# Innovative approaches in pneumococcal vaccine development: Harnessing natural compounds and advanced delivery systems

Monday U Obaji*[1], Akunna P Emeruwa[1,2], Angus N Oli[1], Malachy C Ugwu[1]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Nigeria.
[2]Department of Microbiology and Parasitology, Faculty of Allied Sciences, David Umahi Federal University of Health Sciences, Uburu, Nigeria.

*Submitted: 14th Jan., 2025; Accepted: 15th Jan., 2025; Published online: 28th Feb., 2025*
*DOI: https://doi.org/10.54117/jcbr.v5i1.3*
**Corresponding Author: Monday Uchenna Obaji; mobaji@stu.unizik.edu.ng; +2348069844187*

## Abstract

*Streptococcus pneumoniae* is a leading cause of upper respiratory diseases worldwide, particularly in vulnerable populations such as young children, the aged, and immunosuppressed individuals. Although current pneumococcal vaccines have significantly reduced the global disease burden, limitations such as serotype replacement, variable immunogenicity, and accessibility challenges highlight the need for innovative approaches. This review explores strategies in pneumococcal vaccine development, the potential of protein-based vaccines that target conserved antigens, and recombinant technologies such as reverse vaccinology. The role of natural compounds, including bioactive molecules from *Pleurotus ostreatus* and saponin-based adjuvants, is discussed for their immunomodulatory properties. Advanced delivery systems like ISCOMATRIX and nanoparticle-based platforms are presented as solutions for enhancing mucosal and systemic immunity. This review further highlights the importance of integrating emerging technologies to ensure global accessibility. These innovations hold promise for developing universal and adaptable pneumococcal vaccines that are both effective and affordable for diverse populations.

**Keywords:** *Pleurotus ostreatus*, saponin, adjuvants, ISCOMATRIX, pneumococcal vaccines

## Introduction

*Streptococcus pneumoniae* is a clinical pathogen heavily associated with morbidity and mortality worldwide. This Gram-positive bacterium is responsible for a wide range of diseases, from non-invasive infections to

severe invasive diseases (Iroha *et al.,* 2015). Vulnerable populations, which include children under five, the aged above 65, and immunosuppressed individuals, bear the highest disease burden (Ghia *et al.,* 2019). The World Health Organization (WHO) estimates that pneumococcal diseases account for approximately 300,000 to 500,000 deaths annually in children under five, despite vaccine interventions (WHO, 2016).

Pneumococcal vaccines have significantly reduced disease incidence and mortality compared to pre-vaccine era. Two main types of vaccines widely used for vaccinations against *S. pneumoniae* infections are pneumococcal polysaccharide vaccines 23 (PPSV23) and pneumococcal conjugate vaccine (PCV13). PPSV23 contains purified capsular polysaccharides from 23 pneumococcal serotypes (Xu *et al.,* 2015; Alderson, 2014). It induces T-cell independent immunity, which is less effective in children under two years of age. PCV13 conjugates capsular polysaccharides to a protein carrier, inducing T-cell dependent responses. It provides better protection in infants and young children and reduces nasopharyngeal carriage, indirectly protecting unvaccinated populations through herd immunity (Obaji *et al.,* 2023).

A number of challenges are associated with the use of these vaccines.

1. The current vaccines target specific serotypes, which leaves individuals susceptible to non-vaccine serotypes. The decline in vaccine-included serotypes has led to the emergence of non-vaccine serotypes that lowers the overall effectiveness of vaccination programs (Isturiz *et al.,* 2017).
2. Polysaccharide vaccines do not elicit long-lasting immunity or memory responses in young children.
3. Conjugate vaccines, in turn, are more effective but may still exhibit variability in immune response among different populations.
4. High production costs limit vaccine availability in low-resource areas, where the disease burden is highest.
5. Cold chain requirements further complicate vaccine distribution, particularly in rural and remote areas (Criscuolo *et al.,* 2019).
6. Current vaccines primarily protect against invasive diseases but are less effective against non-invasive conditions such as otitis media and sinusitis, which contribute significantly to the healthcare burden. Nasopharyngeal colonization is a precursor to invasive disease and transmission (Balsells *et al.,* 2017).
7. Current vaccines have limited impact on mucosal immunity, which is crucial for

preventing colonization and subsequent disease.

8. Pneumococcal strains continue to adapt, potentially reducing the efficacy of existing vaccines. 9. Emerging serotypes and antibiotic-resistant strains pose a growing challenge to current vaccination strategies (Prato *etal.,* 2018; Su *et al.,* 2016; Chang *et al.,* 2015).

Protein antigens such as pneumococcal surface protein A (PspA), pneumococcal surface protein C (PspC), and pneumolysin are highly conserved across serotypes (Akbari *et al.,* 2019, Lu *et al.,* 2019). Vaccines based on these antigens can provide broad, serotype-independent protection while also enhancing mucosal and systemic immunity. Mucosal immunity is pivotal in preventing nasopharyngeal colonization, which precedes invasive disease and transmission. Innovations in mucosal vaccine delivery, such as intranasal sprays or oral formulations, could bolster local immune defenses and reduce transmission rates (Shakya *et al.,* 2016; Gupta *et al.,* 2015). Advanced adjuvants, such as saponins or immunostimulatory complexes (ISCOMs), can amplify immune responses, even in populations with weaker immune systems (Yang *et al.,* 2022). Delivery systems like nanoparticles and liposomes improve antigen stability, targeting, and enhance vaccine efficacy. Targeting conserved epitopes within pneumococcal proteins or incorporating multiple antigens in vaccine design could result in universal vaccines, thereby reducing the need for serotype-specific formulations. These innovative approaches to vaccine formulation aim to create thermostable vaccines that do not require stringent cold chain logistics that would be more accessible in remote and resource-limited regions (Ojiako *et al.,* 2019; Lagousi *et al.,* 2019).

**Challenges in Current Pneumococcal Vaccination Strategies**

1. Serotype replacement and vaccine effectiveness: Current pneumococcal vaccines target specific serotypes; however, this selective protection leads to the emergence of non-vaccine serotypes, a phenomenon known as serotype replacement. While vaccination has successfully reduced vaccine-included serotypes, non-vaccine serotypes have become more prevalent, which diminishes the overall effectiveness of immunization programs. This shift necessitates continuous surveillance and vaccine updates to maintain broad protection (Isturiz *et al*., 2017).

2. Limited immunological memory in young children: Polysaccharide vaccines induce an immune response primarily through T-cell-independent mechanisms, which do not elicit long-term immunological memory. This limitation makes young children, whose immune systems rely more on T-cell-dependent responses, particularly vulnerable to pneumococcal infections. Consequently, booster doses or alternative vaccine strategies are required to ensure lasting protection (Mostowy *et al.,* 2017; Tin *et al.,* 2017; Yildirim *et al.,* 2015).

3. Variability in immune response to conjugate vaccines: While conjugate vaccines offer superior immunogenicity compared to polysaccharide vaccines, immune responses vary across populations due to genetic, nutritional, and environmental factors. Differences in host immune profiles may affect vaccine efficacy, highlighting the need for population-specific research and tailored immunization strategies to maximize protection (Golden *et al.,* 2018).

4. High production costs and limited availability: The complex production process of pneumococcal conjugate vaccines leads to high manufacturing costs, limiting accessibility in low-resource settings, where pneumococcal disease burden is highest. Despite financial support from global health organizations, cost-related barriers persist, making vaccine affordability a critical challenge in developing nations (Geno *et al.,* 2015).

5. Cold chain logistics and distribution barriers: Pneumococcal vaccines require strict temperature control throughout storage and distribution. Maintaining a reliable cold chain is particularly challenging in rural and remote areas, where electricity shortages and logistical constraints increase the risk of vaccine degradation (Criscuolo *et al*., 2019). Innovations such as thermostable vaccine formulations or alternative delivery methods are needed to enhance accessibility.

6. Limited protection against non-invasive pneumococcal diseases: While current vaccines effectively prevent invasive pneumococcal diseases (IPD) such as pneumonia and meningitis, they are less effective against non-invasive conditions, which significantly contribute to healthcare burdens (Obaji *et al.,* 2023). Because nasopharyngeal colonization is a precursor to invasive disease and transmission, vaccines that enhance mucosal immunity are required to provide broader protection (Balsells *et al*., 2017).

7. Insufficient impact on mucosal immunity: Current pneumococcal vaccines primarily elicit systemic immunity, providing protection against severe infections but offering limited mucosal immune defense. Since nasopharyngeal colonization serves as a reservoir for pneumococcal transmission and infection, vaccine formulations that strengthen mucosal IgA responses are crucial to reducing overall disease incidence (Kraicer-Melamed *et al.,* 2016; Principi and Esposito, 2018).

8. Bacterial adaptation and evolving strains: *S. pneumoniae* continues to evolve through genetic recombination, altering its surface antigens to evade immune detection. These adaptations may reduce vaccine efficacy over time, requiring continuous updates to vaccine formulations to counteract emerging strains. Surveillance programs must monitor bacterial evolution to inform future vaccine development strategies (Eythorsson *et al.,* 2018; Goncalves *et al.,* 2014).

9. Emergence of antibiotic-resistant strains: The rise of antibiotic-resistant pneumococcal strains presents an additional challenge to vaccination efforts. Resistance to beta-lactams and macrolides complicates treatment options, making effective vaccination strategies even more critical. Future vaccines must incorporate broad-spectrum protection to mitigate the growing threat of antibiotic-resistant serotypes (Prato *et al*., 2018; Su *et al*., 2016; Chang *et al*., 2015).

**Role of Natural Compounds in Immunomodulation**

Natural compounds have gained significant attention for their potential to enhance immune responses, particularly in vaccine formulation. These bioactive substances, derived from plants, fungi, and other natural sources, possess immunostimulatory properties that make them valuable as adjuvants (Alving *et al.,* 2020). For instance, *P. ostreatus*, commonly known as oyster mushroom, is a rich source of bioactive molecules with immunomodulatory effects (Oli *et al.,* 2019). These compounds, including polysaccharides, β-glucans, and proteins, have shown the ability to activate various components of the immune system.

β-glucans from *P. ostreatus* are known for their ability to stimulate innate immune responses. They interact with pattern recognition receptors (PRRs) such as dectin-1 and Toll-like receptors (TLRs) on immune cells, activate macrophages, dendritic cells, and natural killer cells in the process (Oloruntola *et al.,* 2019). These interactions

enhance the secretion of pro-inflammatory cytokines e.g., IL-6 and TNF-α and promote antigen presentation. Proteins derived from *P. ostreatus* have demonstrated adjuvant-like properties by enhancing the activation and maturation of dendritic cells. This increases the production of immunoglobulins and T-helper cell responses (Mendoza, 2015). The bioactive compounds also mitigate excessive inflammation by modulating oxidative stress, which is important for balancing immune activation and preventing tissue damage during vaccine responses. Extracts from *P. ostreatus* can be incorporated into vaccines to boost immunogenicity, particularly in mucosal vaccines where local immune activation is crucial (Oli *et al.,* 2019; Lindequist *et al.,* 2014).

Saponins are another group of compounds with immunomodulatory properties. They are naturally occurring glycosides found in plants like *Quillaja saponaria*, and are among the most studied natural adjuvants due to their potent immunostimulatory properties (Shah *et al.,* 2017). Saponins stimulate both humoral and cellular immune responses by activating dendritic cells, promoting antigen uptake, and enhancing the production of cytokines. They form immunostimulatory complexes (ISCOMs), which facilitate antigen presentation and improve the induction of CD8+ T cell responses (Tessa *et al.,* 2014). Saponin-based adjuvants, such as QS-21 from *Q. saponaria*, are already used in licensed vaccines, including the malaria vaccine and herpes zoster vaccine. They enhance antigen delivery to immune cells, stimulate long-lasting memory responses, and increase the immunogenicity of subunit vaccines, which often lack sufficient intrinsic immunostimulatory activity (Lorent *et al.,* 2014). Other promising natural adjuvants include chitosan, plant-derived lectins, and lipopolysaccharides (LPS) derivatives. They are commonly found in plants and crustacean shells where they drive potent immune responses (Apostólico *et al.,* 2016). Chitosan is biodegradable polymer derived from crustacean shells, known for its ability to boost mucosal immunity and make it suitable for intranasal vaccines. Plant-derived lectins are proteins that bind to carbohydrate residues on immune cells and promote antigen uptake and cytokine production. LPS Derivatives are modified LPS compounds that selectively activate TLR4 to drie strong immune responses without excessive inflammation (El Temsahy *et al.,* 2016; Rosales-Mendoza, and Salazar-Gonzalez, 2014; Arand *et al.,* 2018). These natural adjuvants offer promising

alternatives to traditional vaccine adjuvants as illustrated in table 1.

**Table 1: Other promising natural adjuvants.**

| Natural adjuvant | Benefits | Reference |
|---|---|---|
| Chitosan | Derived from crustacean shells, chitosan is biodegradable and enhances mucosal immunity, making it ideal for intranasal vaccines. | El Temsahy *et al.,* 2016 |
| Plant-derived lectins | These proteins bind to sugar residues on immune cells, promoting antigen uptake and cytokine production. | Rosales-Mendoza, and Salazar-Gonzalez, 2014 |
| LPS derivatives | Modified LPS compounds can selectively activate TLR4, driving potent immune responses without excessive inflammation. | Arand *et al.,* 2018 |

**ISCOM Technology in Vaccine Development**

ISCOMs are an advanced delivery system designed to enhance vaccine efficacy by promoting strong and sustained immune responses. This technology combines antigens with adjuvants in a particulate structure that mimics pathogens, thereby making it highly effective for activating the immune system (Jafar *et al*., 2018). ISCOMs are cage-like nanoparticles composed of antigens, cholesterol, phospholipids, and saponin-based adjuvants. This unique structure enhances antigen stability and delivery to immune cells. They facilitate antigen uptake by antigen-presenting cells (APCs) like dendritic cells and enhance their ability to activate T cells. The saponin adjuvant disrupts endosomal membranes and allows antigens to escape into the cytoplasm and be presented via both MHC-I and MHC-II pathways. This dual presentation stimulates both CD8+ cytotoxic T cells and CD4+ helper T cells (Lorent *et al.,* 2014). The saponin component promotes the production of pro-inflammatory cytokines

while ISCOMs induce robust humoral and cellular immune responses, hence are more suitable for a wide range of pathogens. Their high immunogenicity allows for smaller doses of antigen, which is advantageous in vaccine production and cost-effectiveness. ISCOM technology has been utilized in veterinary vaccines against equine influenza (Soema *et al.,* 2015).

Mucosal immunity plays a critical role in preventing pathogen colonization and transmission, especially for respiratory and gastrointestinal infections. ISCOMATRIX technology represents a refined version of ISCOMs, designed to enhance mucosal vaccine delivery. Similar to ISCOMs, ISCOMATRIX particles consist of cholesterol, phospholipids, and saponins but are formulated for increased stability and compatibility with mucosal surfaces (Verma *et al.,* 2023). This formulation allows for intranasal or oral delivery, bypassing the need for injections and improving vaccine accessibility and compliance. It stimulates mucosal immune cells, such as IgA-producing plasma cells, and provides localized protection at the entry points of pathogens. The particles also trigger systemic immune responses and ensure comprehensive protection. ISCOMATRIX particles are efficiently taken up by mucosal APCs to ensure robust antigen presentation (Jeong and Seong, 2017). These interactions lead to the activation of both Th1 and Th2 pathways, crucial for balanced immunity. ISCOMATRIX technology is being explored for vaccines against respiratory infections and gastrointestinal diseases. It is particularly beneficial in pediatric immunization programs, as children often face challenges with injectable vaccines (Jafar *et al.,* 2018). Unlike aluminum-based adjuvants, ISCOMATRIX particles are effective at inducing cellular immunity and mucosal responses. They also avoid the pro-inflammatory side effects associated with some traditional adjuvants (Paz *et al.,* 2017). Table 2 shows various plant-derived immunostimulatory compounds, their sources, mechanisms of action, and potential applications as vaccine adjuvants. These compounds, including saponins, polysaccharides, and flavonoids, enhance immune responses by promoting antigen uptake, cytokine production, and dendritic cell activation, which make them promising candidates for next-generation vaccines.

**Table 2: Differences between ISCOMATRIX formulations and traditional adjuvants.**

| ISCOMATRIX formulations | Traditional adjuvants | References |
|---|---|---|
| 1. Comprised of antigens, cholesterol, phospholipids, and saponins in nanoparticle form. | 1. Often aluminum-based (e.g., aluminum hydroxide, aluminum phosphate) or oil emulsions. | Jalili *et al.,* 2022; Jafar *et al.,* 2018 |
| 2. Promotes antigen delivery and presentation via both MHC-I and MHC-II pathways, thereby enhancing T-cell and antibody responses. | 2. Primarily induces antibody responses via depot effect; limited impact on T-cell activation. | Paz *et al.,* 2017 |
| 3. Elicits robust humoral and cellular immunity, including cytotoxic T-cell responses. | 3. Strong humoral immunity; limited cellular immune activation. | Caulfield *et al.,* 2014 |
| 4. Effective in inducing mucosal immunity when delivered intranasally or orally. | 4. Ineffective in stimulating mucosal immunity; designed for systemic responses. | Villarini *et al.,* 2017 |
| 5. Nanoparticle structure enhances antigen stability and enables targeted delivery to APCs | 5. Stability depends on antigen-adjuvant binding; may degrade more rapidly *in vivo*. | Paz *et al.,* 2017 |
| 6. Low systemic reactogenicity; saponin components are well-tolerated when formulated properly. | 6. Aluminum adjuvants are safe but may cause localized inflammation or injection-site reactions. | Arand *et al.,* 2018; He *et al.,* 2015 |

**Innovative Pneumococcal Vaccine Candidates**

The development of novel pneumococcal vaccines is critical to overcoming the limitations of existing polysaccharide and conjugate vaccines. Protein-based vaccines and recombinant vaccine technologies represent new strategies for broadening serotype coverage, enhancing immunogenicity, and improving accessibility in low-resource settings. They target conserved pneumococcal proteins that are expressed across multiple serotypes (Pichichero, 2017). These antigens are pivotal for the bacterium's virulence and survival, which makes them ideal candidates for next-generation vaccines. For example, PspA interferes with complement deposition that helps pneumococci evade immune responses (Lu *et al*., 2019; Rodrigues *et al.,* 2018). It is highly conserved across strains and elicits robust immune responses that reduce bacterial colonization and invasive disease. Studies have shown that PspA-based vaccines provide cross-protection against multiple serotypes in animal models, making them suitable for global implementation (Wagner-Muniz *et al.,* 2018; Wang *et al.,* 2018). PspC binds to host factors like complement factor H and secretory IgA and facilitates immune evasion and colonization. As with PspA, PspC is widely conserved among pneumococcal strains. Combining PspC with other protein antigens could enhance vaccine efficacy by targeting multiple virulence mechanisms simultaneously s (Akbari *et al.,* 2019). Pneumolysin is a cytolytic toxin that damages host tissues and promotes inflammation during infection. Toxoid derivatives of pneumolysin have been developed to stimulate immunity without causing toxicity (Terra *et al.,* 2020). Table 3 presents a comparison of natural and synthetic adjuvants, their mechanisms of action, immunogenicity, and safety profiles in vaccine formulations. While natural adjuvants (e.g., saponins and polysaccharides) offer biocompatibility and broad immune activation, synthetic adjuvants (e.g., aluminum salts and TLR agonists) provide precision and stability, which emphasizes the need for optimized combinations to enhance vaccine efficacy.

**Table 3: Protein-based vaccines**

| Protein | Function | Immunogenicity | Vaccine potential | References |
|---|---|---|---|---|
| PspA | Interferes with complement deposition, helping pneumococci evade immune responses. | Highly conserved across strains, eliciting robust immune responses that reduce bacterial colonization and invasive disease. | PspA-based vaccines provide cross-protection against multiple serotypes in animal models | Akbari *et al.,* 2019; Rodrigues *et al.,* 2018 |
| PspC | Binds to host factors like complement factor H and secretory IgA, facilitating immune evasion and colonization. | PspC is widely conserved among pneumococcal strains, providing a broad target for vaccination. | Combining PspC with other protein antigens could enhance vaccine efficacy | Georgieva *et al.,* 2018; Tada *et al.,* 2018 |
| Pneumolysin | a cytolytic toxin that damages host tissues and promotes inflammation during infection | Toxoid derivatives of pneumolysin have been developed to stimulate immunity without causing toxicity | Toxoid versions of pneumolysin fused with other antigens, increase safety and efficacy | Terra *et al.,* 2020 |

Recombinant vaccine technologies leverage genetic engineering to design highly immunogenic and safe vaccine candidates. These approaches enable the production of multi-antigen vaccines and improved delivery methods. The recombinant vaccines can

induce broad immunity against diverse pneumococcal strains by combining multiple conserved protein antigens. Examples are PspA-PspC fusion proteins, which combine the immunogenicity of both antigens to enhance immune response and pneumolysoid-protein fusion toxoids, which increase safety and efficacy (Verhoeven *et al.,* 2014). Reverse vaccinology is an innovative pneumococcal vaccine technology which uses genomic data to identify novel antigens for vaccine development and allows for the rapid design of vaccines targeting emerging pneumococcal strains (Wagner-Muniz *et al.,* 2018; Argondizzo *et al.,* 2015). Another innovative technology in pneumococcal vaccine development is the vector-based vaccines that uses viral or bacterial vectors to deliver pneumococcal antigens and enhance their presentation to the immune system. Further in this category is the DNA vaccines which encode pneumococcal antigens in plasmids and elicit immune responses after being expressed in host cells. Finally, another innovative technology in this regard is the mRNA vaccines, which utilize synthetic mRNA to produce antigens *in vivo*, which enables rapid vaccine production and high immunogenicity. Saponin-based adjuvants enhance cellular and humoral responses, which makes them suitable for recombinant vaccines (Ong *et al.,* 2021).

**Mucosal Vaccination: Bridging Systemic and Local Immunity**

Mucosal immunity is the first line of defense against respiratory pathogens like *S. pneumoniae*, which typically colonize the nasopharyngeal mucosa before progressing to invasive disease. Effective mucosal immune responses can prevent colonization, interrupt transmission, and reduce the risk of systemic infection (Nagai *et al.,* 2019). However, most current pneumococcal vaccines induce systemic immunity through intramuscular administration, with limited effects on mucosal surfaces. Secretory immunoglobulin A (sIgA) is the predominant antibody at mucosal surfaces. It neutralizes pathogens and prevents their attachment to epithelial cells. Pneumococcal-specific sIgA can significantly reduce colonization and subsequent transmission. Pneumococcal colonization is a precursor to invasive diseases (Su *et al.,* 2016). Robust mucosal immunity prevents colonization, thereby reducing the incidence of invasive diseases. Enhanced mucosal responses contribute to herd immunity by minimizing bacterial spread between individuals. If the spread of infection between individuals is prevented, herd

immunity through natural infection is unlikely to develop, as fewer individuals will be exposed to the pathogen and subsequently develop immunity. However, herd immunity can still be achieved through widespread vaccination, ensuring that a large portion of the population becomes immune without requiring direct exposure to the disease. Mucosal vaccination offers a complementary approach to overcome this gap and enhance both local and systemic immunity (Ebensen *et al.,* 2017).

To effectively elicit mucosal immunity, vaccine antigens must be delivered directly to mucosal surfaces. Several innovative strategies have been explored to optimize antigen delivery and immune activation e.g. intranasal delivery directly targets the respiratory mucosa where pneumococcal colonization occurs (Seon *et al.,* 2017). This elicits both mucosal and systemic immunity. It is non-invasive and easy to administer, particularly in pediatric populations. It also promotes the production of sIgA at the site of pathogen entry. Adjuvants are critical for boosting immune responses in mucosal vaccines. Commonly used mucosal adjuvants include cholera toxin (CT) and heat-labile toxin (LT), which are potent inducers of mucosal immunity that enhance antigen uptake and dendritic cell activation. Others are saponin-based adjuvants, which stimulate both humoral and cellular immunity, and chitosan which is a biocompatible polymer that enhances antigen delivery and promotes sIgA production (Pulendran *et al.,* 2021). Nanoparticles encapsulating antigens offer targeted delivery to mucosal tissues and improve stability and uptake by antigen-presenting cells. They protect antigens from degradation in the mucosal environment, facilitate controlled release for prolonged immune stimulation, and increase antigen uptake by M cells and dendritic cells in Peyer's patches. Liposomal and ISCOM technologies enhance antigen stability and delivery to mucosal surfaces while ISCOMATRIX **c**ombines antigens with saponin adjuvants to induce robust mucosal and systemic responses (Rapaka *et al.,* 2021). Live attenuated vaccines or recombinant viral vectors mimic natural infections, efficiently activating mucosal immunity. Oral administration targets the gut-associated lymphoid tissue (GALT), a key site for generating systemic and mucosal immunity (Wong *et al.,* 2019).

**Preclinical and Clinical Evaluation of Novel Vaccines**

The development of novel pneumococcal vaccines involves rigorous preclinical and clinical evaluation to ensure immunogenicity, efficacy and safety. Preclinical studies focus on antigen stability, immune response characterization, and toxicity, while clinical trials in human populations provide insights into vaccine performance under real-world conditions (Geno *et al.,* 2015). These studies serve different purposes. For instance, stability studies ensures that vaccine antigens maintain their structure and function during storage, transportation, and administration. Stability tests, including thermal stress studies, are used to evaluate antigen degradation rates under varying temperature conditions. Advanced delivery systems, such as nanoparticles and liposomes, are often used to encapsulate antigens, to protect them from degradation and extend their shelf life (Lederhofer *et al.,* 2018). The goal of immunogenicity studies is to measure the ability of the vaccine to elicit robust and protective immune responses. Antibody titers e.g., IgG and IgA levels and functional assays, such as opsonophagocytic activity, are used to determine the immune response. On the other hand, cellular immunity is evaluated by measuring cytokine production, T-cell activation, and memory cell generation. Finally, the goal of toxicity studies is to ensure the safety of vaccine components, particularly adjuvants and delivery systems. Acute and chronic toxicity studies are conducted in animal models to evaluate adverse effects. Regulatory guidelines emphasize the need for minimizing reactogenicity without compromising immunogenicity. Recombinant antigens and adjuvants, such as ISCOMs and saponins, have demonstrated low toxicity profiles, supporting their safety for clinical trials (Verma *et al.,* 2023; Facciolà *et al.,* 2022; Shi *et al.,* 2019).

Animal models are essential for studying disease pathogenesis, vaccine-induced immunity, and efficacy before human trials. Among these models**,** mice are widely used for evaluating pneumococcal colonization, immune responses, and protection against invasive diseases. However**,** non-human primates and rabbits offer closer immunological resemblance to humans and are used for confirmatory studies (Haryono *et al.*, 2017). Notably**,** protein-based vaccines targeting PspA and PspC have shown a significant reduction in bacterial colonization and invasive disease in mouse models.

Furthermore, intranasal delivery of recombinant vaccines has demonstrated enhanced mucosal immunity, effectively preventing colonization in animal studies. (Wang *et al.,* 2018; Mukerji *et al.,* 2018).

## Future Directions and Implications for Public Health

The future of pneumococcal vaccine development is shaped by the integration of emerging technologies, innovative strategies to overcome current limitations, and efforts to ensure global accessibility. Reverse vaccinology is a groundbreaking approach that leverages genomic data to identify novel vaccine candidates (Malihe *et al.,* 2020). It identifies proteins shared across multiple serotypes that ensure broad protection. Traditional vaccine development is often slow and reliant on trial-and-error methods. Reverse vaccinology accelerates the discovery process, reducing the time required to bring vaccines from the lab to clinical trials. Genomic analysis also identifies antigens associated with antibiotic-resistant strains, and enables the development of vaccines that specifically target these emerging threats (Argondizzo *et al.,* 2015).

Novel technologies like recombinant DNA methods and microbial fermentation reduce production costs for next-generation vaccines. Thermostable formulations minimize the need for cold chain logistics, lowering distribution costs in remote areas (Fisher *et al.,* 2016). Non-invasive vaccine delivery methods, such as oral or intranasal vaccines, improve uptake by eliminating the need for trained healthcare personnel and sterile injection equipment. Single-dose and combination vaccines reduce the logistical burden of repeated immunization campaigns. Next-generation vaccines that targets conserved antigens and induce robust mucosal immunity can prevent pneumococcal colonization and transmission, hence, leading to a decline in both invasive diseases and antibiotic use (Lagousi *et al.,* 2019).

## Conclusion

Innovative strategies in pneumococcal vaccine development, including protein-based antigens, advanced delivery platforms, and emerging technologies like reverse vaccinology, have the potential to overcome current vaccine limitations, enhance global accessibility, and significantly reduce the burden of pneumococcal disease.

**References**

Akbari, E., Negahdari, B., Faraji, F., Behdani, M., Kazemi-Lomedasht, F., Habibi-Anbouhi, M (2019). Protective responses of an engineered PspA recombinant antigen against Streptococcus pneumoniae. Biotechnology Reports 24, e00385. https://doi.org/10.1016/j.btre.2019.e00385

Alderson, M (2014). Future *Streptococcus pneumoniae* vaccines. *Int. J. Infect Dis*. 21, 25.

Alving C.R, Peachman K.K, Matyas G.R, Rao M, Beck Z (2020). Army liposome formulation (ALF) family of vaccine adjuvants. Expert Rev Vaccines 19(3):279-292. https://doi.org/10.1080/14760584.2020.1745636

Apostólico, J. S., Lunardelli, V. A., Coirada, F. C., Boscardin, S. B., Rosa, D. S (2016). Adjuvants: Classification, Modus Operandi, and Licensing. J. Immunol. Res., 1459394. https://doi.org/10.1155/2016/1459394

Arand, K., Asmus, E., Popp, C., Schneider, D., Riederer, M (2018). The mode of action of adjuvants-relevance of physicochemical properties for effects on the foliar application, cuticular permeability, and greenhouse performance of pinoxaden. J. Agric. Food Chem. 66(23):5770–5777. https://doi.org/10.1021/acs.jafc.8b01102

Argondizzo, A.P., da Mota, F.F., Pestana, C.P., Reis, J.N., de Miranda, A.B., Galler, R., Medeiros, M.A (2015). Identification of proteins in Streptococcus pneumoniae by reverse vaccinology and genetic diversity of these proteins in clinical isolates. Appl. Biochem. Biotechnol. 175, 2124-2165. https://doi.org/10.1007/s12010-014-1375-3

Balsells, E., Guillot, L., Nair, H., Kyaw, M. H (2017). Serotype distribution of Streptococcus pneumoniae causing invasive disease in children in the post-PCV era: A systematic review and meta-analysis. PLoS ONE 12, e0177113. https://doi.org/10.1371/journal.pone.0177113

Caulfield, M.J., Shi, L., Wang, S., Wang, B., Tobery, T.W., Mach, H., Ahl, P.L., Cannon, J.L., Cook, J.C., Heinrichs, J.H (2014). Effect of alternative aluminum adjuvants on the absorption and immunogenicity of HPV16 L1 VLPs in mice. Hum. Vacc. 3, 139-145. https://doi.org/10.4161/hv.3.4.4309

Chang, B., Nariai, A., Sekizuka, T., Akeda, Y., Kuroda, M., Oishi, K., Ohnishi, M (2015). Capsule switching and antimicrobial resistance acquired during repeated

Streptococcus pneumoniae pneumonia episodes. J. Clin. Microbiol. 53, 3318-3324. https://doi.org/10.1128/jcm.01222-15

Chochua, S., Metcalf, B.J., Li, Z., Walker, H., Tran, T., McGee, L., Beall, B (2017). Invasive serotype 35b pneumococci including an expanding serotype switch lineage, United States, 2015-2016. Emerg. Infect. Dis. 23, 922-930. https://doi.org/10.3201/eid2306.170071

Criscuolo, E., Caputo, V., Diotti, R.A., Sautto, G.A., Kirchenbaum, G.A., Clementi, N (2019). Alternative methods of vaccine delivery: An overview of edible and intradermal vaccines. J. Immunol. Res., 8303648. https://doi.org/10.1155/2019/8303648

Ebensen, T., Debarry, J., Pedersen, G.K., Blazejewska, P., Weissmann, S., Schulze, K., McCullough, K.C., Cox, R.J., Guzmán, C.A (2017). Mucosal Administration of Cycle-Di-Nucleotide-Adjuvanted Virosomes Efficiently Induces Protection against Influenza H5N1 in Mice. Front. Immunol. 8, 1223. https://doi.org/10.3389/fimmu.2017.01223

El Temsahy, M.M., El Kerdany, E.D., Eissa, M.M., Shalaby, T.I., Talaat, I.M., Mogahed, N.M (2016). The effect of chitosan nanospheres on the immunogenicity of Toxoplasma lysate vaccine in mice. J. Parasit. Dis. 40, 611-626 https://doi.org/10.1007/s12639-014-0546-z

Eythorsson, E., Sigurdsson, S., Hrafnkelsson, B., Erlendsdottir, H., Haraldsson, A., Kristinsson, K.G (2018). Impact of the 10-valent pneumococcal conjugate vaccine on antimicrobial prescriptions in young children: A whole population study. BMC Infect. Dis. 18, 505. https://doi.org/10.1186/s12879-018-3416-y

Facciolà, A., Visalli, G., Laganà, A., Di Pietro, A (2022). An overview of vaccine adjuvants: Current evidence and future perspectives. Vaccines 10(5):819. https://doi.org/10.3390/vaccines10050819

Fisher, E.L., Otto, M., Cheung, G.Y (2016). A recombinant trivalent fusion protein F1-LcrVHSP70 (II) augments humoral and cellular immune responses and imparts full protection against Yersinia pestis. Front Microbiol 7, 71053. https://doi.org/10.3389/fmicb.2016.01053

Geno, K. A., Gilbert, G. L., Song, J. Y., Skovsted, I.C., Klugman, K.P., Jones, C., Konradsen, H.B., Nahm, M.H (2015). Pneumococcal capsules and their types: Past,

present, and future. Clin. Microbiol. Rev. 28, 871-899. https://doi.org/10.1128/cmr.00024-15

Georgieva, M., Kagedan, L., Lu, Y.J., Thompson, C.M., Lipsitch, M (2018). Antigenic variation in Streptococcus pneumoniae PspC promotes immune escape in the presence of variant-specific immunity. mBio, 9. https://doi.org/10.1128/mbio.00264-18

Ghia, C.J., Dhar, R., Koul, P.A., Rambhad, G., Fletcher, M.A (2019). Streptococcus Pneumoniae as a Cause of Community-Acquired Pneumonia in Indian Adolescents and Adults: A Systematic Review and Meta-Analysis. Clin.Med.Insights.Circ.Respir.Pulm.Med. 13:1179548419862790. https://doi.org/10.1177/1179548419862790

Golden, A.R., Adam, H.J., Karlowsky, J.A., Baxter, M., Nichol, K.A., Martin, I., Demczuk, W., Van Caeseele, P., Gubbay, J.B., Lefebvre, B (2018). Molecular characterization of predominant Streptococcus pneumoniae serotypes causing invasive infections in Canada: The SAVE study, 2011-15. J. Antimicrob. Chemother. 73, 20-31. https://doi.org/10.1093/jac/dky157

Goncalves, V.M., Dias, W.O., Campos, I.B., Liberman, C., Sbrogio-Almeida, M.E., Silva, E.P., Cardoso, C.P., Jr., Alderson, M., Robertson, G., Maisonneuve, J.F (2014). Development of a whole cell pneumococcal vaccine: BPL inactivation, cGMP production, and stability. Vaccine 32, 1113-1120. https://doi.org/10.1016/j.vaccine.2013.10.091

Gupta, P.N (2015). Mucosal vaccine delivery and M cell targeting. Targeted Drug Delivery: Concepts and Design: Springer 313-337. https://doi.org/10.1007/978-3-319-11355-5_9

Haryono, A., Salsabila, K., Restu, W.K., Harmami, S.B., Safari, D (2017). Effect of chitosan and liposome nanoparticles as adjuvant codelivery on the immunoglobulin G subclass distribution in a mouse model. J. Immunol. Res. 2017, 9125048. https://doi.org/10.1155/2017/9125048

He, P., Zou, Y., Hu, Z (2015). Advances in aluminum hydroxide-based adjuvant research and its mechanism. Hum. Vaccin. Immunother. 11, 477-488. https://doi.org/10.1080/21645515.2014.1004026

Iroha, I.R., Chibuko, N.M., Moses, I.B., Ejikeugwu, P.C., Ugbo, E.N., Nwakaeze, E.A., Agumah, N.B (2015).Antibiotic susceptibility

patterns of Streptococcus pneumoniae isolated from the nasopharyngeal mucosa of children in Enugu Metropolis, Nigeria. Int.J.Curr.Microbiol.App.Sci. 4(9): 1-9.

Isturiz, R., Sings, H. L., Hilton, B., Arguedas, A., Reinert, R. R., Jodar, L (2017). Streptococcus pneumoniae serotype 19A: Worldwide epidemiology. Expert Rev. Vaccines. 16, 1007-1027. https://doi.org/10.1080/14760584.2017.1362339

Jafar, M., Ali, B., Zahra, M., Alireza, K., Mohsen, T (2018). Immunization against PR8 influenza virus with chitosan-coated ISCOMATRIX nanoparticles, Artificial Cells, Nanomedicine, and Biotechnology, 46:sup2 587-593. https://doi.org/10.1080/21691401.2018.1464460

Jalili, P., Huet, S., Burel, A., Krause, B.C., Fontana, C., Chevance, S., Gauffre, F., Guichard, Y., Lampen, A., Laux, P (2022). Genotoxic impact of aluminum-containing nanomaterials in human intestinal and hepatic cells. Toxicol. In Vitro, 78, 105257. https://doi.org/10.21203/rs.3.rs-72845/v1

Jeong, H., Seong, B.L (2017). Exploiting virus-like particles as innovative vaccines against emerging viral infections. J. Microbiol. 55, 220-230. https://doi.org/10.1007/s12275-017-7058-3

Kraicer-Melamed, H., O'Donnell, S., Quach, C (2016). The effectiveness of pneumococcal polysaccharide vaccine 23 (PPV23) in the general population of 50 years of age and older: A systematic review and meta-analysis. Vaccine 34, 1540–1550 https://doi.org/10.1016/j.vaccine.2016.02.024

Lagousi, T., Basdeki, P., Routsias, J., Spoulou, V (2019). Novel protein-based pneumococcal vaccines: Assessing the use of distinct protein fragments instead of full-length proteins as vaccine antigens. Vaccines 7, 9. https://doi.org/10.3390/vaccines7010009

Lederhofer, J., van Lent, J., Bhoelan, F., Karneva, Z., de Haan, A., Wilschut, J.C., Stegmann, T (2018). Development of a Virosomal RSV Vaccine Containing 3D-PHAD® Adjuvant: Formulation, Composition, and Long-Term Stability. Pharm. Res. 35, 172. https://doi.org/10.1007/s11095-018-2453-y

Lindequist, U., Niedermeyer, T.H.J., Julich, W.D (2014). The pharmacological potentials of mushrooms. Evid Based complement Alternat Med 2:285-299.

Lorent, J., Lins, L., Domenech, O., Quetin-Lecterecq, J., Brasseur, R., Mingeot-Lectercq, M.P (2014). Domain formation and permeabilization induced the saponin α-hederin and its aglycone heragenin in a cholesterol-containing bilayer. Langmuir, 30, 4556-4569. https://doi.org/10.1021/la4049902

Lu, J., Guo, J., Wang, D., Yu, J., Gu, T., Jiang, C., Kong, W., Wu, Y (2019). Broad protective immune responses elicited by bacterium-like particle-based intranasal pneumococcal particle vaccine displaying PspA2 and PspA4 fragments. Hum. Vaccin. Immunother. 15, 371-380. https://doi.org/10.1080/21645515.2018.1526556

Malihe, M., Zuleeza, A., Lai, T.G., Chit, L.P (2020). Development of Next Generation Streptococcus pneumoniae Vaccines Conferring Broad Protection. Vaccines 8, 132. https://doi.org/10.3390/vaccines8010132

Mendoza, S (2015). A perspective on the use of Pleurotus for the development of convenient fungi-made oral subunit vaccines. Vaccine, 33: 25-33. https://doi.org/10.1016/j.vaccine.2014.10.059

Mostowy, R.J., Croucher, N.J., De Maio, N., Chewapreecha, C., Salter, S.J., Turner, P., Aanensen, D.M., Bentley, S.D., Didelot, X., Fraser, C (2017). Pneumococcal capsule synthesis locus cps as evolutionary hotspot with potential to generate novel serotypes by recombination. Mol. Biol. Evol. 34, 2537–2554. https://doi.org/10.1093/molbev/msx173

Mukerji, R., Hendrickson, C., Genschmer, K.R., Park, S.S., Bouchet, V., Goldstein, R., Lefkowitz, E.J., Briles, D.E (2018). The diversity of the proline-rich domain of pneumococcal surface protein A (PspA): Potential relevance to a broad-spectrum vaccine. Vaccine 36, 6834–6843. https://doi.org/10.1016/j.vaccine.2018.08.045

Nagai, K., Domon, H., Maekawa, T., Hiyoshi, T., Tamura, H., Yonezawa, D., Habuka, R., Saitoh, A., Terao, Y (2019). Immunization with pneumococcal elongation factor Tu enhances serotype-independent protection against Streptococcus pneumoniae infection. Vaccine 37, 160-168. https://doi.org/10.1016/j.vaccine.2018.11.015

Obaji Monday Uchenna, Oli Angus Nnamdi, Ugwu Malachy Chukwudi (2023). Prompt clinical identification and characterization of Streptococcus pneumoniae. World Journal of Pharmaceutical Research. 21 (12) 1-14. https://doi.org/10.20959/wjpr202321-30349

Ojiako, C.M., Okoye, E.I., Oli, A.N., Ike, C.J., Esimone, C.O., Attama, A.A (2019). Preliminary studies on formulation of immune stimulating complexes using saponin from Carica papaya leaves. Heliyon, 5, 2405-8440. https://doi.org/10.1016/j.heliyon.2019.e01962

Oli, A.N., Emeruwa, A.P., Ekwunife, O.I., Okoye, E.L., Anyaoku, C.S., Ibeanu, G.C., Obi, E., Ihekwereme, C.P., Obaji, M (2019). Pleurotus ostreatus extract enhances the phagocytic actions of neutrophils against Streptococcus pneumoniae. Ann Med Health Sci Res.9: 499- 508.

Oloruntola, A., Omotosho, O (2019). Proximate Analysis, Phytochemical Screening and Mineral Content of Pleurotus pulmonarius (Oyster Mushroom) (P20-019-19). Curr Dev Nutr. Jun 13;3(Suppl 1):nzz040.P20-019-19. https://doi.org/10.1093/cdn/nzz040.p20-019-19

Ong, G.H., Lian, B.S., Kawasaki, T., Kawai, T (2021). Exploration of pattern recognition receptor agonists as candidate adjuvants. Front.Cell.Infect. Microbiol 968:745016. https://doi.org/10.3389/fcimb.2021.745016

Paz, L.N.F., Moura, L.M., Feio, D.C.A., Cardoso, M.S.G., Ximenes, W.L.O., Montenegro, R.C., Alves, A.P.N., Burbano, R.R., Lima, P.D.L (2017). Evaluation of in vivo and in vitro toxicological and genotoxic potential of aluminum chloride. Chemosphere, 175, 130-137. https://doi.org/10.1016/j.chemosphere.2017.11.044

Pichichero, M.E (2017). Pneumococcal whole-cell and protein-based vaccines: Changing the paradigm. Expert Rev. Vaccines 16, 1181-1190. https://doi.org/10.1080/14760584.2017.1393335

Prato, R., Fortunato, F., Cappelli, M. G., Chironna, M., Martinelli, D (2018). Effectiveness of the 13-valent pneumococcal conjugate vaccine against adult pneumonia in Italy: A case-control study in a 2-year prospective cohort. BMJ Open 8, e019034. https://doi.org/10.1136/bmjopen-2017-019034

Principi, N., Esposito, S (2018). Development of pneumococcal vaccines over the last 10 years. Expert. Opin. Biol. Ther. 18, 7-17.

Pulendran, B.S., Arunachalam, P., O'Hagan, D.T (2021). Emerging concepts in the science of vaccine adjuvants. Nat Rev Drug Discovery 20(6):454-475. https://doi.org/10.1038/s41573-021-00163-y

Rapaka, R.R., Cross, A.S., McArthur, M.A (2021). Using adjuvants to drive T cell responses for next-generation infectious disease vaccines. Vaccines (2021) 9(8):820. https://doi.org/10.3390/vaccines9080820

Reinke, S., Thakur, A., Gartlan, C., Bezbradica, J.S., Milicic, A (2020). Inflammasome-Mediated Immunogenicity of Clinical and Experimental Vaccine Adjuvants. Vaccines. 8, 554. https://doi.org/10.3390/vaccines8030554

Rodrigues, T.C., Oliveira, M.L.S., Soares-Schanoski, A., Chavez-Rico, S.L., Figueiredo, D.B., Gonçalves, V.M., Ferreira, D.M., Kunda, N.K., Saleem, I.Y., Miyaji, E.N (2018). Mucosal immunization with PspA (Pneumococcal surface protein A)-adsorbed nanoparticles targeting the lungs for protection against pneumococcal infection. PLoS ONE 13, e0191692. https://doi.org/10.1371/journal.pone.0191692

Rosales-Mendoza, S., Salazar-Gonzalez, J. A (2014). Immunological aspects of using plant cells as delivery vehicles for oral vaccines. Expert. Rev. Vaccines 13:737-749. https://doi.org/10.1586/14760584.2014.913483

Shah, R.R., Hassett, K.J., Brito, L.A (2017). Overview of Vaccine Adjuvants: Introduction, History, and Current Status. Methods Mol. Biol. 1494, 1-13. https://doi.org/10.1007/978-1-4939-6445-1_1

Shakya A.K, Chowdhury M.Y, Tao W, Gill H.S (2016). Mucosal vaccine delivery: current state and a pediatric perspective. J Control Release 240:394-413.

Shi, S., Zhu, H., Xia, X., Liang, Z., Ma, X., Sun, B (2019). Vaccine adjuvants: Understanding the structure and mechanism of adjuvanticity. Vaccine. 37: 3167-3178. https://doi.org/10.1016/j.vaccine.2019.04.055

Soema, P.C., Kompier, R., Amorij, J.P., Kersten, G.F (2015). Current and Next Generation Influenza Vaccines: Formulation and Production Strategies. Eur. J. Pharm. Biopharm. 94: 251. https://doi.org/10.1016/j.ejpb.2015.05.023

Su F, Patel G.B, Hu S, Chen W (2016). Induction of mucosal immunity through systemic immunization: phantom or reality? Hum Vaccines Immunotherap. 12:1070-1079. https://doi.org/10.1080/21645515.2015.1114195

Tada, R., Suzuki, H., Takahashi, S., Negishi, Y., Kiyono, H., Kunisawa, J., Aramaki, Y

(2018). Nasal vaccination with pneumococcal surface protein A in combination with cationic liposomes consisting of DOTAP and DC-chol confers antigen-mediated protective immunity against Streptococcus pneumoniae infections in mice. Int. Immunopharmacol. 61:385-393. https://doi.org/10.1016/j.intimp.2018.06.027

Terra, V.S., Plumptre, C.D., Wall, E.C., Brown, J.S., Wren, B.W (2020). Construction of a pneumolysin deficient mutant in Streptococcus pneumoniae serotype 1 strain 519/43 and phenotypic characterisation. Microb. Pathog. 141, 103999. https://doi.org/10.1016/j.micpath.2020.103999

Tessa, M., Kalliope, K., Papadopoulou, Anne, O (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. Crit Rev Biochem Mol Biol. 49(6):439-462. https://doi.org/10.3109/10409238.2014.953628

Tin Tin Htar, M., Stuurman, A. L., Ferreira, G., Alicino, C., Bollaerts, K., Paganino, C., Reinert, R. R., Schmitt, H. J., Trucchi, C., Vestraeten, T (2017). Effectiveness of pneumococcal vaccines in preventing pneumonia in adults, a systematic review and meta-analyses of observational studies. PLoS ONE 12, e0177985. https://doi.org/10.1371/journal.pone.0177985

Verhoeven, D., Perry, S., Pichichero, M.E (2014). Contributions to protection from Streptococcus pneumoniae infection using the monovalent recombinant protein vaccine candidates PcpA, PhtD, and PlyD1 in an infant murine model during challenge. Clin. Vaccine Immunol. 21:1037-1045. https://doi.org/10.1128/cvi.00052-14

Verma S.K, Mahajan P, Singh N.K, Gupta A, Aggarwal R, Rappuoli R, Johri A.K (2023). New-age vaccine adjuvants, their development, and future perspective. Front Immunol. 14: 1043109. https://doi.org/10.3389/fimmu.2023.1043109

Villarini, M., Gambelunghe, A., Giustarini, D., Ambrosini, M.V., Fatigoni, C., Rossi, R., Dominici, L., Levorato, S., Muzi, G., Piobbico, D (2017). No evidence of DNA damage by co-exposure to extremely low frequency magnetic fields and aluminum on neuroblastoma cell lines. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 823:11-21. https://doi.org/10.1016/j.mrgentox.2017.09.001

Wagner-Muniz, D.A., Haughney, S.L., Kelly, S.M., Wannemuehler, M.J., Narasimhan, B (2018). Room temperature stable PspA-based nanovaccine induces protective immunity. Front. Immunol. 9, 325. https://doi.org/10.3389/fimmu.2018.00325

Wong, Y.T., Smardon, C., Shirkhani, K., Amacker, M., Fleury, S., Stegmann, A.J.H (2019). Oral Dispersible Vaccine Comprising Virosomes. International Application No. PCT/EP2019/082940. Available online: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020109485 (accessed on 18 March 2022).

World Health Organization (2016). SAGE working group on pneumococcal conjugate vaccines. Availableonline:https://www.who.int/immunization/policy/sage/sage_wg_pcv_dec2016/n/ (accessed on 18 December 2019).

Xu, Q., Casey, J.R., Pichichero, M.E (2015). Higher levels of mucosal antibody to pneumococcal vaccine candidate proteins are associated with reduced acute otitis media caused by Streptococcus pneumoniae in young children. Mucosal Immunol. 8:1110-1117. https://doi.org/10.1038/mi.2015.1

Yang, J.X., Tseng, J.C., Yu, G.Y., Luo, Y., Huang, C.Y., Hong, Y.R., Chuang, T.H (2022). Recent advances in the development of toll-like receptor agonist-based vaccine adjuvants for infectiousdiseases. Pharmaceutics. 14(2):423. https://doi.org/10.3390/pharmaceutics14020423

Yildirim, I., Kimberly, M., Stephen, I.P (2015). Pneumococcal Disease in the Era of Pneumococcal Conjugate Vaccine. Infect. Dis. Clin. North. Am. 29(4): 679-697. https://doi.org/10.1016/j.idc.2015.07.009