

Streptococcus pneumoniae Serogroups 15 and 33

An Increasing Cause of Pneumococcal Infections in Children in the United States After the Introduction of the Pneumococcal 7-Valent Conjugate Vaccine

Blanca E. Gonzalez, MD,* Kristina G. Hulten, PhD,*† Linda Lamberth, BS,†
Sheldon L. Kaplan, MD,*† Edward O. Mason Jr., PhD,*† and the U.S. Pediatric Multicenter
Pneumococcal Surveillance Group

Background: After the widespread use of the 7-valent pneumococcal conjugate vaccine, replacement serotypes have emerged. Serogroups 15 and 33 have emerged as nonvaccine serotypes causing invasive disease. We describe the clinical characteristics of children with infections caused by these serogroups and determined the genetic relationship of the strains.

Materials and Methods: The United States Pediatric Multicenter Pneumococcal Surveillance Group has prospectively identified children with pneumococcal infections since 1993. Charts were reviewed retrospectively, isolates were serogrouped and serotyped and randomly selected strains were fingerprinted with the use of pulsed field gel electrophoresis. Selected strains were further characterized by multilocus sequence typing.

Results: Between January 1994 and December 2004, 103 children had pneumococcal disease caused by serogroup 15, and 40 children had infections caused by serogroup 33. There was an increase from a mean of 7 cases per year for serogroup 15 in the prevaccine period to 14 cases per year in the postvaccine period and from 2 cases per year for serogroup 33 to 7 cases per year in the same periods. Isolates were susceptible to penicillin and ceftriaxone in both periods. A predominant clone was found in each serogroup representing 60% (30 of 50) of serogroup 15 strains and 83% (24 of 29) of the serotype 33F strains. The serogroup 15 clone comprised strains of serotypes 15B and 15C, whereas the 33 clone contained only serotype 33F strains. One isolate from each of the 15 and 33 clones was characterized by multilocus sequence typing and were found to be ST199 and 100, respectively.

Conclusions: Pneumococcal disease in children caused by penicillin-susceptible clones of serogroups 15 and 33 is increasing in the United States. Clinicians should consider replacement serotypes when encountered with invasive pneumococcal disease in vaccinated children.

Key Words: *Streptococcus pneumoniae*, clonality, invasive infections, replacement serotypes

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The use of the 7-valent pneumococcal conjugate vaccine (PCV7) in the United States since early 2000 has reduced the rate of invasive pneumococcal disease in young children by ~70%, especially in those younger than 24 months of age.^{1,2} Replacement of vaccine serotypes with nonvaccine serotypes in the nasopharynx and middle ear fluid of children and changes in antimicrobial susceptibilities have been documented after the administration of conjugate vaccine.^{3–7} This raises concerns about the increase of invasive disease caused by nonvaccine serotype strains of *Streptococcus pneumoniae*.^{1,8}

The United States Pediatric Multicenter Pneumococcal Surveillance Group has prospectively identified children with systemic pneumococcal infections in both inpatients and outpatients in 8 centers since 1993.⁹ After the introduction of routine administration of PCV7 to infants starting at 2 months of age, we showed an increase of invasive disease caused by *S. pneumoniae* in children 24 months of age or younger caused by nonvaccine pneumococcal serogroups. In particular, serogroups 15 and 33 were more commonly isolated within 2 years of the PCV7 being administered routinely.¹

The purpose of this study was to characterize infections in children caused by *S. pneumoniae* serogroups 15 and 33 in 8 diverse geographic regions and to determine whether the strains were genetically related.

MATERIALS AND METHODS

The United States Pediatric Multicenter Pneumococcal Surveillance Group in 8 centers received approval of each institution's Institutional Review Board to participate. Methods of data and isolate collection were described elsewhere.⁹ Isolates are serogrouped and serotyped by quelling reaction with the use of antisera obtained from the Statens Serum Institut, Copenhagen, Denmark. Susceptibility to penicillin and ceftriaxone was determined by microbroth dilution and categorized according to 2005 Clinical and Laboratory Stan-

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From the *Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine, and the †Infectious Disease Laboratory, Texas Children's Hospital, Houston, TX

E-mail emason@bcm.tmc.edu. Reprints not available.

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dards Institute procedures and breakpoints.¹⁰ Study periods were designated as: prevaccine from January 1994 to December 2000; and postvaccine from January 2001 to June 2004. **Fingerprinting.** We randomly selected strains from both pre- and postvaccine periods. Genomic DNA for pulse field gel electrophoresis (PFGE) was prepared by a modified procedure based on the method of Murray et al¹¹ PFGE was performed with the CHEF DR III system in 0.35× Tris-buffered EDTA according to the Bio-Rad Genepath parameters: with pulse times between 1 and 17 seconds for 18.5 hours, at 14°C at 6V/cm and at a 120-degree angle. PFGE profiles were compared visually and digitally by Pearson correlations/unweighted pair group method with arithmetic mean using the GelComparII computer software (Applied Maths, Kortrijk, Belgium). Relationships between strains were determined based on previously published criteria.¹²

Multilocus sequence type (MLST) was performed according to the instructions posted at the MLST web site (www.mlst.net), and the sequence type was determined with the pneumococcal MLST database located at Imperial College, London, and funded by the Wellcome Trust. χ^2 for trend was used to compare the increase in serogroups 15 and 33 relative to serotypes 1 and 3 (2 other predominant serotypes not included in the 7-valent pneumococcal conjugate vaccine) over the study periods.

RESULTS

Between 1994 and 2004, a total of 103 and 40 patients with pneumococcal disease caused by serogroups 15 and 33, respectively, were identified. Figure 1 shows the distribution of cases caused by these serogroups in both study periods. Serogroups 15 and 33 increased significantly over the study period compared with serotypes 1 and 3 over the same time ($P < 0.005$). Serogroups 15 and 33 combined increased from a mean of 8 cases per year (range, 2–13) in the prevaccine period to a mean of 21 cases/year (range, 12–31) in the postvaccine period. This is compared with serotypes 1 and 3 which was an average of 22 cases/year (range, 11–38) in the

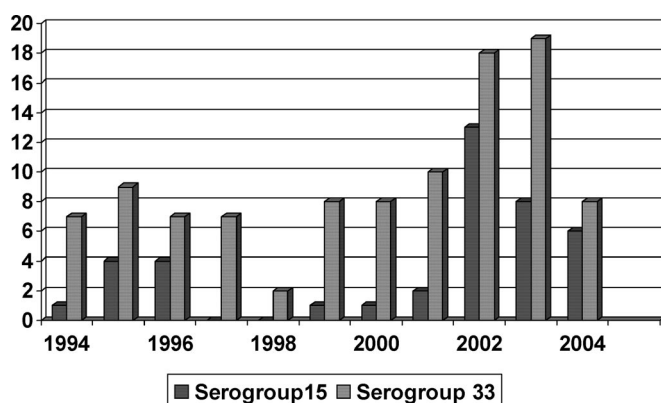


FIGURE 1. Invasive *S. pneumoniae* isolates from children in 8 children's hospitals in the United States. Distribution by year and serogroup. Serotypes 1 and 3 during the comparable period had an average of 7.4 (range, 1–16) and 12.8 (range, 4–20), respectively.

prevaccine period and a mean of 20 cases/year (range, 7–30) in the postvaccine period.

Serogroup 15. Between 1994 and 2004, a total of 103 children had infections caused by *S. pneumoniae* serogroup 15. Forty-eight (47%) cases occurred in the prevaccine period, and 55 (53%) occurred in the postvaccine period. Cases increased from a mean of 6.9 cases/year (median, 7; range, 2–9), between 1994 and 2000 to a mean of 13.7/year (median, 14; range, 8–19) in the period from 2001 to 2004 ($P = 0.01$).

The mean ages for pre- and postvaccine periods were similar (3.5 years versus 2.8 years; $P = 0.29$). No statistical differences between both periods were found regarding race ($P = 0.69$) and gender ($P = 1$). In the pre- and postvaccine period, respectively, 48 and 44% of children had no underlying medical conditions. Table 1 shows the admission diagnosis and the ages at presentation. There was no difference in the disease distribution in the pre- and postvaccine periods. The mean ages at presentation were similar except for the patients with meningitis, who in the prevaccine period were older than in the postvaccine era (9.3 versus 2.3 years; $P = 0.048$) and for bone and joint infections, but numbers for the latter were too small to allow for statistical analysis. Bacteremia remained the most common diagnosis in the postvaccine period, occurring mainly in children younger than 3 years of age.

Two patients in the prevaccine period had received immunization with the polysaccharide pneumococcal vaccine before presentation: a child with IgA deficiency; and a child with human immunodeficiency virus infection. In the postvaccine period, 24 patients had documentation of receiving the 7-valent conjugate vaccine; 4 had 1 dose, 8 had 2 doses, 6 had 3 doses and 6 had 4 doses before presentation. Five children in the postvaccine period had received the polysaccharide pneumococcal vaccine before presentation, 4 with underlying conditions.

Serotype distribution in the prevaccine period was: 15A (10%); 15B (47%); and 15C (43%). In the postvaccine period the distribution was: 15A (13%); 15B (44%); and 15C (43%). Table 1 shows distribution by infection.

Isolates were susceptible to penicillin and ceftriaxone in both periods. In the prevaccine period, 96% (46 of 48) were susceptible to penicillin [minimal inhibitory concentration (MIC), ≤ 0.06] and 4% (2 of 48) nonsusceptible (MIC ≥ 0.1). In the postvaccine period, 85% (47 of 55) of the isolates were susceptible to penicillin, and nonsusceptible strains increased to 15% (8 of 55) ($P = 0.1$). All strains in both periods were fully susceptible to ceftriaxone.

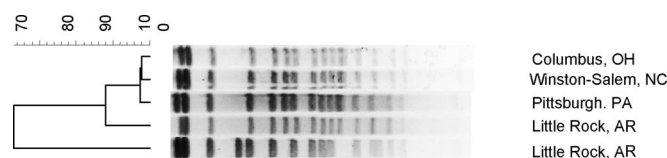
Fingerprinting. Fifty strains were randomly selected for fingerprinting. Ten strains were from the prevaccine period. Six percent (3 of 50) of the strains were serotype 15A, 48% (24 of 50) serotype 15B and 44% (22 of 50) serotype 15C. One isolate was not available for serotyping. A predominant PFGE pattern was found in 30 of 50 (60%) strains (Fig. 2). These clonal strains were either serotype 15B (57%) or serotype 15C (43%); none was serotype 15A. Four of the 10 strains in the prevaccine period displayed the predominant clonal PFGE pattern, and 3 corresponded to the closely related PFGE pattern; while in the postvaccine period, 26 strains displayed the predominant clonal PFGE pattern and

TABLE 1. Clinical Presentation of Patients with *Streptococcus pneumoniae* Serogroup 15 Infections: Distribution of Cases in Pre- and Postvaccine Periods According to Disease and Serotype

Presentation	Prevaccine (n = 48)					Postvaccine (n = 55)					P for Age
	n	Mean Age (yr)	Serotype 15A	Serotype 15B	Serotype 15C	n	Mean Age (yr)	Serotype 15A	Serotype 15B	Serotype 15C	
Bacteremia*	21	3.1	2	8	11	26	2.7	4	9	12	0.7
Bone and joint	2	2.2	1	—	1	1	14.2	—	—	1	—
Meningitis	5	9.3	1	2	2	9	2.3	1	6	2	0.04
Mastoiditis	1	2.3	—	1	—	0	—	—	—	—	—
Otitis†	13	2.5	1	7	4	13	2.1	2	7	4	0.9
Peritonitis	2	3.2	—	1	1	4	5.7	—	—	4	—
Pneumonia	3	0.7	—	2	1	2	1.4	—	2	—	—
Soft tissue	1	6.3	—	1	—	0	—	—	—	—	—

*One isolate not available for typing in postvaccine period.

†One isolate not available for typing in the prevaccine period.

**FIGURE 2.** Cluster analysis of *S. pneumoniae* serogroup 15 isolates recovered from children with invasive infections in 8 United States Children's Hospitals. GelComparII analysis of PFGE results showing predominant *S. pneumoniae* serogroup 15 clone.**FIGURE 3.** Cluster analysis of *S. pneumoniae* serotype 33F isolates recovered from children with invasive infections in 8 United States Children's Hospitals. GelComparII analysis of PFGE results showing predominant *S. pneumoniae* serotype 33F clone.

3 a very closely related pattern. One strain of the predominant clone was selected for MLST characterization and matched to sequence type (ST) 199. Fourteen strains (3 in the prevaccine and 11 in the postvaccine period) had unique PFGE patterns. Serotype 33. Forty patients had infections caused by *S. pneumoniae* serogroup 33. Eleven occurred in the prevaccine period and 29 in the postvaccine period. Cases increased from a mean of 1.6 cases per year (median, 1; range, 0–4) between 1994 and 2000 to a mean of 7.25/year (median, 7; range, 2–13) between 2001 and 2004 ($P = 0.01$).

There were no statistical differences in age ($P = 0.6$), gender ($P = 0.5$) and race ($P = 1$) in children in the pre- and postvaccine period. The majority of the children in the postvaccine period (66%) had no underlying conditions. The most common diagnosis in the prevaccine period was otitis media; whereas in the postvaccine period, bacteremia was the pre-

TABLE 2. Clinical Presentation of Patients with *Streptococcus pneumoniae* Serotype 33F Infections: Distribution of Cases in Pre- and Postvaccine Periods According to Disease

Presentation	Prevaccine (N = 11)	Mean Age (yr)	Postvaccine (N = 29)	Mean Age (yr)
Bacteremia	2	7.2	12	1.7
Bone and joint	0	—	1	10.2
Meningitis	1	13.2	6	2.5
Otitis	6	1.5	2	3.2
Pneumonia	1	3.6	6	4.7
Cellulitis	1	0.6	2	0.3

dominant diagnosis (Table 2). None of the patients in the prevaccine period had received the polysaccharide pneumococcal vaccine. In the postvaccine period, information on vaccination was available for 24 patients. Thirteen had received the 7-valent conjugate vaccine before presentation: 3 patients, 1 dose; 6 patients, 2 doses; 1 patient, 3 doses; and 3 patients, 4 doses. One child additionally received the polysaccharide pneumococcal vaccine. All isolates in the pre- and postvaccine period were serotype 33F.

Antimicrobial Susceptibilities. In the prevaccine period, 100% (11 of 11) of the isolates were fully susceptible to penicillin and ceftriaxone. In the postvaccine period, 90% (26 of 29) of the isolates were susceptible to penicillin ($\text{MIC} \leq 0.06 \mu\text{g/mL}$) and 10% (3 of 29) were nonsusceptible ($\text{MIC} \geq 0.1$); all were susceptible to ceftriaxone.

Fingerprinting. Twenty-nine strains were randomly selected for molecular typing. Five strains were from the prevaccine period. A predominant PFGE pattern (Fig. 3) was exhibited by 24 of 29 (83%) strains, including all 5 strains from the prevaccine period. The remaining strains displayed unique PFGE patterns. One strain belonging to the predominant clone was selected for MLST characterization and matched to ST-100.

DISCUSSION

Several studies have shown an increase in carriage of nonvaccine serogroups in healthy children as an effect of the PCV7, with serogroups 15 and 33 being recognized more frequently.^{3,4,6,7,13} Frazao et al¹³ reported a progressive increase in carriage of these serogroups in children attending day-care centers in Portugal after vaccination with PCV7.

Similarly a nasopharyngeal colonization study in Dallas showed that serogroup 15 was the most common nonvaccine serotype isolated in the children enrolled in their study.³ We previously reported the invasive potential of these replacement serotypes, and the present study shows a predominant clone within each of the serogroup 15 and serotype 33F strains isolated from children with invasive and noninvasive disease.¹ When we compared the increase in number of cases of serogroup 15 and 33 in the pre- and postvaccine periods with serotypes 1 and 3 over the same periods, the increase was significant. Serotype 1 and 3 were selected for this comparison because they are the most prominent causes of disease among the serotypes not included in the 7-valent pneumococcal conjugate vaccine. They remained fairly constant over the entire study, emphasizing the increase seen in serogroups 15 and 33.

We found that bacteremia and meningitis were the most common invasive diseases observed with both serogroups. The major difference found in the pre- and postvaccine periods was the age at which cases of meningitis caused by these serogroups was occurring, with a shift from disease predominantly in teenagers to disease in children younger than 3 years of age. This finding may reflect the replacement of serotypes causing disease occurring in this younger population, who are being vaccinated.

Porat et al¹⁴ reported an increase in serogroup 15 and 33 strains causing otitis media. These strains were highly clonal and had high rates of penicillin nonsusceptibility (49% nonsusceptible for serotypes 15B/C and 82% for serotype 33F). We found that the predominant clones within the serogroup 15 and serotype 33F strains were highly penicillin- and cefotaxime-susceptible. These clones appear to have been present in the United States since 1993 without major changes in PFGE patterns or in penicillin susceptibilities. Our predominant clones were unrelated to those reported by Porat et al¹⁴ as determined by PFGE patterns and antimicrobial resistance. However, a study from Indonesia that examined nasopharyngeal carriage of *S. pneumoniae* in an unvaccinated population found that serogroups 15 and 33 were commonly carried by healthy children in their area. These were penicillin- and ceftriaxone-susceptible like our isolates, but no genetic analysis was reported.¹⁵

In our study, the serogroup 15 clone comprised strains with capsular subtypes 15B and 15C. A close genetic relationship between capsule type B and C strains was also found by Porat et al and has been reported by others.^{14,16,17} Serotypes 15B and 15C differ in that serotype 15B capsule structure contains an *O*-acetyl structure that is absent in serotype 15C. The gene that encodes for the *O*-acetyltransferase (*cps15bM/cps15cM*) was identified by van Selm et al¹⁸ They suggested that variation in the number of pyrimidine thymine-adenine (TA) units within a short repetitive stretch of TA nucleotides in the *O*-acetyltransferase gene caused the difference in the capsular subtype and at the interconversion between 15B and 15C was a result of slipped strand mispairing. This small genetic change as opposed to a larger structural defect is a likely explanation to 15B and 15C capsule switching, given the identical PFGE patterns observed in the majority of 15B and 15C strains included in our study.

Two randomly selected strains from each clone were characterized by MLST and found to be ST-199 for serogroup 15 and ST-100 for serotype 33F. Jefferies et al¹⁹ used MLST to establish relationships between capsular serotypes and genotypes in pneumococcal isolates from various sources. They found a phylogenetic diversity between pneumococci in general and also between strains of the same serotype. In their study, 4 of 9 serotype 15 isolates were ST-199, and 3 of 9 serotype 33F isolates were ST-100. According to their study and the MLST database, ST-199 contains strains of serogroups 15, 19 and 6, which suggests a capsular switch within this sequence type, whereas ST-100 thus far represents only 33F strains. Our study adds to theirs and studies by others²⁰ in suggesting that properties, other than capsule type, associated with a certain clone contribute to the bacterial pathogenic potential.

With the widespread use of the PCV7 and the emergence of replacement serotypes, the pediatrician is left with the query, when presented with a vaccinated child with an invasive pneumococcal infection, of whether this represents a true vaccine failure. The majority of patients in the postvaccine period in our study who developed invasive infections with serogroups 15 and 33 had received 2 or more doses of the PCV7. Underlying medical conditions predisposing to invasive pneumococcal infections were absent in the majority of children who developed invasive infections with serotype 33F and in one-half of the patients with serogroup 15 infections. Thus the role of serotyping in clinical practice is important in distinguishing vaccine failures from disease caused by pneumococcal isolates not of the serotypes in PCV7.

In conclusion, penicillin- and ceftriaxone-susceptible clones of both serogroups 15 and 33 were found in the United States to be causing invasive disease in children. Continuous surveillance is necessary to determine whether these and other serogroups, and possibly certain genotypes, will become important replacements after the widespread use of the 7-valent pneumococcal conjugate vaccine.

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APPENDIX

Members of The United States Multicenter Pneumococcal Surveillance Group: Edward O. Mason Jr., PhD, Sheldon L. Kaplan, MD, Texas Children's Hospital and Baylor College of Medicine, Houston, TX; Ellen R. Wald, MD, Children's Hospital of Pittsburgh and University of Pittsburgh School of Medicine, Pittsburgh, PA; John S. Bradley, Children's Hospital San Diego and the University of California-San Diego School of Medicine, San Diego, CA; William J. Barson, MD, Columbus Children's Hospital and Ohio State University College of Medicine, Columbus, OH; Tina Q. Tan, MD, Ram Yogevev, MD, Children's Memorial Hospital and Northwestern University School, Chicago, IL; Gordon E. Schutze, MD, Children's Hospital and University of Arkansas for Medical Sciences, Little Rock, AR; Laurence B. Givner, MD, Brennan Children's Hospital and Bowman Gray School of Medicine, Wake Forest University, Winston Salem, NC; and Jill Hoffman, MD, Pediatric Infectious Disease Division of Children's Hospital of Los Angeles and the University of Southern California School of Medicine, Los Angeles, CA.