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Antimicrobial Resistance of Invasive Pneumococci in Finland in 1999-2000

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The resistance patterns and macrolide resistance mechanisms of 910 Finnish invasive pneumococci isolated during 1999 and 2000 were studied. Macrolide resistance was detected in 6.9% of isolates. Penicillin resistance was detected in 1.5% of isolates, and penicillin intermediate resistance was detected in 4.0% of isolates. Active macrolide efflux, mediated by the *mef*(A) gene, was the most common macrolide resistance mechanism. Four macrolide-resistant isolates had mutations in rRNA or ribosomal protein L22.

Streptococcus pneumoniae (pneumococcus) is among the major causative agents of several infectious diseases. In addition to upper respiratory tract infections, it is the most common cause of bacteremic pneumonia and, except during outbreak situations, the most common etiologic agent of bacterial meningitis in adult patients.

The prevalence of antimicrobial-resistant pneumococci is on the increase. From a clinical point of view, the resistance of invasive isolates (those from cerebrospinal fluid and blood samples) is of greatest concern, since empirical antimicrobial therapy must be commenced for infections caused by these isolates. To avoid treatment failures, data on the resistance of the most common local causes of meningitis and septicemia should be available.

The two most common mechanisms of macrolide resistance in pneumococci are ribosomal methylation caused by the *erm*(B) gene and active macrolide efflux mediated by the *mef*(A) gene (19, 22). *erm*(B) causes the macrolide, lincosamide, streptogramin B resistance phenotype (MLS_B phenotype), which means that the bacteria are resistant to 14-, 15-, and 16-membered-ring macrolides, lincosamides, and streptogramin B. *mef*(A) causes resistance to 14- and 15- membered-ring macrolides (M phenotype). In many studies, *erm*(B) has been shown to be the most common macrolide resistance gene in Europe, while *mef*(A) has been the most common macrolide resistance gene in North America (1, 4–6, 8–10, 17). Rare macrolide resistance mechanisms in pneumococci are ribosomal mutations and those mediated by the *erm*(TR) methylase gene (18, 20, 21).

In the work described here, the resistance properties of 910 invasive pneumococci isolated in Finland were studied. The resistance mechanisms of macrolide-resistant isolates were detected.

MATERIALS AND METHODS

Invasive pneumococci (isolated from blood and cerebrospinal fluid samples) were consecutively collected during 1999 and 2000 from Finnish clinical microbiology laboratories (n = 910 isolates total). Duplicate isolates were excluded from the study by removing repeat isolates recovered from the same patient within a period 3 months of the time of isolation of the first isolate. There were 471 isolates from 1999 and 439 isolates from 2000. The identification of the strains was confirmed by typical colony morphology and hemolysis on blood agar plates (Oxoid Ltd., Basingstoke, England) supplemented with 5% sheep blood. The strains were further tested for optochin susceptibility (Optochin Disk; Oxoid Ltd.), and to confirm unclear results, some of them were tested with the Slidex Pneumo-Kit (bioMérieux sa, Marcy l'Etoile, France) agglutination test. MIC testing was performed by the agar plate dilution technique (7). The antibiotics tested were erythromycin, azithromycin, spiramycin, levofloxacin, and telithromycin (Aventis Pharma, Romainville Cedex, France); clindamycin, cephalothin, cefaclor, cefuroxime, ceftriaxone, tetracycline, ampicillin, chloramphenicol, penicillin, trimethoprim-sulfamethoxazole, vancomycin, trimethoprim, and rifampin (Sigma-Aldrich Chemie, Gmbh, Steinheim, Germany); ciprofloxacin and moxifloxacin (Bayer AG, Leverkusen, Germany); RP59500 (quinupristin-dalfopristin; Rhone-Poulenc Rorer, Vitry sur Seine Cedex, France); piperacillin-tazobactam (Lederle Laboratories, Pearl River, N.Y.); and meropenem (AstraZeneca Pharmaceuticals, Macclesfield, England). If available, the National Committee for Clinical Laboratory Standards breakpoints were used (11). A control strain, *S. pneumoniae* ATCC 49619, was tested together with the other strains studied.

The macrolide resistance phenotypes were determined by the double-disk method with erythromycin and clindamycin disks (Neo-Sensitabs; A/S Rosco, Taastrup, Denmark), as well as from the MIC data. Resistance gene detection was performed by PCR as described earlier (16). The genes for which the isolates were tested were *erm*(B), *erm*(TR) [a subgroup of *erm*(A)], and *mef*(A). The primers used to detect these genes have been described previously (3, 12, 16). *Escherichia coli* 278 with plasmid pJIR229 [*erm*(B)] (2), *Streptococcus pyogenes* A200 [*erm*(TR)] (15), and *S. pyogenes* A569 [*mef*(A)] (local strain) were used as positive controls in the PCR. For the isolates with negative results by the PCR-based resistance gene detection, sequencing of the genes encoding domain V of the 23S rRNA and ribosomal proteins L4 and L22 was performed with the ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, Calif.). The primers used for sequencing have been presented earlier (13, 21). We also used one new primer whose sequence was specific for the sequence from positions 2417 to 2439: 5′-GCTTTTATCCGTTGAGCGATGGC-3′. The sequences were handled with SeqEd software (Applied Biosystems) and the GCG sequence software package (Wisconsin Package, version 10.1; Genetics Computer Group, Madison, Wis.).

RESULTS

Among the 910 pneumococci tested, 14 (1.5%) were penicillin resistant and 36 were (4.0%) penicillin intermediate. Erythromycin resistance occurred in 63 isolates (6.9%). Tetra-

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TABLE 1. Distribution of MICs for 910 invasive pneumococci recovered in Finland in 1999 and 2000

Antimicrobial drug	MIC (μg/ml)[a]			% Resistance
	50%	90%	Range	
Erythromycin	0.125	0.125	≤0.031–>256	7
Azithromycin	0.5	1	0.063–1>64	7
Telithromycin	≤0.032	≤0.032	≤0.031–2	
Spiramycin	0.5	1	0.25–1>64	
Clindamycin	0.125	0.25	0.031–>64	3
RP59500[b]	1	1	≤0.063–16	
Chloramphenicol	4	4	2–116	2
Penicillin G	0.016	0.032	≤0.008–2	2
Ampicillin	0.063	0.063	≤0.063–8	
Meropenem	≤0.016	≤0.016	≤0.016–0.5	0
Piperacillin-tazobactam	≤0.016	0.032	≤0.016–2	
Cephalothin	0.125	0.25	≤0.063–8	
Cefaclor	1	1	0.25–1>64	4
Cefuroxime sodium	0.063	0.063	≤0.063–16	3
Ceftriaxone	0.016	0.016	≤0.008–2	0.1
Tetracycline	0.25	0.5	0.063–64	6
Ciprofloxacin	2	2	0.5–64	
Levofloxacin	1	2	0.5–16	0.1
Moxifloxacin	0.25	0.5	0.125–2	0
Sulfatrimethoprim	0.25	1	≤0.063–16	8
Vancomycin	0.25	0.25	0.125–0.5	0
Rifampin	0.064	0.064	0.016–64	1

[a] 50% and 90%, MICs at which 50 and 90% of isolates, respectively, are inhibited.
[b] Dalfopristin and quinupristin (70/30).

cycline resistance was detected in 5.7% of the isolates, trimethoprim-sulfamethoxazole resistance was detected in 7.5% of the isolates, and chloramphenicol resistance was detected in 2.1% of the isolates. Only a very few or no isolates were resistant to ceftriaxone, levofloxacin, vancomycin, moxifloxacin, or meropenem (Table 1.)

Fourteen isolates were simultaneously resistant to erythromycin and nonsusceptible to penicillin, while 24 isolates were resistant to both erythromycin and tetracycline. The rate of resistance to erythromycin increased from 5.9% in 1999 to 8.0% in 2000, and the rate of resistance to tetracycline increased from 5.3 to 6.2%. The rate of penicillin nonsusceptibility, however, decreased from 7.2 to 3.6%, and the decrease was seen in both penicillin resistance and intermediate resistance.

The most common macrolide resistance phenotype was the M phenotype, which was detected among 51% (32 of 63) of the erythromycin-resistant isolates. Twenty-four isolates (38%) were of the constitutive MLS_B phenotype, and seven isolates were of the macrolide and streptogramin B resistance phenotype (MS phenotype). The resistance mechanisms of all macrolide-resistant ($n = 63$) and intermediately resistant ($n = 1$) isolates were studied. As expected, the isolates of the M phenotype had the *mef*(A) gene and the isolates of the MLS_B phenotype had the *erm*(B) gene. *erm*(TR) was not detected in any of the isolates.

None of the genes were detected in the isolates of the MS phenotype. Isolate 560 was found to have a 12-bp insertion (TCCGTCCACGTG) after position 277 in the gene encoding the L22 ribosomal protein, leading to 4-amino-acid insertion VRPR (valine, arginine, proline, arginine). The macrolides MICs for this isolate were comparably low, but the telithromycin MIC was 2 μg/ml (Table 2). Three isolates had A2059G mutations in alleles encoding for 23S rRNA; isolate 927 had A2059G mutations in three of four alleles, isolate 1341 had A2059G mutations in two alleles, and isolate s502 had a A2059G mutation in one allele. For these three isolates, the erythromycin MICs related to the number of mutated alleles: the higher the number of alleles, the higher the erythromycin MIC. Point mutations leading to a 1-amino-acid change were seen in genes encoding for both L4 (four isolates) and L22 (one isolate) ribosomal proteins. However, the relevance of these mutations remained unclear, as they were not at the positions that are known to confer resistance.

DISCUSSION

Penicillin resistance among invasive pneumococci is not common in Finland. Penicillin resistance was detected in only 1.5% of the isolates, and intermediate resistance was detected in 4.0%. Trimethoprim-sulfamethoxazole resistance was the most common type of resistance among the isolates tested, occurring in 7.5% of the isolates; and erythromycin resistance was the second most common, occurring in 6.9% of the isolates. Resistance to tetracycline and chloramphenicol occurred in a few percent of the isolates, but the drugs often used against invasive pneumococcal infections, such as ceftriaxone, vancomycin, and meropenem, have remained effective. Rifampin resistance occurred in very few isolates. Rifampin is a great inducer of mutation-derived resistance, which is the reason why it is not used as a monotherapy in the treatment of patients.

According to our previous study of penicillin-nonsusceptible pneumococci, the MLS_B phenotype is the most common mac-

TABLE 2. Ribosomal mutations in pneumococci isolated from blood

Strain	Mutation location		No. of mutated alleles (23S)[c]	MIC (μg/ml)					
	L22 gene[a]	23S rRNA gene[b]		Erythromycin	Azithromycin	Spiramycin	Clindamycin	Telithromycin	Chloramphenicol
502		A2059G	1	8	64	4	0.25	≤0.031	4
134I		A2059G	2	64	>64	>64	1	0.063	2
927		A2059G	3	128	>64	>64	1	≤0.031	2
560	12-bp insertion			2	8	16	0.125	2	4
ATCC 49619[d]				0.125	0.5	0.5	0.125	≤0.031	2

[a] Gene encoding ribosomal protein L22.
[b] Genes encoding 23S rRNA.
[c] Total number of alleles, 4.
[d] Control strain.

rolide resistance phenotype in Finland (12). Interestingly, in the present study, the majority of the macrolide-resistant isolates were of the M phenotype. This is of note, since the MLS_B phenotype has proven to be dominant in several earlier European studies, while the M phenotype has been shown to be dominant in North America (1, 4–6, 8–10, 17). Recently, however, a study from Germany showed that the M phenotype was the most common phenotype among invasive pneumococci in that country as well (14).

Seven macrolide-resistant isolates had the MS phenotype and did not carry any of the genes studied. For the three isolates with mutations in 23S rRNA, erythromycin MICs were between 8 and 128 μg/ml, depending of the number of mutated alleles. The telithromycin MICs for those isolates were low. Interestingly, for the isolate with a 12-bp insertion in the gene encoding ribosomal protein L22, the telithromycin MIC was as high as 2 μg/ml. This is of note, as none of the other mutations detected in this study or in a previous study (13) led to telithromycin MICs this high. In the study by Tait-Kamradt et al. (20), the telithromycin MIC was 3.12 μg/ml for a pneumococcal isolate with an 18-bp insertion in the gene encoding ribosomal protein L4. Interestingly, in both of these cases, similar kinds of mutations were behind the high MICs.

In Finland, treatment guidelines for meningitis recommend the use of ceftriaxone as the first-line empirical treatment. For defined cases of pneumococcal meningitis, penicillin G is considered the drug of choice for the treatment of infections caused by penicillin-susceptible strains. Penicillin is also regarded as the first-line therapy for defined cases of pneumococcal sepsis or pneumonia. The resistance patterns of invasive pneumococcal isolates detected in this study indicate that these guidelines are still valid in Finland.

The question of empirical treatment for community-acquired pneumonia remains difficult, as penicillin is not effective against *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, the two important microbes causing atypical pneumonia. If one of these agents is suspected, macrolides are recommended as the first-line choice for outpatients with pneumonia in Finland. According to this study and data from the Finnish Study Group for Antimicrobial Resistance, both penicillin and macrolide resistance are more common among pneumococci from noninvasive infections (mainly respiratory tract isolates) than among those from invasive infections. In 2000, the rate of penicillin nonsusceptibility was 8% and the rate of macrolide resistance was 11% among all pneumococci in Finland (Finnish Study Group for Antimicrobial Resistance, unpublished data). Typically, macrolide resistance is of a higher grade than penicillin resistance in pneumococci. In particular, the *erm*(B) methylase gene and some of the ribosomal gene mutations cause very high grade resistance. Therefore, treatment failures are to be expected if macrolide-resistant pneumococcal pneumonia is treated empirically with macrolides. This may lead to bacteremia and invasive disease. The answer to treatment may well be in the new drugs, such as telithromycin and the new fluoroquinolones, which are still effective against the main causative agents of community-acquired bacterial pneumonia (7, 12, 13).

In conclusion, invasive pneumococci were comparably susceptible in Finland in 1999 and 2000. Trimethoprim-sulfamethoxazole and macrolide resistance were the most common, while penicillin nonsusceptibility remained uncommon and multiresistance was rare. Active efflux was the most common macrolide resistance mechanism. Four isolates were found to have ribosomal mutations conferring macrolide resistance. The results of this study indicate that most of the drugs conventionally used against pneumococcal infections are still effective against invasive diseases caused by pneumococci in Finland.

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