The Emergence of *Streptococcus pneumoniae* Resistant to Macrolide Antimicrobial Agents: A 6-Year Population-Based Assessment

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From 1994 through 1999, the available isolates (4148 isolates) from active population-based surveillance of invasive pneumococcal disease in metropolitan Atlanta were serotyped and were tested for antimicrobial susceptibility. Macrolide-resistant isolates were studied for the presence of *ermAM* (a ribosomal methylase gene), *mefE* (a macrolide efflux gene), and *tetM* (the class M tetracycline resistance gene). Macrolide resistance increased from 16% of all invasive isolates in 1994 to 32% in 1999. Of the macrolide-resistant pneumococcal isolates studied, 99% contained genomic copies of *mefE* or *ermAM*. Isolates with *ermAM* were mainly serotypes 6B, 23F, 14, or 19F and contained *tetM*; *mefE*-associated isolates were predominantly serotypes 14, 6A, or 19F, and most did not contain *tetM*. The frequency of the *ermAM*-mediated phenotype in invasive *Streptococcus pneumoniae* remained stable over the 6-year surveillance. However, the *mefE*-mediated phenotype increased from 9% in 1994 to 26% of all isolates in 1999 and was noted in new serotypes. By 1999, 93% of the *mefE*-containing strains had minimum inhibitory concentrations $\geq$8 μg/mL. Dissemination of the *mefE* determinant accounted for the rapid increase in the rate of macrolide resistance in our *S. pneumoniae* population.

*Streptococcus pneumoniae* is a leading etiologic agent of bacteremia, bacterial meningitis, pneumonia, bronchitis, sinusitis, and otitis media [1–4]. In 1997, 61,800 cases of invasive pneumococcal disease occurred in the United States (23/100,000 population) with 6100 deaths (2/100,000 population). Rates among children <5 years old and adults ≥65 years old were 50 and 46/100,000 population, respectively [5]. In addition to its propensity for causing pneumococcal disease among very young and older age groups, *S. pneumoniae* is also a major cause of infections in individuals with human immunodeficiency virus infection, sickle cell disease, splenectomy, and other conditions associated with humoral immune deficiency or dysfunction [6–8].

Although drug-resistant *S. pneumoniae* were noted outside the United States in the 1970s and 1980s, antimicrobial-resistant *S. pneumoniae* strains have emerged in large numbers only recently in the United States. From 1979 to 1987, 0.02% of pneumococcal isolates identified in a nationwide surveillance program were resistant to penicillin [9]. Subsequent studies, including our current Atlanta surveillance, have found that >30% of *S. pneumoniae* isolates are not fully susceptible to penicillin [5, 10–12].

Erythromycin and other macrolides are now recommended and commonly used in the treatment of pneumonia and other upper respiratory infections [13, 14]. Well-tolerated oral macrolide suspensions of the “newer” macrolides (e.g., azithromycin and clarithromycin) introduced in the 1990s are widely prescribed [15–18]. Between 1993 and 1995, azithromycin and clarithromycin use was reported in one study to have increased by >30% [19]. Thus, the development of macrolide-resistant pneumococci might be anticipated.

Erythromycin-resistant (Ery*) isolates are also resistant to clarithromycin and azithromycin [15, 16]. To assess and better understand the basis for the emergence of macrolide resistance in *S. pneumoniae*, we studied invasive pneumococcal infections and pneumococcal isolates collected during a 6-year, active, population-based surveillance in a defined human population.

Materials and Methods

Surveillance. From 1994 through 1999, all cases of *S. pneumoniae* from normally sterile sites were identified from the 8-county metropolitan Atlanta area (Georgia Health District 3). Surveillance was done as part of the Georgia Emerging Infections Program, a Centers for Disease Control and Prevention (CDC)-sponsored program for active, population-based surveillance in the United States. The specific population-based surveillance methods have been described elsewhere [1]. In brief, all hospitals and laboratories in Georgia Health...
District 3 were monitored prospectively for isolates of invasive \textit{S. pneumoniae} from normally sterile sites (e.g., blood and cerebrospinal fluid), and isolates were collected. Audits were done monthly to assure completeness of reporting. Viable \textit{S. pneumoniae} isolates were retrieved from 83\% of invasive pneumococcal cases during the study period. Case report forms were completed for all patients identified. Isolates from nonresidents of the 8-county area defining Georgia Health District 3 were eliminated. Duplicate isolates were excluded so that each isolate represented an individual case. Additional demographic information was obtained from 1997 census estimates for Georgia Health District 3 (population 2.78 million).

**Culture conditions and antimicrobial susceptibility testing.** The MICs of cefotaxime, chloramphenicol, clarithromycin, erythromycin, penicillin, and tetracycline were determined by the broth microdilution method \cite{1, 20}. Customized MIC panels of antimicrobial agents were Sensititre panels (Radiometer America, Westlake, \textit{OH}) from 1994 to 1996, Pasco panels (\textit{D\text{\textregistered}co, Detroit}) in 1997, and 96-well MIC panels (PML Microbiologicals, Wilsonville, \textit{OR}) in 1998 and 1999. The following concentrations of antimicrobial agents (expressed in micrograms per milliliter) were prepared in lyophilized panels: cefotaxime, 0.015–8; chloramphenicol, 1–32; clarithromycin, 0.06–8; erythromycin, 0.06–16 (0.06–8 in 1994–1996); penicillin, 0.015–8; and tetracycline, 0.25–32. Cultures for preparing the inoculum were grown on sheep’s blood agar (Becton Dickinson Microbiology Systems, Cockeysville, \textit{MD}). A suspension of cells equal to 0.5 McFarland turbidity standard was prepared in Mueller-Hinton broth (Becton Dickinson Microbiology Systems) with a turbidimeter (A-just; Abbott Laboratories, Abbott Park, \textit{IL}), according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS), and 10 \textmu L of this suspension was added to 10 mL of cation-adjusted Mueller-Hinton broth supplemented with 5\% lysed horse blood \cite{21}. The MIC panels were incubated for 20–24 h at 35\°C in CO\textsubscript{2}. The panels were read with a mirror reader (Microtiter; Dynatech, Chantilly, \textit{VA}). The MIC was defined as the lowest concentration of drug that inhibited growth. To ensure consistency among test results, we tested MICs of a strain of \textit{S. pneumoniae}, ATCC 49619, with known drug-susceptibility patterns each day that surveillance isolates were tested.

With the exception of erythromycin, the susceptibility standards for each drug were defined according to NCCLS break points \cite{21}. In 1996, the NCCLS standards for erythromycin were changed from \(>1\) to \(<4 \mu g/mL\) (intermediate) and \(\geq4 \mu g/mL\) (resistant) to 0.5 \mu g/mL (intermediate) and \(\geq1 \mu g/mL\) (resistant) \cite{22}. We used the 1996 definitions for all isolates (including those from 1994–1996) but designated an additional category, “high-level resistance,” to note pneumococcal isolates with erythromycin MICs \(\geq4 \mu g/mL\).

**Detection of \textit{ermAM}, \textit{mefE}, and \textit{tetM}.** The 2 known mechanisms of macrolide resistance in pneumococci, \textit{ermAM} (a ribosomal methylase gene) and \textit{mefE} (a macrolide efflux gene), were screened by use of polymerase chain reaction (PCR) and Southern blot analysis; \textit{tetM} (the class M tetracycline resistance gene) was also assessed because of the association between \textit{ermAM} and conjugative/conjugative transposons that carry \textit{tetM}. Crude preparations of genomic DNA from isolates with high-level erythromycin resistance were obtained by boiling bacterial pellets for 10 min in 100 \textmu M NaCl, 10 \textmu M Tris-HCl (pH 8.3), 1 \textmu M EDTA, and 1\% Triton X-100. Primer sets KG1F (5'-TTTGAAACAGTAAAGGCATC-3') and KG1R (5'-GTTCCGTTACTTTGTGCGGTTT-3'), KG5F (5'-AGTATCATTAATCACTGTGC-3'), and KG3R2 (5'-TTTTCTCTGGACTAAAGTG3') or CM9 (5'-CGAATTCTGAACAGGGGATACGGG-3') and KG6R (5'-TCCACATAATCGAGAAGCGG-3') were used to define a 551-bp region of \textit{ermAM}. 345-bp region of \textit{mefE} \cite{23}, and 630-bp region of \textit{tetM}, respectively. KG17R (5'-CTTCAAGGGTCAAATGGCTCG-3') was also paired with KG5F to amplify \textit{mefE}, resulting in a 1355-bp product.

PCR amplification consisted of 35 cycles with a 30-s denaturation at 95\°C, a 30-s anneal at 60\°C, and extension for 1 min at 72\°C in a thermal cycler (GeneAmp PCR System 9600; Applied Biosystems, Foster City, \textit{CA}). Each reaction contained 50 \textmu M KCl, 10 \textmu M Tris-HCl (pH 8.3), 200 \mu M each deoxyribonucleoside triphosphate, 2.5 \textmu U Taq DNA polymerase (Applied Biosystems), 1.5 \textmu M MgCl\textsubscript{2}, and 1.5 \mu M of each primer. After electrophoresis, PCR products were visualized through 1.2\% agarose gel and ethidium-bromide staining.

Gels that were used for Southern transfer were subjected to a 1-h acid depurination (0.25 M HCl) that was followed by a 30-min base treatment (1.5 M NaCl and 0.5 M NaOH) and neutralization (1.5 M NaCl and 0.5 M Tris-HCl). Gels were equilibrated in transfer buffer (2\times saline-sodium citrate (SSC) [0.15 M NaCl and 0.015 M sodium citrate]) before overnight capillary blotting \cite{24}. DNA was fixed to a nylon membrane by UV cross-linking and prehybridized for 2 h at 65\°C. Membranes were hybridized overnight at 65\°C with specific probes in 5\times SSC, 1\% blocking reagent (Boehringer Mannheim, Indianapolis), 0.1\% N-Lauroylsarcosine, and 0.02\% SDS. Chemiluminescence was detected (Genius 3; Boehringer Mannheim), according to the kit manufacturer’s instructions.

Specific probes were made by random-primed labeling PCR products, \textit{mefE}, \textit{ermAM}, and \textit{tetM}. Primers KG5F (5'-AGTATCATTAATCACTGTGC-3') and KG17R (5'-CTTCAAGGGTCAAATGGCTCG-3') were used to generate the \textit{mefE} PCR product. The \textit{ermAM} and \textit{tetM} PCR products were produced as described above. The products were labeled with digoxigenin (Genius 2; Boehringer Mannheim), according to the kit manufacturer’s instructions.

**Statistical analysis.** \(P\) values were calculated by Fisher’s 2-tailed exact test, \(Y\)’s \(x^2\) test, or \(x^2\) for linear trend. Calculations were done with the Epi Info statistical program (version 6.0a; CDC, Atlanta).

**Results**

From 1994 through 1999, 4974 separate invasive infections were detected by isolation of \textit{S. pneumoniae} from blood, cerebrospinal fluid, or other sterile sites; 4148 isolates were available for further study. The number of cases occurring each year varied from 717 to 871. The incidence of invasive pneumococcal disease in Atlanta ranged between 28 and 32/100,000 population (mean, 31/100,000 per year). Nineteen percent of invasive pneumococcal disease from 1994–1999 (tables 1 and 2). Overall, penicillin resistance (MIC, \(\geq2 \mu g/mL\)) was found in 618 (15\%) of 4148 isolates tested, with an additional 769 (19\%) of 4148 isolates exhibiting an intermediate MIC to penicillin (\(\geq0.12–\leq1 \mu g/mL\)). Among 4081
isolates that were serotyped, serotype 14 was the most common cause of invasive disease, found in 21%, and was followed by serotypes 4 (9%), 23F (9%), 6B (9%), 9V (8%), 19F (6%), and 6A (6%). Serotypes 18C, 19A, 12F, and 1 accounted for 14% of all isolates, and serotypes 22F, 13A, 7F, 16, and 9N comprised 10% of isolates. Seventeen isolates were nontypeable. Eighty-seven percent of isolates were of serotypes covered in the currently licensed 23-valent polysaccharide vaccine, whereas 66% had serotypes covered by the 7-valent conjugate vaccine. In children <5 years old, 82% of the isolates had serotypes included in the 7-valent conjugate vaccine. Assuming that cross-protection extends to serotypes 6A and 19A, 93% of the pediatric cases would have been covered.

Decreased susceptibility to erythromycin (MIC, ≥0.5 μg/mL) was found in 963 (23%) of 4148 invasive isolates of *S. pneumoniae* (table 1). Of all 4148 isolates, 11 (0.3%) exhibited intermediate susceptibility to erythromycin (MIC, 0.5–2 μg/mL) and 137 (3%) were resistant, with MICs ≥1 but <4 μg/mL. High-level erythromycin resistance (MIC, ≥4 μg/mL) was found in 815 (20%) of all isolates: 146 had MICs of 4–8 μg/mL, 308 had MICs >8 and ≤16 μg/mL, and the remaining 361 had MICs ≥16 μg/mL.

For the invasive pneumococcal isolates, erythromycin resistance did not significantly differ on the basis of race or sex, although proportions of resistance were slightly higher for whites and males. Fifty-two percent of the erythromycin-resistant isolates occurred in children <5 years old. In contrast, 17% of the EryR strains were isolated from adults ≥65 years old. Compared with the 2 urban counties (Fulton and Dekalb) that make up the city of Atlanta, the 6 suburban counties were more likely to have residents infected with EryR isolates (30% vs. 18%; *P < .001*). The proportions of erythromycin resistance for blood and cerebrospinal fluid were similar: 23% (894/3959) and 29% (41/140), respectively.

During the surveillance period, the percentage of isolates resistant to erythromycin (figure 1) and the MICs to erythromycin (table 1) gradually increased for invasive isolates from 16% (97/596) in 1994 to 31% (223/709) in 1999. The percentage of all isolates with high-level erythromycin resistance (MIC, ≥4 μg/mL) increased with time, from 13% (75/596) in 1994 to 31% (218/709) in 1999 (*P < .001*). By 1999, 70% (155/223) of all EryR isolates had MICs ≥16 μg/mL (table 1).

Nitry-four percent (878/937) of all EryR isolates examined were serotype 14, 6B, 6A, 23F, 19F, or 19A (table 2). Forty-four percent (374/845) of serotype 14 isolates, 56% of serotype 6A (139/250), 47% of serotype 6B (170/361), 24% of serotype 19A (31/133), and 25% of serotype 23F (91/367) were resistant to erythromycin. Seventy-eight percent (734/937) of the EryR isolates were of serotypes included in the 7-valent conjugate vaccine.

EryR pneumococcal isolates also exhibited decreased susceptibility to penicillin: 84% (801/952) had MICs ≥0.12 μg/mL, and 66% (629/952) had MICs ≥1 μg/mL. Similarly, 44% of EryR strains were not susceptible to cefotaxime (MICs, ≥1 μg/mL), compared with 6% cefotaxime resistance in isolates sensitive to erythromycin. Overall, ~30% of EryR isolates from 1994 to 1999 were clindamycin resistant (MIC, ≥1 μg/mL; table 1).

To better understand the basis for the rapid emergence of macrolide resistance in *S. pneumoniae* in our population, we studied all high-level EryR pneumococcal isolates consecutively recovered in metropolitan Atlanta from January 1994 through February 1996 (n = 164) and high-level EryR isolates recovered in 1998 (n = 172). Erythromycin resistance was associated with 2 generally exclusive mechanisms: *mefE* and *ermAM*. Ninety-seven percent of the EryR isolates contained *mefE* or *ermAM*. Ninety-seven percent of the EryR isolates contained *mefE* or *ermAM* as a single copy in the pneumococcal genome, and an additional 1.5% had both. The distribution of *mefE* and *ermAM* in the 1994–1996 group was approximately equal: 44% possessed *ermAM* without *mefE*, 54% were positive for *mefE* and not *ermAM*, and 1% (n = 2) had both markers (figure 2A). One percent was negative for both *mefE* and *ermAM*. Among the high-level EryR isolates from 1998, *mefE* or *ermAM* were detected as a single copy in 75% and 22% of isolates, respectively (figure 2B). Three (1.7%) isolates from 1998 possessed both markers.

The mefE-positive strains were more likely than the *ermAM*-containing strains to be susceptible to penicillin (92% vs. 77%, respectively; *P < .001*). The difference was largely the result of *mefE* in penicillin-susceptible isolates of serotypes 14, 6, and...
Table 2. No. of erythromycin-resistant isolates by serotype in Atlanta, 1994–1999.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Total</th>
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<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107</td>
<td>0</td>
<td>0</td>
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<td>73</td>
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</tr>
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<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25</td>
<td>0</td>
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<td>63</td>
</tr>
<tr>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0</td>
<td>2 (1)</td>
<td>19</td>
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<tr>
<td>31</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>6A</td>
<td>111</td>
<td>0</td>
<td>139 (6)</td>
<td>250</td>
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<tr>
<td>6B&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0</td>
<td>170 (112)</td>
<td>361</td>
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<tr>
<td>7F&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>65</td>
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<td>0</td>
<td>53</td>
</tr>
<tr>
<td>9V&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2</td>
<td>20</td>
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<td>0</td>
<td>38</td>
<td>153</td>
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<tr>
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<td>1</td>
<td>66 (13)</td>
<td>261</td>
</tr>
<tr>
<td>22A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91</td>
<td>1</td>
<td>1 (1)</td>
<td>93</td>
</tr>
<tr>
<td>23B&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>91 (45)</td>
<td>367</td>
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<tr>
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<tr>
<td>35B</td>
<td>24</td>
<td>0</td>
<td>1 (1)</td>
<td>25</td>
</tr>
<tr>
<td>Nontypeable</td>
<td>172</td>
<td>0</td>
<td>7 (4)</td>
<td>24</td>
</tr>
<tr>
<td>Other&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124</td>
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<tr>
<td>Total</td>
<td>3132</td>
<td>11</td>
<td>937 (229)</td>
<td>4081</td>
</tr>
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</table>

NOTE. Nos. in parentheses are no. of clindamycin-resistant isolates. Susceptible, MIC, <0.25 μg/mL; Intermediate, MIC, 0.50 μg/mL; Resistant, MIC, ≥1.00 μg/mL.
<sup>a</sup>Serotypes included in the polysaccharide vaccine Pneumovax 23 (Merck, West Point, PA).
<sup>b</sup>Serotypes included in the 7-valent conjugate vaccine Prevnar (Wyeth-Lederle Vaccines, Philadelphia).

19A. The tetracycline, clindamycin, and chloramphenicol resistance levels of erm<sub>AM</sub>-containing strains were 96%, 98%, and 61%, respectively (figure 2). In contrast, Ery<sup>a</sup> isolates with the mef<sub>E</sub> determinant were unlikely to be tetracycline, clindamycin, or chloramphenicol resistant (12%, 3%, and 5% resistance, respectively). No significant differences were found between mef<sub>E</sub>- and erm<sub>AM</sub>-containing strains by race, sex, or residence of the infected patients.

The marked increase in Ery<sup>a</sup> invasive <i>S. pneumoniae</i> in our population from 1994 through 1999 was due to the mef<sub>E</sub>-associated determinant. As determined by molecular typing of isolates from 1994–1996 and 1998 or by isolate phenotype (Ery<sup>a</sup>/clindamycin-susceptible or -resistant), resistance due to erm<sub>AM</sub> was unchanged throughout the 6-year surveillance (i.e., it remained at 6% of all invasive isolates). In contrast, mef<sub>E</sub>-associated resistance increased from 9% of all invasive pneumococcal isolates in 1994 to 26% of isolates in 1999 (<i>P</i> < .001; figure 1). The increase in Ery<sup>a</sup> isolates containing mef<sub>E</sub> accounted for the overall increase in macrolide resistance to 31% of pneumococcal isolates in 1999. In addition, the MIC of mef<sub>E</sub>-associated isolates to erythromycin increased from 1995 to 1999 (<i>P</i> < .001). In 1995, only 21% (19/92) of mef<sub>E</sub>-associated isolates had MICs ≥8 μg/mL to erythromycin. By 1999, 94% (172/184) of the mef<sub>E</sub>-containing strains had MICs ≥8 μg/mL, and 63% (116/184) had MICs ≥16 μg/mL.

Most of the increase in mef<sub>E</sub>-associated macrolide resistance observed during the surveillance was found in serotypes 14, 19F, and 23F. From 1995 through 1999, the mef<sub>E</sub> phenotype increased from 29% to 61% of all serotype 14 isolates, from 7% to 25% of all serotype 19F isolates, and from 7% to 20% of all serotype 23F isolates. Slightly more than half of the serotype 6A isolates had the mef<sub>E</sub> phenotype throughout the study period. In addition, other serotypes had mef<sub>E</sub>: 6% of serotype 4 isolates and 9% of serotype 9V isolates were Ery<sup>a</sup> (mef<sub>E</sub>-associated) in 1998 (vs. 0% in 1995). These data suggest that mef<sub>E</sub> is rapidly spreading in <i>S. pneumoniae</i> in Atlanta. In contrast, erm<sub>AM</sub> remained stable during the surveillance and was mostly confined to serotypes 6B, 23F, 14, and 19F.

Discussion

The rapid emergence of antimicrobial resistance in <i>S. pneumoniae</i> [25–27] has greatly complicated the choice of empiric treatment regimens for suspected pneumococcal infections [13, 14]. Resistance to penicillin, cephalosporins, and other β-lactam antibiotics has increased the use of erythromycin and “newer” macrolides, such as azithromycin and clarithromycin [17, 18, 28]. However, this approach (i.e., use of macrolides) now appears to be compromised by the rapid emergence of macrolide resistance in <i>S. pneumoniae</i>. In the 7 sites of active bacterial core surveillance (population 16 million), including Atlanta and sites in states other than Georgia, erythromycin resistance of invasive <i>S. pneumoniae</i> isolates was 275 (12%) of 2365 isolates in 1997 and 306 (12%) isolates in 2001.
of 2546 isolates in 1998. We found that erythromycin resistance in invasive pneumococcal isolates recovered in Atlanta has increased from 16% in 1994 to >31% in 1999, and erythromycin resistance in these isolates was associated with the presence of \( \text{ermAM} \) or \( \text{mefE} \) (or both). Although antimicrobial prescribing practices and demographics may vary regionally, the higher erythromycin levels observed in Atlanta, compared with levels in the other sites, require further study.

Erythromycin-ribosomal methylase (the \( \text{ermAM} \) gene product) methylates highly conserved adenine residues in the peptidyl transferase center, domain V, of newly synthesized 23S rRNA. This methylation blocks the binding of macrolide, lincomamide, and streptogramin B antibiotics (MLSB phenotype) [29]. Erythromycin MICs for pneumococci containing \( \text{ermAM} \) are typically \( \geq 64 \mu g/mL \) [30–33].

Conjugative/composite transposons that carry the \( \text{ermAM} \) in \( S. \ pneumoniae \) are characterized by the presence of \( \text{tetM} \) and often coharbor a chloramphenicol-resistance determinant [34–36]. \( \text{Tn917} \), a nonconjugative transposon, encodes only the \( \text{ermAM} \) erythromycin resistance and is rarely detected as an
isolated element in *S. pneumoniae*. However, Tn917 is found within a Tn916-like transposon as a part of a composite element, Tn872 [30]. Ninety-six percent of the *ermAM*-containing invasive isolates that we studied contained *tetM* and were EryR, clindamycin resistant, tetracycline resistant, and chloramphenicol sensitive or resistant. Of interest, the percentage and distribution of *ermAM*, and thus of these conjugative transposons, was stable in *S. pneumoniae* during the 6-year surveillance period and was largely limited to serotypes 6B, 23F, 14, and 19F. The *ermAM* mechanism of macrolide resistance has been documented in *S. pneumoniae* since the 1980s and appears to have reached an equilibrium in our population [30–33].

In 1996, a macrolide efflux mechanism, *mefE*, was described in strains of *S. pneumoniae* that were lacking an *ermAM* determinant. These strains were reported to express an M phenotype without lincomamide or streptogramin B resistance [37, 38]. Pneumococci containing *mefE* have MICs to erythromycin and other 14- and 15-membered macrolides from 1 to 32 μg/mL [30–33]. Since *mefE* did not emerge until the 1990s, it is likely to be some time before this determinant stabilizes within the pneumococcal population.

Ninety-seven percent of macrolide-resistant *S. pneumoniae* in our study had genomic copies of *mefE* or *ermAM*, and an additional 1.5% had both. Although the proportion of *mefE* to *ermAM* in macrolide-resistant *S. pneumoniae* varies, other studies have also reported that >97% of macrolide-resistant pneumococcal isolates contain *mefE* or *ermAM* [16, 32, 39, 40]. Thus, *mefE*-associated macrolide efflux and *ermAM* methylation are currently the major mechanisms of macrolide resistance in *S. pneumoniae*. However, macrolide-resistant pneumococcal isolates that were *mefE*- and *ermAM*-negative were found in our surveillance. Similar macrolide-resistant pneumococcal isolates that are *mefE*– and *ermAM*-negative have been described by others and suggest the potential for the emergence of additional mechanisms of macrolide resistance in *S. pneumoniae* [32, 39, 40].

The mechanisms behind the rapid dissemination of *mefE*-associated macrolide resistance are unknown. The clonal expansion and global spread of multidrug-resistant pneumococcal clones is one explanation for the dissemination of pneumococcal antimicrobial resistance. Intercontinental spread of multidrug-resistant pneumococcal clones has been documented [41–43]. For example, the spread of erythromycin resistance via a serotype 14 clone from the United Kingdom to Sweden and Australia [44] was recently noted. The relationship of *ermAM* with pneumococcal serotypes 6B, 23F, 14, or 19F and of *mefE* with serotypes 14, 6A, or 19F supports this model. Alternatively, horizontal transfer of genetic elements among different pneumococcal strains and selection via antimicrobial use may result in the rapid dissemination of antimicrobial resistance. In preliminary studies of our isolates (K.G. and D.S.S., unpublished data), both clonal expansion and horizontal transfer account for the rapid emergence of *mefE*. Clonal expansion and horizontal transfer of other pneumococcal antimicrobial resistance determinants have been described in other populations [31, 45].

Serotype 6A accounts for 18% of the *mefE*-associated erythromycin resistance in children <5 years old. Although this serotype is not included in current vaccines, serotype 6B is included in the 7-valent conjugate vaccine and is likely to provide cross-protective antibodies [46]. The importance of pediatric vaccination in the prevention of pneumococcal macrolide resistance should be emphasized, since 52% of erythromycin resistance occurred in children <5 years old. This age group represented the source of 35% of all invasive pneumococcal isolates in metropolitan Atlanta. In contrast, adults ≥65 years old accounted for 17% of the observed erythromycin resistance and for 19% of all invasive cases.

The clinical significance of low-level macrolide resistance has been questioned [17]. Large numbers of clinical failures in the treatment of respiratory pneumococcal infections have not been reported with the use of macrolides. Newer agents, such as clarithromycin, are reported to achieve levels up to 480 μg/mL at epithelial surfaces and in alveolar macrophages, which suggests that they could be effective in respiratory infections caused by macrolide-resistant organisms [47]. However, the rapid increase in the numbers of high-level macrolide-resistant pneumococcal strains and the corresponding increase in MICs to erythromycin to levels ≥16 μg/mL in *mefE*-containing isolates suggest that macrolide clinical failures may increase.

This study found that *mefE* is rapidly increasing in *S. pneumoniae*, whereas *ermAM*-mediated resistance remained stable. The percentage of *mefE*-associated macrolide-resistant invasive *S. pneumoniae* isolates nearly tripled from 1994 through 1999. By 1999, ~26% of all invasive *S. pneumoniae* isolates recovered in the Atlanta population had *mefE*-associated macrolide resistance. The prevalence of the *mefE* determinant increased in serotypes 14, 19F, and 23F and appeared in new serotypes (e.g., serotypes 4 and 9V) during the surveillance. Rapid increases in *mefE*-containing *S. pneumoniae* have also been reported from South Africa [48] and Canada [32]. The trend of increasing MIC values for erythromycin in isolates associated with *mefE* is also of concern.

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