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Vaccination against Pathogenic Bacteria: An Insight into Polysaccharide and Conjugate Vaccines

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ABSTRACT

Background: The cellular glycocalyx, a dense and complex coating of glycans, surrounds the surface of cells and serves as a critical interface between the cell and its external environment. Within this glycocalyx, the intricate glycans play essential roles in a variety of biological processes, including mediating cell-cell interactions, facilitating bacterial pathogenicity, and providing protection against environmental stressors such as desiccation, immune responses, and antimicrobial agents. The polysaccharides found on the outer surface of bacterial cells are particularly noteworthy due to their high degree of conservation across species and their easy accessibility. These characteristics make them excellent targets for immunological purposes, as they can be readily recognized by the immune system. As a result, bacterial polysaccharides and their repetitive units have been extensively studied and utilized as antigens in the development of vaccines with antibacterial properties. These vaccines leverage the unique structural features of polysaccharides to elicit robust and specific immune responses, offering a promising strategy for combating bacterial infections and enhancing public health.

Conclusion: In conclusion, it is important to emphasize that the topic explored in this article is vast, and the research field has experienced rapid growth in recent decades with ongoing advancements. Given the breadth of this field, it is challenging to cover the entire spectrum of polysaccharide-based bacterial vaccines targeting all bacterial pathogens. Additionally, due to inherent limitations, it was not feasible to include all research on polysaccharide and glycoconjugate vaccine development for a comprehensive set of bacterial pathogens. Therefore, only a subset of common bacteria and related vaccine development efforts have been evaluated in this discussion. Despite these limitations, the progress made in this area underscores the potential of polysaccharide-based vaccines as a powerful tool in the fight against bacterial infections.

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Introduction

Pathogenic organisms, such as bacteria, pose the greatest peril to human existence. Currently, there exist two principal methodologies for addressing infections. One strategy consists of the provision of antimicrobial or antiviral and antiparasitic compositions to individuals. The alternative solution involves safeguarding our crowd from infectious agents through the implementation of vaccination. Among these two tactics, vaccination has proven to be effective, cost-efficient, and endring, and it has contributed to the containment and even eradication of numerous infectious ailments (1, 2). The vaccination strategy has become increasingly appealing due to the swift development of bacterial resistance to numerous antibiotics in recent decades. The inception of antibacterial vaccines based on carbohydrates was initially provided by Avery and Heidelberger in the 1920s. These scholars discovered the immunogenicity of pneumococcal capsular polysaccharides and their crucial feature in the serotype-specificity of diverse strains (3, 4). However, the achievements in antibacterial vaccine development, including those based on carbohydrates, were halted in the 1950s at the same time as the remarkable triumph of antibiotics. Nevertheless, in recent decades, the advancement of antibacterial vaccines has surged owing to the rise in bacterial resistance to antibiotics. On the whole, carbohydrate-based antibacterial vaccines have made substantial contributions to the enhancement of human health and well-being. Regrettably, polysaccharide vaccines primarily exhibit efficacy in adult populations, while they demonstrate negligible effectiveness in infants and under-five-year-old children, who are at high risk. The primary reason for this ineffectiveness lies in the inability of polysaccharides alone to induce potent T-cell-dependent immune responses in these individuals (5). Carbohydrate-based conjugate vaccines (known as glycoconjugate vaccines) have emerged as a solution for this issue. The underlying concept that underpins these vaccines involves the formation of a covalent bond between a carrier molecule, which is typically a protein with high immunogenicity, and carbohydrate antigens. This bonding process serves to augment the immunogenicity of the antigens and elicit immune responses that are dependent on T cells (6, 7). These vaccines have proven to be effective in both adults and children, and they are widely utilized. However, notwithstanding the notable progressions in the enhancement of conjugate immunizations extracted from bacterial polysaccharides, these immunizations still exhibit particular restrictions. The primary challenge lies in the complexity of the polysaccharides produced by bacteria, which makes it difficult to ensure quality control and efficient combination with carrier proteins (8). Currently, significant efforts are being made to synthesize oligosaccharides comprising the repeating units of polysaccharides in bacteria and to utilize them for developing antibacterial conjugate vaccines, which are oligosaccharide-based (9). These conjugate vaccines, which can be synthetic or structurally known as carbohydrate antigens, offer numerous benefits, including controlled conjugation chemistry and product quality, reduced contamination, the ability to conduct structure-activity relationship studies, and optimization of vaccines (10, 11).

Carbohydrate-based vaccines

The general structure of carbohydrate-based vaccines

The principal carbohydrate-based vaccines that have licenses are pneumococcal, meningococcal, *Haemophilus influenzae* type b (Hib), and *Salmonella typhi* (*S. typhi*). In this category, carbohydrate antigens are separated from microbial cultures and then conjugated to the proteins of the carrier. Unlike their remarkable effectiveness versus the respective pathogens, lots of important issues exist in vaccine development, containing complex extraction methods,

heterogeneous mixtures, the attendance of cellular parts as impurities, and uncontrolled and irreproducible protein conjugation chemistry (12). To deal with these problems, vaccines can be produced pure and homogeneous through chemical synthesis, and these alternative vaccine designs can be even safer and more effective.

The general structure of conjugate vaccines

Glycoconjugate vaccines typically consist of three parts: an antigen, which is a carbohydrate, a carrier molecule, and a linker. The first step in the vaccine production process is identifying the desired antigen that is expected to be antigenic, protective, and present on the cell surface to help immune recognition. The next stage involves selecting an appropriate carrier molecule that must be immunostimulatory so the carbohydrate antigen will be more immunogenic. In the end, for the carbohydrate antigen and the carrier molecule, which should be controlled by conjugation chemistry and relevant reaction qualification, a linker is recognized (13).

Advancements in carbohydrate chemistry have made complex oligosaccharides to synthesize on a large scale. Quimi-Hib was the first vaccine developed for Hib; it was developed in Cuba using a synthetic antigen conjugated to a toxoid through a spacer. Compared to isolated biological vaccines, synthetic vaccines offer advantages such as an antigenic structure with a spacer arm, homogeneity, high reproducibility, greater purity, and better safety profiles (13, 14).

The third kind of glycoconjugate vaccine contains synthetic carbohydrate antigens and transports with synthetic peptides. The majority part of vaccines produced for cancers and viruses fall into this category (15). Nevertheless, no entirely synthetic vaccine has been commercially produced yet. Even the promising candidates couldn’t pass the preclinical stage.

Bacterial carbohydrate antigens

Bacteria express isolated polysaccharides, including polysaccharide capsules, lipopolysaccharides (LPS), and extracellular polysaccharides. Polysaccharide capsules and LPS are protected and located on the surface of bacterial cells, making them valuable immunological goals for vaccine development. Nonetheless, bacterial polysaccharides are particular to species and even to strains, and it must be mentioned that a vaccine isolated from a particular bacterial polysaccharide is only applicable to a particular bacterium and strain. To make a useful vaccine to deal with infectious diseases, multivalent features are required (4).

Nevertheless, polysaccharides of bacteria could be influential for vaccine construction; they shouldn’t be optimal immunogens necessarily. Large polysaccharides could be effective in the interaction of carrier molecules in the resulting glycoconjugates with immune cells. Furthermore, while bacterial polysaccharides are large and complex, they are generally formed of small repeating units of monosaccharides or oligosaccharides. The immune system may only recognize certain unique epitopes of a polysaccharide, often the non-reducing terminal epitope or epitopes containing one to several repeating units instead of the entire glycan (16).

Thus, the length of glycans is always a concern in designing carbohydrate-based vaccines, and it is not necessarily true that "the longer the glycans, the better." Additionally, there seems to be no golden standard regarding the optimal glycan length as an antigen; the best oligosaccharide alternative for any specific polysaccharide antigen may need to be evaluated individually (12). Other factors, such as structural uniqueness, conformational changes, and the stability of carbohydrate epitopes that may affect immunogenicity and antigenicity, should also be considered in the design and development of carbohydrate-based antibacterial vaccines (11, 17).

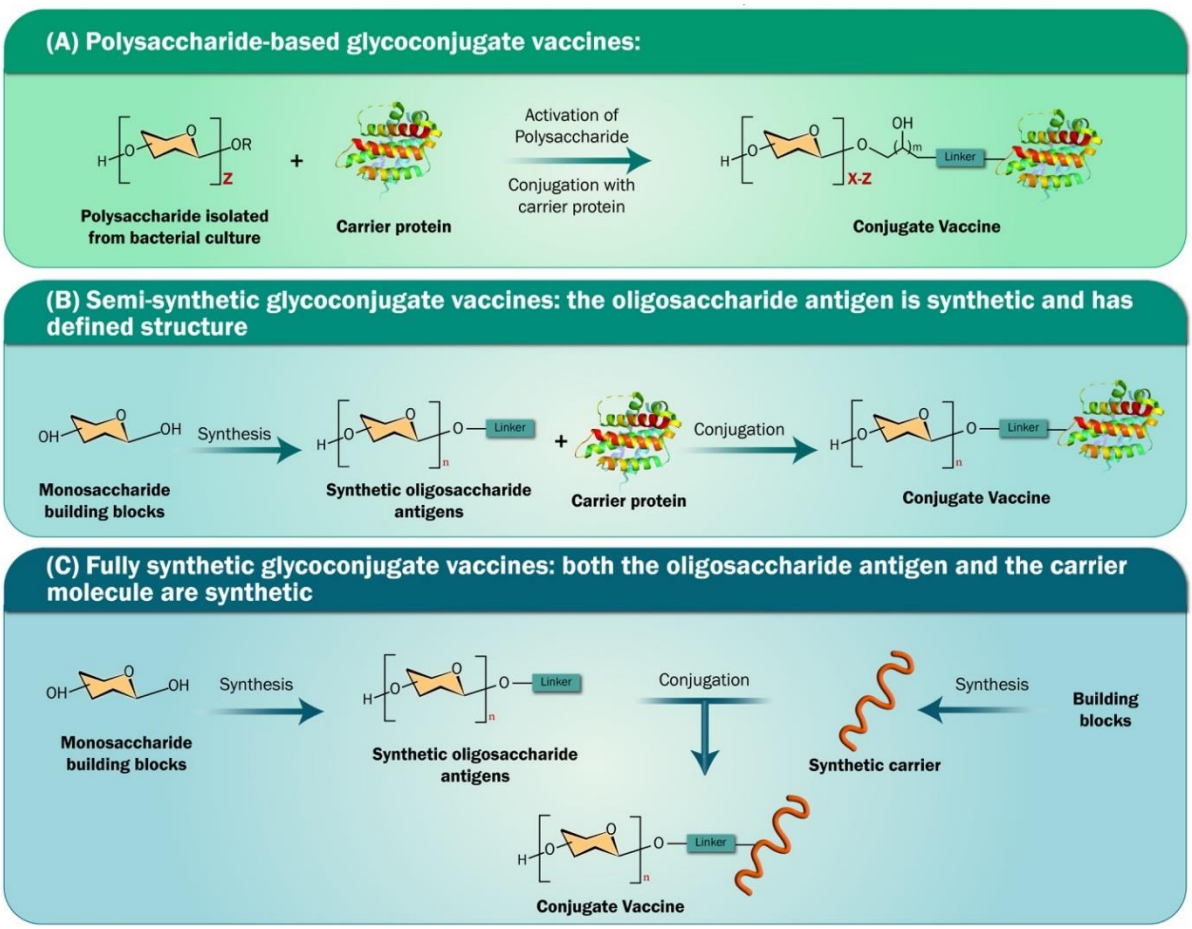

Fig 1. Three main categories of vaccines, which are glycoconjugate. (a) Polysaccharide-based glycoconjugate vaccines. (b) Semi-synthetic glycoconjugate vaccines containing synthetic oligosaccharide antigens and a well-defined structure. (c) Entirely synthetic glycoconjugate vaccines are composed of synthetic oligosaccharide antigens and synthetic carrier molecules.

Carrier molecules

In glycoconjugate vaccines, carrier molecules have a crucial figure in stimulating the immune system and increasing immune reactions, particularly T-cell-dependent reactions, to targeted carbohydrate antigens. Therefore, the carrier molecule must include T-cell helper epitopes and be immunologically active. Other essential characteristics of a vaccine carrier include safety, low-cost production, and consistent quality.

Among different vaccine carriers, proteins are the most widely utilized for developing antibacterial vaccines. Proteins possess numerous functional groups, such as amino, thiol, carboxylic, and hydroxyl groups, enabling different conjugation techniques. Currently, several common carrier proteins are utilized in licensed bacterial vaccines, including mutant cross-reacting combinations (CRM197) from diphtheria toxin, diphtheria toxoid (DT), tetanus toxoid (TT), meningococcal outer membrane protein complex (OMPC), and *H. influenzae* protein D (HiD). In addition to these established carriers, many other carrier proteins are undergoing preclinical research or clinical trials (15, 18).

Table 1. Some carrier proteins are utilized in various vaccines to target different bacteria (1).

Microorganism	Carrier protein
C. difficile	TcdA_B2
	TcdB_GT (CRM197-PSII)
E. coli	K 100-HCH
	K100-TT
GBS	GBS III PS/rCTB
	GBS type V/CRM197
	GBS80
Hib	Hib-CRM197
	Hib-N6
	Hib- N10
	Hib- N19
	Hib- HCH
	Hib-CT
	Hib-TT
	Hib-ADH
K. pneumoniae	11 octasaccharides/KLH
	11 octasaccharides/BSA
N. meningitidis	Hib- N19
	CRM197
	spr 96/2021
	spr1875
	Upec-5211
	Orf3526
Salmonella	COPS/CRM197/*S. Enteritidis*
	PADRE/O-SP *S. typhimurium*
Shigella	O-SP/rEPA *C. diphtheriae*
	CRM9/ADH
	S. flexneri 2a O-SP/rEPAsucc
S. aureus	*S. aureus* type 8 capsular polysaccharide/DT with SPDP linker
S. pneumoniae	PS/TT conjugates
	PnPS 14 conjugated/BSA
	PnPS-TT
	PnPS 6A-TT

It is important to note that carrier proteins can also induce particular antibody reactions that can repress the immune reaction to the conjugated carbohydrate antigens (19). Repeated immunizations with vaccines including the same carrier protein may decrease vaccination effectiveness. Therefore, research aimed at discovering new carrier proteins or non-protein carrier molecules is essential.

Linkers in glycoconjugate vaccines

Carbohydrate antigens should be covalently linked to carrier molecules to exhibit high immunogenicity. The selection of an appropriate linker between carbohydrate antigens and carrier proteins is dependent on lots of factors. At first, the linker must be non-immunogenic. Thus, it does not induce unwanted antibodies or immune responses. It has also been shown that strong immune answers to the linker may suppress immune responses to the carbohydrate antigen (21-23). Next, the linker should possess two or more functional groups since it needs to connect two distinct biomolecules: the carbohydrate antigen and the carrier protein. Additionally, conjugation responses could be highly useful and selective, as this may be helpful to decrease complexity and heterogeneity, leading to the creation of a desirable and stable glycoconjugate antigen.

The loading of antigens in glycoconjugate vaccines can significantly impact their immunological properties (24). Many linkers and corresponding conjugation methods have been developed for producing glycoprotein conjugates from glycans. The choice of a suitable linker and conjugation technique for the vaccine is very determined by the structure of the carbohydrates and sometimes by the carrier protein, too. For polysaccharide antigens, their activation methods and resulting functional groups dominate the conjugation approach because the derivatized sugar units in polysaccharide antigens typically act directly as linkers (25).

For synthetic oligosaccharide antigens, introducing different functional groups during the synthetic course is flexible to facilitate particular attachment forms and conjugation techniques. Generally, amino and thiol groups in carrier proteins can often be utilized for attaching carbohydrate antigens. Activated acyl groups, aldehydes, or alkenes are also introduced to carbohydrate antigens to enable their coupling with proteins through N-acylation, chemoselective

reduction, etc. Conjugation methods such as click reactions have also been reported (26).

In summary, selecting suitable carbohydrate antigens is crucial in developing antibacterial glycoconjugate vaccines. For polysaccharide antigens, their activation methods to facilitate conjugation with carrier proteins are also serious because they determine not only the characteristics (e.g., structural integrity and molecular size) of the conjugated carbohydrate antigens but even the linker shape and bond, conjugation chemistry, antigen loading, chemical stability, product quality, and biological actuality compatibility. Consequently, the properties of both the linker and carrier protein significantly influence the immunological properties of the resulting glycoconjugate vaccines. The mentioned factors could be considered and optimized when a recent glycoconjugate vaccine is going to be designed and developed.

Immune response to carbohydrate-based vaccines

Bacterial polysaccharides are promising goals for the development of glycoconjugate vaccines against infectious diseases (21). A major issue with polysaccharide vaccines, or carbohydrate antigens in general, is their low immunogenicity, which results in only weak antibody responses, particularly in children. This limitation primarily arises from their incompetence to be appropriately presented to T cells. Polysaccharide vaccines mostly interact with B cells to arouse rapid and desired innate immune reactions and produce non-specific IgM antibodies that are T cell-independent and have little affinity for carbohydrate antigens (11, 27). T cell-independent responses are weaker and shorter-lived compared to acquired immune responses and even repeated vaccinations do not generate sufficiently strong immune responses to combat pathogens effectively (28).

Furthermore, the covalent linkage of carbohydrates to immunogenic proteins containing T cell epitopes may change them from T cell-independent antigens to T cell-dependent antigens, thereby inducing acquired immune reactions that involve both B and T cells. Acquired immunity is robust and durable but takes longer to develop compared to innate immunity. In acquired immunity, antigen-presenting cells (APCs), such as dendritic cells, play a crucial role. APCs present glycoprotein antigen epitopes to T cells, facilitating cross-linking of T cell immunogens with B cell receptors, thus initiating signaling processes for acquired immune responses. Stimulating signals from dendritic cells and macrophages help activate and mature B cells into plasma cells that secrete particular antibodies.

Other B cells are produced independently, with marginal zone B cells and non-circulating mature B cells being isolated in the spleen and other lymphoid tissues. In particular, glycoconjugate vaccines are internalized by APCs and processed in endosomes, where the resulting carbohydrate epitopes are presented alongside peptides generated from protein digestion to T cells. These epitopes bind to carbohydrate epitopes recognized by T cell receptors on CD4+ T cells, with secreted signaling cytokines aiding in the maturation of B cells and the production of high-affinity IgG antibodies specific to carbohydrates, as well as the generation of memory B cells (29).

Various methodologies are available for estimating the extent of the immune reaction triggered by vaccines, encompassing the assessment of innate, humoral, cellular, and cytokine reactions. Nevertheless, the approach most frequently employed involves measuring the level of antibodies. Several factors, such as age, gender, genetics, geographical location, and other environmental and behavioral factors, can potentially influence a vaccine's capacity to provoke an antibody response. Moreover, the type of vaccine employed can also exert an impact on the vaccine response (30).

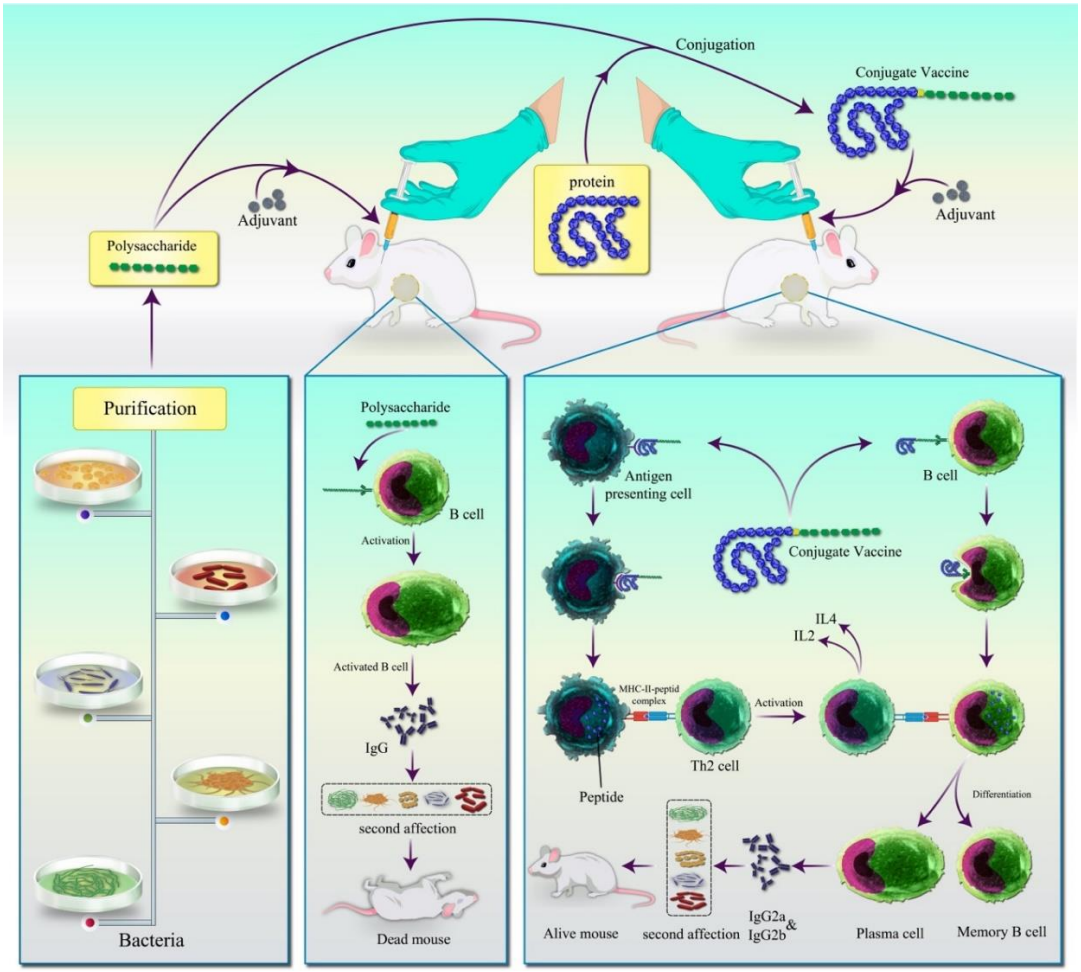

Fig 2. The immune mechanism of the polysaccharide vaccine and the diversity from conjugate vaccine. Polysaccharide vaccines induce the activation of marginal zone B-cells, which will undergo rapid cell division at the site of infection and subsequently develop into short-lived plasma cells. The conjugate vaccine is internalized by the antigen-presenting cells, leading to the digestion of the conjugate molecules and the acquisition of glycan peptides. These glycan-peptides subsequently bind to MHC-II and are presented to cells for recognition, so triggering the secretion of cytokines IL-4 and IL-2. Consequently, this process facilitates the maturation of homologous B cells into memory B cells and the production of IgG antibodies.

Polysaccharide-protein conjugate vaccines demonstrate enhanced immunogenicity, as they possess the ability to induce durable protection. Conversely, polysaccharide vaccines solely elicit transient antibody responses that are independent of T-cell activation. The preservation of these vaccines has proven to be a formidable undertaking due to a multitude of factors, including the lack of suitable animal models, limited understanding of the underlying conservation mechanism, or inadequate commercial interest (31)

Principle of conjugate vaccines

Considerable endeavors have been channeled towards the advancement of glycoconjugate vaccines with antibacterial properties, employing

naturally occurring polysaccharides as antigens. This is predominantly due to the inherent benefits conferred by conjugate vaccines in comparison to their polysaccharide counterparts. In the following, some licensed polysaccharide and glycoconjugate vaccines and promising synthetic vaccine candidates that are at the moment undergoing clinical trials will be discussed.

Streptococcus pneumoniae

Infections caused by *Streptococcus pneumoniae* (*S. pneumoniae*) pose a significant global challenge. The mentioned gram-positive bacteria primarily lead to aggressive diseases such as pneumonia, sepsis, meningitis, and otitis media in infants. *S. pneumoniae* is classified into 97 serotypes based on the structure of its capsular polysaccharides. Among these, 20 high-risk serotypes account for over 90% of pneumococcal infections.

To decrease the global disease burden, pneumococcal vaccination is essential, particularly in developing countries (32).

The B Group of *Streptococcus*

From the recent 1970s, the B Group of *Streptococcus* (GBS), a gram-positive bacterium, has been a main concern for bacterial infections in newborns and pregnant women. This disease leads to lots of severe conditions including sepsis, meningitis, pneumonia, and miscarriage, with *Streptococcus agalactiae* being one of the strains primarily responsible for these complications (34). Globally, about 18% of pregnant women are colonized by GBS, significantly increasing the risk of infection in both pregnant women and newborns (35).

Table 2. Polysaccharide-based vaccines targeting different serotypes of *S. pneumoniae*

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Native capsular polysaccharides of pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F	CRM_{197}	Licensed	Prevnar 20®, Pfizer	Adults (18 years and older)	(1)
Native capsular polysaccharides of pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	CRM_{197}	Licensed	Prevnar 13®, Wyeth Pharmaceuticals	Children (6 weeks to 17 years), Adults (18 years and older)	(1)
Native capsular polysaccharides of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, 23F	CRM_{197}	Licensed	Prevnar®, Wyeth/Pfizer	Children (over 9 years)	(1)
Native capsular polysaccharides of pneumococcal serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	Protein D, TT, DT	Licensed	Synflorix®, GSK	Children (over 5 years)	(1)
CP from 23 serotypes of *S. pneumoniae*	-	Licensed			(2, 3)

Native capsular polysaccharides of pneumococcal serotypes including:1-2-3-4-5-6B-7F-8-9N-9V-10A-11A-12F -14 -15B -17F -18C -19F -19A -20 -22F -23F -33F	-	Licensed	PNEUMOVAX23, Merck & Co.	Children (over 2 years), Adults (over 50 years at risk for pneumococcal disease)	(2)
Capsule-multivalent	rEPA	Phase I	Limmatech Biologics	May be aimed at various age groups	(3)
Native capsular polysaccharides of pneumococcal serotypes including:1-2-3-4-5-6A-6B-7F-8-9N-9V-10A-11A-12F-14-15B-177-18C-19A-19F-20B-22F-23F-33F	Rhizavidin	Phase II	Affinia	Children	(1)
Synthetic oligosaccharides	TT	Phase II	Alopex	Children	(1)
Native capsular polysaccharides of pneumococcal serotypes including:1-5-14-6B-18C-19F-23F	TT	Phase III	QuimiVio, CIGB	Children	(1)
Capsule-serotype 4	piuA	Preclinical	Academic-UCL/LSHTM UK	Children under 5 years old	(3)
Oligosaccharides (capsular polysaccharide) of pneumococcal serotypes:2,3,5,8,14	CRM_{197}	Preclinical	-	-	(2)
Tetrasaccharide of pneumococcal serotype 1	CRM_{197}	Preclinical	-	-	(2)
Hexasaccharide of pneumococcal serotype 2	CRM_{197}	Preclinical	-	-	(2)
Tetrasaccharide of pneumococcal serotype 3	CRM_{197}	Preclinical	-	-	(2)
Tetrasaccharide of pneumococcal serotype 3	BSA	Preclinical	-	-	(2)
Hexasaccharide of pneumococcal serotype 3	CRM_{197}	Preclinical	-	-	(2)
Hexasaccharide of pneumococcal serotype 3	TT	Preclinical	-	-	(2)
Tetrasaccharide of pneumococcal serotype 3 and 14	Bacteriophage Qβ	Preclinical	-	-	(2)
Tetrasaccharide (capsular polysaccharide) of pneumococcal serotype 4	CRM_{197}	Preclinical	-	-	(2)
Oligosaccharide (capsular polysaccharide) of pneumococcal serotype 5	CRM_{197}	Preclinical	-	-	(2)
Tetrasaccharide (capsular polysaccharide) of pneumococcal serotype 8	CRM_{197}	Preclinical	-	-	(2)

Hexasaccharide (capsular polysaccharide) of pneumococcal serotype 14	BSA	Preclinical	-	-	(2)
Tetrasaccharide (capsular polysaccharide) of pneumococcal serotype 14	Pneumococcus surface adhesin A	Preclinical	-	-	(2)
Repeated unit (capsular polysaccharide) linked with aliphatic spacer of pneumococcus serotype 14	CRM_{197}	Preclinical	-	-	(2)
Chimeric antigen (capsular polysaccharide) consisting of a repeated unit of pneumococcus serotypes¬19A and¬19F	CRM_{197}	Preclinical	-	-	(2)
PS14, PS23F	Hc	Preclinical	-	-	(4)

CRM197: Non-toxic diphtheria toxin mutant; TT: Tetanus toxoid; DT: Diphtheria toxoid; Protein D: conserved surface protein from non-typed *Haemophilus influenzae*; BSA: Bovine serum albumin; HC: tetanus toxin native C-fragment

Table 3. Vaccines against GBS.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Capsular polysaccharides of serotypes Ia, Ib, II, III, IV, V	CRM_{197}	Phase II	Pfizer	Children	(1, 2)
Types Ia, Ib, II, III, and V CPS	TT	Phase II	-	-	(5)
Types VI and VIII	Alpha C protein	Preclinical	-	-	(6)

Preventive measures tried to reduce the risk of invasive GBS disease in newborns have focused on the prophylactic administration of antibiotics during labor. However, while prophylactic antibiotic treatment during labor is effective for early-onset GBS disease, it cannot prevent late-onset GBS disease. As a result, effective vaccines against GBS are very efficient (36).

The A Group of *Streptococcus*

The A Group of *Streptococcus* (GAS) known as a gram-positive bacterium that leads to a vast range of clinical syndromes and severe infections, including pharyngitis, cellulitis, pyoderma, necrotizing fasciitis, sepsis, pneumonia, and streptococcal toxic shock syndrome. More than half a million human pass out each year due to infections caused by GAS (38). The economic burden of these infections is significant in both developed and developing countries. GAS infections are typically treated with antibiotics; however, drug resistance is becoming a serious problem. Lots of virulent components of GAS have been identified as potential targets for the development of vaccines against it (39).

Staphylococcus aureus

Staphylococcus aureus (*S. aureus*), a gram-positive opportunistic bacterium, which caused a wide range of diseases, including osteomyelitis, sepsis, abscesses, endocarditis, toxic shock syndrome, bacteremia, pneumonia, and infections

related to intravenous devices, skin, and prosthetics in humans . It has a high prevalence in both community and hospital settings. In recent decades, hospital-acquired *Staphylococcus* infections have significantly increased (40-60%) (40).

Since the mid-1940s, the incidence of penicillin-resistant *S. aureus* infections in hospitals has risen, marking the first emergence of antibiotic resistance by this bacterium and the establishment of community-acquired infections. The prevalence of methicillin-resistant strains (MRSA), as well as vancomycin-resistant and clindamycin-resistant strains, has complicated infection control efforts. Vancomycin is the recommended treatment for serious infections caused by MRSA; however, reduced sensitivity, side effects, and high rates of clinical failure limit its effectiveness. Linezolid and daptomycin are also effective treatments but are associated with drug toxicity issues (41).

Post-surgical *S. aureus* infections have a high mortality rate, and survivors often need an additional 13 to 17 days in hospital, significantly growing healthcare costs. Consequently, there is an urgent need for vaccines targeting this pathogen to help reduce associated hospital care costs (42).

Staphylococcus epidermidis

The *Staphylococcus* genus comprises more than 40 species and 20 subspecies, and it is categorized into two primary groups: coagulase-positive and coagulase-negative staphylococci. *S. aureus*, a member of the coagulase-positive group, and *Staphylococcus epidermidis* (*S. epidermidis*), a member of the coagulase-negative group, are the most frequently encountered species in their respective groups (52). *S. epidermidis* is a normal constituent of the skin flora and an opportunistic pathogen. It possesses the capability to induce subacute and chronic infections, particularly among medical devices, hospitalized patients, and individuals with compromised immune systems (53). Because of its substantial mortality rate and significant financial burden on the healthcare system, it is regarded as a global healthcare concern (54).

Haemophilus influenzae type b (Hib)

Hib is a gram-negative bacterium that exists in two distinct forms, namely encapsulated and unencapsulated. It bears responsibility for a vast array of grave ailments, including epiglottitis, pneumonia, sepsis, and meningitis. Before the implementation of Hib immunizations in the United States, Hib stood as the primary causative factor of bacterial meningitis incidents in children under five years old. Currently, a variety of commercially available Hib vaccines, primarily based on carbohydrates, can be found in the market (62).

Neisseria meningitidis

Meningococci, *Neisseria meningitidis* (*N. meningitidis*), a bacterium of the Gram-negative classification, can colonize the upper respiratory path of humans. The presence of this bacterium in carriers may lead to a partial defense against disease, as colonization stimulates the production of antibodies in the mucosal lining. It has been observed that approximately 10-15% of the general population and over 35% of individuals residing in confined communities harbor this bacterium without exhibiting any symptoms. The transmission of meningococci occurs via direct contact with the respiratory secretions of carriers. These bacteria possess the capability to breach the protective mucosal barrier and enter the bloodstream, resulting in invasive diseases such as bacteremia, septicemia, and meningitis. The fatality rate for such cases is estimated to be around 10-15%. It is worth noting that invasive diseases are predominantly caused by meningococci that express the capsule. Vaccination stands as the most cost-effective measure in the management of diseases caused by capsular bacteria (64).

Table 4. Polysaccharide-based vaccines targeting GAS.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups
Branched oligosaccharides (cell wall) containing one, two, and three repeat units from different serotypes of Group A *Streptococcus*	Inactive mutant of C5a peptides and ScpA (ScpA193)	Preclinical	-	-
Oligomannoside fragments from different serotypes of Group A *Streptococcus*	Gold nanoparticles	Preclinical	-	-
Group-specific CPS	TT	Preclinical	-	-

Table 5. Vaccines against *S. aureus*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Synthetic oligosaccharides	TT	Phase II	Alopex	Children	(1)
Capsular polysaccharides types 5 and 8	Recombinant exoprotein A detoxified from *P. aeruginosa*; Diphtheria toxoid	Phase III	-	-	(7)
Capsule-Type 5 and 8	rEPA	Preclinical	GlycoVaxyn	Infants and young children	(3)
Capsular polysaccharides types 5 and 8	Alpha hemolysin, manganese transport system membrane protein, LysM domain protein	Preclinical	-	-	(8)
Capsular polysaccharides types 5 and 8	CRM197, MntC Clumping A	Preclinical	-	-	(9)
dPIA (75%)	TT	Preclinical	-	-	(10)
dPIA	ClfA factor	Preclinical	-	-	(11)
PIA	Recombinant SesC protein	Preclinical	-	-	(12)
dPIA (85%)	DT	Preclinical	-	-	(13)
CP8	ETA	Preclinical	-	-	(14)
dPIA (15%)	DT	Preclinical	-	-	(15)

Hla-MntC SACOL0723: Alpha-hemolysin, manganese transporter system membrane protein, LysM domain protein; dPIA: deacetylated PIA; PIA: polysaccharide Intercellular adhesin; rSesC: recombinant *S. epidermidis* surface exposed rSesC protein; ETA: *P. aeruginosa* exotoxin A

Klebsiella

Klebsiella pneumoniae (*K. pneumoniae*), a gram-negative, encapsulated, rod-shaped bacterium is a part of the intestinal flora in humans and an opportunistic pathogen that can cause hospital-acquired infections. This bacterium is responsible for pneumonia, bloodstream infections, surgical site infections, and urinary tract infections. Such infections typically occur in individuals with severe comorbidities or underlying risk factors. Recently, *K. pneumoniae* has been recognized as the most common pathogen associated with mortality from infectious causes in children below the age of five. Its high rate of asymptomatic colonization is often combined with multidrug resistance (MDR), posing a potential issue for individuals with significant comorbidities or other risk factors for infection (66, 67).

Shigella

In developing countries, shigellosis is known as a prevalent and destructive diarrheal disease, particularly affecting children under the age of five (44).

Shigella is a gram-negative bacterium with 50 serotypes, including four distinct groups: *Shigella dysenteriae* (*S. dysenteriae*) (15 serotypes), *Shigella flexneri* (*S. flexneri*) (15 serotypes), *Shigella boydii* (*S. boydii*) (19 serotypes), and *Shigella sonnei* (*S. sonnei*) (1 serotype). The strains of S. dysenteriae and *S. flexneri* are other infectious and aggressive, whereas *S. sonnei* has lower pathogenicity compared to the others (69). Among these, S. flexneri 2a is considered the most dangerous and is one of the primary causes of shigellosis (70).

Shigella infection leads to the expression of Shiga toxin, which may lead to severe intestinal symptoms with numerous complications in humans. Nevertheless, recently, no vaccine is available against *Shigella* (70).

Escherichia coli

Escherichia coli (*E. coli*) is known as a uropathogen and the main reason of lower urinary tract infections (UTIs). UTIs pose a serious health risk and can lead to life-threatening systemic infections, accounting for approximately 7 million incipient care visits and 1 million emergency room visits each year in the United States. These infections also have significant secondary consequences, including economic burdens, recurrent episodes, and excessive antibiotic use. Women and older adults are particularly susceptible, with recurrent infections being common in these populations.

A secure and useful vaccine could address this widespread health issue and combat emerging antibiotic resistance associated with *E. coli* infections (72).

Burkholderia mallei and *Burkholderia pseudomallei*

Burkholderia pseudomallei (*B. pseudomallei*) and *Burkholderia mallei* (*B. mallei*) are highly dangerous gram-negative bacteria that pose an earnest threat to human and animal life. *B. pseudomallei* and *B. mallei* are found sporadically in tropical and subtropical regions of the world, and if left untreated, the mortality rate for infected patients can reach up to 50%. *B. pseudomallei* causes severe melioidosis, while *B. mallei* leads to glanders, both of which can result in death (73).

Diagnosis of these diseases is challenging because their clinical symptoms can range from skin abscesses to acute pulmonary infections and rapid-onset septicemia. Additionally, both bacteria are assorted as select agents by the Centers for Disease Control and Prevention (CDC) because of their intence infectivity of them through inhalation, light infectious doses, and potential misuse as biothreat agents. While antibiotics are an efficient way to deal with these infections, they are not completely effective because of drug resistance.

Table 6. Polysaccharide-based vaccines targeting *S. epidermidis*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
PIA and Gly-TA	-	Preclinical	-	-	(16)
PIA	rSesC protein	Preclinical	-	-	(17-20)
Capsular polysaccharide (Poly-β-1,6-N-succinylGlcN)	-	Preclinical	-	-	(17)
PIA	-	Preclinical	-	-	(21, 22)

Table 7. Vaccines against Hib.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Native polysaccharides	TT	Licensed	ActHIb®, Sanofi Pasteur	Children (2 months to 5 years)	(1)
Native polysaccharides	TT	Licensed	Hiberix®, GSK	Children (15 months to 4 years)	(1)
Medium-length capsular polysaccharides	OMP	Licensed	PedaxHib®, Merck	Children (2-71 months)	(1)
Capsular oligosaccharides	CRM_{197}	Licensed	HibTiter®, Pfizer	Children (2-71 months)	(1)
Capsular oligosaccharides	CRM_{197}	Licensed	VaxemHib®, Novartis	Children (2 months to 4 years)	(1)
Synthetic oligosaccharides from capsular polysaccharides (average 7 repeating units)	TT	Licensed	Quimi-Hib®, CIGB, Cuba	Children	(23)
Capsular polysaccharides	TT	Licensed	Pentacel, Sanofi Pasteur	Children (6 weeks to 4 years)	(2)
Capsular polysaccharides	Complex outer membrane protein	Licensed	VAXELIS, MCM Vaccine	Children (6 weeks to 4 years)	(2)

OMP: The outer membrane proteins of *Neisseria meningitidis* serotype B

At the moment, there is no approved preventive vaccine for the mentioned diseases. Thus, developing effective countermeasures to deal with these infections, such as vaccines, is of utmost importance (74).

Vibrio cholera

Cholera, known as an acute watery diarrheal infection, originated from the pathogenic gram-negative bacterium *Vibrio cholerae* (*V. cholerae*). Cholera leads to widespread epidemics and is particularly prevalent in sub-Saharan Africa and

Table 8. Polysaccharide-based vaccines targeting Meningococci.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Capsular oligosaccharides of meningococcal serotypes A/C/W/Y	CRM_{197}	Licensed	Menveo®, GSK	Children (from 2 months), adults (over 55 years)	(1)
Capsular polysaccharides of meningococcal serotypes A/C/W/Y	DT	Licensed	Menactra®, Sanofi Pasteur	Children (from 9 months), adults (over 55 years)	(1)
Medium-length capsular polysaccharides of meningococcal serotypes A/C/W/Y	TT	Licensed	Nimenrix®, Pfizer	Children (from 12 months), adults (over 55 years)	(1)
Capsular oligosaccharides of meningococcal serotype C strain C11	CRM_{197}	Licensed	Menjugate®	Children (from 2 months), adults, elderly (over 64 years)	(1)
Capsular oligosaccharides of meningococcal serotype C	CRM_{197}	Licensed	Meningite®, Pfizer	Children (from 6 weeks), adults, elderly (over 64 years)	(1)
De-O-acetylated polysaccharides of meningococcal serotype C strain C11	TT	Licensed	NeisVac-C®, Pfizer	Children (from 2 months), adults, elderly (over 64 years)	(1)
Medium-length capsular polysaccharides (100-200 kDa) of meningococcal serotype A	TT	Licensed	MenAfriVac®, Ser. Ins. India	Children (from 1 year), adults (over 29 years)	(1)
Capsular polysaccharides of meningococcal serotypes A/C/W/Y	-	Licensed	Menomune-A/C/Y/W-135, Sanofi Pasteur	Children (over 2 years)	(2)
Capsular polysaccharide of meningococcal serotype W	TT	Licensed	MenQuadfi, Sanofi Pasteur	Children (over 2 years)	(2)
Synthetic oligosaccharides	TT	Phase 2	Alopex	Children	(1)
Oligosaccharides and glycolipids (capsular polysaccharide) of meningococcal serogroup C	MPL	Preclinical	-	-	(24)
Oligosaccharides (capsular polysaccharide) of meningococcal serogroup A	TT	Preclinical	-	-	(2)
Oligosaccharides of meningococcal serogroup A	CRM_{197}	Preclinical	-	-	(2)
Oligosaccharides of meningococcal serogroup C	TT	Preclinical	-	-	(2)
Oligosaccharides of meningococcal serogroup X	CRM_{197}	Preclinical	-	-	(2)

Oligosaccharides (lipopolysaccharide) of meningococcal serogroup C	DT	Preclinical	-	-	(2)

MPL: Monophosphoryl lipid A

Table 9. Polysaccharide-based vaccines targeting *Klebsiella*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
O antigen oligosaccharides of *Klebsiella* 4V	ETA	Phase 1	LMTB-GSK	Children	(1)
24 valent CPS mix	-	phase I	VAX-24	-	(25)
Synthetic oligosaccharides of *K. pneumoniae*	TT	Phase 2	Alopex	Children	(1)

Table 10. Polysaccharide-based vaccines targeting *Shigella*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Synthetic oligosaccharides of *S. flexneri* 2a, 4V	CRM_{197}	Phase I	LMTB-GSK	Children	(1)
Oligosaccharides (polysaccharide-O-lipopolysaccharide) of *S. flexneri* serotype 2a	TT	Phase I	Pasteur Institute	Children	(2)
Oligosaccharides of *S. flexneri* 2a and 4V	EPA	phase I	Flexyn2a	-	(26)
Capsule-Type 1 of *S. dysenteriae*	rEPA	phase I	Limmatech Biologics	Infections	(3)
Capsule- 2a of *S. flexneri*	rEPA	phase I	Limmatech Biologics	Children	(3)

Table 11. Polysaccharide-based vaccines targeting *E. coli*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
O-antigen-ExPEC serotypes 01, 02, 06, 025	rEPA	Phase Ib	Limmatech Biologics/J&J	Adults and children	(3)

Synthetic oligosaccharides	TT	Phase 2	Alopex	-	(1)
Oligosaccharides of pathogenic *E. coli* extraintestinal serotypes O1A, O6A, O18A, O25B, O2, O4, O8, O15, O16, O75	EPA	Phase 3	Johnson & Johnson	Children	(1)

Table 12. Polysaccharide-based vaccines targeting *B. mallei* and *B. pseudomallei*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Oligosaccharides (capsular polysaccharide) of *B. pseudomallei*	Non-toxic diphtheria toxin domain Hc	Preclinical	-	-	(2)
Disaccharides (polysaccharide-O-lipopolysaccharide) of *B. mallei* and *pseudomallei*	CRM_{197}	Preclinical	-	-	(2)
Oligosaccharides (capsular polysaccharide) of *B. mallei* and *pseudomallei*	CRM_{197}	Preclinical	-	-	(2)
O-PSII of *B. pseudomallei*	AcrA	Preclinical	Government/ Academic- DRDC/ University of Alberta Canada	Adults and children	(3)

Table 13. Polysaccharide-based vaccines targeting *V. cholerae* .

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Oligosaccharides of *V. cholerae* O139	BSA	Preclinical	-	-	(2)
Oligosaccharides of *V. cholerae* O1 serotype Inaba	BSA	Preclinical	-	-	(2)
O139 CPS	Diphtheria toxin mutantCRMH21G	Preclinical			(25)

Table 14. Polysaccharide-based vaccines targeting *Salmonella* .

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
Capsular polysaccharide Vi of *S. typhi*	-	Licensed	Typhim Vi, Sanofi Pasteur	Children (over 2 years)	(2)
Capsular polysaccharide Vi of *S. typhi*	TT	Licensed	Typbar-TCV	Children (6 months), adults (up to 45 years)	(27)
Capsular polysaccharide Vi of *S. typhi*	TT	Licensed	PedaTyph™ Bio-Med	Children (6 months to 12 years)	(28)
Combined Typhoid-Hepatitis A Vaccine	-	Licensed	TYPHIM Vi, Typherix, Hepatyrix, VIVAXIM, Sanofi Pasteur	Children (over 2 years) and adults	(28)
Vi	-	licensed	Vi CPS vaccines	from 2 years old up to adults	(29)
Oligosaccharides (polysaccharide-O-lipopolysaccharide) of *S. enteritidis*	Bacteriophage Qβ	Preclinical	-	-	(2)
Oligosaccharides of *S. paratyphi* A	Bacteriophage Qβ	Preclinical	-	-	(2)
Capsular polysaccharide Vi of *S. typhi*	EPA	Preclinical	-	All age groups	(30)

Table 15. Polysaccharide-based vaccines targeting *Enterococcus*.

Polysaccharide	Carrier Protein	Clinical Stage	Trade Name, Manufacturer	Target Age Groups	References
DHG	-	Preclinical	-	-	(31)
DHG	SagA PpiC	Preclinical	-	-	(32)

DHG: diheteroglycan; PpiC: peptidyl-prolyl cis-trans isomerase; SagA: secreted antigen A

South Asia, posing a significant public health challenge in many places around the world (75). Based on the World Health Organization (WHO), in 2018, 34 countries reported a total of 1,227,391 cholera cases and 5,654 deaths, resulting in a mortality rate of 0.6% (76).

Actual cholera vaccines are oral vaccines, which are either live attenuated or inactivated all cells with or without cholera toxin subunit B (77). Although existing vaccines can help control outbreaks of infection. But there are some limitations, for instance, they may not efficiently

protect young populations and those facing lots of health stresses in many endemic cholera countries (78). Thus, it is essential to work on emerging and efficient vaccines that may provide high and long-lasting immunity.

Salmonella

Typhoid fever is a bacterial disease originating prevalent in lots of low- and middle-income countries. In high-income areas, it is primarily associated with travel from the gram-negative bacterium *Salmonella enterica* (*S. enterica*) serovar Typhi and is to endemic regions, and vaccination rates among travelers are low. Because of bacterial resistance, even antibiotics can't be efficient in managing typhoid fever (79). The high incidence of antibiotic resistance, combined with its limited host range in humans, indicates that improving sanitation and vaccination are the best strategies for control and eradication (80).

Enterococcus

Enterococci are opportunistic bacteria which stand as the second most commonly occurring form of gram-positive pathogens, accounting for the majority of hospital-acquired infections. Certain strains of enterococci are also employed as probiotic agents in certain instances, such as for the management of antibiotic-induced diarrhea, irritable bowel syndrome, and various other gastrointestinal disorders. Some strains of enterococci possess anti-cancer, hypocholesterolemic, and immune-modulating properties. In certain circumstances, the mutualistic association between enterococci and their host can be disrupted, resulting in the onset of severe illnesses. Due to the limited availability of new antimicrobial drugs and the resistance of enterococci to current antibiotic alternatives, both passive and active immunotherapies have been proposed as potential approaches for preventing and treating infections caused by this opportunistic pathogen (85).

Conclusion

Bacterial surface polysaccharides, being conserved and accessible, are ideal vaccine targets. These polysaccharides have been widely used as antigens to develop antibacterial vaccines. However, the vast field is rapidly evolving, making it difficult to cover all bacterial pathogens. This discussion focuses on a subset of common bacteria and related vaccine efforts, highlighting the progress in this area.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zanjan University of Medical Sciences with the ethical code IR.ZUMS.AEC.1402.036.

Conflict of interest

None.

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