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COMMENTARY

The Ongoing Genetic Adaptation of *Streptococcus pneumoniae*

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ABSTRACT *Streptococcus pneumoniae* has demonstrated a remarkable ability to adapt during the conjugate vaccine era. The increasing incidence of serotype 35B disease and emergence of a multidrug-resistant clone reported in this issue of the *Journal of Clinical Microbiology* (L. Olarte et al., J Clin Microbiol 55:724–734, 2017, https://doi.org/10.1128/JCM.01778-16) underscore the limitations of pneumococcal vaccines that target the polysaccharide capsule.

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Despite the availability of conjugate vaccines, *Streptococcus pneumoniae* continues to be a formidable pathogen, causing meningitis, bacteremia, and pneumonia, as well as other respiratory infections. Nearly 500,000 children under 5 years of age die from pneumococcal pneumonia each year, and most of these fatalities occur in resource-poor areas of Asia and Africa (1). In the United States, where access to vaccination is much greater and vaccination of children is mandated, the widespread use of protein-conjugated pneumococcal polysaccharide vaccine led to a 90% decline in invasive pneumococcal disease in children below 5 years of age and a 50% decline in adults between 1998 and 2015 (2). The greatest impact of the conjugate vaccine has been observed among those with the highest rates of immunization—young children. The decline in invasive pneumococcal disease noted in adults, in whom vaccine use has been more limited, is evidence of indirect or "herd" immunity: conjugate vaccine prevents the carriage of vaccine types, and eradication of carriage in infants and young children eliminates the source of spread of pneumococci to adults.

The success reported in the United States prompted international donations to fund increased pneumococcal conjugate vaccine distribution to low-income countries (3). However, global pneumococcal vaccine coverage was only 37% in 2015, suggesting that more assistance is needed for low- and middle-income countries (4).

Prior to vaccines, the most significant advance in fighting pneumococcal disease was the introduction of penicillin in the 1940s. Reliable β-lactam activity against *Streptococcus pneumoniae* persisted until 1965, when penicillin nonsusceptibility (MIC of $\geq$0.12 μg/ml) in a clinical isolate was first reported (5). In 1977, multidrug-resistant (MDR) strains (nonsusceptible to penicillin and at least 2 additional drug classes) causing bacteremia in four patients and asymptomatic carriage in $>$100 individuals were initially recognized in South Africa (6). The prevalence of antimicrobial resistance among pneumococci in the United States remained low until the 1990s and then began to increase (7). By 2002-2003, penicillin-nonsusceptible and MDR isolates comprised 34% and 22% of the pneumococcal population, respectively (8, 9).

Along with the emergence of antimicrobial resistance in the United States, increased treatment failures of children with meningitis were observed (10). Because pneumococcal strains remained relatively susceptible to achievable levels of β-lactams, albeit at higher concentrations, infections that did not require crossing of the blood-brain barrier continued to respond well to standard therapy (11). A polysaccharide vaccine had been introduced decades earlier for use in military settings (12), with current

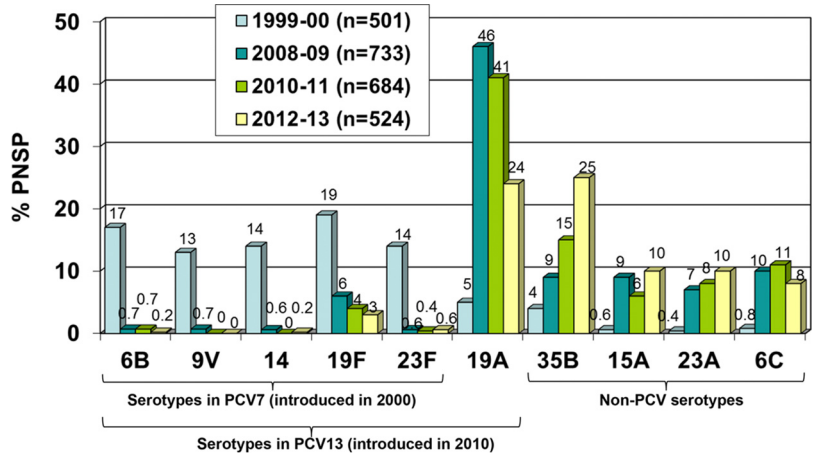

FIG 1 Changing proportions of serotypes of penicillin-nonsusceptible (MIC of ≥0.12 μg/ml) *Streptococcus pneumoniae* (PNSP) isolates in the United States during the pneumococcal conjugate vaccine (PCV) era (14, 15).

recommendations for use in elderly and immunocompromised individuals, but a new type of vaccine was needed to stimulate an immune response in children less than 2 years of age. The heptavalent polysaccharide protein conjugate vaccine (PCV7) was introduced in 2000 and included the most common serotypes among the penicillin-nonsusceptible *S. pneumoniae* (PNSP) population causing invasive disease (13). By 2008-2009, only 5% of the clinical isolates in the United States had serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F), but the prevalence of a nonvaccine serotype, 19A, had increased to 22% of all clinical isolates, encompassing 46% of PNSP and 58% of MDR strains (14). A new pneumococcal conjugate vaccine (PCV13) with added coverage of 19A and other serotypes (1, 3, 5, 6A, and 7F) was approved by the FDA in 2010. By 2012-2013, PCV13 was having the desired effect, with serotype 19A strains falling to 10% of the overall population (24% of PNSP and 39% of MDR strains) (15).

As serotype 19A strains decreased, another pneumococcal serotype increased in prevalence: the nonvaccine serotype 35B (15). Newly identified pneumococci, such as 19A or 35B, that appear after vaccination are called "replacement strains." They appear to occupy the ecological niche that, before vaccination, was occupied by a vaccine strain, although it remains unclear why a pneumococcus fills that niche, whereas other nasopharyngeal streptococci do not do so. Longitudinal surveillance, including invasive and noninvasive clinical isolates from patients of all age groups at ≥43 U.S. medical centers, has tracked serotype 35B since 1999-2000, when it comprised 2% of all isolates; it then increased from 4% in 2008-2009 to 9% of all isolates in 2012-2013 (14, 15). The percentage of serotype 35B strains from children aged 0 to 5 years, the primary recipients of PCV13, was higher than its percentage among other age groups (15% versus 8%) in 2012-2013. Serotype 35B also grew to represent a greater portion of the isolates not susceptible to penicillin, from 4% in 1999-2000 to 9% in 2008-2009 and 25% in 2012-2013 (14, 15). These changes in pneumococcal serotypes of PNSP before and after conjugate vaccine introduction are summarized in Fig. 1.

The percentage of serotype 35B strains not susceptible to penicillin increased from 81% in 2008-2009 to 96% in 2012-2013; multidrug resistance was less common but also increased, from 0.03% (2/78 isolates) to 15% (20/136 isolates) (15). The Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance identified serotype 35B as the most common nonvaccine serotype causing MDR invasive disease in 2013 (16). These findings suggested that a serotype 35B antimicrobial-resistant pneumococcal clone was emerging in response to PCV13 (17), just as had occurred with serotype 19A after PCV7 was introduced (18).

In this issue of the *Journal of Clinical Microbiology*, Olarte and colleagues provide new information regarding genetic events that have contributed to the increasing

prevalence of serotype 35B invasive disease (19). From 1994 to 2014, their longitudinal surveillance program collected 5,420 isolates from patients at 8 U.S. pediatric hospitals who had invasive pneumococcal disease. The investigators identified 78 serotype 35B isolates that they further characterized using a multilocus sequence typing (MLST) scheme based on 7 housekeeping genes. Most of the 78 invasive serotype 35B isolates (55%) were collected after the introduction of PCV13, when the percentage of MDR isolates grew to 28%; only one MDR serotype 35B isolate had been noted before 2010 (19). The majority of serotype 35B isolates were sequence type 558 (ST558) (69%) or single-locus variants (SLV) of ST558 (12%), and all of the 35 isolates collected during the pre-PCV13 era could be clustered into a clonal complex (CC), CC558. More diversity among serotype 35B isolates was recognized in the post-PCV13 period, with nine CC156 strains (8 ST156 and 1 SLV156) detected starting in 2011 that were all MDR. Although a few CC156 isolates with a serotype 35B serotype have been reported previously, the increasing prevalence of an MDR CC156 clone documented by Olarte et al. (19) is new and a cause for concern.

From 2011 to 2014, the pediatric surveillance program also collected 473 isolates causing otitis media and identified 48 noninvasive serotype 35B isolates for comparison to the sequence types of invasive serotype 35B isolates from the same time period (19). The susceptibility profiles and genetic composition of invasive and noninvasive serotype 35B isolates were similar (19).

How does the pneumococcus continue to avoid elimination after vaccination and evolve into new MDR clones? The capacity of *S. pneumoniae* to acquire new genetic information was first demonstrated in 1928 by Frederick Griffith (20), who injected live unencapsulated pneumococci into the peritoneal cavity of mice along with killed serotype 3 (encapsulated) organisms (21). The live unencapsulated organisms that had been derived from a serotype 2 pneumococcus acquired the capacity to make type 3 capsular polysaccharide, rendering them pathogenic. In 1944, Oswald Avery (22) showed that nucleic acids, not proteins, were responsible, thereby demonstrating the chemical nature of genetic material. The process of acquiring DNA, called transformation, is demonstrable in other species but is a characteristic of pneumococci. It requires immediate physical contact between the DNA of one type and a living bacterium of another type.

The nasopharynx of young children is a perfect environment for encouraging transformation. Infants and young children tend to be colonized by large numbers of pneumococci, and more than one serotype may colonize simultaneously (23). Thus, it is no surprise that pneumococci of one serotype are able, by transformation, to acquire DNA from pneumococci of another serotype, thereby "switching" capsules. Since the conjugate vaccine is directed against capsular polysaccharide, it will have no effect against a strain of pneumococcus that has acquired a non-vaccine-type polysaccharide.

The MLST profile of the MDR serotype 35B^{ST156} isolates reported by Olarte et al. (19) was documented earlier in other serotypes. ST156 was a predominant penicillin-resistant serotype 9V clone (Spain 9V-3) circulating in the United States prior to the introduction of PCV7 (24). Beall et al. observed ST156 among 177 different STs (83 clonal sets) represented in a collection of 1,476 invasive isolates from 1999, 2001, and 2002 Active Bacterial Core surveillance (24). Twenty-five of the 83 clonal sets were associated with more than one serotype; ST156 isolates were associated with serotypes 9V, 9A, 14, 19F, and 11A (24). CC156 was one of only three clonal complexes with new serotype associations in the post-PCV7 period (24). The acquisition by ST156 of genetic material encoding the production of type 35B capsule is the latest example of pneumococcal capsular switching to escape vaccine pressure. The ability of this antimicrobial-resistant strain to acquire a serotype not recognized by the 13-valent conjugate vaccine illustrates a basic biologic principle: bacteria have ways of escaping mammalian host responses. Although Olarte et al. found that serotype 35B strains caused the majority of cases of invasive pneumococcal disease in their surveillance program, only 8 isolates were ST156 (19).

There are two special concerns about serotype 35B. The 35B polysaccharide is not contained in either the 23-valent polysaccharide vaccine or the 13-valent conjugate vaccine, suggesting that fully vaccinated adults or children will not be protected against it. Furthermore, many of the 35B isolates, especially those that are ST156, have reduced susceptibility to penicillin and other antibiotics, indicating a need for clinicians to remain cautious as they select dosages of β-lactams or as they prescribe other agents, such as macrolides or tetracyclines. Interestingly, the relative antimicrobial resistance of serotype 35B might give the organism a survival advantage over more-susceptible strains in children who undergo antibiotic therapy (25).

The changing epidemiology of *S. pneumoniae* observed during the conjugate vaccine era has fostered the development of new methods for serotyping and the application of genotyping tools like MLST that enhance our understanding of this fascinating organism. However, there is a compelling need for more active surveillance programs outside the United States to better understand the epidemiology of strains in other countries where the disease burden of pneumococcal disease is much higher. Without active surveillance, how can we be sure that the conjugate vaccines launched in Asia and Africa adequately cover the antimicrobial-resistant serotypes of pneumococci circulating in those countries? It would be tragic if high mortality from pneumococcal pneumonia persists in this region despite the noble efforts under way to increase global vaccine coverage.

This increasing prevalence of invasive pneumococcal disease caused by the non-vaccine serotype 35B in the United States and the emergence of a new MDR clone due to capsular switching show, once again, the need for a pneumococcal vaccine that targets a conserved protein (26). Vaccine candidates include a protein that has a role in pathogenicity, such as pneumolysin, or one that is exposed on the bacterial surface and whose antibody would enhance ingestion and killing by white blood cells. Phase I clinical trials of vaccines delivering pneumococcal surface protein A (PspA) and pneumolysin have been completed (27). Studies investigating pneumococcal pilus proteins, a serine-threonine protein kinase (StkP), and cell wall separation protein (PcsB) as vaccine antigens have shown protection in animal models (27). Hopefully, these research efforts will yield a safe and effective vaccine targeting a new protein antigen that is independent of the local epidemiology of circulating strains. Producing vaccines to protein-conjugated polysaccharide capsules to fight an organism with >90 interchangeable serotypes conjures up the well-known image of a dog chasing his/her own tail.

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