Haemophilus Influenzae type b Vaccines: Current Trends and Future Outlook

Jian Kim

Abstract

More than 10 million children under the age of 5 die annually due to *Haemophilus influenzae* type b (Hib), and it can be concluded that infants and children are patient groups at particular risk.[1] Among Hib, certain serotypes such as Nontypeable *Haemophilus Influenzae* (NTHi) are particularly difficult to treat as they are harder to detect and have high mutation rate as they spread from the nasopharyngeal. Despite the success of current Hib vaccines, vaccine candidates against NTHi continue to be a challenge as the current conjugate vaccines do not work effectively against the nontypeable strains.[4,5,6] By examining the effectiveness of existing vaccines, this review article emphasizes the need for continued research to expand vaccine development for both typeable and nontypeable strains of Hib to mitigate the Hib-related diseases.

Introduction

As the usage of vaccines develop immunological memory, prevent co-infections, and have far-reaching effects for the society's health, it has been considered as an effective means of immunization to prevent future diseases. Loss of control of bacterial and viral infections can lead to fatal diseases, which is one of the major reasons that vaccines are implemented.[7,8] That said, our lives depend tremendously on our ability to control bacterial and viral populations that cause diseases. It is demonstrated in Figure 1 how the vaccine immediately decreases the disease's trends.

Any type of infection that is caused by the pleomorphic gram-negative coccobacillus called *Haemophilus influenzae* is called a Haemophilus influenzae disease.[1,2] *Haemophilus influenzae*, originally known as the Pfeiffer's bacillus as Richard Pfeiffer first discovered it during an influenza pandemic in 1892, is a bacterial pathogen that leads to subsequent diseases. It can easily be transmitted by the inhalation of direct airborne respiratory droplets.[1,9] The incubation period, the duration between the infection and the manifestation of the disease, is unknown. However, it is suspected to take a few days for the symptoms such as the diseases mentioned in the previous sentence.[1,18] At the beginning of the 20th century, there were many casualties from the diseases that originated from the Haemophilus influenzae.[10,11] This paper will specifically discuss about the Hib, which can lead to severe secondary infections.

Hib is categorized as encapsulated and non-encapsulated strains. There are six encapsulated serotypes which are from 'a' to 'f', which all contain distinct capsular polysaccharides. These are the main cause of lower respiratory infections such as flu, viral bronchiolitis, and pneumonia.[52] Non-encapsulated strains are nontypeable, meaning that they are not reactive with typing antisera — Antisera are prepared to combat specific diseases and provide passive immunity. It also means that they lack a polysaccharide capsule.[52] To highlight, the difference between an encapsulated and non-encapsulated strains are in the pathogenic mechanism.[12,13]

Among the six capsular types from type a to type f, type b is more invasive than others. Thus, Hib may cause secondary airway mucosal infections that are life threatening like meningitis (an infection of the lining of the brain and spinal cord), epiglottitis (an infection along

the air passage from the throat to the lungs), and pneumonia (an infection of the lungs).[12,14] These are highly morbid diseases as they lead on to hearing loss, seizures, loss of limbs, and intellectual disabilities.[15-17]

The rise of Hib is especially unsettling to many as infants and children under the age of 5 are exclusively more susceptible to this infection and subsequent secondary infections by 95%, even though Hib usually affects the upper respiratory tract of both children and adults. It is also known that adults above the age of 65 and pregnant women are more susceptible to Hib.[1,3] American Indians and Alaska natives, and people with certain medical conditions such as sickle cell disease, human immunodeficiency virus (HIV) infection, asplenia, antibody and complement deficiency states, patients with cancer receiving chemotherapy, radiotherapy, and in post bone marrow transplant states are also susceptible to the disease as well.

Nontypeable *Haemophilus influenzae* is an emerging pathogen that is derived from the change of the serotype of Hib. NTHi is the main cause of otitis media in infants and children, sinusitis in children and adults, and non-bacteremic pneumonia in the elderly.[1,20] Respiratory pathogens like NTHi continue to affect the host and exacerbate other respiratory problems, and depend on the host to get the necessary elements they need to maintain their growth and to be resistant to traditional antibiotic treatments. Even after the host has been treated with specific vaccines to counter the capsular *H.influenzae,* NTHi causes infections. The amount of children and adults infected to diseases due to NTHi is rising, and it is an invariable problem in both developed and non industrialized nations as it causes respiratory tract infections. The encapsulated *H. influenzae* utilizes proteins like protein H and Haemophilus surface fibrils (Hsf), but these features do not exist in NTHi subtypes. There are a few ways in which *H. influenzae* attach to the host cell and cause damage to the host. NTHi uses mechanisms such as attaching to the surface of the epithelial cells, accessing the underlying extracellular matrix layer, and invading certain serum factors to affect a group of proteins. This allows NTHi to be connected to each other and enter to the host cells with ease. Conjugate vaccines, polysaccharide antigen conjugated to a carrier molecule, are used to counter Hib, but it does not successfully work against NTHi as there is a lack of a polysaccharide capsule in NTHi strains.

Today there is still a significant disease burden of Hib for pediatric populations, and current research has focused on understanding the structure of Hib (Fig 2), with a focus on the Hib pili as it may contribute to the onset of disease. Overall, additional research into Hib prevention is crucial in order to decrease the significant global disease burden.[1,28] This paper will explore currently used vaccines and the development strategies for the prevention of Hib.

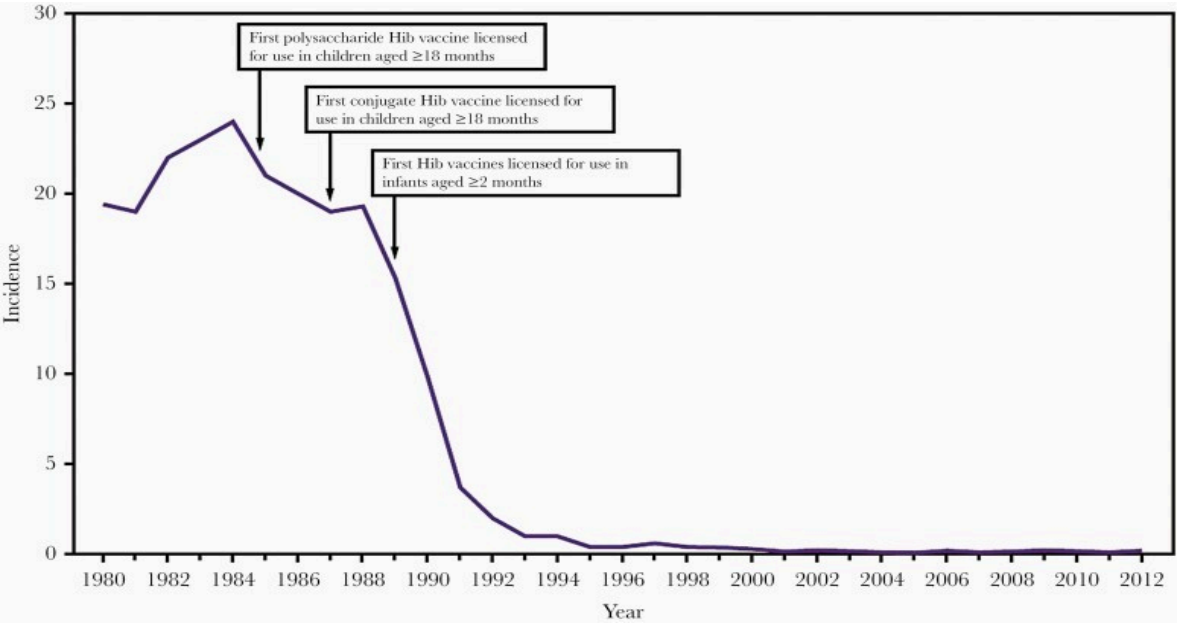

Figure 1. Impact of *Haemophilus influenzae* type b (Hib) vaccines on incidence per 100 000 children <5 years old in the United States, 1980–2012 (Gilsdorf, 2021)[19]

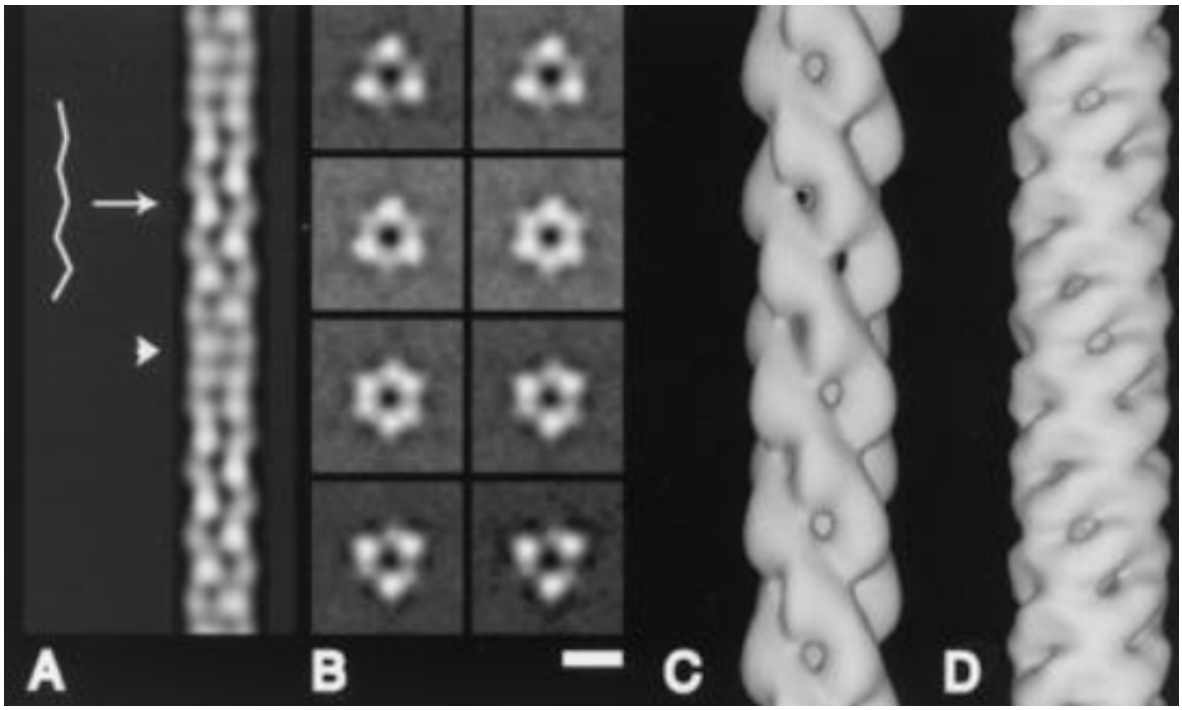

Figure 2. The picture on the left shows the (A) helical axis in a zigzag appearance of a negatively charged pili, (B) cross sections, (C) Surface area, and (D) 3D shape. Regarding the structure of Hib pili, it is concluded that Hib pili is similar to pili class 1, and is strongly associated with p-pili. There is no association between Hib pili and E.coli or the Type IV virus. (X.Mu, 2002)[28]

Vaccine development

To ensure that the vaccines can provoke an immune response, the vaccine approval process by the Food and Drug Administration (FDA) is composed of methodological and precise steps. Once the vaccine shows promising results, clinical trials are conducted to further determine its efficacy. The steps are described in the following sentences. First, the researchers advance an Investigational New Drug (IND) application to the FDA.[29,30] This process includes data from animal studies, information on manufacturing technology, and assessments of vaccine quality, all of which are crucial for demonstrating the short-term and long-term efficacy of the vaccine. The clinical development stage consists of the following three phases. Phase 1 involves administering the trial vaccine to a group of 20 to 100 people. During this phase, researchers collect data on the vaccine's safety in humans. This phase allows the researchers to gather information about the side effects and the effectiveness in creating an immune response. The way vaccines function is by injecting an antigen of a disease and mimicking it, stimulating the immune system.[30,40] Therefore, the vaccine can likely bring side effects, and it is a signal from your body that it is starting to build immunity against a disease. In Phase 2, the participant group expands to 100 to 300 individuals, selected based on shared characteristics such as age and physical health. This phase is important as it emphasizes the representativeness of the participants by including people from diverse backgrounds. Researchers gather more comprehensive data on the vaccine's side effects, risks, and its ability to trigger an immune response. Phase 3 is when the clinical trial expands to 1000 to 3000 people. It is also when the researchers corroborate how well the vaccines function and the availability of side effects to ensure the use of it. After the FDA approves a vaccine, the vaccine enters Phase 4. This phase involves ongoing studies to monitor the vaccine’s safety and effectiveness for a long period.

In this section, we will discuss the current FDA-licensed monovalent, combination vaccines, and conjugate vaccines. ActHIB (PRP-T) is one of the three monovalent vaccines licensed by the FDA in the U.S., specifically designed for use in infants as young as 6 weeks old.[23,31] Combination vaccines, such as Vaxelis (DTaP-IPV-Hib-HepB) and Pentacel (DTaP-IPV/Hib), protect against multiple diseases simultaneously, allowing children to receive several vaccines in one dose. Among many combination vaccines, Vaxelis and Pentacel are notable options that include the Hib vaccine.

Additionally, conjugate vaccines are formulated to induce a strong immune response against bacterial capsular polysaccharides (CPCs). These high molecular weight polysaccharides (simple sugars) allow many pathogens to evade the immune system. By concealing cell-surface components that would otherwise trigger an immune response, these pathogens can cause infections in various human tissues, including the gut, respiratory tract, urinary tract, and other host tissues.[32,33]

1. **ActHIB (PRP-T)**

ActHIB is a Polyribosylribitol Phosphate-Tetanus Conjugate (Hib) Vaccine, containing a polysaccharide from the outside of the Hib bacterium conjugated to tetanus toxoid protein. Tetanus is a deadly infectious disease that is caused by Clostridium Tetani, a type of anaerobic bacteria that they thrive in the environment without oxygen.[34-36] ActHIB, a vaccine developed by Sanofi Pasteur Inc. in France, was approved by the FDA on September 27, 1996.[36-38] This vaccine is designed so that it prevents the invasive diseases caused by Hib and the secondary

infections by intramuscular injections. As this vaccine is a monovalent vaccine, it is designed to only combat Hib and the invasive diseases from Hib; it won't protect against other types of bacteria.[34,39] ActHIB vaccine is also immunogenic in children with sickle cell anemia, a disease that can make the host more susceptible to Hib.[53]

ActHIB is designed for 4 doses (0.5 mL each) for children from 2 months through 5 years of age: A three-dose primary series administered at 2, 4, and 6 months of age. A single booster dose administered at 15-18 months of age. The Centers for Disease Control and Prevention (CDC) states that "A child 7 to 11 months of age should receive 2 doses of ActHIB Vaccine at 8-week intervals and a booster dose at 15 to 18 months of age. A child 12 to 14 months of age should receive 1 dose of ActHIB Vaccine followed by a booster 2 months later.[2,28] Specifically, ActHIB vaccine is a solution for injection as a single-dose vial of lyophilized powder to be reconstituted with the supplied 0.4% Sodium Chloride diluent.[53] It is not approved for use in individuals 6 years of age and older.

There were over 110,000 infants and children in Canada, the United States, Finland, France, Chile, Israel, and the United Kingdom for the clinical trials. In Sanofi's product monograph, it was said that "In clinical trials where 921 infants were given the vaccine at 2, 4 and 6 months, a titre of at least 0.15 µg/mL was achieved after dose 3 in 99% and a titre of at least 1.00 µg/mL in 93%. The weighted GMT achieved was 7.0 µg/mL (95% confidence limits are 3.4 - 14.2 µg/mL). Protective levels of anti-PRP developed after the second dose in 92.8% of these infants." These insightful aspects of this clinical trials is that as the number of doses rise, the vaccine efficacy rises. However, it is known that not a lot of children and adults do not have access to vaccines. This is proven by a study in India, which showed that nationwide usage of vaccines is needed to cover the diffusion of Hib. The important note to take away from this study was that the role of private sector is undefined, and that the universal spread of Hib could wane if the vaccinations were prevalent.[43,44] Therefore, it is unlikely for one to get all three vaccines, and many individuals do not have the immunity to this.[43,45] To add, the protective level of getting until the second dose is 92.8%, which means that 7920 individuals will not get the protection.

In a clinical study in Tennessee to discover the immunogenicity of the ActHIB vaccine compared to PedvaxHIB and HibTITER in the ability to have protective antibody levels in infants. Table 3 displays how the ActHIB vaccine is more effective than the other vaccines as the Geometric Mean Concentration (GMC) (mcg/mL) is the highest post third immunization at 7 months, which suggest the vaccine's ability to generate a strong immune response.[53]

Some of the side effects are irritability, sleepiness, loss of appetite, and swelling.[41,42] Individuals with severe allergies should not use this vaccine, as convulsions are listed as a potential side effect. If Guillain-Barré syndrome occurred within 6 weeks of the prior vaccine containing tetanus toxoid, the patient should not take the ActHIB vaccine. To add, there are immune system disorders such as Anaphylaxis and other allergic reactions.[53]

MK,	N*	Geometric Mean Concentration (GMC) (mcg/mL)			Post Third Immunization % ≥1.0 mcg/mL
		Pre-Immunization at 2 months	Post Second Immunization at 6 months	Post Third Immunization at 7 months	
PRP-T† (ActHIB vaccine)	65	0.10	0.30	3.64	83%
PRP-OMP‡ (PedvaxHIB®)	64	0.11	0.84	N/A	50%§
HbOC¶ (HibTITER®)	61	0.07	0.13	3.08	75%

* N = Number of children
† *Haemophilus influenzae* type b Conjugate Vaccine (Tetanus Toxoid Conjugate)
‡ *Haemophilus influenzae* type b Conjugate Vaccine (Meningococcal Protein Conjugate)
§ Seroconversion after the recommended 2-dose primary immunization series is shown
¶ *Haemophilus influenzae* type b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate)
N/A = Not applicable in this comparison trial although third dose data have been published

Figure 3. Anti-PRP Antibody Responses Following a Two or Three Dose Series of a Haemophilus influenzae type b Vaccine at 2, 4, 6 Months of Age - Tennessee.

2. Vaxelis (DTaP-IPV-Hib-HepB)

Vaxelis [(Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine): DTaP-IPV/Hib] is a hexavalent vaccine that is designed to provide immunization against: diphtheria, tetanus, pertussis, poliomyelitis, *Haemophilus influenzae* type b, and hepatitis B. As vaxelis is not a monovalent vaccine, an ActHIB vaccine component is combined through reconstitution for intramuscular injection as well. To add, as it is a combined vaccine, it was aimed to protect people from diphtheria, tetanus, pertussis, poliomyelitis, and Hib. Diphtheria Toxoid protects against diphtheria, which causes respiratory issues. Tetanus Toxoid prevents tetanus, which is a bacterial infection caused by muscle stiffness. Acellular Pertussis Antigens protects from pertussis, which is a contagious respiratory disease. Inactivated Poliovirus is in Vaxelis to protect against poliomyelitis, a viral infection that can cause paralysis. Hib prevents infections caused by Hib bacteria such as meningitis and pneumonia. Lastly, Hepatitis B Surface Antigen provides immunity against hepatitis B, which can lead to chronic liver diseases of liver cancer. The conjugation with the ActHIB component significantly helps stimulate the immune response to protect from Hib. In fact, the presence of conjugate protein carriers are crucial as the polysaccharide capsule makes an effective vaccine.[53]

The FDA approved Vaxelis for use as a four-dose series in infants and children at ages 2, 4, 6, and 15 — 18 months in June of 2008. The child will need 3 shots of a 0.5 mL intramuscular injection: one shot at 2 months old, one shot at 4 months old, and one shot at 6 months old. The efficacy of the vaccine increases with the number of doses, as shown in figure 3. Vaccines use different carriers, and the polysaccharide helps immunize Hib as it creates the production of antibodies against Hib.[47,48]

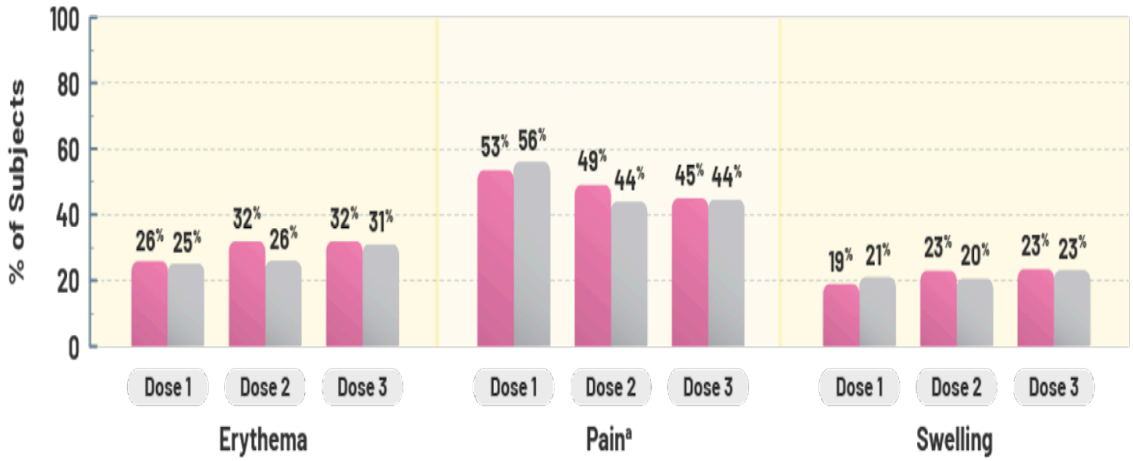

Figure 4. It is shown how the number of patients who showed side effects such as Erythema, Pain, and swelling decreased with the additional number of doses of Vaxelis. (Sanofi, 2023)

In a clinical study, consisting of more than 6800 children to test if it is suitable for premature individuals, the following results were shown: **"**Overall, 160 infants were considered premature (DTaP-IPV-Hib-HepB = 111 Control = 49). The incidence of adverse events (AEs) for DTaP-IPV-Hib-HepB was comparable between overall and premature populations for all AEs days 1-15 post vaccination (Overall = 96.3%; Premature = 97.3%;), solicited injection-site AEs days 1-5 post vaccination (Overall = 84.1%; Premature = 75.5%), and solicited systemic AEs days 1-5 post vaccination (Overall = 93.7%; Premature = 94.5%). A high percentage of premature infants mounted protective immune responses to antigens contained in DTaP-IPV-Hib-HepB vaccine. Response rates in preterm infants for all antigens (80-99%) were in a similar range to all infants (80-99%) for both DTaP-IPV-Hib-HepB and control vaccines." (Wilck, 2020). This result shows how the rate of the incidence of adverse events is pretty low. The immune responses were clearly shown, however to a satisfactory amount. The following do support that Vaxelis is safe for infants.[43,49]

However, Vaxelis also has concerns. It is suggested if Guillain-Barré syndrome occurred within 6 months of a prior vaccine containing tetanus toxoid, the syndrome might aggravate. Additionally, the FDA mentioned that "The solicited adverse reactions following any dose were irritability (≥55%), crying (≥45%), injection site pain (≥44%), somnolence (≥40%), injection site erythema (≥25%), decreased appetite (≥23%), fever ≥38.0°C (≥19%), injection site swelling (≥18%), and vomiting (≥9%)." Therefore, there are some concerns on hypersensitivity which could be severe allergic reactions like anaphylaxis.[53]

3. Pentacel (DTaP-IPV/Hib)

Pentacel is a combination vaccine that is designed to protect against diphtheria, tetanus, pertussis, poliomyelitis, and Hib. Consisting of DTaP and conjugated toxoid, Pentacel is targeted to children 6 weeks to 4 years old for the prevention of Hib. It is approved for 3-dose series, recommended schedule at 2, 4, 6 years of age.[49,50] Specifically, it was shown that infants treated with the vaccine had a low possibility of getting fever, vomiting, and drowsiness compared to the ones who did not take the vaccine.[50,51]

In a clinical study, presented in the figure below, it was shown how Pentacel is a notable vaccine against Hib. It confirms the safety of Pentacel when it is administered according to the standard vaccination schedule, which is the ages 2, 4, and 6 years. This study also demonstrates how Pentacel can be safely administered alongside vaccines such as PCV7, Hepatitis B, MMR, and Varicella. Overall, the results indicate that Pentacel is a safe vaccine for infants according to the recommended schedule.[55]

However, the safety and efficacy of Pentacel in infants less than 6 weeks of age have not been established, and no data are available. A problem with this vaccine is that it has a low effectiveness to people with a weakened immune system. They are at higher risk of polio, Hib, and tetanus even after they receive the vaccine. Skibinski Et al. mentioned about Addition of pneumococcal or meningococcal vaccines to DTaP-based vaccines and providing a New carrier protein strategies for conjugate vaccines is going to be innovative.[55]

Study	Pentacel	Control Vaccines	Concomitantly Administered Vaccines
494-01	2, 4, 6 and 15 months	HCPDT + POLIOVAX + ActHIB at 2, 4, 6, and 15 months	7-valent pneumococcal conjugate vaccine* (PCV7) at 2, 4, and 6 months in a subset of participants† Hepatitis B vaccine at 2 and 6 months‡
P3T06	2, 4, 6, and 15-16 months	DAPTACEL + IPOL + ActHIB at 2, 4, and 6 months; and DAPTACEL + ActHIB at 15-16 months	PCV7* at 2, 4, and 6 months Hepatitis B vaccine at 2 and 6 months‡
494-03	2, 4, 6, and 15-16 months	None	PCV7* at 2, 4, and 6 months in all participants; and at 15 months in a random subset of participants Hepatitis B vaccine at 2 and 6 months (if a dose was previously administered)‡ or at 2, 4, and 6 months (if no previous dose) Measles, mumps, rubella vaccine§ (MMR) and varicella§ vaccine at 12 or 15 months in random subsets of participants
5A9908	15-18 months¶	None	None

Figure 5. Clinical Safety Studies of Pentacel: Vaccination Schedule.

Vaccine development for NTHi

NTHi poses a significance in public health for numerous reasons. Primarily, NTHi is the major cause of respiratory infections, including acute otitis media (AOM) and cystic fibrosis. In adults, NTHi may cause chronic bronchitis and chronic obstructive pulmonary disease (COPD) that is the fourth leading cause of global mortality. Specifically, the ability of NTHi to form biofilms in the respiratory tract of adults with COPD causes chronic infections. As NTHi is a major cause of mortality, a vaccine that targets NTHi needs to be developed.[56]

The only vaccines that can target the NTHi strains are the conjugate vaccines. This is important as the NTHi strains are not detectable through the other types of vaccines discussed

earlier. If the strain is not targeted, it will cause respiratory and invasive diseases. After the conjugate vaccines were developed, NTHi strains were less frequently detected in respiratory tract infection. Therefore, there has been a decline in the interest to find other serotypes of *H. Influenzae.*

The development of the vaccine started with the discovery of a polysaccharide of a pathogen polyribosylribitol phosphate (PRP) in the 1970s in Finland.[21,22] Then, it was found out that conjugate vaccines are more capable of inducing an immune response. Polysaccharide vaccines are important as they help activate B cells, a type of cell that creates antibodies. Children under 18 months of age have poorly developed B cells, therefore have poor immunogenicity as well. To that end, people utilized the conjugate vaccine more. Hib conjugate vaccine was in fact the first licensed conjugate vaccine in the USA in 1987.[23,24] In the 1980s, Hib protein conjugate vaccines were developed, and this improved the immunogenicity of the PRP polysaccharide. Despite advancements in the development of a reliable vaccine for Hib, there have been no vaccines developed for nontypeable strains of *H. influenzae* to date.[25,26] The reason that it is still not yet developed is because the NTHi strains differ phenotypically and also genotypically, meaning they have different traits. Moreover, NTHi is known for its heterogeneity, and this has been the major hindrance when developing a vaccine.[26,27]

One of the most prevalent studies regarding the vaccine that can combat Hib by detecting NTHi strains is by Akkoyunlu Et al. It is about how LPD (lipoprotein D) and PDm(The non acylated form of protein D) are conjugated to Hib to test the protein D - conjugate PRP vaccine's potential of protection. When tested with rats, it was shown how ActHIB was successful in invoking immunization and protecting the individual. It was shown how sera, which produces passive immunity, was in the largest amount when it was successful against a Hib strain and an NTHi strain.[44,46] Therefore, the usage of lipoprotein would help improve ActHIB. As the concern is the universal usage of the vaccine and the efficacy expecting less than 3 doses, it is important to develop the vaccine by the inclusion of LPD.

Discussion

Vaxelis, ActHIB, and Pentacel can protect the body from Hib, but they are ineffective against NTHi strains. However, recent studies have shown the improved efficacy of Hib vaccines when Protein D (PD), a surface-exposed lipoprotein that is found in all H.influenzae strains, is added. PD also showed protective immunity in animal clinical trials, demonstrating that PD can protect against NTHi-induced otitis media in rats and chinchillas. The efficacy of NTHi vaccines may be due to PD's enzymatic activity that involves transferring choline from host cells to the bacterial lipopolysaccharide (LOS). However, one of the challenges in NTHi vaccine development is addressing biofilm formation as it is very resistant to immune responses and antibiotics. PD may not fully address biofilm formation and needs to be further evaluated. The future directions should be towards combining PD with other effective antigen to further enhance the vaccine effectiveness.

Conclusion

This paper introduced the severe problems of Hib and the effectiveness of the approved vaccines. After all, it can be concluded that the emergence of vaccines such as Vaxelis, Pentacel, and ActHIB significantly decreased the Hib infection rate.[34-36] There have been many countries with robust vaccination programs, actively promoting these vaccines and future studies. However, the persistence of non-typeable strains of Hib and the sporadic outbreaks still remain and vaccines should be advanced as there should be more innovative strategies to fight against Hib and its tremendous effects. As mentioned above, more studies should focus on protein D and complementary antigen to enhance the effectiveness of the vaccine.

To this day, Hib, which can cause meningitis, epiglottitis, and pneumonia, is still not completely prevented due to NTHi. After decades of global vaccine use, impressive control of Hib invasive disease has been achieved through mass vaccination. However, the disease is still prevalent and has severe consequences. High vaccination coverage has been achieved in most parts of the world and the number of cases remains low, although there are still a few countries where Hib vaccination has not been implemented in the National Immunization Program (NIP).

The burden of disease and associated mortality in these countries is very high, and introduction of mass vaccination should be considered. It is clear that vaccines should be enhanced as the vaccines were not effective at identifying nontypeable strains, especially in young people. In order to make vaccines against NTHi, the conjugate vaccines mentioned previously should be enhanced to increase the effectiveness. In order to do this, there needs to be more studies of corresponding suitable carrier proteins and antigens specific to NTHi. Multivalent vaccines should be designed to protect multiple serotypes as well.

References

(1) Khattak, Z. E.; Anjum, F. Haemophilus Influenzae Infection. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(2) Mäkelä, P. H. Unencapsulated Haemophilus Influenzae--What Kind of Pathogen? *Eur J Clin Microbiol Infect Dis* **1988**, *7* (5), 606–609. https://doi.org/10.1007/BF01964236.

(3) Bröker, M. Burden of Invasive Disease Caused by Haemophilus Influenzae Type b (Hib) in Africa. *Minerva Pediatr* **2008**, *60* (3), 337–342.

(4) Slack, M. P. E. A Review of the Role of Haemophilus Influenzae in Community-Acquired Pneumonia. *Pneumonia (Nathan)* **2015**, *6*, 26–43. https://doi.org/10.15172/pneu.2015.6/520.

(5) Murphy, T. F.; Faden, H.; Bakaletz, L. O.; Kyd, J. M.; Forsgren, A.; Campos, J.; Virji, M.; Pelton, S. I. Nontypeable Haemophilus Influenzae as a Pathogen in Children. *Pediatr Infect Dis J* **2009**, *28* (1), 43–48. https://doi.org/10.1097/INF.0b013e318184dba2.

(6)
Levine, O. S.; Lagos, R.; Muñoz, A.; Villaroel, J.; Alvarez, A. M.; Abrego, P.; Levine, M. M. Defining the Burden of Pneumonia in Children Preventable by Vaccination against Haemophilus Influenzae Type b. *Pediatr Infect Dis J* **1999**, *18* (12), 1060–1064. https://doi.org/10.1097/00006454-199912000-00006.

(7)
Doherty, M.; Buchy, P.; Standaert, B.; Giaquinto, C.; Prado-Cohrs, D. Vaccine Impact: Benefits for Human Health. *Vaccine* **2016**, *34* (52), 6707–6714. https://doi.org/10.1016/j.vaccine.2016.10.025.

(8)
Nandi, A.; Shet, A. Why Vaccines Matter: Understanding the Broader Health, Economic, and Child Development Benefits of Routine Vaccination. *Hum Vaccin Immunother* **2020**, *16* (8), 1900–1904. https://doi.org/10.1080/21645515.2019.1708669.

(9)
Brueggemann, A. B.; Jansen van Rensburg, M. J.; Shaw, D.; McCarthy, N. D.; Jolley, K. A.; Maiden, M. C. J.; van der Linden, M. P. G.; Amin-Chowdhury, Z.; Bennett, D. E.; Borrow, R.; Brandileone, M.-C. C.; Broughton, K.; Campbell, R.; Cao, B.; Casanova, C.; Choi, E. H.; Chu, Y. W.; Clark, S. A.; Claus, H.; Coelho, J.; Corcoran, M.; Cottrell, S.; Cunney, R. J.; Dalby, T.; Davies, H.; de Gouveia, L.; Deghmane, A.-E.; Demczuk, W.; Desmet, S.; Drew, R. J.; du Plessis, M.; Erlendsdottir, H.; Fry, N. K.; Fuursted, K.; Gray, S. J.; Henriques-Normark, B.; Hale, T.; Hilty, M.; Hoffmann, S.; Humphreys, H.; Ip, M.; Jacobsson, S.; Johnston, J.; Kozakova, J.; Kristinsson, K. G.; Krizova, P.; Kuch, A.; Ladhani, S. N.; Lâm, T.-T.; Lebedova, V.; Lindholm, L.; Litt, D. J.; Martin, I.; Martiny, D.; Mattheus, W.; McElligott, M.; Meehan, M.; Meiring, S.; Mölling, P.; Morfeldt, E.; Morgan, J.; Mulhall, R. M.; Muñoz-Almagro, C.; Murdoch, D. R.; Murphy, J.; Musilek, M.; Mzabi, A.; Perez-Argüello, A.; Perrin, M.; Perry, M.; Redin, A.; Roberts, R.; Roberts, M.; Rokney, A.; Ron, M.; Scott, K. J.; Sheppard, C. L.; Siira, L.; Skoczyńska, A.; Sloan, M.; Slotved, H.-C.; Smith, A. J.; Song, J. Y.; Taha, M.-K.; Toropainen, M.; Tsang, D.; Vainio, A.; van Sorge, N. M.; Varon, E.; Vlach, J.; Vogel, U.; Vohrnova, S.; von Gottberg, A.; Zanella, R. C.; Zhou, F. Changes in the Incidence of Invasive Disease Due to Streptococcus Pneumoniae, Haemophilus Influenzae, and Neisseria Meningitidis during the COVID-19 Pandemic in 26 Countries and Territories in the Invasive Respiratory Infection Surveillance Initiative: A Prospective Analysis of Surveillance Data. *Lancet Digit Health* **2021**, *3* (6), e360–e370. https://doi.org/10.1016/S2589-7500(21)00077-7.

(10)
Taubenberger, J. K.; Hultin, J. V.; Morens, D. M. Discovery and Characterization of the 1918 Pandemic Influenza Virus in Historical Context. *Antivir Ther* **2007**, *12* (4 Pt B), 581–591.

(11)

Heinz, E. The Return of Pfeiffer's Bacillus: Rising Incidence of Ampicillin Resistance in Haemophilus Influenzae. *Microb Genom* **2018**, *4* (9), e000214. https://doi.org/10.1099/mgen.0.000214.

(12)
Verma, R.; Khanna, P.; Chawla, S.; Bairwa, M.; Prinja, S.; Rajput, M. Haemophilus Influenzae Type b (Hib) Vaccine: An Effective Control Strategy in India. *Hum Vaccin* **2011**, *7* (11), 1158–1160. https://doi.org/10.4161/hv.7.11.17683.

(13)
Haemophilus influenzae - PAHO/WHO | Pan American Health Organization. https://www.paho.org/en/topics/haemophilus-influenzae (accessed 2023-09-17).

(14)
Scholz, H.; Noack, R. [Hemophilus influenzae infection and their prevention by vaccination]. *Kinderarztl Prax* **1993**, *61* (6), 189–191.

(15)
Hersi, K.; Gonzalez, F. J.; Kondamudi, N. P. Meningitis. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(16)
Guerra, A. M.; Waseem, M. Epiglottitis. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(17)
Torres, A.; Cilloniz, C.; Niederman, M. S.; Menéndez, R.; Chalmers, J. D.; Wunderink, R. G.; van der Poll, T. Pneumonia. *Nat Rev Dis Primers* **2021**, *7* (1), 25. https://doi.org/10.1038/s41572-021-00259-0.

(18)
Rowley, A. H.; Wald, E. R. The Incubation Period Necessary for Detection of Bacteremia in Immunocompetent Children with Fever. Implications for the Clinician. *Clin Pediatr (Phila)* **1986**, *25* (10), 485–489. https://doi.org/10.1177/000992288602501001.

(19)
Jr, G. Hib Vaccines: Their Impact on Haemophilus Influenzae Type b Disease. *The Journal of infectious diseases* **2021**, *224* (12 Suppl 2). https://doi.org/10.1093/infdis/jiaa537.

(20)
Hunt, B. C.; Xu, X.; Gaggar, A.; Swords, W. E. Nontypeable Haemophilus Influenzae Redox Recycling of Protein Thiols Promotes Resistance to Oxidative Killing and Bacterial Survival in Biofilms in a Smoke-Related Infection Model. *mSphere* **2022**, *7* (1), e0084721. https://doi.org/10.1128/msphere.00847-21.

(21)

Wen, S.; Feng, D.; Chen, D.; Yang, L.; Xu, Z. Molecular Epidemiology and Evolution of Haemophilus Influenzae. *Infect Genet Evol* **2020**, *80*, 104205. https://doi.org/10.1016/j.meegid.2020.104205.

(22) Saleh, A.; Qamar, S.; Tekin, A.; Singh, R.; Kashyap, R. Vaccine Development Throughout History. *Cureus 13* (7), e16635. https://doi.org/10.7759/cureus.16635.

(23) *About Hib Vaccine (Haemophilus Influenzae Type b Vaccine) | CDC*. https://www.cdc.gov/vaccines/vpd/hib/hcp/about-vaccine.html (accessed 2023-08-08).

(24) Goldblatt, D. Conjugate Vaccines. *Clin Exp Immunol* **2000**, *119* (1), 1–3. https://doi.org/10.1046/j.1365-2249.2000.01109.x.

(25) Kadry, N. A.; Porsch, E. A.; Shen, H.; St. Geme, J. W. Immunization with HMW1 and HMW2 Adhesins Protects against Colonization by Heterologous Strains of Nontypeable Haemophilus Influenzae. *Proc Natl Acad Sci U S A* **2021**, *118* (32), e2019923118. https://doi.org/10.1073/pnas.2019923118.

(26) Cerquetti, M.; Giufrè, M. Why We Need a Vaccine for Non-Typeable Haemophilus Influenzae. *Hum Vaccin Immunother* **2016**, *12* (9), 2357–2361. https://doi.org/10.1080/21645515.2016.1174354.

(27) Behrouzi, A.; Vaziri, F.; Rahimi-Jamnani, F.; Afrough, P.; Rahbar, M.; Satarian, F.; Siadat, S. D. Vaccine Candidates against Nontypeable Haemophilus Influenzae: A Review. *Iran Biomed J* **2017**, *21* (2), 69–76. https://doi.org/10.18869/acadpub.ibj.21.2.69.

(28) Mu, X.-Q.; Egelman, E. H.; Bullitt, E. Structure and Function of Hib Pili from Haemophilus Influenzae Type b. *J Bacteriol* **2002**, *184* (17), 4868–4874. https://doi.org/10.1128/JB.184.17.4868-4874.2002.

(29) Commissioner, O. of the. *The Drug Development Process*. FDA. https://www.fda.gov/patients/learn-about-drug-and-device-approvals/drug-development-process (accessed 2023-09-19).

(30) *How Vaccines are Developed and Approved for Use | CDC*. https://www.cdc.gov/vaccines/basics/test-approve.html (accessed 2023-09-19).

(31)
Pinkbook: Haemophilus influenzae (Hib) | CDC. https://www.cdc.gov/vaccines/pubs/pinkbook/hib.html (accessed 2023-04-07).

(32)
Cress, B. F.; Englaender, J. A.; He, W.; Kasper, D.; Linhardt, R. J.; Koffas, M. A. G. Masquerading Microbial Pathogens: Capsular Polysaccharides Mimic Host-Tissue Molecules. *FEMS Microbiol Rev* **2014**, *38* (4), 660–697. https://doi.org/10.1111/1574-6976.12056.

(33)
Haemophilus b Conjugate Vaccines for Prevention of Haemophilus influenzae Type b Disease Among Infants and Children Two Months of Age and Older Recommendations of the ACIP. https://www.cdc.gov/mmwr/preview/mmwrhtml/00041736.htm (accessed 2023-09-20).

(34)
Medical Definition of Tetanus. RxList. https://www.rxlist.com/tetanus/definition.htm (accessed 2023-09-20).

(35)
Medical Definition of Clostridium. RxList. https://www.rxlist.com/clostridium/definition.htm (accessed 2023-09-20).

(36)
ActHIB (Haemophilus b Conjugate Vaccine): Uses, Dosage, Side Effects, Interactions, Warning. RxList. https://www.rxlist.com/acthib-drug.htm (accessed 2023-09-20).

(37)
Centers for Disease Control and Prevention (CDC). FDA Approval of a Haemophilus b Conjugate Vaccine Combined by Reconstitution with an Acellular Pertussis Vaccine. *MMWR Morb Mortal Wkly Rep* **1996**, *45* (45), 993–995.

(38)
ActHIB (Haemophilus b Conjugate Vaccine): Uses, Dosage, Side Effects, Interactions, Warning. RxList. https://www.rxlist.com/acthib-drug.htm (accessed 2023-09-20).

(39)
Research, C. for B. E. and. ActHIB. *FDA* **2022**.

(40)
Siegrist, C.-A. Mechanisms Underlying Adverse Reactions to Vaccines. *J Comp Pathol* **2007**, *137 Suppl 1*, S46-50. https://doi.org/10.1016/j.jcpa.2007.04.012.

(41)

Hib/MenC vaccine side effects. nhs.uk. https://www.nhs.uk/conditions/vaccinations/hib-men-c-booster-side-effects/ (accessed 2023-09-20).

(42) Fortunato, F.; Martinelli, D.; Lopalco, P. L.; Prato, R. Safety Evaluation of the DTaP5-IPV-Hib-HepB Vaccine: A Review. *Expert Opin Drug Saf* **2022**, *21* (3), 295–302. https://doi.org/10.1080/14740338.2022.2007882.

(43) Moro, P. L.; Jankosky, C.; Menschik, D.; Lewis, P.; Duffy, J.; Stewart, B.; Shimabukuro, T. T. Adverse Events Following Haemophilus Influenzae Type b (Hib) Vaccines in the Vaccine Adverse Event Reporting System (VAERS), 1990-2013. *J Pediatr* **2015**, *166* (4), 992–997. https://doi.org/10.1016/j.jpeds.2014.12.014.

(44) Nix, E. B.; Hawdon, N.; Gravelle, S.; Biman, B.; Brigden, M.; Malik, S.; McCready, W.; Ferroni, G.; Ulanova, M. Risk of Invasive Haemophilus Influenzae Type b (Hib) Disease in Adults with Secondary Immunodeficiency in the Post-Hib Vaccine Era. *Clin Vaccine Immunol* **2012**, *19* (5), 766–771. https://doi.org/10.1128/CVI.05675-11.

(45) Sharma, A.; Kaplan, W. A.; Chokshi, M.; Hasan Farooqui, H.; Zodpey, S. P. Implications of Private Sector Hib Vaccine Coverage for the Introduction of Public Sector Hib-Containing Pentavalent Vaccine in India: Evidence from Retrospective Time Series Data. *BMJ Open* **2015**, *5* (2), e007038. https://doi.org/10.1136/bmjopen-2014-007038.

(46) Akkoyunlu, M.; Melhus, A.; Capiau, C.; van Opstal, O.; Forsgren, A. The Acylated Form of Protein D of Haemophilus Influenzae Is More Immunogenic than the Nonacylated Form and Elicits an Adjuvant Effect When It Is Used as a Carrier Conjugated to Polyribosyl Ribitol Phosphate. *Infect Immun* **1997**, *65* (12), 5010–5016.

(47) Dhillon, S.; Keam, S. J. DTaP-IPV/Hib Vaccine (Pentacel). *Paediatr Drugs* **2008**, *10* (6), 405–416. https://doi.org/10.2165/0148581-200810060-00008.

(48) *Pentacel (Tetanus Toxoid Conjugate): Uses, Dosage, Side Effects, Interactions, Warning*. RxList. https://www.rxlist.com/pentacel-drug.htm (accessed 2023-09-20).

(49) *Licensure of a Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus, and <I>Haemophilus</I> b Conjugate Vaccine and Guidance for Use in Infants and Children*. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5739a5.htm (accessed 2023-09-20).

(50) *Federal Advisory Committee Votes to Include Newly Licensed Pentacel® Vaccine in Vaccines for Children Program*. https://www.news.sanofi.us/press-releases?item=137050 (accessed 2023-08-08).

(51) Dhillon, S.; Keam, S. J. DTaP-IPV/Hib Vaccine (Pentacel). *Paediatr Drugs* **2008**, *10* (6), 405–416. https://doi.org/10.2165/0148581-200810060-00008.

(52) Wen, S., Feng, D., Chen, D., Yang, L., & Xu, Z. (2020). Molecular epidemiology and evolution of *Haemophilus influenzae*. *Infection, Genetics and Evolution*, *80*, 104205. https://doi.org/10.1016/j.meegid.2020.104205

(53) Research C for BE and. ActHIB. *FDA*. Published online October 14, 2022. Accessed July 22, 2024. https://www.fda.gov/vaccines-blood-biologics/vaccines/acthib

(54) Research C for BE and. VAXELIS. *FDA*. Published online April 13, 2023. Accessed July 22, 2024. https://www.fda.gov/vaccines-blood-biologics/vaxelis

(55) Research C for BE and. Pentacel. *FDA*. Published online February 28, 2023. Accessed July 22, 2024. https://www.fda.gov/vaccines-blood-biologics/vaccines/pentacel

(56) Behrouzi A, Vaziri F, Rahimi-Jamnani F, et al. Vaccine Candidates against Nontypeable Haemophilus influenzae: a Review. *Iran Biomed J*. 2017;21(2):69-76. doi:10.18869/acadpub.ibj.21.2.69