

Incidence of macrolide resistance in *Streptococcus pneumoniae* after introduction of the pneumococcal conjugate vaccine: population-based assessment

David S Stephens, Susu M Zughailer, Cynthia G Whitney, Wendy S Baughman, Lawrence Barker, Kathryn Gay, Delois Jackson, Walter A Orenstein, Kathryn Arnold, Anne Schuchat, Monica M Farley, and the Georgia Emerging Infections Program

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Department of Medicine, Emory University School of Medicine (Prof D S Stephens MD, S M Zughailer PhD, K Gay VMD, Prof W A Orenstein MD, Prof M M Farley MD) and Research Service, VA Medical Center (D S Stephens, W S Baughman MSPH, M M Farley), Atlanta, GA, USA; Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, and National Immunization Program, Centers for Disease Control and Prevention, Atlanta, GA, USA (C G Whitney MD, L Barker PhD, D Jackson MS, A Schuchat MD); and Georgia Department of Human Resources, Division of Public Health, Atlanta, GA, USA (K Arnold MD)

Correspondence to: Prof David S Stephens, Department of Medicine, Emory University Hospital, 1364 Clifton Road, NE, Atlanta, GA 30322, USA
dstep01@emory.edu

Summary

Background The prevalence of macrolide resistance in *Streptococcus pneumoniae* has risen in recent years after the introduction of new macrolides and their increased use, especially in young children. We assessed emergence of macrolide-resistant invasive *S pneumoniae* disease in Atlanta, GA, USA, before and after the licensing, in February 2000, of the heptavalent pneumococcal conjugate vaccine for young children.

Methods Prospective population-based surveillance was used to obtain pneumococcal isolates and demographic data from patients with invasive pneumococcal disease. We calculated cumulative incidence rates for invasive pneumococcal disease for 1994–2002 using population estimates and census data from the US Census Bureau. The rates for 2000–02 were calculated from 2000 census data.

Findings The incidence of invasive pneumococcal disease in Atlanta fell from 30·2 per 100 000 population (mean annual incidence 1994–99) to 13·1 per 100 000 in 2002 ($p < 0\cdot0001$). Striking reductions were seen in children younger than 2 years (82% decrease) and in those 2–4 years (71% decrease), age-groups targeted to receive pneumococcal conjugate vaccine. Significant declines were also noted in adults aged 20–39 (54%), 40–64 (25%), and 65 years and older (39%). Macrolide resistance in invasive *S pneumoniae* disease in Atlanta, after increasing steadily from 4·5 per 100 000 in 1994 to 9·3 per 100 000 in 1999, fell to 2·9 per 100 000 by 2002. Reductions in disease caused by *mefE*-mediated and *erm*-mediated macrolide-resistant isolates of conjugate-vaccine serotypes 6B, 9V, 19F, and 23F, and the vaccine-associated serotype 6A were also recorded.

Interpretation Vaccines can be a powerful strategy for reducing antibiotic resistance in a community.

Introduction

Decreased susceptibility of *Streptococcus pneumoniae* to macrolide antibiotics has emerged worldwide.^{1–7} In Atlanta, GA, USA, between January, 1994, and December, 1999, the prevalence of macrolide-resistant *S pneumoniae* rose to more than 25% of invasive pneumococcal isolates. Similar increases were noted in other parts of the USA, Canada, Europe, Africa, and Asia.^{1–7} Increases in the prevalence of macrolide resistance emerged after the introduction of new macrolides (azithromycin, clarithromycin) and their widespread use, especially in children younger than 5 years.¹ 95–98% of macrolide resistance in pneumococci results from two major mechanisms—erythromycin-ribosomal methylation encoded by *ermAM* (*B*)⁸ and macrolide efflux encoded by *mef*-containing elements.^{9–11} In Atlanta, and much of the rest of the USA, the rapid increase in pneumococcal macrolide resistance during the 1990s was caused by the clonal expansion and horizontal transfer of the *mefE*-containing insertion element.^{12,10} Overall, macrolide resistance associated with *mefE* or *erm* has been predominantly reported in pneumococcal serotypes 14, 19F, 19A, 6B, 6A, and 23F.

In February, 2000, a seven-valent pneumococcal conjugate vaccine containing capsular polysaccharide serotypes 4, 6B, 14, 23F, 19F, 9V, and 18C linked to

Crm₁₉₇ was approved for use in young children in the USA.^{12,13} The pneumococcal conjugate vaccine was in general use in Atlanta in children in both the public and private sectors by the end of 2000. It was predicted to be protective against vaccine-related serotypes 6A and possibly 19A, as well as serotypes included in the vaccine. However, beginning in August 2001, shortages of the vaccine complicated its introduction and prompted modified recommendations.¹⁴ We assessed the effect of the seven-valent pneumococcal conjugate vaccine on macrolide resistance of invasive *S pneumoniae* using an established population-based active surveillance system and molecular typing of pneumococcal isolates.

Methods

Surveillance and data collection

Prospective population-based surveillance for invasive pneumococcal disease has been done under active bacterial core surveillance (ABCs) of the Georgia Emerging Infections Program in metropolitan Atlanta since 1994. Pneumococcal isolates and demographic data from patients with invasive pneumococcal disease were obtained from all hospitals and laboratories in Georgia health district 3—the core eight-county metropolitan Atlanta region with a 2000 census population of 3·1 million.

The methods by which surveillance and isolate data were obtained have been described elsewhere.^{1,2,7} Briefly, all patients with invasive pneumococcal disease (defined as growth of pneumococci from normally sterile sites—eg, cerebrospinal fluid or blood) who resided in the surveillance area were identified by active, prospective, laboratory-based surveillance. Case-report forms and available sterile-site isolates of *S pneumoniae* were obtained. Laboratory audits were done monthly to assess accuracy of case identification.

We measured coverage with the pneumococcal conjugate vaccine in Atlanta by means of a continuing national immunisation survey.¹⁵ We assessed the proportions of children aged 19–35 months in Fulton or DeKalb, the two central counties of HD-3, who had received one, at least two, at least three, or at least four doses of vaccine for each quarter of 2000, 2001, 2002, and the first two quarters of 2003.

Laboratory tests

We assessed antimicrobial susceptibility of isolates according to guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS).^{2,16} Isolates not susceptible to erythromycin (minimum inhibitory concentration, MIC ≥ 0.5 mg/L) were further classified as having either M (*mef*) or MLS_B (*erm*) phenotype. Isolates that were resistant to erythromycin but susceptible to clindamycin were classified as M phenotype, and those that were resistant to both erythromycin and clindamycin were classified as MLS_B phenotype.^{1,2,11}

Subsets of erythromycin-resistant isolates (MIC 1 to ≥ 64 mg/L), identified consecutively by surveillance in 1994–96 and in 1998–2002, were studied¹⁰ for the presence of *mef*, *erm*, and other determinants of macrolide resistance. 41% of available isolates (including every third isolate) in 1994–96, 50% of isolates (every other isolate) in 1998, and all resistant isolates in 1999–2002 were investigated. Nucleotide primers¹⁰ to *mef* and *erm* were used in PCR to amplify 345 bp of *mefE* and 551 bp of *ermAM*. We used an additional set of primers, MEF-F (5'-

CATATGGGCAGGGCAAGCAG-3') and MEF-R (5'-GCAATCACAGCACCCAATACG-3'), to amplify a 505 bp *mefE*-specific product. Southern hybridisations were also done.¹⁰ A 1680 bp template spanning most of *mefE* and the next open reading frame, *mel*,¹⁰ was generated by PCR with primers KG17R and KG5F.¹⁰ Serotyping was undertaken as described elsewhere.^{2,7}

Statistical analysis

We did statistical analyses using EpiInfo2000 version 1.1.2. The χ^2 test was used for comparisons of proportions.¹³ We calculated cumulative incidence rates for 1994 to 2002 using population estimates and census data from the US Census Bureau. The rates for 2000 to 2002 were calculated from 2000 census data. Vaccine serotypes were 4, 6B, 14, 23F, 19F, 9V, and 18C; vaccine-related serotypes were 6A and 19A. To calculate serotype and antibiotic-resistance rates we assumed, as previously described,¹³ that the distribution for cases with missing serotype or antimicrobial-sensitivity data was the same as that of isolates with this information available. To verify the results, we repeated comparisons of rates with only cases with complete data included. The rates are reported as cases per 100 000 population.

Role of the funding source

This work was supported by a VA Merit Grant (DSS) and the Centers for Disease Control and Prevention. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

6695 separate invasive pneumococcal infections were identified in Atlanta during the surveillance period from 1994 to 2002. Of all cases identified, 83% of isolates were viable and available for further testing in 1994, 85% in 1995, 81% in 1996, 82% in 1997, 86% in 1998, 85% in 1999, 84% in 2000, 87% in 2001, and 77% in 2002. Recurrent infections (defined as isolation of invasive pneumococci more than 7 days after the first or previous episode) represented 5% of total cases during the surveillance period and were counted as separate infections. 95% of isolates were from blood; 3.5% from cerebrospinal fluid, or cerebrospinal fluid and blood; and 1.5% from other sites (eg, joint, pleural fluid). The mean annual incidence of invasive pneumococcal disease between 1994 and 1999 was 30.2 per 100 000 population (range 27.6–31.7 per 100 000; figure 1, table 1). The incidence fell to 22.3 per 100 000 in 2000 ($p < 0.0001$), decreased in 2001 to 18.4 per 100 000 ($p < 0.0001$), and further fell in 2002 to 13.1 per 100 000 ($p < 0.0001$), a 57% overall decrease in 2002 compared with the mean annual incidence during 1994–99.

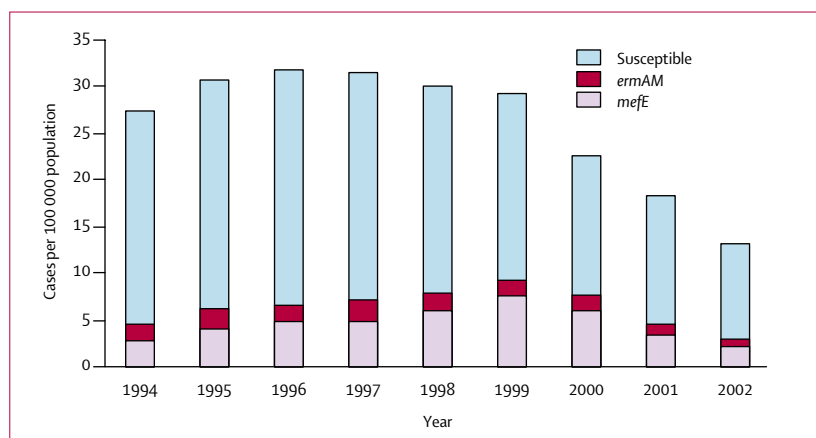


Figure 1: Incidence of invasive *S pneumoniae* in metropolitan Atlanta, 1994–2002

In February, 2000, pneumococcal conjugate vaccine was recommended for all children aged 2–23 months and for certain children (eg, those with sickle-cell disease, sickle haemoglobinopathies, HIV infection, other chronic diseases, and other immunocompromising disorders) aged 24–59 months.¹² The recommended vaccine schedule was doses at 2, 4, and 6 months, with the final dose given at age 12 months or older. Vaccine shortages led to modification of these recommendations.¹⁴ Pneumococcal conjugate vaccine coverage in Atlanta during 2000–2003, is shown in figure 2. By 2003, 58–67% of children aged 19–35 months had received at least three doses of conjugate vaccine, 77–80% had received two doses, but less than 30% had received four doses.

The decline in the incidence of invasive pneumococcal disease in Atlanta was most striking in children younger than 2 years of age (figure 3, table 1), with an 82% decrease from a mean of 278 per 100 000 in 1994–99 to 50

per 100 000 in 2002 ($p < 0.0001$), and in children aged 2–4 years, with a 71% decline from 46 per 100 000 in 1994–99 to 13.3 per 100 000 in 2002 ($p < 0.0001$) (figure 3, table 1). The proportion of pneumococcal disease in children younger than 2 years declined from a mean of 28% (range 24.7%–32.6%) in 1994–99 to 10.6% in 2002. Furthermore, we noted a fall in the incidence of invasive pneumococcal disease of 39% (from mean 73.5 per 100 000 in 1994–1999 to 44.6 per 100 000 in 2002, $p = 0.0006$) in the population aged 65 years and older; 54% ($p < 0.0001$) in 20–39-year-old adults; and 25% ($p = 0.007$) in adults 40–64 years of age (figure 3, table 1). In African-Americans, the mean yearly incidence of invasive pneumococcal disease was 50.3 per 100 000 between 1994 and 1999 and declined by 63% to 18.6 per 100 000 ($p < 0.0001$) in 2002. The incidence in white people was 18.2 per 100 000 (mean 1994–99) and declined by 54% to 8.4 per 100 000 in 2002 ($p < 0.0001$).

	1994	1995	1996	1997	1998	1999	2000	2001	2002
Total number of cases*	717	827	866	871	857	855	699	578	413
Incidence	27.6	31.0	31.7	31.4	30.1	29.4	22.3	18.4	13.1
Macrolide resistant	4.5	6.0	6.5	7.2	7.9	9.3	7.7	4.6	2.9
<i>mef</i>	2.9	4.1	4.9	4.8	6.0	7.7	6.2	3.5	2.1
<i>erm</i>	1.5	1.9	1.6	2.3	1.8	1.5	1.5	1.1	0.8
<2 years									
Number of cases	178	230	253	238	281	235	177	70	45
Total incidence	219.3	274	307.4	285.4	323.2	260	190.8	78.6	50
Macrolide resistant	55.6	74.5	108	112.7	126	134.1	98.4	24.2	20.2
<i>mef</i>	43.6	58.8	86.9	89.3	103.2	115.4	89.6	15.7	17.5
<i>erm</i>	12.0	15.7	21.0	23.4	22.8	18.3	8.8	8.5	2.7
2–4 years									
Number of cases	61	54	76	55	42	56	38	39	18
Total incidence	49.8	43.2	62.0	44.2	32.8	44.2	28.4	30.2	13.3
Macrolide resistant	12.8	12.4	13.9	11.7	11.2	17.4	10.3	9.1	2.8
<i>mef</i>	6.9	6.7	10.9	6.8	7.5	14.7	8.6	7.5	2.8
<i>erm</i>	5.9	5.7	3.0	4.9	3.7	2.7	1.7	1.6	0
5–19 years									
Number of cases	23	35	38	42	21	28	16	24	21
Total incidence	4.3	6.2	6.8	7.3	3.6	4.7	2.5	3.9	3.4
Macrolide resistant	0.2	0.6	0.9	1.1	0.6	0.8	1.1	0.3	0.6
<i>mef</i>	0.2	0	0.6	0.6	0.6	0.8	1.1	0.3	0.4
<i>erm</i>	0	0.6	0.3	0.4	0	0	0	0	0.2
20–39 years									
Number of cases	160	166	171	169	144	139	135	104	76
Total incidence	16.6	16.9	17.4	17.2	14.4	13.9	12.6	9.8	7.3
Macrolide resistant	1.6	1.6	1.7	2.3	2.6	3.3	2.8	2.3	2.1
<i>mef</i>	1.1	1.1	1.1	1.3	1.8	2.5	1.8	1.9	1.6
<i>erm</i>	0.5	0.5	0.6	1.0	0.8	0.8	1.0	0.4	0.5
40–64 years									
Number of cases	156	188	180	207	204	245	207	205	158
Total incidence	21.9	25.1	23.4	26.1	24.5	28.3	22.9	22.9	18.7
Macrolide resistant	2.5	4.1	3.6	3.8	4.6	5.8	7.3	5.2	3.4
<i>mef</i>	1.7	2.7	2.6	2.5	3.2	4.2	5.2	4.2	2.6
<i>erm</i>	0.8	1.4	1.0	1.3	1.4	1.6	2.1	1.0	0.8
≥65 years									
Number of cases	139	154	148	160	165	152	126	136	95
Total incidence	69.9	76.6	72.2	77.4	75.7	68.9	55.7	60.6	44.6
Macrolide resistant	11.6	19.2	12.0	15.8	17.4	18.3	16.4	15.5	7.3
<i>mef</i>	7.3	12.2	7.8	8.2	12.9	16.7	12.6	11.5	4.5
<i>erm</i>	4.3	7.0	4.2	7.6	4.5	1.6	3.8	4.0	2.8

*Number of cases in the eight-county metropolitan Atlanta. Data are cases per 100 000 population.

Table 1: Incidence of invasive pneumococcal disease in metropolitan Atlanta by year, age-group, macrolide resistance, and mechanism of resistance

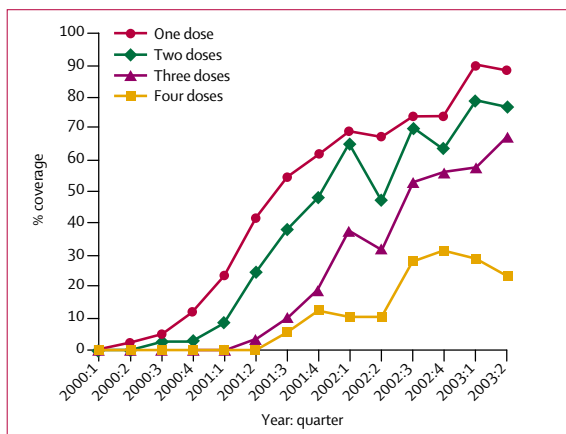


Figure 2: Pneumococcal conjugate vaccine coverage in Atlanta, 2000–2003
Percentage of children aged 19–35 months in two central metropolitan Atlanta counties (Fulton and DeKalb) of HD-3 who had received at least one, two, three, or four doses of the vaccine. Data are expressed by quarter of 2000, 2001, 2002, and the first half of 2003.

5589 isolates (83% of all invasive cases) were available for serotyping and antimicrobial-susceptibility testing. In the 1990s, rapid increases in the incidence of disease due to macrolide-resistant invasive *S pneumoniae* were reported in Atlanta, from 4.5 per 100 000 in 1994 to 9.3 per 100 000 in 1999 (figure 1, table 1). However, between 1999 and 2002, the incidence of macrolide-resistant

disease fell by 69% ($p < 0.0001$), from 7.7 per 100 000 in 2000 to 4.6 per 100 000 in 2001 and to 2.9 per 100 000 in 2002. The incidence of disease due to macrolide-resistant *S pneumoniae* in children younger than 2 years, after increasing steadily during 1994–99 from 55.6 per 100 000 to 134 per 100 000 in 1999 (figure 3, table 1), fell by 85% by 2002 (98.4 per 100 000 in 2000, 24.2 per 100 000 in 2001, and 20.2 per 100 000 in 2002, $p < 0.0001$). The rate of macrolide resistance also substantially declined in children aged 2–4 years and in adults aged 65 years and older (figure 3, table 1). Rates of macrolide resistance in invasive isolates fell in both African-American (from 13.6 per 100 000 in 1999 to 3.5 per 100 000 in 2002, $p < 0.0001$) and white populations (from 6.8 per 100 000 in 1999 to 2.2 per 100 000 in 2002, $p < 0.0001$).

The incidence of MLS_B (*erm*)-associated macrolide resistance in invasive pneumococcal isolates fluctuated between 1.5 per 100 000 and 2.3 per 100 000 from 1994 to 1999, but declined 57% to 0.8 per 100 000 population by 2002 (figure 1, table 1), $p = 0.006$. The incidence of M(*mef*)-associated resistance in invasive pneumococcal isolates steadily increased from 1994 to 1999 (from 2.9 per 100 000 to 7.7 per 100 000), but fell significantly by 2002 to 2.1 per 100 000 population, $p < 0.0001$ (figure 1, table 1). The change represented a 73% decline in the incidence of *mef*-associated invasive pneumococcal disease in 2002 compared with 1999.

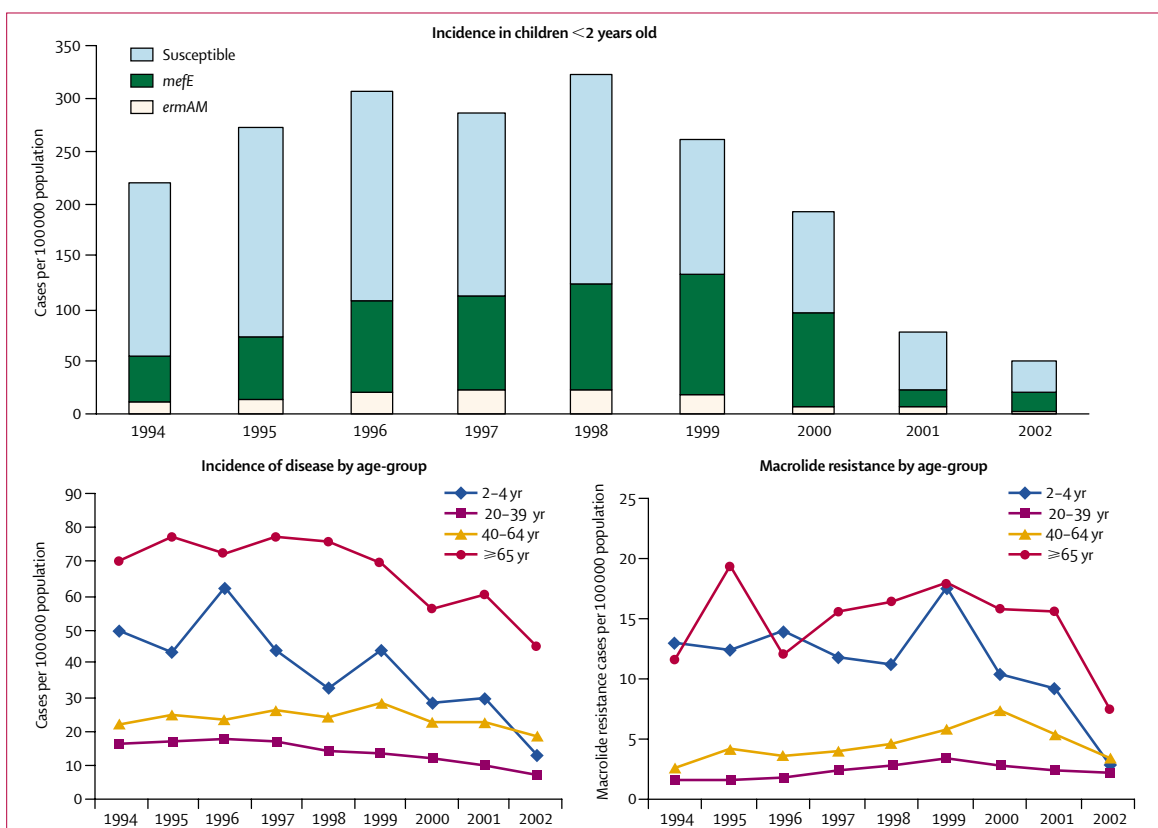


Figure 3: Incidence of invasive *S pneumoniae* disease by age-group, in metropolitan Atlanta 1994–2002

To confirm the macrolide-resistance mechanisms, pneumococcal surveillance isolates were studied for *mefE*, *ermAM*, or other mechanisms. Overall, more than 99.2% of all macrolide-resistant pneumococcal isolates assessed were positive for *mefE* or *ermAM* and were associated with the M or MLS_B phenotype (1.9% of these isolates were positive for both *mefE* and *ermAM*). No other *erm* determinants (eg, *mefA*, or mutations of ribosomal protein or 23S rRNA) were identified in the small proportion (<1%) of the macrolide-resistant population of invasive *S pneumoniae* that lacked *mefE* or *ermAM*. As expected, invasive isolates positive for *erm* were all resistant at MIC greater than 64 mg/L. During surveillance the MIC of *mefE*-positive strains increased (table 2). In 1995, 20% (19/93) of *mefE* strains had MIC to erythromycin of 8 mg/L or more. By 1999, the proportion of *mefE*-positive strains that had MIC of 8 mg/L or more was 94% (172/184). Although the incidence of *mefE*-associated macrolide resistance decreased between 2000 and 2002, in 2000 84.8% (139/164), in 2001 89% (85/96), and in 2002 89% (46/52) of *mefE*-positive pneumococcal strains recovered from invasive pneumococcal disease had MIC of 8 mg/L or more and 64% (33/52) had MICs of 16 mg/L or more.

The presence of *erm* or *mefE* was also investigated by individual serotypes (figure 4, tables 3 and 4). Over three-quarters of all invasive pneumococcal isolates collected between 1994 and 1999 in Atlanta were one of the nine conjugate vaccine or vaccine-related serotypes: 4, 14, 6A, 6B, 9V, 18C, 19A, 19F, or 23F; and serotypes 14, 6B, 9V, 19F, 23F, 6A, and 19A accounted, between 1994 and 1999, for 85–96% of the *mefE*-positive strains. The proportion of *mefE* strains that were serotype 14 increased steadily in the 1990s; by 1999 serotype 14 accounted for more than half of the *mefE* isolates and 61% of all serotype 14 isolates were positive for *mefE* (figure 4); *erm* was predominantly found in 6B, 14, 23F, and 19F isolates. By contrast, less than 2% of serotypes 4 and 18C isolates were macrolide resistant (table 3). By 2002, major reductions in the number and percentage of vaccine or vaccine-related invasive pneumococcal serotypes were noted; 43.6% of cases were now vaccine or vaccine-associated serotypes and serotype 14 now represented 19.6% of the *mefE*-containing invasive pneumococcal isolates (figure 4).

Substantial declines were noted by 2002 in the incidence of vaccine serotypes 14 (92%), 6B (53%), 19F (84%), and 23F (76%) and the incidence of macrolide resistance in these serotypes (table 3, figure 4). Falls in incidence were seen also for serotypes 4 and 18C but *mefE*-mediated or *erm*-mediated macrolide resistance remained uncommon in these serotypes. The incidence of vaccine-related serotype 6A and macrolide-resistant 6A isolates also decreased ($p=0.02$), but there was no significant change in the incidence of vaccine-related 19A, or in the overall incidence of non-vaccine serotypes 1, 3, 16, 10, 11, 12F, 22F, 33F, 35B, 7F, 8, 13, 20, 9N, 15A,B,C,F, 17A, or 18A,F (7.4 per 100 000, mean 1994–1999 [range 6.0–9.2 per 100 000], and 6.0 per 100 000 population in 2002).

	Total number of isolates	Number of isolates with MIC <8 µg/mL	Number (%) of isolates with MIC >8 µg/mL
1995	93	74	19 (20%)
1996	97	67	30 (31%)
1997	140	42	98 (70%)
1998	162	34	128 (79%)
1999	187	15	172 (94%)
2000	164	25	139 (85%)
2001	96	11	85 (89%)
2002	52	6	46 (89%)

Table 2: MIC to erythromycin of *mefE*-containing *S pneumoniae*, metropolitan Atlanta, 1995–2002

The incidence of invasive pneumococcal disease due to *erm*-resistant serotypes 6B, 14, 23F, and 19F declined substantially (table 3). We noted decreased pneumococcal invasive disease due to *mefE*-containing strains of the vaccine-associated serotypes 19F, 23F, 9V, 6B, and 6A. However, the rapid reduction in *mefE*-associated macrolide resistance and the percentage of isolates resistant to macrolides in Atlanta was led by the rapid decrease in the incidence of invasive pneumococcal disease caused by serotype 14 (table 4). Between 1999 and 2002, *mefE*-mediated, macrolide-resistant, serotype-14-associated invasive disease declined 90% from 4.2 per 100 000 to 0.42 per 100 000 population in Atlanta, $p<0.0001$. *mefE*-mediated, macrolide-resistant, serotype-14 disease in children younger than 2 years of age fell by 96% from 74.9 per 100 000 in 1999 to 2.7 per 100 000 in 2002 ($p<0.0001$) (table 3).

The decline in the incidence of macrolide-resistant serotype 14 was most striking in African-Americans,

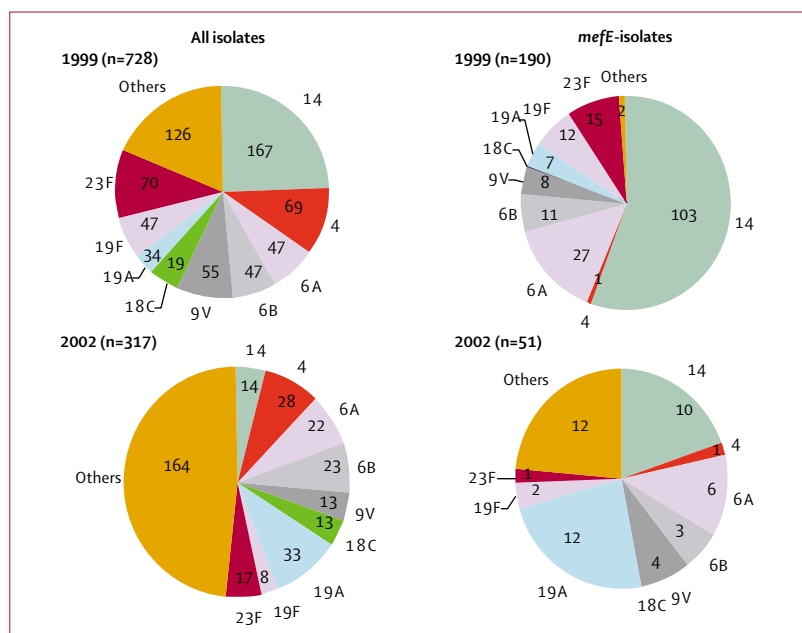


Figure 4: Distribution of invasive *S pneumoniae* by serotype

Numbers inside each pie section represent number of cases caused by the serotype listed outside the pie chart; designated serotypes include the seven serotypes in the heptavalent conjugate vaccine, plus vaccine-related serotypes 6A and 19A; "others" refers to non-conjugate vaccine serotypes.

	1994	1995	1996	1997	1998	1999	2000	2001	2002
All vaccine serotypes									
Total number of cases	446	511	605	543	564	594	487	316	151
Total incidence	17.4	19.0	22.2	19.6	19.7	20.7	15.7	10.1	5.7
Macrolide resistant	3.4	4.3	5.2	5.8	6.1	7.6	6.7	3.1	1.3
<i>mef</i>	2.1	2.6	3.7	3.5	4.4	6.1	5.4	2.2	0.8
<i>erm</i>	1.3	1.7	1.5	2.3	1.7	1.5	1.3	0.9	0.5
Serotype 4									
Total number of cases	60	62	83	84	83	83	62	54	36
Total incidence	2.3	2.3	3.0	3.0	2.9	2.8	2.0	1.7	1.1
Macrolide resistant	0	0	0.1	0.1	0.2	0.1	0	0.1	0.04
<i>mef</i>	0	0	0.1	0.1	0.2	0.04	0	0.1	0.04
<i>erm</i>	0	0	0	0	0.04	0.04	0	0	0
Serotype 14									
Total number of cases	134	154	179	149	195	201	164	70	18
Total incidence	5.2	5.7	6.5	5.5	6.8	6.9	5.2	2.2	0.6
Macrolide resistant	1.3	2.0	2.5	2.4	3.3	4.5	3.7	1.3	0.4
<i>mef</i>	1.3	1.8	2.3	2.0	2.8	4.2	3.5	1.2	0.4
<i>erm</i>	0.04	0.2	0.2	0.4	0.5	0.3	0.2	0.1	0.04
Serotype 6B									
Total number of cases	74	76	85	78	70	59	64	53	30
Total incidence	2.8	2.8	3.1	2.7	2.2	1.9	2.0	1.6	0.9
Macrolide resistant	1.7	1.3	1.4	1.3	1.0	1.0	0.9	0.9	0.4
<i>mef</i>	0.4	0.4	0.3	0.2	0.3	0.4	0.5	0.2	0.1
<i>erm</i>	1.3	0.9	1.1	1.1	0.7	0.5	0.5	0.6	0.3
Serotype 9V									
Total number of cases	62	61	83	82	53	69	46	44	17
Total incidence	2.4	2.2	3.0	2.9	1.8	2.2	1.4	1.3	0.5
Macrolide resistant	0.05	0	0.1	0.2	0.1	0.3	0.2	0.3	0.1
<i>mef</i>	0.04	0	0.1	0.2	0.1	0.3	0.2	0.2	0.1
<i>erm</i>	0	0	0.04	0.04	0	0.04	0	0.04	0
Serotype 18C									
Total number of cases	18	22	37	24	43	41	36	29	17
Total incidence	0.7	0.8	1.3	0.8	1.5	1.4	1.2	0.9	0.5
Macrolide resistant	0	0	0.04	0.1	0.1	0	0	0	0
<i>mef</i>	0	0	0.04	0.04	0.1	0	0	0	0
<i>erm</i>	0	0	0	0.04	0	0	0	0	0
Serotype 19F									
Total number of cases	38	52	63	48	56	56	50	30	10
Total incidence	1.5	1.9	2.3	1.7	1.9	1.9	1.6	0.9	0.3
Macrolide resistant	0.1	0.2	0.6	0.6	0.6	0.6	0.7	0.2	0.1
<i>mef</i>	0.1	0.1	0.5	0.6	0.4	0.5	0.5	0.1	0.1
<i>erm</i>	0	0.1	0.04	0.04	0.2	0.1	0.2	0.1	0.04
Serotype 23F									
Total number of cases	56	82	75	78	62	83	64	37	22
Total incidence	2.2	3.1	2.7	2.8	2.1	2.9	2.0	1.1	0.7
Macrolide resistant	0.2	0.6	0.3	0.9	0.6	1.1	1.1	0.3	0.1
<i>mef</i>	0.2	0.2	0.2	0.3	0.4	0.6	0.7	0.2	0.04
<i>erm</i>	0	0.4	0.1	0.6	0.2	0.5	0.4	0.1	0.1
Serotype 6A									
Total number of cases	38	52	39	46	62	55	48	41	32
Total incidence	1.4	1.9	1.4	1.6	2.1	1.9	1.5	1.4	1.0
Macrolide resistant	0.8	1.1	0.8	1.0	1.1	1.1	0.8	0.6	0.2
<i>mef</i>	0.8	1.0	0.8	1.0	1.0	1.1	0.7	0.6	0.2
<i>erm</i>	0	0.1	0	0	0.1	0	0.1	0	0
Serotype 19A									
Total number of cases	42	34	26	29	28	22	24	36	43
Total incidence	1.6	1.3	0.9	1.0	0.9	0.7	0.7	1.1	1.3
Macrolide resistant	0.1	0.3	0.2	0.2	0.4	0.3	0.1	0.4	0.6
<i>mef</i>	0.1	0.3	0.2	0.2	0.4	0.3	0.1	0.4	0.5
<i>erm</i>	0	0	0	0	0	0	0	0.03	0.1
All non-vaccine serotypes									
Total number of cases	177	226	199	255	202	175	141	196	188
Total incidence	6.8	8.4	7.3	9.2	7.1	6.0	4.5	6.2	6.0
Macrolide resistant	0.3	0.4	0.3	0.2	0.3	0.2	0.2	0.4	0.7
<i>mef</i>	0	0.04	0.2	0.04	0.1	0.1	0.1	0.2	0.5
<i>erm</i>	0.3	0.4	0.2	0.2	0.2	0.1	0.1	0.2	0.2

Data are cases per 100 000 population

Table 3: Incidence of invasive pneumococcal disease in metropolitan Atlanta by year, serotype, macrolide resistance, and mechanism of resistance

	1994	1995	1996	1997	1998	1999	2000	2001	2002
All age-groups									
Number of cases	134	154	179	149	195	201	164	70	18
Macrolide-resistant cases	35	52	69	69	94	131	118	42	14
Macrolide resistant (%)	26.1	33.7	38.5	46.3	48.2	65.2	71.9	60.0	77.7
<i>mef</i> (%)	25.3	31.1	35.2	37.5	37.9	60.7	67.1	52.8	66.6
<i>erm</i> (%)	0.8	2.6	3.3	8.8	10.3	4.6	4.8	7.2	11.1
<2 years									
Macrolide-resistant cases	14	25	36	40	53	71	62	6	3
<i>mef</i> incidence	17.8	30.1	39	46.8	57.7	74.9	65.6	4.8	2.7
<i>erm</i> incidence	0	0	3.0	1.5	4.0	3.9	0	1.2	0
2–4 years									
Macrolide-resistant cases	6	5	7	3	5	11	8	6	1
<i>mef</i> incidence	4.9	2.9	6.0	0	2.8	7.4	5.2	4.2	0.9
<i>erm</i> incidence	0	0.9	0	2.9	0.9	0.9	0.8	0	0
5–19 years									
Macrolide-resistant cases	0	0	2	1	2	2	4	1	0
<i>mef</i> incidence	0	0	0.4	0.2	0.4	0.4	0.5	0.1	0
<i>erm</i> incidence	0	0	0	0	0	0	0	0	0
20–39 years									
Macrolide-resistant cases	4	2	5	5	8	6	12	8	2
<i>mef</i> incidence	0.3	0.2	0.4	0.4	0.5	0.6	0.8	0.6	0.2
<i>erm</i> incidence	0	0	0.1	0.1	0.3	0	0.2	0.1	0
40–64 years									
Macrolide-resistant cases	5	12	9	10	12	24	23	16	4
<i>mef</i> incidence	0.5	1.3	0.8	0.7	1.2	2.3	2.1	1.6	0.4
<i>erm</i> incidence	0.2	0.3	0.3	0.4	0.1	0.5	0.4	0.1	0.1
≥65 years									
Macrolide-resistant cases	6	11	11	9	15	17	9	7	4
<i>mef</i> incidence	3.0	4.1	5.4	2.9	4.5	7.5	4.1	2.5	1.7
<i>erm</i> incidence	0	1.1	0	1.2	2.2	0	0	0.5	0

Data for incidences are cases per 100 000 population.

Table 4: Incidence of macrolide resistance in pneumococcal serotype 14 isolates by year and age-group

from 9.6 per 100 000 (1994–99) to 0.7 per 100 000 in 2002. Significant declines in the incidence of macrolide resistance in serotypes other than 14 were also noted (table 4) in isolates from invasive pneumococcal disease in people aged older than 5 years. Overall, macrolide resistance declined 27% in invasive conjugate-vaccine serotypes in unvaccinated older children and adults. Although the incidence of serotype-14 disease fell significantly, the percentage of serotype-14 isolates recovered that were macrolide-resistant remained high; in 2002, 78% of serotype-14 isolates were macrolide resistant (table 4). Furthermore, the incidence of macrolide resistance in non-vaccine serotypes (0.30 per 100 000, mean 1994–99) more than doubled in 2002 (0.7 per 100 000; $p=0.02$; table 3).

Discussion

The incidence of invasive pneumococcal disease in Atlanta fell between 1994–99 and 2002. Striking reductions were seen in children targeted to receive pneumococcal conjugate vaccine and in adults older than 20 years. Macrolide resistance in invasive *S pneumoniae* disease, after increasing steadily between 1994 and 1999, was substantially reduced by 2002. Reductions in disease caused by *mefE*-mediated and *erm*-mediated macrolide-resistant isolates of conjugate vaccine serotypes 6B, 9V, 19F, and 23F, and the vaccine-associated serotype 6A were

also recorded. The emergence of macrolide-resistant *mefE*-positive *S pneumoniae*,¹⁷ probably driven by macrolide use, was due to clonal expansion of MEGA-positive strains and horizontal spread to unrelated strains.¹⁰ *MefE* is related to a family of efflux pumps that includes *mefA*, which is also found in *S pneumoniae*,¹⁸ especially in Europe, as well as in group A¹⁹ and group B streptococci.²⁰ High rates of macrolide resistance in pneumococci also occur due to *ermAM*⁸ or mutations in ribosomal proteins (eg, L4, L22) or 23S rRNA (domains II and V);²¹ however, *ermAM* remained stable and ribosomal mutations accounted for 1% or less of macrolide resistance in invasive *S pneumoniae* in Atlanta and North America.

Emergence of pneumococcal macrolide resistance has been complicated by significant increases in the MIC to erythromycin of *mefE*-positive strains in invasive *S pneumoniae* with over 80% now having MIC of 8 mg/L or more. Although the clinical significance of resistance to the new macrolides is controversial,²² clinical failures due to *mefE*-positive pneumococci have been reported with rising frequency.^{23,24} These failures include *mefE*-positive pneumococcal isolates from patients with MIC of 8 mg/L and 16 mg/L. Allelic variations of the MEGA element,¹⁰ contribution of an *msrA* homologue found on the MEGA element,¹⁰ or differences in efflux efficiency in different pneumococcal encapsulated (serotype) backgrounds could influence the MIC of *mefE*-positive strains. The

increasing MIC of *mefE*-positive pneumococci compromise the choice of macrolides as empirical treatment for suspected serious pneumococcal infections.

The decline in macrolide resistance in *S pneumoniae* in Atlanta between 2000 and 2002 was the result of introduction of the pneumococcal conjugate vaccine; the vaccine had both direct and significant herd immunity effects as shown by the striking decline in vaccine serotypes in children younger than 2 years and those aged 2–4 years, candidates for the pneumococcal conjugate vaccine; significant declines in disease caused by vaccine serotypes in adults who were not vaccinated with conjugate vaccine; the decline in disease caused by the vaccine-associated serotype 6A; and the absence of reductions in non-vaccine serotypes in both children and adults. Moreover, these changes are not explained by variation in the incidence of overall pneumococcal disease recorded in Atlanta from 1994 to 1999 before the introduction of the vaccine. These conclusions are also lent support by similar declines in the incidence of invasive pneumococcal disease due to conjugate vaccine serotypes at other surveillance sites,¹³ and continued decreases in 2003 in Atlanta.

Other explanations (eg, decreases in rates of antimicrobial use, emergence and spread of different serotypes, expanded use of the pneumococcal polysaccharide vaccine, or changes in at-risk populations) do not explain the reported reductions in invasive pneumococcal disease or macrolide resistance. Overall antimicrobial prescribing by office-based physicians for children and adolescents younger than 15 years decreased significantly between 1992 and 2000,²⁵ but use of macrolides, especially new longer-acting agents, continued to increase, paralleling the rises in pneumococcal macrolide-resistance rates reported up to 2000; and use of these agents in Atlanta and throughout the USA has remained high. Use of pneumococcal polysaccharide vaccine in adults aged 65 years and older and in those with underlying risks rose in the 1990s, but rates in Atlanta and nationally have not substantially changed since 1999^{26,27} and thus do not account for the declines noted during 2000–02 in adult populations. Highly active antiretroviral therapy (HAART), introduced in 1996 for the treatment of HIV/AIDS, might also be predicted to influence the rates of invasive pneumococcal disease in this high-risk adult population; there were about 9000 adults with AIDS in 2002 in Atlanta. However, the rates of pneumococcal disease in adults did not change significantly until after 2000 and the number of newly diagnosed AIDS patients in Atlanta increased between 2000 and 2003. Fluctuations in the incidence of serotypes causing pneumococcal disease are seen with the clonal spread of different serotypes. As an example, disease due to serotype 14 increased in Atlanta from 1994 to 1999 and *mefE*-mediated macrolide resistance in this serotype rose rapidly. However, variation in the overall incidence of invasive pneumococcal disease in the 6 years before introduction

of the conjugate vaccine was within 15% of the mean incidence. The striking decreases in the incidence of overall pneumococcal disease and of conjugate-vaccine serotypes only seen from 2000 to 2002 were highly significant and not explained by serotype fluctuation.

Pneumococcal disease disproportionately affects certain populations (eg, African-Americans).¹³ In Atlanta, African-Americans constituted a third of the study population and half of all patients with invasive pneumococcal disease. The reductions in incidence of pneumococcal disease and macrolide resistance were striking in African-Americans after introduction of the pneumococcal conjugate vaccine, an effective strategy for reducing the burden of disease and resistance in this high-risk population. The reasons for the disproportionate declines in invasive disease in African-Americans are not clear. A large herd immunity effect of the vaccine in the African-American population, possibly due to high density households in urban Atlanta or focused targeting in African-Americans 2–4 years of age in the vaccine catch-up schedule, are possibilities to be investigated.

The decline in overall macrolide resistance was the result of the decrease in incidence of pneumococcal disease due to the vaccine serogroups frequently associated with macrolide and other antibiotic resistance (eg, *mefE* in serotype 14). Declines in resistance to penicillin, other β -lactam antibiotics, and to other antibiotics have also been noted because of the decline in these serogroups.¹³ However, we did not find evidence for a slowing of the spread of *mefE*-associated macrolide resistance among pneumococci. The rate of macrolide resistance in serotype-14 isolates was 78% in 2002, the rate of macrolide resistance in 19A isolates increased, and there were significant increases in the incidence of *mefE* resistance in invasive *S pneumoniae* of non-vaccine serotypes. These data suggest that the selective pressure for macrolide resistance continues in our population and that the beneficial effect of the pneumococcal conjugate vaccine on macrolide resistance might be short-lived if concurrent measures to promote appropriate antibiotic use are ignored. In Atlanta up to 2002, serotype replacement with non-vaccine serotypes as a cause of invasive pneumococcal disease was not seen. However, trends in the incidence and macrolide resistance of certain serotypes (19A, 33F) are of concern and serotype replacement has been reported in other settings.

The decline in invasive pneumococcal disease and macrolide resistance occurred despite substantial shortages of pneumococcal conjugate vaccine. Between August, 2001, and May, 2003, shortages affected coverage of children.¹⁴ As in Atlanta (figure 2), in the whole USA only 37% of children in birth cohorts aged 19–35 months were fully vaccinated (four doses) but 68% had received three or more doses by 2003.²⁸ Despite the restrictions in coverage, the impressive declines in adult cases who were not vaccinated with the conjugate vaccine indicate a large herd immunity effect of the pneumococcal conjugate

vaccine. Significant herd immunity effects were also seen in a South African study with a pneumococcal conjugate vaccine,²⁹ and the vaccines decrease rates of pneumococcal pneumonia and otitis media caused by vaccine serotypes.^{28–30} Continued surveillance will be necessary to monitor future trends in pneumococcal-disease incidence and the long-term effects on antibiotic resistance.

The substantial rise in erythromycin resistance due to efflux in *S pneumoniae* and the subsequent fall paralleling the introduction of the heptavalent pneumococcal conjugate vaccine show both the rapid selection and spread of an antibiotic resistance determinant in pneumococci and the opportunity for effective vaccines that effect transmission to reduce rates of disease and antibiotic resistance. However, selective pressure for antibiotic resistance continues and vaccines must be combined with programmes that emphasise appropriate antibiotic use.

Contributors

D Stephens participated in the study concept, data analysis, and design and writing of the report; S M Zughairer was involved in molecular typing, data analysis, writing of the summary, and manuscript preparation and revision; C G Whitney participated in data collection, writing of the report, and critical review; W S Baughman, D Jackson, and L Barker participated in data collection and analysis; K Gay developed molecular typing methods and characterised strains before introduction of the vaccine; W Orenstein obtained data for immunisation coverage in young children in Atlanta by quarter and by number of doses received and reviewed the presentation of the data; K Arnold participated in surveillance data collection, interpretation, and writing of the report; A Schuchat was involved in the design and critical review of the study; and M M Farley was involved in planning, data collection, data analysis, and manuscript review.

Conflict of interest statement

We declare that we have no conflict of interest.

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