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# PNEUMOCOCCAL POLYSACCHARIDES – ADVANCING RESEARCH AND DEVELOPMENT

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**Carbohydrates in the form of capsular polysaccharides and lipopolysaccharides constitute a major component of the cellular surface for many bacterial species.[1] In *Streptococcus pneumoniae*, capsular polysaccharides function as major virulence factors that protect the bacterium from phagocytosis by host immune cells. Within this species, capsular polysaccharides exhibit enormous structural diversity, resulting in significant differences in immunogenicity and antigenicity. In turn, this has presented numerous challenges toward the prevention and serological analysis of pneumococcal infections.[2] In the present article, we will discuss the importance of capsular polysaccharides in *S. pneumoniae* infection and will describe the use of purified serotype-specific polysaccharides in developing and evaluating pneumococcal vaccines, verifying novel immunoassays, and tracking bacterial disease epidemiology.**

*S. pneumoniae* is a major human pathogen known to cause both invasive and non-invasive infections such as pneumonia, meningitis, bacteremia, otitis media, and sinusitis.[2,3] More importantly, this bacterium is a leading cause of vaccine-preventable morbidity and mortality worldwide.[4] In the United States, pneumococcal pneumonia is estimated to result in 150,000 hospitalizations annually with 5-7% of cases resulting in death.[5] Moreover, the Global Disease Burden study from 2018 reported that *S. pneumoniae* was the leading cause of lower respiratory infection morbidity and mortality globally and contributed to more deaths than all other etiologies combined in 2016.[6]

This pneumococcal pathogen causes disease through the production of numerous virulence factors, including pneumolysin, pneumococcal surface proteins, and capsular polysaccharides.[2] Of these, the capsular polysaccharides—which are polymeric, surface-exposed carbohydrate molecules that encapsulate the bacteria—are the most important virulence factors as they shield the bacteria from neutrophil clearance. Currently, more than 95 different capsular serotypes have been identified, each distinguishable by serological response, variations in chemical structure, and related genetic mutations.[2,7-13] These unique differences can associate with distinct epidemiological properties, including variations in carriage or prevalence of disease. For example, capsular types with higher ratios of charge to carbon may be physically larger, making them more resistant to neutrophil clearance.[14,15]

Because of their importance in pneumococcal pathogenicity, capsular polysaccharides have been components in a number of serotype-specific vaccines. In 1983, a 23-valent pneumococcal polysaccharide vaccine (PPV23, Pneumovax23®; Merck Sharp & Dohme Corp.) was developed; this vaccine contained the pooled capsular polysaccharides purified from 23 different serotypes.[16] Due to poor immunogenicity in infants, PPV23 was not approved for use in children younger than 2 years of age. In 2000, after nearly two decades without a suitable vaccine for juveniles, a 7-valent pneumococcal conjugate vaccine (7vPnC, Prevnar®; Wyeth Pharmaceuticals LLC, a subsidiary of Pfizer Inc.) was developed and licensed for use in infants and young children in the United States. This vaccine was created through the covalent coupling of a protein carrier to the purified polysaccharides from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.[17] To extend vaccine coverage to additional serotypes, a 13-valent pneumococcal conjugate vaccine (13vPnC, Prevnar 13®; Wyeth Pharmaceuticals LLC, a subsidiary of Pfizer Inc.) was later licensed for pediatric and adult use. 13vPnC is composed of the same serotype-conjugates included in 7vPnC as well as polysaccharide conjugates for serotypes 1, 3, 5, 6A, 7F, and 19A.[18,19]

While widespread use of these vaccines has resulted in a reduction in pneumococcal pneumonia in targeted populations, non-vaccine serotypes have increased over time.[20] To address this problem, a 15-valent pneumococcal conjugate vaccine (PCV15, Vaxneuvance™; Merck Sharp & Dohme Corp.) and a 20-valent pneumococcal conjugate vaccine (20vPnC, Prevnar 20®; Wyeth Pharmaceuticals LLC, a subsidiary of Pfizer Inc.) were developed and recently approved for use in adults aged 18 years and older.[21] These vaccines build upon the efficacy of 13vPnC through the incorporation of additional serotypes to provide further coverage for serotypes associated with pneumococcal disease worldwide. PCV15 includes serotypes 22F and 33F, which are among the serotypes known to cause invasive pneumococcal disease following the widespread use of 13vPnC.[21] 20vPnC includes the addition of serotypes 8, 10A, 11A, 12F, 15B, 22F, and 33F, which have been associated with high case–fatality rates, antibiotic resistance, and meningitis.[22]

In addition to their role in pneumococcal vaccine development, purified polysaccharides are also necessary for evaluating how effectively a vaccine can induce an immune response. Currently, the gold standard for measuring immunogenicity is via a standardized enzyme-linked immunosorbent assay (ELISA) protocol designed for the quantitation of human IgG antibodies specific for *S. pneumoniae* capsular polysaccharides.[23] The protocol for this assay, which was developed in 2000 by representatives from academia, government, industry, and the World Health Organization (WHO), is provided as a training manual containing the standard operating procedures for the preparation, execution, and analysis of the ELISA.[24] In this assay, ELISA plates are first coated with purified, serotype-specific capsular pneumococcal polysaccharides (ATCC, Manassas, VA). Following adsorption of the individual polysaccharides, dilutions of absorbed human sera are then added to the ELISA plates. The serotype-specific antibodies that remain bound to the purified pneumococcal polysaccharides are then detected and quantitated using an anti-human IgG antibody conjugated with alkaline phosphatase.[24]

In recent years, capsular polysaccharides have been similarly used in the development and evaluation of novel immunoassays. Though the WHO ELISA protocol provides a standardized, accurate method for the evaluation of vaccine efficacy, it has come under scrutiny as it is laborious and consumes large volumes of serum. To this end, the development of novel multiplex immunoassays that allow for the simultaneous measurement of specific antibodies against various pneumococcal polysaccharides have been explored.[25-29] For example, a 2012 study by Klein et al. described the development and characterization of a multiplex bead-based antibody quantification assay (MBIA) based on Luminex xMAP technology (Luminex, Austin, TX) that required the conjugation of purified serotype-specific capsular pneumococcal polysaccharides (ATCC, Manassas, VA) to fluorescent beads. In this assay, the polysaccharide-bead conjugate mixture was incubated with various dilutions of pre-adsorbed serum. Following incubation, bound antibodies were quantified using phycoerythrin-labeled goat anti-human reporter antibodies.[25] As compared to the traditional ELISA protocol, the MBIA procedure was able to provide a more efficient, cost-effective means of analyzing small volumes of serum.

Along with their function in detecting and enumerating anti-pneumococcal antibodies, the combinatorial use of capsular polysaccharides in immunoassays can be used for tracking the epidemiology of serotype-specific pneumococcal infections following vaccination. The gold-standard method for tracking invasive *S. pneumoniae* infection has occurred through traditional culture-based assays. However, good-quality samples are not always available, culturing and serological analyses can be fairly time consuming and laborious, and the detection of viable pneumococci can be hampered by use of antibiotics.[30,31] To assess the positive impact of novel pneumococcal conjugate vaccines, serotype-specific diagnostics can provide valuable data on how effectively the vaccine reduces disease in the population and can assist in monitoring for the potential emergence of rare serotypes.

Pfizer Inc, a global leader in the biopharmaceutical industry committed to producing quality products that make an impact on health worldwide, is collaborating with ATCC, a leading provider of standards for the global scientific community, to ensure that researchers worldwide have access to purified, high-quality polysaccharides. The supply of pneumococcal polysaccharides is essential in continuing to drive pneumococcal research, predicting future epidemiological shifts, and combating this deadly pathogen.

Overall, capsular polysaccharides are bacterial antigens that play a significant role in pneumococcal pathogenicity. By targeting these polymeric carbohydrates using polysaccharide or polysaccharide conjugate vaccines, the disease burden of *S. pneumoniae* serotypes covered by these vaccines has been reduced significantly. Availability of purified pneumococcal polysaccharides has also facilitated the development of serological assays and diagnostics and improved the understanding of disease epidemiology.

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