Shifting Genetic Structure of Invasive Serotype 19A Pneumococci in the United States

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(See the editorial commentary by Tyrrell, on pages 1345–7.)

Background. Following 7-valent conjugate vaccine introduction in the United States in 2000, invasive serotype (sero19A) pneumococcal disease (IPD) emerged rapidly. Sero19A IPD incidence increased slightly during 2005–2008 (from 2.3 cases to 2.5 cases per 100,000 population), whereas sero19A penicillin resistance (defined as a minimum inhibitor concentration [MIC] ≥ 2 μ g/mL) increased significantly (from 28.7% to 43.7%). To better understand changes, we characterized sero19A isolates recovered during 2004–2008.

Methods. We performed antimicrobial susceptibility testing on all 2767 sero19A IPD isolates identified through the Centers for Disease Control Active Bacterial Core surveillance during 2004–2008. We genotyped 1804 (96.3%) of 1874 sero19A isolates recovered during 2005–2007 and all 148 year 2008 sero19A isolates from children <5 years of age.

Results. Resistant clonal complex (CC) 320/271^{19A} increased from 20.9% (115 of 550) to 32.9% (208 of 633; $P < .001$) of IPD isolates during 2005–2007, which paralleled increased sero19A penicillin resistance (from 28.7% [163 of 567 isolates] to 39.5% [261 of 661 isolates]; $P < .001$). Total IPD due to 320/271^{19A} increased during 2005–2007 and increased from 2.1 to 3.6 cases per 100,000 population during 2005–2008 in children <5 years of age. The penicillin-susceptible/intermediate, putative vaccine-escape CC695^{19A} increased from 7.5% (41 of 550) to 13.6% (85 of 633) of sero19A isolates during 2005–2007 ($P = .002$).

Conclusions. Sero19A rates may have plateaued; however, clonal shifts are increasing resistance. Increased IPD caused by CC320/271^{19A} and CC695^{19A} could reflect additional selective advantages in addition to resistance.

Nearly a decade after its introduction in the United States, the 7-valent conjugate vaccine (PCV7) has continued to reduce invasive pneumococcal disease (IPD) among people of all ages [1]. There has been a smaller, yet significant, increase in IPD caused by non-PCV7 serotypes, especially IPD caused by serotype 19A (sero19A) [1–4]. Consistent with the lack of protection

afforded by PCV7 against sero19A IPD [5–6], carriage of sero19A has also increased [7–9].

We previously reported annual increases in IPD due to sero19A during the early post-PCV7 period, with a 3-fold increase evident between 1998 and 2005 [2, 4]. Concurrently, there was a 5-fold increase in the proportion of antimicrobial-resistant sero19A isolates. Increased antimicrobial resistance was primarily due to the emergence of sero19A strains within clonal complex (CC) 320/271 [4].

CC320/271 comprises closely related, globally distributed, and multiply antimicrobial-resistant sero19F and sero19A strains [4, 10]. Two sequence types (STs) within CC320/271, ST271 and ST236, are possible ancestral genotypes of this complex [11–13]. The oldest strains in the multilocus ST (MLST) database from CC320/271 are sero19F strains from 1993 [12], potentially suggesting that sero19A strains of CC320/271 occurred more recently through capsular switching.

Received 27 August 2010; accepted 20 November 2010.

Potential conflicts of interest: none reported.

Presented in part: 50th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Illinois, 17 September 2010; and the Cold Spring Harbor-Asia Emerging Infectious Diseases Conference, Suzhou, China, 21 October 2010.

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The Journal of Infectious Diseases 2011;203:1360–8

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2011.

0022-1899 (print)/1537-6613 (online)/2011/20310-0001\$14.00

DOI: 10.1093/infdis/jir052

CC320/271^{19A} isolates were not detected within Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance (ABCs) during 1999 (pre-vaccine implementation), whereas CC320/271^{19F} isolates were common [14, 15]

Two additional 19A CCs of major importance include CC199 and CC695 [4]. Within CC199, ST199 is widely distributed and circulates as either sero19A or sero15B/C. CC199 remained the most prevalent sero19A CC in 2005, representing 60% of invasive sero19A isolates [4]. The majority of CC199^{19A} strains recovered in the United States were intermediately penicillin-resistant (penicillin minimum inhibitory concentrations [MICs] of 0.12–1.0 µg/mL) [2, 4]. A strain of ST199^{19A} was involved in a capsular switch event with a sero4 recipient strain (ST695⁴), which resulted in a progeny strain of ST695^{19A} [17]. This switch from a vaccine to nonvaccine capsular type represented the first vaccine escape event implicated to have occurred within the post-PCV7 implementation period. Five isolates of ST695^{19A} were first identified in 2003–2004 [2]; by 2005, ST695^{19A} had diverged, and additional sero19A progeny variants were detected, with CC695 increasing in prevalence to become the third most common sero19A CC [4, 17]. Like CC199, CC695^{19A} strains are intermediately penicillin resistant, because ST199^{19A} donated both the capsular (*cps*) locus and adjacent penicillin-binding protein genes.

Since 2005, the proportion of IPD caused by sero19A has stabilized, whereas the proportion of invasive sero19A strains that are antimicrobial-resistant has increased [1]. Therefore, our purpose here was to determine whether more-recent changes within the sero19A population genetic structure had occurred that contributed to the increase in antimicrobial resistance. Here, we show that the two predominant CCs, CC199 and CC320/271, continued to change in relative proportion as causes of IPD: the moderately antimicrobial-resistant CC199 decreased in prevalence, whereas the highly resistant CC320/271 continued to increase, as did the moderately resistant vaccine-escape CC695.

MATERIALS AND METHODS

Surveillance

We previously provided a detailed analysis of the 1998–2005 period, covering the surveillance population that was under continuing surveillance [4]. The CDC ABCs areas expanded (as described below) in the period 2004–2008. Here, we describe data for the complete surveillance population during 2004–2008. A case of IPD was defined as detection of pneumococci in a normally sterile site specimen from an individual in the surveillance population [1, 6]. Areas under surveillance since 1999 had a total population of 18–19 million individuals. Areas under continuous surveillance during 2004–2008 included all or portions of 10 states with a combined population ranging from ~27.4 million (2004) to 28.9 million (2008) individuals [18].

Serotyping and Antimicrobial Susceptibility Testing

Isolates were serotyped by latex agglutination and the Quellung reaction using typing antisera prepared in the CDC *Streptococcus* Laboratory. MICs were determined by the broth microdilution method [16]. Isolates with penicillin MICs of ≥ 2 µg/mL and 0.12–1.0 µg/mL were considered to be resistant and intermediately resistant to penicillin, respectively [16]. These breakpoints generally reflect PBP gene alterations that confer penicillin MICs above the basal typical pneumococcal MIC of $\leq .03$ µg/mL [19]. Erythromycin and clindamycin MICs ≥ 1 µg/mL indicated resistance to macrolides and lincosamides, respectively. Isolates with cefotaxime and/or ceftriaxone MICs ≥ 2 µg/mL were considered to be resistant to third-generation cephalosporins, although current guidelines indicate that these MICs indicate resistance only when associated with meningitis [16].

MLST Procedure and Partial MLST Profiling

MLST was performed [20] with modifications [4]. For year 2005 data, complete allelic profiles were previously presented for 528 of the 550 results shown here [4], with 22 isolates assigned to CCs according to known associations of sero19A IPD isolates of specific resistance phenotypes with 2–6 allele partial MLST profiles. CCs were assigned on the basis of full 7-allele MLST identifiers for 108 of the 1254 genotyped year 2006–2007 sero19A IPD isolates. For 1092 of the 1254 year 2006–2007 isolates genotyped, CCs were assigned based upon known sero19A IPD isolate associations with *xpt-ddl* combinations and antimicrobial resistance profiles. The remaining 54 isolates were assigned CCs using 3–6 allele profiles. We also assigned all 148 year 2008 pediatric (<5 years) sero19A isolates to CCs using *xpt-ddl* combinations and antimicrobial resistance profiles. For unusual allelic/MIC profile associations, complete MLST profiles were obtained, MICs were repeated and the serotypes were verified using PCR (to verify sero19A and to check for contamination with non-19A serotypes) [21, 22]. During year 2005–2007 sero19A IPD isolate analysis, we found 65 new STs, 28 of which were from 2006–2007 isolates. This information and strain information for 22 known STs that were not previously associated with sero19A was deposited in the MLST database. Supplementary Table 1 depicts MLST and MIC data for the 1804 sero19A isolates recovered during 2005–2007.

Statistical Analyses

Annual incidence rates were calculated for 1999–2008, using population estimates from the US Census Bureau. To calculate serotype-specific incidence rates, cases with missing serotype information were redistributed by age and race, assuming that the frequency was the same as that for cases with serotype information. Data were analyzed with SAS, version 9.1 (SAS Institute), and Epi Info, version 6.04b (CDC). *P* values were generated using the χ^2 test; 95% confidence intervals (CIs) were

Figure 1. A, Overall serotype 19A (sero19A) invasive pneumococcal disease (IPD) rates and proportion of disease due to penicillin-susceptible (minimum inhibitory concentration [MIC] ≤ 0.06 $\mu\text{g/mL}$), intermediate (MIC = 0.12–1.0 $\mu\text{g/mL}$), and resistant (MIC ≥ 2 $\mu\text{g/mL}$) serotype 19A in 1999 and 2004–2008 based upon Active Bacterial Core Surveillance Data. After 1999, the surveillance areas were expanded; only the years 2004–2008 reflect the same catchment populations. B, Age-specific sero19A IPD rates over the period 1999–2008.

calculated. A P value of $< .05$ was considered statistically significant.

RESULTS

Pneumococcal IPD Surveillance During 2004–2008

From 2004–2008, 3517, 3908, 3922, 4012, and 4094 IPD cases, respectively, occurred among surveillance site residents. Pneumococcal isolates were characterized from 88%–90% of these cases.

Sero19A IPD during 2004–2008

Figure 1 illustrates the previously described increase (from 0.7 to 2.3 cases per 100,000 population) in sero19A IPD between 1999 and 2005 [4] (the previously reported sero19A IPD rate during 2005 was slightly higher due to considering only areas under continuous surveillance during 1998–2005). During 2006–2008, the sero19A IPD rate stabilized at 2.5–2.6 cases per 100,000

individuals (Figure 1A). During 2005–2008, the overall IPD rate due to all serotypes combined changed little, within the range of 13.9–14.5 cases per 100,000 population [1, 23].

Consistent with the overall IPD epidemiology, young children and older adults had the highest rates of sero19A IPD. Sero19A IPD rates among infants < 2 years of age increased from 10.8 to 15.6 cases per 100,000 individuals during 2004–2007 ($P = .011$), followed by a decrease to 11.6 cases per 100,000 population in 2008 ($P = .034$) [Figure 1B]. Adults ≥ 80 years of age were the next most susceptible individuals to sero19A IPD, with rates ranging from 4.9–9.4 cases per 100,000 population during 2004–2008 (a significant increase in 2006, compared with 2004 [$P < .001$], but not significant decrease in 2008, compared with 2006). Within 5 of the 7 age groups, the sero19A IPD rate decreased in 2008, compared with 2007, and only the 50–64-year-old age group showed small annual increases.

Increasing Penicillin-resistant Sero19A IPD

From 1999–2004, penicillin-resistant sero19A IPD increased from 5.3% of sero19A cases (6 of 114 case isolates) to 20.4% of sero19A cases (83 of 408 case isolates; $P = .0002$) [Figure 1A]. Between 2004 and 2005, the percentage of penicillin-resistant sero19A cases increased to 28.7%. During 2005–2008, although the overall sero19A IPD rate stabilized, the proportion of penicillin-resistant sero19A IPD isolates increased incrementally up to the maximum percentage of 43.7% in 2008 ($P = .01$).

Sero19A Clonal Complexes from 2005 Onward

Table 1 shows all CCs (defined as identical or closely related MLST types shared by 2 or more isolates within a given year) and all individual STs determined during 2005–2007. The 10 most frequently occurring CCs from year 2005 sero19A isolates [4] were also detected among year 2006 and 2007 sero19A isolates (Table 1), with the proportions of the 7 minor CCs (CCs 230,1339,156,81,338,292, and 63) fluctuating little over this period. Although the 3 predominant CCs (199, 320/271, and 695) remained consistent, in that they collectively comprised 86.7%–87.3% of sero19A isolates recovered during each of the 3 years (Figure 2A), these individual CCs also exhibited the most change in their proportions of the sero19A genetic structure in 2007, relative to 2005 (Table 1; Figure 2A). Although CC199 accounted for 58.9% (324 of 550) of sero19A isolates during 2005, it accounted for only 40.3% (255 of 633) of sero19A isolates during 2007. Conversely, CC320/271 accounted for only 20.9% (115 of 550) of sero19A isolates during 2005, but increased to 32.9% (208 of 633) of sero19A isolates during 2007 ($P < .001$) [Figure 2A]. During 2008, CC320/271 accounted for 49.3% (73 of 148) of IPD isolates recovered from children < 5 years of age (Figure 2B). Year 2007 was the first period for which a CC (CC320/271) accounted for more sero19A IPD than CC199 within any age group. We observed this for the < 5 -year-old age group during

Table 1. Genotype Distribution of Invasive Serotype 19A Isolates Within Individual Clonal Complexes During 2005–2007

Clonal complex	Serotypes determined during 2005–2007 through full MLST (no. sequence typed) ^a	Total isolates assigned to complex based upon full or partial MLST, % of total serotype 19A isolates			No. of penR isolates observed within complex, % of penR serotype 19A isolates		
		2005	2006	2007	2005	2006	2007
199	199(174),667(64),1756(16),1673(13),1936(11), 668(11), 2269(5),799(3),2081(2), 2220(2), 2273(2),2344(2), 4179(2),416,419,645, 680,876, 2343, 2366,2380, 2381,2392,2540, 2542,2584, 2586, 2734,3426, 4025,4177, 4178,4608,4613, 4615, 5087	324 (58.9)	348 (56.9)	255 (40.3)	14 (8.8)	15 (7.6)	15 (6.1)
320/271	320(87),1451(12),2514(4), 271(3),236(2),2271(2), 202, 1464,2267,2347,2383,2393, 2588,4180 ,4609,4614, 4616,4698	115 (20.9)	140 (22.5)	208 (32.7)	112 (70.4)	138 (69.7)	202 (82.1)
695	695(76),2365(8), 2363,899	41 (7.5)	56 (8.9)	86 (13.6)	0	0	0
230	230(10),276(7),1274(2),2013	17 (3.1)	17 (2.7)	22 (3.5)	5 (3.1)	4 (2.0)	3 (1.2)
1339	1339(6),2268(3),2270(2),2382, 2541,4176	15 (2.7)	22 (3.5)	9 (1.4)	14 (8.8)	20 (10.1)	8 (3.3)
156	156(3),557(2),2733(2),4026(2), 4610(2),2367,4023, 4024,4181, 4182,4612,4618	8 (1.5)	15 (2.4)	12 (1.9)	5 (3.1)	13 (6.6)	9 (3.7)
81	2346(6),81,2583	8 (1.5)	6 (.96)	8 (1.3)	8 (5.0)	6 (3.0)	8 (3.3)
338	338(2),172(2),2345,2585,2735	7 (1.3)	6 (.96)	8 (1.3)	0	1 (.5)	0
292	292(3)	4 (.73)	2 (.32)	3 (.47)	0	0	0
63	63,1621,2543	3 (.55)	5 (.80)	5 (.79)	0	0	0
1201	1201(2)	2 (.36)	0	0	0	0	0
193	193	0	1 (.2)	3 (.47)	0	0	0
62	62,4607	0	0	2 (.32)	0	0	0
66	66,73	0	0	2 (.32)	0	0	0
Unrelated STs from single isolates	2005: 690,1797,2272,2348, 2364,2539 2006: 4619, 558,2943 2007: 4617,1390, 1518,4611, 392, 816, 1927,2272,2705, 2988	6 (1.1)	3 (.48)	10 (1.6)	1 (.06)	1 (.5)	1 (.4)
Total isolates genotyped		550	621	633	159	198	246
19A IPD rate ^b		2.56	2.60	2.54	0.74	0.82	0.99

NOTE. MLST, multilocus sequence type; penR, penicillin resistant; ST, sequence type.

^a The number for which complete 7-allele profiles were determined. If no number is provided, only 1 complete 7 allele profile was obtained. Underlined STs were either first discovered or first documented within serotype 19A at <http://www.mlst.net/> as a result of this surveillance during 2005–2007.

^b Based upon projection of isolates characterized to total 19A isolates recovered in surveillance. The surveillance areas were the same during 2005–2007. The surveillance populations during 2005, 2006, and 2007 were estimated to be 27.8, 28.2, and 28.6 million, respectively.

2007–2008 and the 5–17-year-old age group during 2007 (data not shown; data were not available for the 5–17-year-old age group for 2008). The post-vaccine serotype switch variant CC695^{19A} comprised the third largest CC and also showed annual increases among IPD isolates, from 7.5% of sero19A isolates during 2005 to 9.0% during 2006 and 13.9% during 2007 ($P = .002$) [Figure 2A]. Isolates recovered from children <5 years old during 2005–2008 varied little in total clonal composition from the cumulative data from all age groups,

with the 3 major complexes accounting for 86.2%–87.8% of the isolates (data not shown).

Increased IPD Due to Antimicrobial-resistant CC320/271 Sero19A

More than 98% (455 of 463) of CC320/271^{19A} isolates collected during 2005–2007 were penicillin resistant, accounting for 75.2% (455 of 605) of the recovered penicillin-resistant sero19A isolates. Resistant CC320/271 comprised ~20% of sero19A

Figure 2. A, Prevalence of the 3 major invasive serotype 19A pneumococcal disease (Sero19A IPD) genotypes during 2005–2007. B, Sero19A IPD rates for all ages combined (2005–2007), and for the <5-year-old age group (2005–2008) due to each of the 3 most frequently occurring Sero19A clonal complexes during the years 2005–2007.

isolates during 2005 (Figure 3A), with this proportion increasing marginally to ~22% during 2006 (Figure 3B). During 2005–2006, CC320/271 accounted for 70%–71% of all penicillin-resistant sero19A isolates. A substantial increase in the proportion of penicillin-resistant CC320/271 was evident during 2007, during which these isolates accounted for ~32% of sero19A isolates (Figure 3C) and 82.3% (205 of 249) of penicillin-resistant sero19A isolates. Concurrent with the increase of penicillin-resistant ($\text{MIC} \geq 2.0 \mu\text{g/mL}$) CC320/271, there was a marked decrease in the proportion of penicillin-susceptible/intermediate ($\text{MIC} < 0.03\text{--}1.0 \mu\text{g/mL}$) CC199^{19A} isolates (from ~56% of sero19A isolates during 2005 to ~40% during 2007) (Table 1, Figure 2A). Interestingly, the third-largest CC, CC695, comprised solely of penicillin-susceptible/intermediate isolates, differed from CC199 in that it increased in its proportion of sero19A isolates in 2007, relative to 2005 (Figure 2A).

Among penicillin-resistant sero19A isolates recovered during 2005–2007, the majority were also resistant to macrolides (91%–96% during each year), lincosamides (67%–79%), third-generation cephalosporins (80%–84%), tetracycline (70%–86%), and to all 4 of these classes (58%–70%) [Figure 3A–C]. During each of these years, most (91%–95%) of the sero19A isolates that were resistant to these 5 classes of antimicrobials were within CC320/271. The only other complex that shared such

multidrug-resistance was CC81, the clonal identifier of the widely distributed Spain^{23F}-1 [13]; 21 of 22 isolates expressed resistance to all 5 of these classes, but there were only 6–8 CC81 isolates recovered during each of these years (Table 1). Supplementary Table 1 contains the MIC data (for 16 antimicrobials) for the 1804 genotyped sero19A isolates that were collected during 2005–2007.

New Sero19A Variants Suggestive of Recent Serotype Switch Events

Ten of the 11 CCs or STs detected during 2005 that represented multiple isolates were also found during 2006–2007 [Table 1]. Several STs were encountered that previously lacked documentation associating them with sero19A (Table 2). ST62 is the major ST found within invasive sero11A in the United States [14, 15], but ST62^{19A} and its double-locus variant ST4607^{19A} were recovered in 2 different states from children. ST100 is typical of invasive sero33F isolates in the United States [14, 15]. A double-locus variant of ST100, ST2705^{19A}, was recovered from 1 isolate obtained from an infant in 2007. CC193^{19A}, normally associated with sero17F [15], was detected during 2006–2007 in 4 states (3 isolates from adults and 1 isolate from a child). Other newly discovered STs were also more characteristic of non-sero19A serotypes. For example, ST4619^{19A} is a single-locus variant of ST191, the major ST of invasive sero7F within the United States and elsewhere [12, 14, 15], and the isolate was recovered from an adult.

Three STs unrelated to other STs in this study were associated with single penicillin-resistant ($\text{MIC} > 2 \mu\text{g/mL}$) isolates. These included ST558^{19A}, which is normally associated with penicillin-nonsusceptible invasive sero35B (Table 2) [23, 24], ST1518, which is described from 3 different serogroup 6 isolates [12], and the double-locus variant (ST2539^{19A}) of PMEN clone CSR^{19A}-11 [13].

DISCUSSION

Multiple factors in the years after the introduction of PCV7 may have resulted in sero19A becoming the prevalent invasive serotype in the United States. Although increases in IPD caused by antimicrobial-susceptible strains within sero19A were also apparent [2, 4], the multidrug-resistant CC320/271 increased most dramatically. During 2005–2007, CC320/271 continued its increase, with a concurrent decrease in the nonresistant major CC199, indicating that antimicrobial resistance was potentially the single strongest factor overall in sero19A emergence [25]. Although the sero19A IPD rate slightly decreased between 2005 and 2008, cases due to antimicrobial-resistant sero19A continued to increase. The majority (>70%) of penicillin-resistant sero19A isolates recovered during 2007–2008 were also resistant to erythromycin, clindamycin, cefotaxime, and tetracycline (data not shown). CC320/271 accounted for the majority of

Figure 3. Association of invasive serotype 19A (sero19A) clonal complexes expressed as percentage of total Sero19A isolates, with resistance to ≥ 1 of 5 antimicrobials (penicillin [MIC ≥ 2 μ g/mL], erythromycin, clindamycin, cefotaxime, and tetracycline) during 2005 (A), 2006 (B), and 2007 (C). "Other penR CCs" correspond to CC1339, CC81, CC156, and CC230 which primarily comprise isolates with a penicillin MIC of ≥ 2 μ g/mL. "Other" corresponds to all other genotypes not mentioned in the 3 categories directly above. cefR, cefotaxime resistant (MIC ≥ 2 μ g/mL); cliR, clindamycin resistant; eryR, erythromycin resistant; MIC, minimum inhibitory concentration; penR, penicillin resistant.

multidrug-resistant isolates during 2005–2007 (Figure 3A–C), and its prevalence continued to increase among children < 5 years of age in 2008 (Figure 2B); thus, the sustained increase in resistant sero19A during 2008 is probably also largely accountable to increased CC320/271 IPD across all age groups.

Perhaps surprisingly, little fluctuation was observed during 2005–2007 in the proportions of highly resistant sero19A complexes other than CC320/271 (CC1339, CC81, and CC156),

which are also composed primarily of penicillin-resistant isolates. CC320/271 is much more prevalent and is resistant to more classes of antimicrobials than is minor invasive sero19A CC156, which was described in a localized spike in the occurrence of infections due to a highly resistant sero19A strain [26]. Although CC156 sero19A isolates recovered through our surveillance are macrolide-resistant due to the possession of *mef*(A) [4], they are not resistant to lincosamides and streptogramin B

Table 2. Multilocus Sequence Types (MLSTs) With a New Serotype 19A Association Found Within Active Bacterial Core Surveillance (ABCs) Invasive Pneumococcal Disease Isolates Recovered During 2005–2007

ST	Serotypes listed in MLST database	Observations pertaining to invasive pneumococcal strains encountered in ABCs
62	11A	Major serotype 11A genotype. New SLV ST4607 also found during year 2007 serotype 19A characterization.
66	9N, 14, 19F	Major serotype 9N genotype.
73	15A, 15F	Not previously observed. DLV of ST66.
236	19F	Common multidrug-resistant serotype 19F genotype; PMEN clone Taiwan ^{19F} -14. DLV of major serotype 19A genotype ST320.
271	19F	Common multidrug-resistant serotype 19F genotype. SLV of major serotype 19A genotype ST320.
292	15A	Common antimicrobial-susceptible serotype 15A genotype.
392	17F, 23F	Typically found as serotype 17F in the United States.
557	9V, 14, 6B	SLV of ST156, most common serotype 9V genotype.
558	29, 35B	Representative of penicillin-nonsusceptible serotype 35B
668	nontypeable	Previously found within single nontypeable isolate. SLV of ST199.
690	6A	Minor serotype 6A genotype
816	10	Typically found within serotype 10A in the United States.
876	14	Not previously observed in the United States. SLV of ST199
899	4	Typically found as serotype 4. SLV of ST695 and predicted ancestor of CC695.
1390	6A, 6C	Common serotype 6C genotype
1797	7C	Previously encountered in single-serotype 7C isolate.
2705	33	DLV of major serotype 33F genotype ST100.
2988	19F	Not previously observed in the United States.

NOTE. STs available at <http://www.mlst.net/>. DLV, double-locus variant; PMEN, Pneumococcal Molecular Epidemiology Network; SLV, single-locus variant; ST, sequence type; US, United States.

antimicrobials, because this CC generally lacks the *erm*(B)-encoded 23S ribosomal RNA methylase. CCs 320/271 and 81 displayed the most multidrug resistance within sero19A during 2005–2007, with *erm*(B)-mediated resistance to both erythromycin and clindamycin. [4].

Both antimicrobial-resistant and antimicrobial-susceptible CC199^{19A} isolates caused less disease in 2007 than during 2006 (Table 1; compare Figure 3C to 3B), suggesting that antimicrobial resistance is not necessarily enough to make a clone successful in transmission and disease. Conversely, the continued increase of CC695^{19A} during 2005–2007 is also interesting. CC695^{19A} have penicillin MICs (0.06–.12 µg/mL) that are above the normal wild-type levels of susceptibility typical of CC695⁴ (<0.03 µg/mL) and some isolates also expressing erythromycin resistance (Supplementary Table 1). According to molecular evidence, ST695^{19A} is the product of a post-vaccine ST695⁴ recipient and a ST199^{19A} *pbp2x-cps* locus-*pbp1a* donor [17]. The success of CC695^{19A} could be entirely related to selective advantages conferred by the acquisition of altered *pbp* genes and the sero19A *cps* locus but may also be due to other genomic determinants of the sero4 recipient.

Whether serotype switch events have greatly impacted the increased sero19A IPD rates observed in the post-PCV7 era, serotype switching has undeniably played a major role in the evolution of the current invasive sero19A genetic structure in the

United States. Indeed, >95% of recovered sero19A isolates are represented by CCs that circumstantially appear to have originated within other serotypes (ie, serotypes 4, 7F, 9V, 11A, 14, 15A, 15B/C, 17F, 19F, 23F, 33F, and 35B). This is true not only for STs comprising the 10 CCs representing multiple sero19A isolates during each year of 2005–2007 (Table 1; STs 199, 320/271, 695, 230, 1339, 156, 81, 338, 292, and 63) but is also true for the majority of STs observed to occur only within single unrelated isolates during 2005–2007 (STs 690, 1797, 558, 2943, 1518, 392, 816, and 1927). When considering the 10 sero19A CCs by order of prevalence, we observe a common theme in that each complex appears potentially to have existed first within serotypes other than 19A. ST199 was not only the prevalent genotype representing intermediately penicillin-resistant sero19A in the pre- and post- PCV7 era but is also the major genotype represented by penicillin-susceptible invasive sero15B/C in the United States [14, 15]. Presumably ST199 was first associated with penicillin-susceptible pneumococci, possibly indicative of a sero15B/C progenitor strain. ST320 was first documented within multidrug-resistant sero19F strains and is highly related to STs 271 and 236, which are commonly found in antimicrobial-resistant sero19F [13–15]. ST695 was first documented as the major genotype among penicillin-susceptible sero4 invasive pneumococci in the United States [14, 15], and ST695^{19A} variants first appeared in the post-PCV7 era [2, 4].

ST230 was first associated with sero14 in 1996 and was subsequently found expressing alternative serotypes, including 19A [12]. ST1339 was first identified among sero19A strains in the post-PCV7 period [2,4] and is a double locus variant of PMEN clone North Carolina^{6A}-23 (ST376) [27], which represented the prevalent antimicrobial-resistant invasive sero6A strain in the United States [14, 15]. ST81 was first described within sero23F isolates characterized in Spain during the early 1980s, has disseminated globally [28], and accounted for large fractions of invasive sero23F and sero19F invasive isolates recovered during the pre-PCV7 period in the United States [14]. ST156 was first identified in Spain [29] and France [30], representing the widely disseminated multidrug-resistant sero9V strain that is also the most prevalent sero9V genotype in the United States [14, 15]. ST338 was first described from sero23F isolates recovered in Portugal [31] and is also the genotype of the major invasive sero23A penicillin-nonsusceptible strain recovered within the United States [15, 32]. Among pneumococcal isolates collected by ABCs, ST292 and ST63 have previously only been observed from sero15A strains [15].

We have found the general approach of matching current partial MLST data to our previous complete MLST analysis to be reliable, even for minor complexes. From completed MLST genotyping (7 alleles) of >700 sero19A ABCs isolates, we observed that CCs within sero19A, defined by all isolates sharing at least 4 allelic identities (and the vast majority sharing 6–7 alleles) can usually be predicted in our IPD surveillance by their *xpt-dll* combination and antimicrobial susceptibility profiles [2, 4, 14, 15]. For example, during 1999–2005, we fully genotyped 341 CC199 sero19A isolates and, of these, 304 possessed the *xpt4-dll14* combination. The CC199 strains containing *xpt4-dll14* represented 14 different MLST profiles, all of which were either identical to or a single-locus variant of ST199. We have not found the *xpt4-dll14* combination within non-CC199 sero19A genotypes. Similarly, we found that 92% of 115 CC320/271 sero19A isolates contained *xpt20-dll1*. These 106 isolates represented 7 different MLST profiles, all of which were either identical to or single-locus variants of ST320.

Although 3 genotypes accounted for 87%–88% of invasive sero19A IPD during 2005–2007, the overall genetic structure of sero19A is complex and ever-changing. Although their numbers were small, the appearance of several additional genotypes within sero19A during 2006–2007, especially those associated with penicillin resistance, could be reflective of how quickly the serotype is diversifying. The inherent clonal diversity of sero19A potentially provides a reservoir for it to flourish under differing environmental conditions. Intense immunologic pressure against prevalent strains by PCV7 possibly provided a niche advantageous for certain sero19A CCs, such as CC199, CC320/271, and CC695. Continued antimicrobial pressure may have had an additional role, particularly in the continued expansion of the highly-resistant CC320/271. The overall slight decrease of 19A

IPD during 2007–2008 was due to a decrease in IPD due to penicillin-susceptible and intermediately resistant isolates (Figure 1A). It is likely that this reflects a continued decrease in the major CC199^{19A} (see Figure 2B, where a decrease in CC199 IPD is apparent in all age groups during 2006–2007, and its continued marked decrease is observed during 2007–2008 among children <5 years of age). It is possible that this change has been effected through partial replacement of the CC199^{19A} clonal complex in the pediatric nasopharyngeal reservoir through increased carriage of other nonvaccine serotypes. Alternatively, this decrease may represent the development of naturally acquired population-level immunity, a phenomenon that likely plays a role in temporal trends in serotype-specific incidence, even in the absence of vaccination. CC1339, CC81, and CC156 are representative of lineages associated with PCV7-targeted serotypes that flourished before PCV7 implementation. It is unknown why these antimicrobial-resistant sero19A variants have not significantly emerged, whereas the penicillin-intermediate CC695^{19A} has shown relatively successful expansion over the years 2002–2007 [2, 4]. It is hoped that the implementation of PCV13 will soon eliminate all sero19A strains as significant causes of IPD.

Supplementary Data

Supplementary data are available at <http://jid.oxfordjournals.org> online.

Funding

This project was funded by the CDC's Emerging Infections Program, the CDC Antimicrobial Working Group, and the National Vaccine Program Office.

Acknowledgments

We are grateful to CDC/NCIRD/DBD/RDB *Streptococcus* Laboratory members for serotyping, antimicrobial susceptibility testing, isolate storage, and many other tasks that made this report possible. We acknowledge and appreciate the sequencing expertise of Teresa Street and Rory Bowden at the University of Oxford. We thank Dr. James Jorgensen and his laboratory group for antimicrobial susceptibility testing of all isolates other than those recovered in Georgia and Minnesota. We are grateful to the Minnesota Department of Public Health laboratory for pneumococcal serotyping and susceptibility testing of all isolates recovered in Minnesota. The contributions of members of the Active Bacterial Core Surveillance/Emerging Infections Program Network made this work possible: California Emerging Infections Program: Gretchen Rothrock, Pam Daily, Susan Brooks, Joelle Nadle, Mirasol Apostol; Connecticut Emerging Infections Program: Susan Petit, M. Zachariah Fraser, Nancy Barrett; Georgia Emerging Infections Program: Paul Malpiedi, Wendy Baughman, Kathryn E. Arnold; Maryland Emerging Infections Program: Kim D. Holmes, Elisabeth A. Vaeth; Minnesota Emerging Infections Program: Catherine Lexau, Lindsey Leshner, and Lori Triden; New York Emerging Infections Program: Bridget Anderson, Shelley Zansky, Glenda Smith, Christine Long; Oregon Emerging Infections Program: Mark Schmidt, Karen Stefonek; Tennessee Emerging Infections Program: Brenda Barnes, Terry McMinn; Centers for Disease Control and Prevention: Chris Van Beneden, Tami H. Skoff, Carolyn Wright. We acknowledge the use of the pneumococcal multilocus sequence type database, which is located at Imperial College London and is funded by the Wellcome Trust, and we thank its curator, Cynthia Bishop, for supplying designations of new alleles and allelic profiles.

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