b]	Spain, The Netherlands, Iceland, USA, Australia	1988–1999	P, E, T	Multiresistant Spanish serotype 6B clone (Spain^{6B}-2)
PW 1026	81	Italy	**23F**	Worldwide	1984–2001	P, E, T	Multiresistant Spanish serotype 23F clone (Spain^{23F}-1)
PW 2243	355	Germany	**23F**	Germany[e]	1999	P	

[a] The serotype observed in the present study is given in boldface; other information was obtained from the MLST database (www.mlst.net).
[b] The present study is the first to describe the serotype 23F variant of the multiply resistant Spanish serotype 6B clone (Spain^{6B}-2).
[c] Clones as assigned by the pneumococcal molecular epidemiology network (31).
[d] As available in the MLST database by December 2004.
[e] Isolated from a 4-year-old child in Germany as part of a nationwide study (48).
[f] P, penicillin G; E, erythromycin; T, tetracycline.
[g] ND, not determined.

levofloxacin resistance. In total, 14 different alterations could be detected in *gyrA* (Ser81Phe, Ser81Tyr, Glu85Lys, Glu85Gly, Ser114Gly), *gyrB* (Gly486Glu), *parC* (Ser79Phe, Lys137Asn, Asp83Tyr, Asp83Gly, Ser79Tyr, Asn91Gly), and *parE* (Ile460Val, Asp435Asn). The *parC* Asp83 alteration has only recently been described among six isolates from the United States (4) and two strains from Italy (33) and may contribute to a slight increase in the levofloxacin MIC. When this alteration was seen in combination with a *gyrA* alteration (Ser81), the levofloxacin MIC increases to 8 μg/ml (strains PW 239 and PW 836). The *parC* Lys137Asn alteration, which was found to be widespread among European isolates, has also been observed among multidrug-resistant *S. pneumoniae* isolates in the international SENTRY study (23); but as it was also observed among levofloxacin-susceptible strains in Korea, the effect on fluoroquinolone resistance may be low (25). The *parC* Asn91Gly change has previously been found in *S. pneumoniae* and may be a consequence of the uptake of DNA from viridans group streptococci (3, 4). This change was detected in one isolate that showed five alterations in the QRDR and a serotype exchange from serotype 6B to serotype 23F, underscoring the possibility that this strain may be highly competent and capable of gene exchange with other streptococci. A single or double alteration in *parC* leads to a lower level of levofloxacin resistance, and some strains remained levofloxacin intermediate (levofloxacin MIC = 4 μg/ml) but susceptible to moxifloxacin (strains PW 1443, PW 802, and PW 1601). In contrast, three strains (strains PW 603, PW 931, and PW 1026) showed high-level levofloxacin and ciprofloxacin resistance in combination with a relatively high moxifloxacin MIC, even though they had only one alteration in *parC* and remained the wild type for *gyrA*. The latter may indicate the existence of other unknown mechanisms of fluoroquinolone resistance which require further investigation.

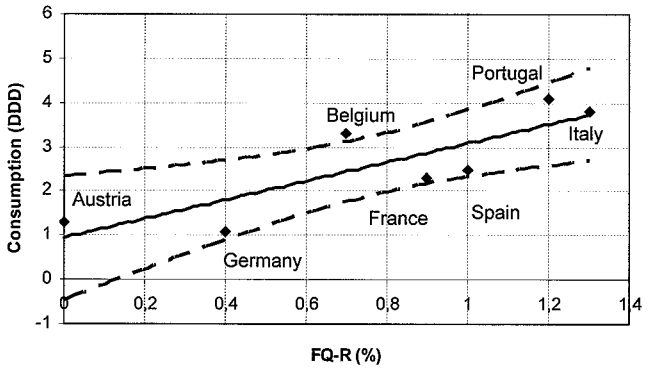

FIG. 1. Regression analysis of fluoroquinolone consumption and fluoroquinolone resistance in eight European countries. The analysis demonstrates a correlation between the resistance rate and the rate of consumption of fluoroquinolones ($r^2 = 0.76$). The correlation is within the 95% confidence interval (dashed lines) for all countries except Belgium. FQ-R, level of fluoroquinolone resistance (in percent); consumption is defined as the DDD per 1,000 inhabitants; data were obtained from the European Study of Antibiotic Consumption (see the text).

In summary, the present study showed that antibiotic resistance rates are highly variable in eight different Western European countries. Furthermore, the distribution of macrolide resistance phenotypes and genotypes varied between these countries. Presently, strains with 23S rRNA mutation or alterations in the ribosomal protein L4 do not play an important role in the spread of macrolide resistance in Western Europe. The spread of fluoroquinolone resistance to multiple-antibiotic-resistant clones is alarming, and further dissemination of these clones throughout Europe may be expected. Europe-

wide surveillance to monitor the further spread of these antibiotic resistant *S. pneumoniae* strains is warranted.

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