Streptococcus pneumoniae (the pneumococcus) represents one of the leading bacterial infectious agents of respiratory tract infections (RTI) in humans, including acute otitis media, sinusitis, acute exacerbations of chronic bronchitis, and community-acquired pneumonia (5), and it is the main cause of mortality of patients affected by the latter disease (19). This pathogen is also frequently involved in life-threatening infections of the central nervous system; e.g., in Poland, it is responsible for 20.9% of the reported cases of bacterial meningitis (42). Recently, the situation has been aggravated worldwide by the appearance and spread of pneumococcal strains that have acquired resistance to several classes of antimicrobials, including β-lactams (2). Resistance to penicillin and other β-lactam antibiotics in pneumococci is associated with modifications of genes encoding penicillin-binding proteins, enzymes involved in peptidoglycan synthesis that are molecular targets for β-lactams (4). In clinical isolates, resistance arises from the acquisition of foreign DNA from either viridans streptococci (11, 24) or other penicillin-nonsusceptible S. pneumoniae (PNSP) (7, 21) and, thus, from the formation of the so-called mosaic pbp genes. The acquisition of these resistance determinants by some clones further drives their spread under the selective pressure of extensive antibiotic consumption. Recently, 22 international clones further drives their spread under the selective pressure of extensive antibiotic consumption. Recently, 22 in-
multiple clones that combine resistance to other classes of antimicrobials (multidrug resistance) have been recognized (28) (see www.sph.emory.edu/pmen). Two such clones were originally identified in Poland in the mid-1990s and were designated Poland23F-16 and Poland6B-20 (37).

The epidemiological situation concerning the penicillin nonsusceptibility of S. pneumoniae isolates in Poland has remained largely unstudied up to now, particularly at the molecular level. The aim of our study was therefore to characterize the population of PNSP strains isolated in the country from various infections with the use of molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST), as well as to investigate resistance determinants by PCR-restriction fragment length polymorphism (PCR-RFLP) of pbp genes and particularly clones in the context of β-lactam susceptibility phenotypes. (Parts of this work were presented at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 14 to 17 September 2003.)

MATERIALS AND METHODS

Bacterial isolates, susceptibility testing, DNA isolation, and serotyping. Eight hundred eighty-seven pneumococcal isolates were collected from 1998 to 2002 in 63 medical centers in 53 towns all over Poland as a part of a continuous surveillance system. These isolates were derived from 260 (29.3%) female and 496 (55.9%) male patients; otherwise, the gender was not reported. Twenty-four (2.7%) isolates were obtained from patients under 2 years of age. Additionally, 22 PNSP isolates obtained from 13 centers from 1995 to 1997 were included in the analysis. Altogether, the isolates were derived from the following clinical specimens: sputum (553 isolates), bronchoalveolar lavage (141), cerebrospinal fluid (126), and blood (54), with the remainder being obtained from other sources (pleural fluid, eye swab, sinus, and pus from middle ear). Isolates were reidentified on the basis of colony morphology, susceptibility to optochin (bioMérieux, Marcy l’Etoile, France), and bile solubility. Susceptibility to penicillin (Sigma Chemical Company, St. Louis, MO) was tested by the microdilution method and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) with breakpoints of 0.12 μg/ml and 2 μg/ml for nonsusceptible and resistant phenotypes, respectively (30). Isolates with MICs within the range of 0.12 μg/ml to 1 μg/ml were considered intermediate to penicillin. All PNSP isolates identified were also tested...
against amoxicillin (SmithKline Beecham Pharmaceuticals, Worthing, United Kingdom), ceftriaxone (Sigma), cepafine (Bristol-Myers Squibb, New Brunswick, NJ), and meropenem (AstraZeneca, Macclesfield, United Kingdom) using CLSI guidelines. Total bacterial DNA was purified using the Genomic DNA Prep Plus kit (A&A Biotechnology, Gdynia, Poland). Serotyping was performed by PCR with primers specific for genes responsible for the biosynthesis of the 14, 19F, and 23F types of capsular polysaccharide (25). Isolates that were negative in those PCRs, all the isolates with a suspected serotype switch, and randomly selected representatives of the pneumococci serotyped by PCR were subjected to conventional immunological serotyping by capsule swelling reactions at the Statens Seruminstitut, Copenhagen, Denmark.

The collection of the study isolates from 1998 to 2002 was used for two other analyses. The majority of the RTI isolates were screened for nonsusceptibility to ciprofloxacin, and susceptibilities and serotypes of five isolates that were both ciprofloxacin- and penicillin-nonsusceptible were reported previously (38). The isolates from cerebrospinal fluid and blood of patients with meningeval signs were subjected to a separate study in which 18 PNSP isolates were identified. Susceptibility profiles and serotypes of the meningitis-related isolates were described elsewhere previously (39).

PFGE, MLST, and PCR-RFLP of pbp genes. DNA purification, its restriction digestion with the Smal enzyme (Fermentas, Vilnius, Lithuania), and PFGE of the resulting DNA fragments were performed as described previously (26). Isolates sharing the same PFGE pattern were considered to be of the same PFGE type and subtype, whereas isolates with one to three band differences were classified into distinct subtypes of the same type. Isolates differing by more than three bands were reported as being separate types. The MLST analysis was performed according to a standard procedure involving the amplification and sequencing of seven housekeeping genes, as previously proposed by Enright and Spratt (12). The Internet-accessible database (www.mlst.net) was used for assigning allele numbers and, on the basis of the resulting allelic profiles, the sequence types (STs) of isolates. eBURST analysis (14) software (available at www.mlst.net) was used to estimate the relationships among the isolates and to construct a population snapshot, applying the definition according to which members of a clonal complex share six out of seven MLST loci (27, 41), i.e., which ones constitute single-locus variants (SLVs). The fingerprints of pbp1a, pbp2b, and pbp2x genes were determined by PCR-RFLP with gene-specific primers (7, 10, 29), followed by HinfI restriction enzyme (Fermentas) digestion and electrophoresis in 2% agarose gels (SeaKem; BMA, Rockland, ME). The resulting RFLP patterns were compared to their counterparts obtained for wild-type S. pneumoniae strain R6 (ATCC 27336). Each unique pattern was assigned a single Araible number, and particular combinations of these patterns were assigned to the isolates in the following order: pbp1a-pbp2a-pbp2b.

In addition, the pneumococcal ATCC strains ATCC 70069 (Spain23F-1), ATCC 700670 (Spain23F-2), ATCC 700671 (Spain19F-3), ATCC 700906 (Taiwan19F-15), ATCC BAA-343 (Poland23F-16), ATCC BAA-612 (Poland49F-20), and ATCC BAA-661 (Sweden59F-25) were used for reference purposes in the PFGE analysis and PCR-RFLP of pbp genes. When a PCR-RFLP pattern of any of these strains differed from those found in the study group, it was designated "other" without additional numbering.

PFGE and MLST analyses of five ciprofloxacin- and penicillin-nonsusceptible isolates from RTI and of 18 penicillin-nonsusceptible isolates from patients with meningitis were reported previously (38, 39); however, PFGE was repeated and reinterpreted in this work because of the different context of the overall collection of the isolates.

Statistical analyses. The diversity index was calculated as described previously by Grundmann et al. (18). The chi-square test with 95% confidence intervals was applied to assess the differences in nonsusceptibility frequencies in time and age groups; the two-tailed Spearman coefficient (r) was used in the analysis of correlation between antibiotic consumption and PNSP level, allowing a 1-year lag period between the consumption data and the PNSP prevalence.

RESULTS

Susceptibility to penicillin and other β-lactams. Altogether, 131 PNSP isolates from 39 centers in 32 towns were analyzed in this study, including 22 isolates from 1995 to 1997 and 109 isolates from 1998 to 2002 (12.3% of the isolates from this period). As shown in Fig. 1, the frequency of PNSP remained relatively stable over the period from 1998 to 2001 (8.1 to 11.7% of isolates) but then significantly increased in 2002 (20.3%; P = 0.03). The majority of PNSP isolates were derived from sputum samples (63 isolates; 48.1%), bronchoalveolar lavages (23 isolates; 17.6%), and cerebrospinal fluid (17 isolates; 13.0%). Fifteen isolates (11.5%) were obtained from children below 2 years of age; penicillin nonsusceptibility was therefore significantly associated with this age group (P < 0.001; chi-square test). The gender distribution did not differ from that for the entire collection; i.e., 32.1% and 55.7% of isolates were from female and male patients, respectively. The penicillin MIC50 and MIC90 values, calculated for all the isolates from the period from 1998 to 2002, were ≤0.015 μg/ml and 0.5 μg/ml, respectively. The majority of PNSP isolates (89 isolates; 67.9%) were resistant to penicillin (MIC range, 2 to 8 μg/ml); all these isolates, together with four isolates with intermediate penicillin resistance, were also resistant to ceftriaxone. Resistance to amoxicillin, ceftriaxone, and cepafine was rare and occurred in 10, 8, and 2 isolates, respectively. As mentioned above, susceptibilities of ciprofloxacin-nonsusceptible isolates and meningitis-related isolates were described previously (38, 39).

Serotypes, PFGE types, and STs of PNSP. Four serotypes, serotypes 6B, 9V, 14, and 23F, characterized the vast majority (85.5%) of all PNSP isolates (30, 20, 23, and 39 isolates, respectively). The other observed serotypes were serotypes 19A, 19F, 6A, 11A, 15A, and 24F. Four isolates were nontypeable (rough). The general coverages of the 7-valent and 23-valent vaccines were 87.8% and 88.6%, respectively, and in the group of isolates from children below 2 years of age, the 7-valent vaccine coverage was 93.3%.

PFGE analysis revealed the presence of 80 different DNA banding patterns, which were grouped into 27 types (Tables 1 and 2); 7 of these types were split further into 60 subtypes. The four predominant PFGE types (at least five isolates each) (9, 35), types 1, 4, 11, and 12, comprised 99 isolates (75.6%).

Among 34 STs found in the MLST analysis (Tables 1 and 2), 13 STs characteristic for 15 isolates were new. Of these STs, seven represented novel combinations of known alleles (STs 1010, 1019, 1027, 1032, 1049, 1051, and 1052), and six profiles contained new alleles (STs 1473, 1476, 1482, 1505, 1506, and 1507). Isolates of six main STs (STs 81, 116, 315, 143, and 272) with at least five isolates each constituted 70.2% of the PNSP group, and when their SLVs were also considered, the ratio increased to 81.7%. The diversity index was equal to 89.5%.
<table>
<thead>
<tr>
<th>Pneumococcal clone</th>
<th>ST&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Allelic profile&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Serotype</th>
<th>PFGE type(s)</th>
<th>No. of isolates</th>
<th>Site(s) of isolation&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Yr of isolation</th>
<th>pBP profile&lt;sup&gt;e&lt;/sup&gt;</th>
<th>MIC (μg/ml)&lt;sup&gt;f&lt;/sup&gt;</th>
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<tr>
<td>Spain&lt;sup&gt;38F,1&lt;/sup&gt;</td>
<td>81</td>
<td>4-4-2-4-1-1</td>
<td>23F</td>
<td>1a</td>
<td>20</td>
<td>Sputum, BAL, CSF, blood</td>
<td>1997-2002</td>
<td>1-1</td>
<td>PEN AMX CXM CRO FEP MEM</td>
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<td>Spain&lt;sup&gt;6B,2&lt;/sup&gt;</td>
<td>90</td>
<td>5-5-1-2-6-3-4</td>
<td>6B</td>
<td>3a</td>
<td>3</td>
<td>CSF, sputum</td>
<td>1999, 2001</td>
<td>1-2-other</td>
<td></td>
</tr>
<tr>
<td>Spain&lt;sup&gt;3V,3&lt;/sup&gt;</td>
<td>156</td>
<td>7-11-10-1-6-8-1</td>
<td>9V</td>
<td>4a</td>
<td>18</td>
<td>Sputum, BAL, CSF, sinus</td>
<td>1995, 1999, 2000-2002</td>
<td>1-1</td>
<td></td>
</tr>
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<td>Taiwan&lt;sup&gt;23F,15&lt;/sup&gt;</td>
<td>242</td>
<td>15-29-4-21-30-1-14</td>
<td>23F</td>
<td>7</td>
<td>4</td>
<td>Sputum</td>
<td>2000</td>
<td>3-1-3</td>
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</tr>
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<td>173</td>
<td>7-13-8-1-10-6-36</td>
<td>23F</td>
<td>9</td>
<td>1</td>
<td>Blood</td>
<td>1998</td>
<td>4-1-4</td>
<td></td>
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<td>Sweden&lt;sup&gt;43A,25&lt;/sup&gt;</td>
<td>63</td>
<td>2-5-36-12-17-21-14</td>
<td>15A</td>
<td>14</td>
<td>1</td>
<td>Sputum</td>
<td>1994</td>
<td>Other-12-4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> New STs and alleles are underlined.

<sup>b</sup> In the order aroE-gldh-gki-recP-spi-xpt-ddl.

<sup>c</sup> BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

<sup>d</sup> Susceptibilities, serotypes, and STs of meningitis-related isolates were described in reference 39, and ciprofloxacin-nonsusceptible isolates (single isolates of ST81, ST276, and ST1477 and two isolates of ST156) were described in reference 38.

<sup>e</sup> In the order pBP1a-pBP2x-pBP2b.

<sup>f</sup> NT, nontypeable; other, pattern unique to an ATCC clone.

<sup>g</sup> In the order penicillin; AMX, amoxicillin; CXM, cefuroxime; CRO, ceftriaxone; FEP, cefepime; MEM, meropenem.
<table>
<thead>
<tr>
<th>ST</th>
<th>Allelic profile</th>
<th>Serotype</th>
<th>Other countries of isolation</th>
<th>PFGE type</th>
<th>No. of isolates</th>
<th>Site(s) of isolation</th>
<th>Yr of isolation</th>
<th>MIC (g/ml)</th>
<th>PEN</th>
<th>AMX</th>
<th>CXM</th>
<th>CRO</th>
<th>FEP</th>
<th>MEM</th>
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<tr>
<td>490</td>
<td>2-13-9-1-6-19-14</td>
<td>6A</td>
<td>Bulgaria, Finland, Greece, Greenland, Sweden</td>
<td>6A</td>
<td>15</td>
<td>Sputum</td>
<td>2002</td>
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<td>0.12</td>
<td>0.25</td>
<td>0.12</td>
<td>0.25</td>
<td>0.12</td>
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<td>1019</td>
<td>7-8-1-10-15-14</td>
<td>6A1</td>
<td>BAL</td>
<td>2002</td>
<td>15</td>
<td>BAL, blood, CSF, pleural fluid, sputum</td>
<td>2002</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
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<tr>
<td>135</td>
<td>7-5-4-12-6-20-46</td>
<td>6B</td>
<td>Germany, Spain</td>
<td>2002</td>
<td>18</td>
<td>Eye, blood</td>
<td>2002</td>
<td>0.25</td>
<td>0.12</td>
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<tr>
<td>1473</td>
<td>2-19-2-17-1-28-168</td>
<td>19A</td>
<td>BAL, sputum</td>
<td>2001</td>
<td>8-WT-WT</td>
<td>Blood</td>
<td>1999</td>
<td>0.5</td>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
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<tr>
<td>1027</td>
<td>10-41-47-1-6-14-2</td>
<td>19F</td>
<td>Sputum</td>
<td>1999</td>
<td>5-18-9</td>
<td>CSF</td>
<td>1999</td>
<td>0.5</td>
<td>0.03</td>
<td>0.12</td>
<td>0.03</td>
<td>0.12</td>
<td>0.06</td>
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<tr>
<td>277</td>
<td>1-19-2-17-1-28-168</td>
<td>19A</td>
<td>The Netherlands</td>
<td>2001</td>
<td>8-1-WT</td>
<td>Sinus</td>
<td>2001</td>
<td>1</td>
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<td>1</td>
<td>8</td>
<td>1</td>
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<td>19A</td>
<td>The Netherlands</td>
<td>2001</td>
<td>8-1-WT</td>
<td>Sputum</td>
<td>2001</td>
<td>1</td>
<td>0.5</td>
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<td>319</td>
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<td>The Netherlands</td>
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<td>1</td>
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<tr>
<td>317</td>
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<td>19A</td>
<td>The Netherlands</td>
<td>2001</td>
<td>NT-19-10</td>
<td>Sinus</td>
<td>2001</td>
<td>1</td>
<td>0.5</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

New STs and alleles are underlined.

In the order aroE-gdh-gki-recP-spi-xpt-ddl.

BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

Susceptibilities, serotypes, and STs of meningitis-related isolates were described in reference 39, and ciprofloxacin-nonsusceptible isolates (single isolates of ST81, ST276, and ST1477 and two isolates of ST156) were described in reference 38.

In the order pbp1a-pbp2x-pbp2b. NT, nontypeable; other, pattern unique to an ATCC clone.

PEN, penicillin; AMX, amoxicillin; CXM, cefuroxime; CRO, ceftriaxone; FEP, cefepime; MEM, meropenem.

TABLE 2. Clinical characteristics, serotypes, PFGE profiles, and STs of other PNSP isolates.

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The eBURST analysis showed the presence of 7 clonal complexes and 11 singletons (Fig. 2). The comparison of the isolates with the international multiresistant pneumococcal clones revealed that the isolates harboring allelic profiles that were identical to those of seven clones (Spain23F-1, Spain6B-2, Spain9V-3, Taiwan23F-15, Poland23F-16, Poland6B-20, Sweden15A-25) or their SLVs constituted the majority of the PNSP isolates (94 isolates; 71.8%) (Table 1 and Fig. 2A). Among the 37 remaining isolates (Table 2 and Fig. 2B), 19 isolates belonged to ST143 and serotype 14, and only these isolates showed penicillin resistance among the isolates that were not closely related to the international clones. As mentioned above, serotypes and STs of 5 ciprofloxacin-nonsusceptible PNSP isolates from patients with RTI and 18 penicillin-nonsusceptible isolates from patients with meningitis and their classification into the international clones have been reported previously (38, 39).

**Polymorphism of the pbp genes in PNSP.** Three pbp genes, *pbp1a*, *pbp2x*, and *pbp2b*, were studied by PCR-RFLP, yielding 10, 22, and 12 different restriction patterns, respectively (Tables 1 and 2). Some of the isolates produced wild-type (WT) RFLP patterns of the genes (22, 2, and 35 isolates, respectively), and in nine isolates, *pbp1a* was nontypeable (NT) due to the lack of an amplification product. For each of the three genes studied, a certain pattern (designated polymorph 1 in each case) was clearly predominant among the isolates (54.2% for *pbp1a*, 52.8% for *pbp2b*, and 54.2% for *pbp2x*), and some of the patterns were found in isolates unrelated by MLST (summarized in Table 3). Altogether, 29 different combinations of single-gene polymorphs (excluding those with nontypeable *pbp1a*) were discerned among the isolates, with the 1-1-1 profile being the most prevalent (65 isolates; 49.6%).

**The Spain23F-1 group.** The Spain23F-1 group contained 25 isolates, most of which belonged to ST81 and its two SLVs that represented novel allelic profiles (ST1051 and ST1476) (Table 1). Serotype 23F was characteristic for the majority of the isolates; however, three isolates possessed serotype 19F or 19A. Of the two PFGE types discerned, type 1 was split into numerous subtypes (1a to 1p), with the subtype 1a pattern being identical to that of the ATCC Spain23F-1 strain. This group was uniform with respect to the *pbp* fingerprint (profile 1-1-1), which was the same as that for the reference ATCC strain. The isolates were resistant to penicillin (MIC, 2 μg/ml) and cefuroxime, intermediate to meropenem, susceptible or intermediate to ceftriaxone and cefepime, and susceptible to amoxicillin.

**The Spain6B-2 group.** The Spain6B-2 group was represented by three isolates of ST90 and serotype 6B and a single PFGE type with two subtypes, one of which also characterized the ATCC Spain6B-2 strain (Table 1). The isolates shared their *pbp1a* and *pbp2x* RFLP patterns with the reference strain; however, they all produced another *pbp2b* pattern. The β-lac-
tam susceptibility phenotypes of these isolates were the same as those of the Spain23F-1 representatives.

The Spain9V-3 group. Out of 25 isolates that belonged to the Spain9V-3 group, most were of serotype 9V, four were of serotype 14, and a single isolate was rough (Table 1). All of the isolates belonged to ST156, except for a single ST557 isolate. Two PFGE types were observed, one of which was further differentiated into several subtypes (4a to 4i), with subtype 4a also being characteristic for the ATCC Spain9V-3 strain (Fig. 3). The majority of the isolates shared their pbp fingerprints with the Spain23F-1 clone, with the exception of a single isolate with the unique pbp1a pattern 2 and with a penicillin MIC of 1 µg/ml. In general, susceptibility to β-lactams in this group was the same as that among the Spain23F-1 isolates. Apart from a single isolate from 1995 and 2 isolates from 1999, 22 other representatives of this group were recovered in 2000 or later (P = 0.001), with 9 isolates being recovered in 2002 (Fig. 1).

The Taiwan23F-15 group. Two isolates of ST242 and serotype 23F represented the Taiwan23F-15 group (Table 1). One of the isolates shared the PFGE pattern (PFGE type 7) and pbp fingerprint (profile 3-1-3) with the ATCC Taiwan23F-15 strain, whereas the other isolate varied in pbp2x (profile 3-3-3) and belonged to a different PFGE type. Both isolates were resistant to penicillin and cefuroxime, intermediate to ceftriaxone, and susceptible to cefepime. Over, 10 isolates were resistant to amoxicillin, 6 were resistant to ceftriaxone, and 2 were resistant to cefepime.

The Poland6B-20 group. The Poland6B-20 group included 24 isolates of serotype 6B, classified into the major, probably ancestral, ST315 or one of its five SLVs (STs 316, 606, 1052, 1052, 1505) (Table 1). In the PFGE analysis, two types were present, one of which had several subtypes (12a to 12i). Similarly to the Poland23F-16 group, divergence of the pbp fingerprints (four profiles) correlating with various levels of resistance was observed. The majority of the isolates were intermediate to penicillin (MIC, 0.12 µg/ml) and showed modifications only in the pbp2x pattern; these isolates were uniformly susceptible to the other β-lactams tested. The group also included two isolates with increased penicillin MICs (0.5 µg/ml) and modified pbp1a (profile 7-11-WT) as well as three penicillin-resistant isolates with modifications of pbp2b and/or pbp1a patterns (6-10-WT and NT-7-3). These isolates showed cefuroxime resistance and increased MICs of other β-lactams, and they all differed from the remaining isolates by the type of the pbp2x pattern modification.

The Sweden15A-25 group. A single isolate of ST63 and serotype 15A shared PFGE type 14 with the ATCC Sweden15A-25 strain but differed from it by the pbp1a PCR-RFLP pattern (Table 1). The isolate, similarly to the reference strain, was intermediate to penicillin and susceptible to other β-lactams.

The ST143 group. All 19 isolates belonging to the ST143 group were of serotype 14 (Table 2). In the MLST analysis (Table 2), the major ST of these isolates, ST143, represented a double-locus variant of ST156 (in the gdh and recP loci), characteristic of the Spain9V-3 clone. In addition, this group contained two SLVs of ST143, ST790, and ST1477. Two PFGE types, types 6 and 4, the latter with 10 subtypes (4f and 4j to 4t) that belonged to the same PFGE type, type 4, as the representatives of Spain9V-3 were discerned. Although the majority of the isolates shared their pbp profile (1-1-1) with Spain23F-1 and Spain9V-3, three other variants of pbp2x were found in single isolates (profiles 1-6-1, 1-11-1, and 1-17-1). The group shared β-lactam susceptibility phenotypes with the three Spanish clones. Some of the isolates showed elevated MICs of cefuroxime (up to 16 µg/ml) but without obvious correlation to their pbp fingerprints. Although pneumococci of the ST143 group have been present in Poland since at least 1994 (37), they became more frequent from 2000 onwards (P = 0.025), following the same trend as that seen for Spain9V-3 (Fig. 1).

Other isolates. The remaining 18 PNSP isolates (Table 2 and Fig. 1B) showed a significant degree of variability and included isolates of seven serotypes plus three rough isolates. Molecular analysis discerned 13 STs, 14 PFGE types, and nine combinations of pbp patterns among these isolates; for seven isolates, the complete pbp profiles were not determined due to the lack of pbp1a amplification. In the eBURST analysis, three clonal groups, ST490/1049, ST276/230/319, and ST317/1473, as well as six singletons, STs 1019, 135, 1010, 1625, 1027, and 277 (novel STs are underlined), were observed. All these isolates were intermediate to penicillin and susceptible to other β-lactams, except for two isolates of ST276 and single isolates of ST490 and ST1019, which were also cefuroxime resistant.
DISCUSSION

The first clinical isolates of pneumococci with reduced susceptibility to penicillin (MIC, 0.06 µg/ml) appeared in Australia in the 1960s (20) and were followed by resistant isolates, reported first in South Africa in 1977 (3). Since the 1980s, the prevalence of PNSP has been constantly increasing worldwide. In the recent Alexander Project involving 26 countries, the mean frequency of penicillin nonsusceptibility among isolates from 1998 to 2000 was estimated to be 31.7%, with 18.2% resistant and 13.5% intermediate isolates, and there were significant differences among particular countries (22). The overall rate of 12.3% PNSP and 8.9% resistant isolates from 1998 to 2002 placed Poland among countries with moderate resistance frequencies. However, a significant increase was observed in 2002, which clearly paralleled the increase in β-lactam consumption. As shown by the recent data from the European Surveillance of Antimicrobial Consumption Project, the consumption of penicillins and cephalosporins increased in Poland from 5.79 and 1.81 defined daily doses (DDDs) per 1,000 inhabitants per day, respectively, to 11.2 and 2.29 DDDs in the period from 1997 to 2001 and dropped slightly in 2002 to 9.86 and 2.04 DDDs, respectively (www.esac.ua.ac.be) (17). When our preliminary susceptibility data for 2003 (16.5% PNSP) were included, antibiotic consumption and PNSP prevalence correlated with each other (r = 0.83; P = 0.04) (A. Skoczynska et al., unpublished). In Poland, penicillins and cephalosporins constitute the main types of antimicrobials used in outpatient treatment (56.3%), and the level of consumption of these antimicrobials in 2002 was very similar to that reported in Spain (17), which presumably promoted the spread of resistance (1).

The dissemination of representatives of two related clones, Spain9V-3 and ST143, appears to be responsible for the increase in resistance observed in 2002, while the frequencies of other clones remained relatively stable (Fig. 1). These two expanding clones differ by two loci in MLST and usually have other clones remained relatively stable (Fig. 1). These two clones, the specific clonal structure of Polish PNSP, which resulted in very good coverage by the 7-valent conjugated vaccine. Therefore, the introduction of childhood vaccination program should hopefully lead to a reduction in the frequency of resistance (44), especially along with more prudent antimicrobial use, as seen recently in Spain (32). However, the circulation of non-vaccine-type PNSP, such as 24F/ST230 (33, 43) and Sweden23A-25, as well as capsule switching may compromise the effect of the vaccine in the future and highlights the need for continuous surveillance of circulating PNSP isolates.

REFERENCES


