Vaccine and vaccination as a part of human life: in view of Covid-19

KB. Megha, PV. Mohanan*

Toxicology Division, Biomedical Technology Wing,
Sree Chitra Tirunal Institute for Medical Sciences and Technology (Govt. of India),
Poojapura, Trivandrum 695 012, Kerala, India.

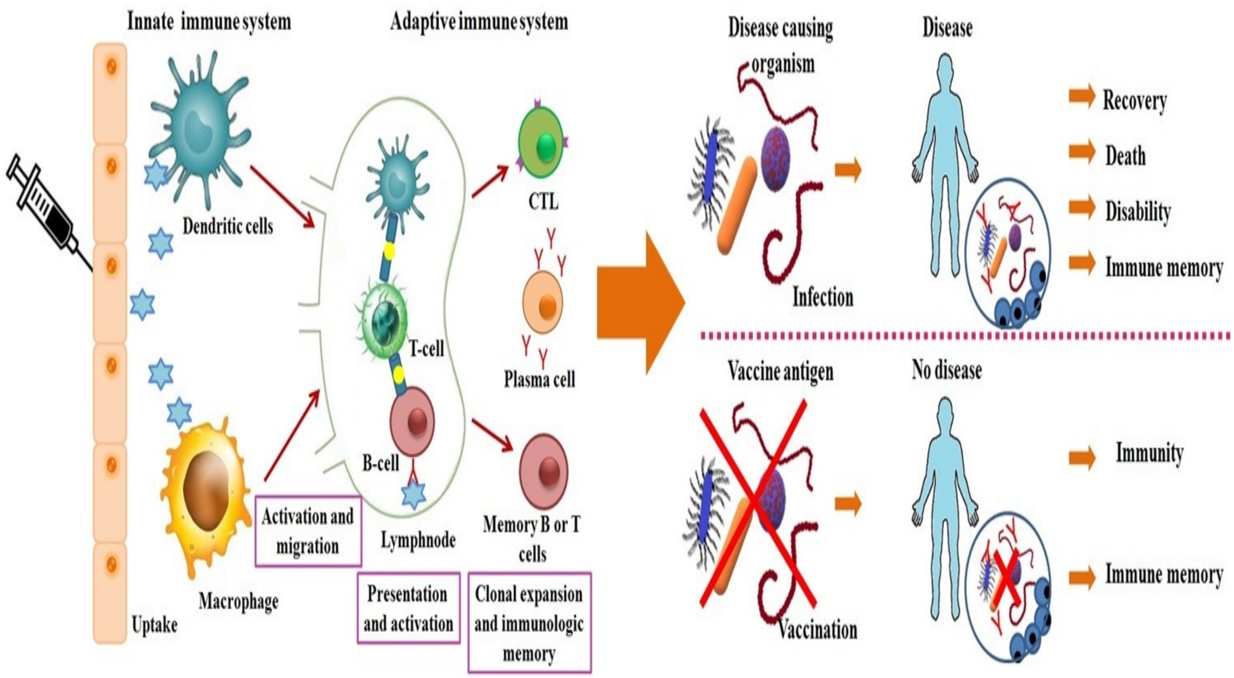
*Corresponding author: Dr. P.V. Mohanan

Email:mohanpv10@gmail.com, mohanpv@sctimst.ac.in
Phone: 91-471-2520266, Fax: 91-471-2341814

Abstract
Vaccination created a great breakthrough towards the improvement to the global health. The development of vaccine and their practice made a substantial decrease and control in infectious diseases. The abundance and emergence of new vaccines has facilitated targeting populations to alleviate and eliminate contagious pathogens from their innate reservoir. However, along with the infections like malaria and human immunodeficiency virus (HIV), effective immunisation remains obscure and imparts a great challenge unto the realm of science. The vaccines developed after utilizing plant based system supported technology comprises the incorporation of the preferred genes to express the specific protein (antigen) for a particular disease condition into the genome of plant tissues using several techniques. Though plant-supported vaccines propose several benefits to the vaccine industry, still there remain challenges that limit the rate of effective production of vaccines of this third-generation. A novel Corona virus SARS-CoV-2 reason for causing Corona virus diseases 2019 (COVID-19) crashed the human population and rapidly spread round the world within the half of 2020 created a worldwide epidemic. The need for establishing a protected and compelling COVID-19 immunization is a global requirement to end this pandemic. Even though there exist lot of limitations, continuous efforts has put forward so as to develop highly competent and effective vaccine for many human and animal linked diseases due to its unlimited prospective. This review article focus on the historical outlook and the development of the vaccine as it is a crucial area of research where the life of the human is saved from various potential diseases.

Keywords: COVID-19, vaccine, immunization, herd immunity, transmission

Graphical abstract



1. Introduction

The term 'vaccine' was originated from Latin '*Variolae vaccinae*' successively after Edward Jenner demonstrated the prevention of cowpox in 1798. Vaccines are considered as a biological preparation that has the ability to enhance immunity, for disease prevention (prophylactic vaccine) or for treatment (therapeutic vaccine). Immunization is considered as the ultimate achievement to public health care system during 20th century, according to the Centers for Disease Control and Prevention (CDC) [1]. The vaccines are normally administered in their liquid form either by injection, rather oral or intra-nasal routes. Immunity refers to the capability of the human body to distinguish and tolerate the indigenous material as self to the body and to recognize and eliminate the foreign material as non-self. The ability to discriminate microbes as foreign substance by the immune system provides protection towards infectious diseases. Generally immunity indicated by the occurrence of antibody to a specific organism or closely related organism. Active and passive are the two basic mechanisms to acquire immunity. The active immunity provides protection that are produced by the persons own immune system. Usually this type of immunity lasts for many years or for a lifetime. Passive immunity enables effective protection by products produced from animals or humans and transferred to another human usually by injection but wanes within weeks or months.

Vaccines are usually effective but rarely provide permanent or complete protection from infectious diseases [2]. They generally comprises of either the whole/entire disease causing organism or their constituents that can induce antigenic response. They are produced by attenuation by growing the disease causing organism under sub optimal conditions which lessen their disease causing ability. The entire pathogenic organisms were inactivated using thermal or chemical methods. Some vaccines are developed from components of pathogens such as nucleic acid or from specific proteins or polysaccharides. Another type of vaccine is inactivated toxins from toxin producing microbes. The effectiveness of the polysaccharide vaccine in young children was increased using conjugation of polysaccharides with proteins. Vaccination made greatest advances in public health that impacted for the human permanence and healthiness.

2. Corona virus disease 2019 (COVID-2019) pandemic

The novel beta-corona virus family member SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) is the causative for COVID-19 pandemic. The disease mainly spreads through the respiratory droplets from the infected person. As of 15 December 2020, 70 million cumulative cases have been reported globally with 1.6 million deaths as reported by the World Health Organization [3]. Until now, no particular treatment strategy has been demonstrated to be useful against the COVID-19. The mutation that occurs to the viral genome leads to the antigenic shift and drift, and it keeps spreading from one population to the other. These susceptible mutations ultimately generate unusual subtypes that let the virus to get away from the immune system even after the vaccine administration [4]. Scientists across the world are joining hands to introduce an innovative approach to remodel drugs, expand vaccines or devices to hinder/obstruct the progression of this devastating pandemic. It is therefore much anticipated that the vaccine should be appropriate for all age groups including pregnant ladies, and lactating mother preferably ought to give a quick onset of defense with a single dose and should persist the protection for at least one year of administration.

Vaccines are generally inherent in a complex multi-scale system which includes clinical, biological, behavioral, social, environmental and economical relationships [5]. The action of vaccines is by making our immune system more organized and co-ordinate to identify and remember the foreign pathogenic microbes. Thereby vaccination aids in the generation and storage of antigenic specific memory cells. In future, the frequent susceptibility to the actual disease can make our immune system quickly respond to opsonize the bacteria or viruses more effectively. The benefits of vaccination, one of the most economic public health interventions, have not wholly reached target beneficiaries in many low and middle income countries [6]. According to WHO, vaccination imparts an important and successful means to prevent infectious diseases. Due to infectious disease the mortality rate among children can be reduced by the massive immunization plan that mainly depends on the accessibility of the highly economic and immunologically protective vaccines against most dreadful infectious conditions [7]. There are so many strategies and assured properties associated with the making of a vaccine (figure 1).

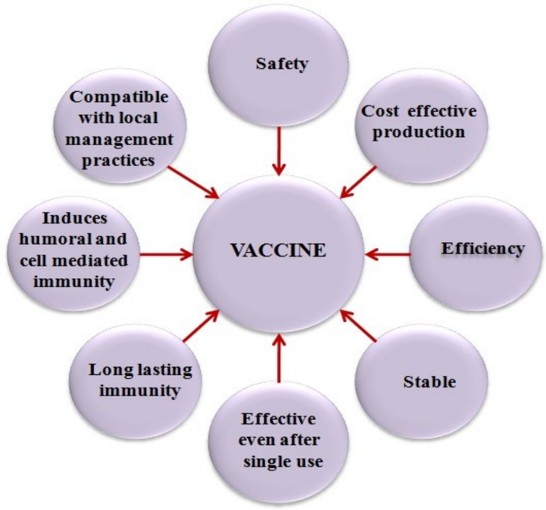


Figure 1: Characteristics of a vaccine

Vaccines are generally unique and are administered to large groups of typically healthy individuals which besides include infants and children too. It is really unsatisfactory that when vaccine itself can induce side effects which creates burden even though the illness itself can exhibit severe fatal side effects. The vaccination should provide a much economical approach thereby reduce childhood disease burden, rather compared with clean water and improved sanitation facility that definitely can reduce transmission of disease but require time consuming and expensive infrastructure investments [8].

3. Vaccine against Covid-19: Present status of development

Covid-19 is an ailment brought about by the serious intense respiratory syndrome caused by corona virus 2 (SARS-CoV-2). SARS-CoV-2 was first recognized in the city of Wuhan, China, in December 2019, after a group of patients with pneumonia of obscure reason were accounted for to the World Health Organization (WHO). The episode was pronounced a general wellbeing crisis of global worry on 30th January 2020, and the malady brought about by SARS-CoV-2 was authoritatively named COVID-19 on eleventh February 2020. Subsequent to surveying the flare-up and following transmission of the infection in numerous different nations around the world, on eleventh March 2020 the WHO declared COVID-19 a pandemic. This implies the infection has spread around the world, and it is the first time that a corona virus has led to a pandemic. The virus mainly spread through respiratory droplets from the infected personals. Corona viruses are structurally pleomorphic, enveloped virus attributed with fringes of projections comprising of S protein on their outer surface. Their genome is operationally functioned with positive sense of ssRNA complexed with nucleocapsid (N) protein which forms helical nucleocapsids. The four structural proteins present in SARS-COV2 virus are spike (S), nucleocapsid (N), envelope (E) and membrane (M) proteins encoded at 3'end of the viral genome (figure 2) [9].

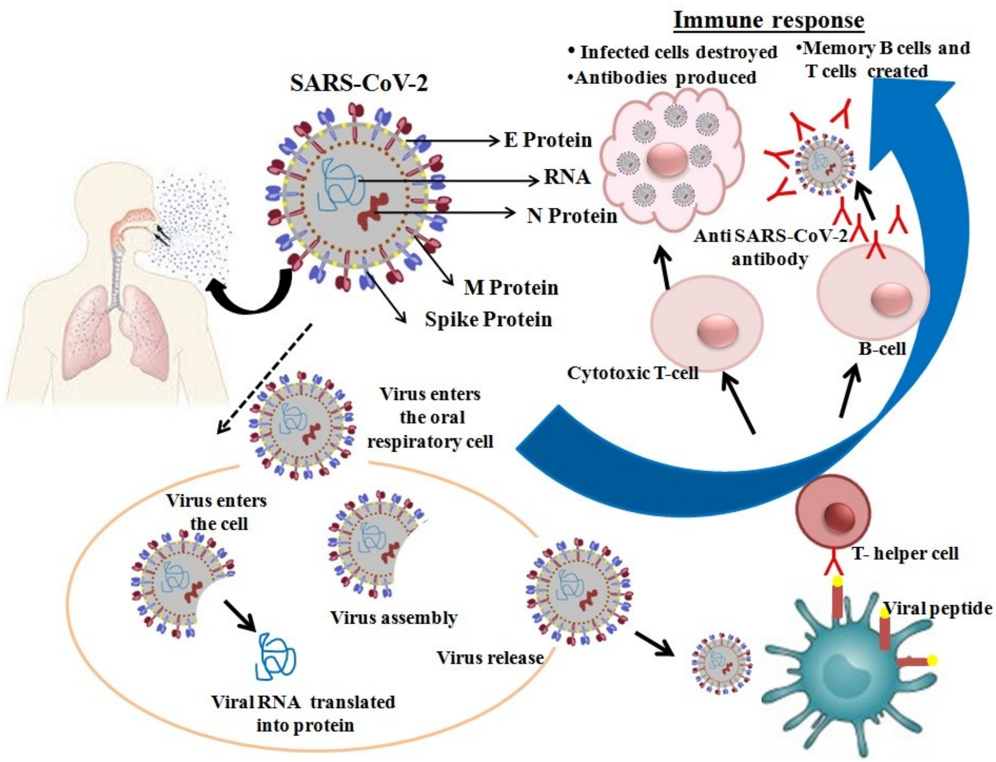


Figure 2: Transmission of SARS-CoV-2 causing COVID-19.

The urgent need for vaccine development against SARS-CoV2 virus was due to the pandemic announcement of COVID-19 disease by WHO. The succeeding widespread mortalities and morbidities in most countries alarmed researchers and scientists to stood together to eradicate this deadly pandemic. Even tough for normal development of vaccine, it would take more than 10 years instant but the vaccine against SARS- COV2 goes at really quick pace making a breakthrough in development of vaccine through several research institutions and vaccine manufactures to put an end to the fast spreading pandemic. Due to this epidemic situation, the whole process of vaccine development were shortened together with the clinical trial phase too, so that the vaccine has to get fast tracked within 16-18 month duration without reducing its efficiency and efficacy. The simultaneous promotion of many vaccines is expected to occur by the beginning of 2021. There are 164 candidate vaccines under development process, of which 24 vaccines are in highly advanced stages of development [10].

The most challenging research task obtained in the laboratory for this potential vaccine is evidence of clinical safety and efficacy. The most challenging research task obtained in the laboratory for this potential vaccine is evidence of clinical safety and efficacy. According to the latest report from Ministry of Health and family welfare, Govt. of India on December 2020, the SARS-CoV2 vaccine production landscape in the world reveals that there are 58 candidates under clinical trial vaccines and an additional 203 vaccines in different early stages of development [11, 12]. There are many institutions that are committed in developing the Covid-19 vaccine, including many academic/research and vaccine manufacturing companies in India. In association with Oxford University and Astra Zeneca, the Serum

Institute of India is conducting a Phase 3 trial of ChAdOx1 nCoV-19 vaccine in around 1600 safe subjects aged 55 years. Once the phase 3 trial with a single dose of vaccine has been successfully completed the company will commence mass manufacturing [10].The first vaccine is developed in collaboration with National Institute of Virology, Pune (Indian Council of Medical Research) and previously in process of developing an inactivated vaccine called 'Covaxin'. This vaccine has successfully completed the trials in animal models such as mice, guinea pigs and rabbits and remarkably exhibited strong immunological response of the inactivated vaccine; apparently Phase 1-2 clinical trials has already started in several institutions in India. In the development of every new vaccine, including SARS-CoV2 has to face several challenges.

The rapid drift and several genomic alterations undergone in the new SARS-CoV2 virus have been identified. The safety and effectiveness of the vaccine can only be reviewed when a huge number of factors are considered in various ethnic and geographical locations. Regardless of the availability of the safe and effective vaccine, the impartial distribution to the most vulnerable will be the foremost challenge. The next principal challenge to be fulfilled is the logistics of procurement, their safe dispensing, efficient storage depot, unbroken cold chain facility and their administration at community level. For the perfect accomplishment of COVID-19 vaccine, it should be able to apply for humans in routine, so that it should disrupt the spreading of pandemic from person to person and also defend against both clinical diseases as well as viral transmission.

4. Importance of immunization and vaccination

Vaccines can protect our existence and avoid diseases and disabilities, moreover signify good worth between health mediations. Due to the progression in medical sciences, vaccination protected children from many infectious and contagious diseases. One of the greatest attainments is the eradication of polio. Immunization assists getting protected from dreadful ailments and furthermore prevents spreading of the sickness. In order to introduce immunological memory and thereby defend against the effects of infection, immunization is an approach of stimulating the host's defense in case of a particular pathogen.

4.1 Children

Immunization is the principle health intervention used to reduce child mortality. Low paces of immunization not just leave many young children at danger for different serious vaccine-preventable diseases yet additionally serve as an indicator of inadequacies in getting other preventive medical care administrations [13]. Due to the significance of immunization, it is vital that the executions of the program against vaccine preventable illnesses are checked intently. Also, one confronts of this program is defaulting immunization – neglecting to receive the recommended vaccination at the suggested time. The implementation of childhood vaccination by World Health Organization's (WHO) reduced childhood mortality. They included the early day's vaccine series including DTP, MMR, Hib, hepatitis B, varicella and polio vaccines.

In vaccinated children the long term sequelae related with certain childhood illness such as neurological impairments, hearing loss and various other physical disabilities can be avoided. In children, constant or recurrent infections in early childhood can lead to poor stunted growth, which in turn adversely affects the adult health, cognitive capacity and finally facing the economic productivity [14-16]. For example, in children due to measles infection will wipe out the already existing antibodies to different pathogens in months following infection period and made their health state more vulnerable, prone to multiple infections and possibly leads to death.

4.2 Adults

In adults, immunization protects themselves from numerous acute contagious diseases and their associated complication which varies from inherited rubella syndrome to Hepatitis B and malignancy connected with Human papilloma virus. The elder adults were advised to receive yearly influenza and pneumococcal polysaccharide vaccines, with decennial tetanus diphtheria boosters recommended for all adults. For some individuals additional vaccines are also suggested for their precise occupational, behavioral, or travel exposures as well as for several chronic disease conditions. There exist several challenging concerns associated with vaccine safety and acceptance, vaccine cost and investment, constancy and safety measures of vaccine supply, approaches for accomplishing more adolescents and adults, and enhanced awareness for pandemics of influenza. The safety of the vaccine is considered to be a major public concern and with this regard the requirement for vaccine-induced protection have been related to recent outbreaks of vaccine-preventable diseases such as measles [17] and higher rates of exemptions from school-entry vaccine requirements [18,19]. In the H1N1 influenza pandemic reported in 2009-2010, vaccine safety monitoring was the foremost preference [20, 21].

5. History of vaccine

Till now, vaccination is regarded as the most expected efficient as well as cost effective interventions for prophylactic precaution against numerous infectious or contagious diseases [22]. During 15th century, the first evidence of purposeful attempt made to induce immunity was accomplished by the Chinese and Turks. Various reports supported and suggested that dried crusts obtained from the small pox pustules were either inserted into small scratches in the skin or inhaled into nostrils to attain immunity against smallpox by a technique called variolation. The positive effect of variolation was examined in 1718 by Lady Mary Wortley Montagu on their native resident population and also imparted the technique in their children. Edward Jenner, the English physician is considered as the founder of vaccinology as he notably improved the technique of variolation and tested by him and observed the fact that milkmaids were immune to the fatal disease small pox after exposure to cowpox infection. Later Jenner performed the clinical trials and broadcast the result outcome to the world [23, 24]. By the end of 1980, the worldwide eradication of smallpox was attained by the introduction of variolation in 17th century and followed by the concerted vaccination programs made it a complete success [25].

However, despite of the remarkable achievements of Jenner and due to lack of sufficient knowledge about microbiology, it took 8 decades to pass for the next step towards the history of vaccine which happened in the experimental laboratory of Louis Pasteur. The term 'vaccine' was coined by Louis Pasteur towards the respect of Jenner's significant insight. The concept of attenuation was most specifically formulated by Pasteur and his colleagues confirmed its effectiveness first with the diarrheal disease in chickens caused by *Pasteurella multocida* [26], the anthrax an infectious bacterial disease in sheep and most horrible rabies virus in animals and humans [27]. During the last decade of nineteenth century, there was a tremendous development in vaccine technology. The key development methods to inactivate whole bacteria for the making of vaccine, the antitoxin production and the understanding of serum components (antibodies) capable of neutralizing toxins or inhibiting bacterial growth led a great breakthrough in the history of vaccine production. Later, during the last years of 19^{th} and the initial years of the 20^{th} century, inactivated whole vaccine for plague [28], typhoid [29] and cholera [30] were developed and examined.

The eminent workers responsible for unraveling, and developing the 'concept of serum antibodies' were Emil von Behring, Shibasaburo Kitasato, Alexandre Yersin, Almworth Wright, Emile Roux, and Paul Ehrlich. During 1923, Alexander Glenny and Barbara Hopkins demonstrated that, due to the action of formalin the diphtheria toxin can be converted into a toxoid [31]. During the initial years of the twentieth century, Calmette and Guerin introduced the more effective technique of serial cultivation of a pathogen by *in vitro* or in unnatural hosts and they passaged 230 times bovine tuberculosis bacteria in artificial media containing bile to achieve an attenuated strain to defend against human tuberculosis-BCG vaccine [32].

In 1926, a 'killed vaccine' was developed for whooping cough using whole *Bordetella pertussis* and followed in 1927 led to the development of tetanus toxoid and in late 1940's tetanus toxoid was combined with diphtheria and pertussis (DTP) as children vaccine. In the era of 1950's put forward the expansion of poliovirus vaccine, in which both an inactivated vaccine and live vaccine were developed. The former by Jonas stalk [33] in 1954 and latter by Alfred Sabin [34] (1961), an oral polio vaccine were easy to deliver and eliminated the spread of polio. In 1960's, three attenuated vaccine were developed- for measles (1963) by Samuel Katz and John Enders [35], for mumps (1967) by Maurice Hilleman [36], and for rubella virus (1970) by various workers [37-39]. While in 1971 MMR single vaccine were developed after combining measles, mumps, and rubella vaccines. In 1964 the killed rabies vaccine was developed by administering in the abdomen with 30 painful shots and finally in 1980 a newer version was introduced with five shots to be given in arm to protect from fatal rabies. The 1980 witnessed the birth of two important approaches for vaccine development by application of conjugation in bacterial capsular polysaccharides to proteins and by means of genetic engineering. The conjugate vaccines was developed using a part of the bacterial cell wall to develop a safe antigen for pneumococcal, meningococcal, and Haemophilus influenzae type b (Hib). These vaccines protected from infections in blood, life threatening meningitis, and a variety of pneumonia. Towards the last decades of the 20th century, Sellards and Laigret [40] serially passaged yellow fever virus in mice and later by Theiler and Smith [41] more successfully attenuated yellow fever virus in chicken embryo tissues.

The initial vaccine emerged through genetic engineering was against hepatitis B virus which was licensed in 1986 by an antigen cloned rather grown and hepatitis A was developed in 1990 as a killed vaccine. Three primary vaccines were developed by reassortment: live and inactivated influenza [42, 43] as well as one of the two rotavirus vaccines [44]. The chickenpox vaccine for children was licensed in 1995 and the first DTaP (1996) vaccine got approved by combining merely parts or fractions of *B. pertussis* organism with diphtheria and tetanus which considerably diminished pertussis induced death following DTP vaccination. By the development of influenza vaccine in 2000 made a remarkable reduction in premature death. The foremost therapeutic vaccine derived from blood cell infusions were approved in 2010 for prostate cancer. The discovery of Hepatitis C virus by the Nobel Laureates of 2020 directed towards a landmark achievement in the current battle against viral diseases. This will allow the rapid development of antiviral drugs and vaccines directed against hepatitis C which greatly improves global health and hoping for the complete eradication of the virus from the world population [45]. By employing the novel reverse vaccinology, a multi-component recombinant vaccine was developed and commercialized against meningococcus in 2013. The much advancement in structural biology and reverse vaccinology could be able to describe more effective antigens, while systems biology probably resolve to understand of how modification in the expression level of specific genes associate with protecting immune responses[46-49]. Therefore, the approach enhances our knowledge of how to induce specific immune responses and, thus, the development of highly specific and potent novel vaccines.

6. Classification of vaccines

The progress and improvement of vaccines against many diseases causing organism denotes a key innovation in the history of modern medicine. The conventional vaccine strategy has relied on basically two types of microbial compositions of which one to generate vaccine for immunization or rather to produce a protective immune response. During the initial phase, living infectious microbes which are prepared in their weaker stage that are incapable to induce disease was used as vaccine. In later stage of vaccine preparations, inert, inactivated or subunit groups of antigens were used. However, with the recent and considerable progress in the field of molecular biology contributed much advanced alternative strategies that enhance the development of vaccines. There exist numerous approaches to design and develop vaccines against various microbes. They mainly depend on the fundamental information available about the microbes including the mechanism of infection in the host and the immune response exhibited by them. Following are some of the types of the vaccines based on their course of development.

- Live-attenuated vaccines
- Inactivated vaccines
- Recombinant subunit vaccines
- DNA vaccines
- Conjugate vaccines
- Toxoid vaccines

6.1 Live attenuated vaccine

Live-attenuated vaccine contain of a newly adapted version of pathogenic microorganisms that has been attenuated or made weak by culturing it in the *in vitro* conditions as a result they has lost its pathogenicity (figure 3). Mainly they are accomplished by serially growing the pathogenic microbes in a deviant host such as by *in vitro* tissue culture technique, embryonated eggs, and *in vivo* animals models used for multiple passages or generations. Majority of the traditional vaccines that are currently administered in humans and animals are raised in an unnatural host. The vaccine developed against 17D strain of yellow fever was developed by passaging the virus in mice and subsequently in chick embryos. In case of polio vaccine, viruses were continuously passaged in monkey kidney cells and afterward in chick embryo and measles in chick embryo fibroblast [50].

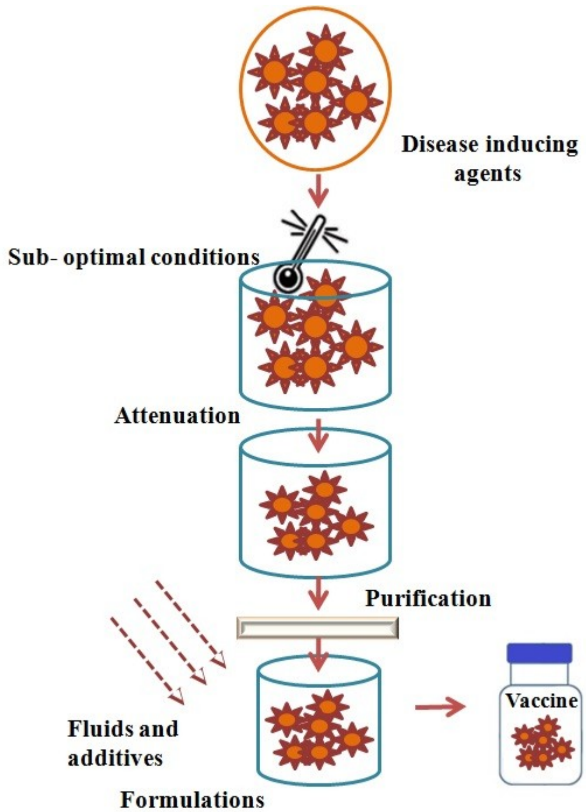


Figure 3: Live attenuated vaccine

The live-attenuated vaccines were prepared after attenuating the viral strain thereby making them completely devoid to induce pathogenicity or without virulence but are highly competent to trigger a protective immunological response. The examples of presently available live attenuated vaccines against viral infections comprises of cowpox, MMR, influenza, oral polio vaccine and yellow fever. The vaccines comprise for BCG, tuberculosis and oral typhoid is live-attenuated bacterial vaccine. One of the major advantages of the live-attenuated vaccine for virus is relatively easy to develop but more complicated to generate for bacteria due to the presence of several genes. However, utilizing the benefits of recombinant DNA technology might help in the removal of several key genes. The mechanism of live-attenuated vaccine is similar to that of natural infection without causing any infection but elicit a better immunological response conferring immunity for lifelong with one or two doses. One of the major disadvantages of the attenuated vaccine is that the reversion of virulence after secondary mutation which might lead to disease progression. People who are immune-compromised, with weak or damaged immune system and in pregnancy cannot receive the live vaccine. Another drawback of the live-attenuated vaccine is that, it requires strong cooling system to stay them effective and highly skilled health care workers which limits their widespread use. It would create extra cost while conducting a massive immunization program.

6.2 Inactivated vaccines

The inactivation of the antigen is typically done by using heat or chemicals like formaldehyde or by radiation (Figure 4). After the chemical exposure, the multiplication capability of the pathogen was hindered but has to retain the structural immunogenic intactness as that of its original natural or basic appearance. It is very essential to maintain the structural integrity of antigenic epitopes of surface antigens. Therefore, inactivated whole organism vaccine ensures the protection by directly evoking the humoral and cell mediated immunological response against the pathogen.

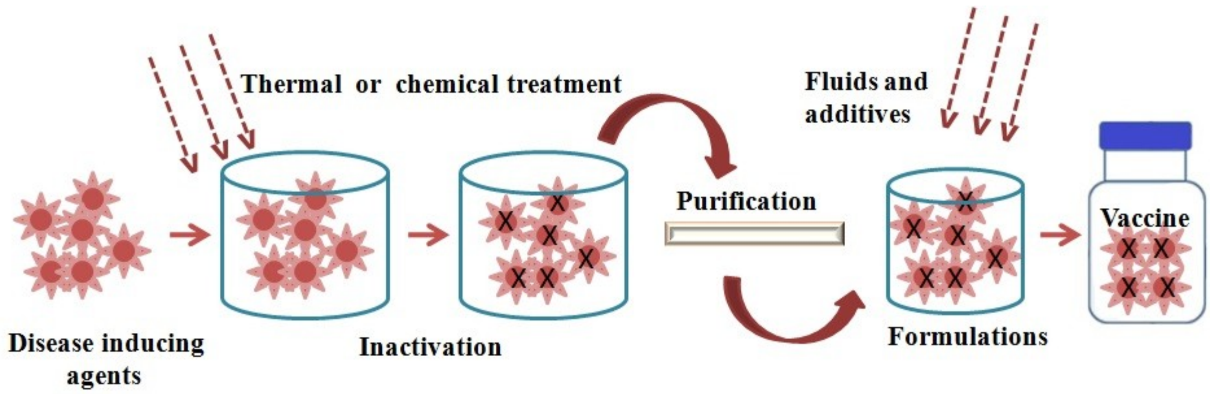


Figure 4: Inactivated vaccine

Examples of presently accessible inactivated vaccines for viruses are polio, influenza, hepatitis A and rabies. The vaccines for pertussis, typhoid, plague, and for cholera comes under the category of whole inactivated bacterial vaccine. The greater advantages of inactivated vaccine than live vaccine are that, they are further steady and safer as it contains deceased microbes that cannot mutate back or revert to their pathogenic/virulent state. These vaccines generally do not require cold storage facility as well as shifting in freeze- dried form thus making them much more economical and can be made easily accessible to the people. Most inactivated vaccine induces weaker immunological responses than live vaccine. Therefore they require boosters of multiple doses to maintain their potential immunological response. Moreover, too much treatment for inactivation of pathogen might devastate immunogenicity, while inadequate treatment exposure can build infectious virus capable of inducing diseases. Also there exists a risk towards allergic reactions due to the occurrence of unrelated structural particles of microbes in the body. An assessment of live-attenuated and inactivated whole virus vaccine is illustrated in Table 1.

Table 1: Comparison between live attenuated and inactivated whole virus vaccine

Features	Live	Dead
Dose	Low	High
No. of doses	Single	Multiple
Need for adjuvant	No	yes
Duration of immunity	Many years	short
Antibody response	IgG	IgA IgG
Cell mediated immunity	Good	Poor
Reversion to virulence	Possible	Not possible

6.3 Recombinant subunit vaccine

The immense progress achieved in biotechnology has made to recognize the peptide site encircling the most important and potential antigenic sites of viral antigens. Therefore, as an alternative of using the whole pathogenic microbe for immunization only the major subunits components of antigens which are more prevalent to induce immunologic response are sorted and used as vaccine (figure 5). Only the specific antigenic determinants of antigens were used for the development of this type of vaccines so that it significantly lowers the adverse risks associated and the chances of virulence reversal could be completely abolished. The vaccine against influenza virus *Haemophilus influenzae* A and B and hepatitis B surface antigen are examples of subunit vaccine.

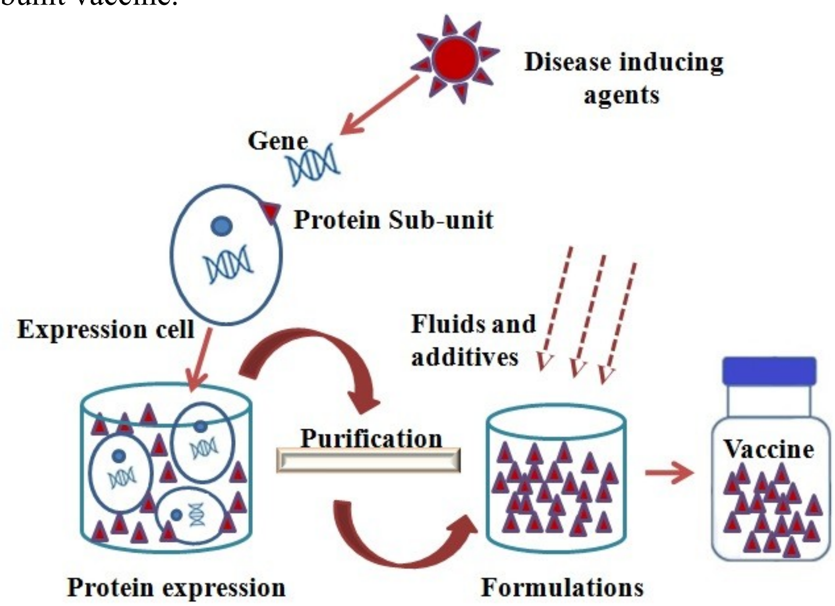


Figure 5: Recombinant subunit vaccine

6.4 DNA vaccines

One of the greatest achievements in the vaccine technology is the development of the DNA vaccines. The DNA vaccine development requires the direct placement of a plasmid into the appropriate tissue site holding entire gene expression cassette that encodes alone with unique antigens to which the necessary immune response is essential [51]. Immunization using DNA helps in stimulating effectively both the humoral and cellular immune response to antigenic proteins. Genes encoding specific antigens are expressed, and their gene products would undergo glycosylation and alterations in post-translational modifications comparable to natural infections. It has been practiced for many years by utilizing the genetic material to transport the genes for several therapeutic purposes. (Figure6) [52].

DNA vaccines are considered as the third generation vaccine that underwent the immunization process to a new stage of technology. The usage of DNA vaccine encodes almost the entire gene for all the significant antigens. DNA vaccine for pathogenic microorganism would induce a powerful antibody response towards antigen released by the cells. The main advantage of the DNA vaccine is that it cannot induce as they consist of only copies of few genes of pathogens and not the whole microbe. Moreover, DNA vaccines are comparatively simple and less expensive to plan and develop. This vaccine can be administered directly into the body using a needle or needleless device by applying high-pressure to enter the DNA coated microscopic gold particles directly into the cells. The DNA naked vaccine against herpes and influenza virus were tested in humans.

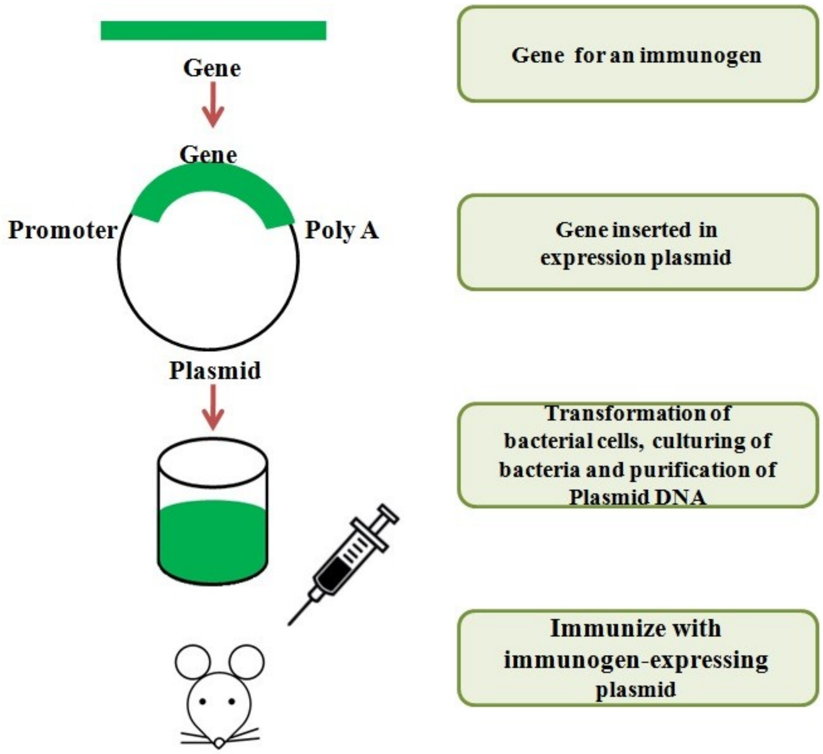


Figure 6: Development of DNA vaccine

6.5 Conjugate vaccine

Some pathogenic bacteria possess a polysaccharide outer envelope and generally mimics similar to human polysaccharides. So infant's immature immune system and also in younger children could not recognize or respond to the encountered infection [53]. The conjugate vaccines were developed by chemically attaching the polysaccharide to a strong T-cell stimulating antigen such as tetanus and diphtheria toxoids (figure 7). This leads to the enhanced stimulation of the immature immune system against the linked protein and polysaccharide providing sufficient protection against disease causing organism. Examples of the conjugate vaccines include influenza vaccine (HiB), for pneumococcal and meningococcal.

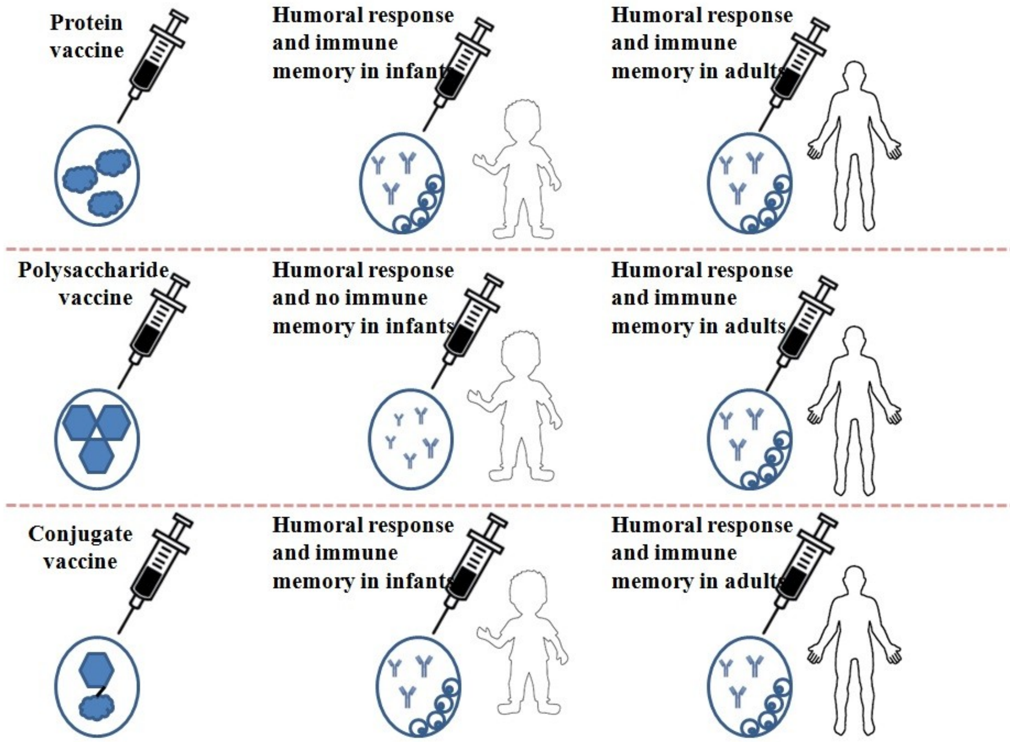


Figure 7: Development of conjugate vaccine

6.6 Toxoid vaccines

Bacterial toxins are generally termed as toxoids secreted as exotoxins by pathogenic microbes which are able to produce disease symptoms after getting into our body. Toxoid vaccines are prepared from purified bacterial exotoxin. By the application of heat or chemical treatment the toxicity of the purified exotoxins are made suppressed or inactivated without harming the capability to trigger immunogenicity. Such detoxified exotoxins can be used as vaccines. The immunization with toxoids produces anti-toxoid antibodies that comprise the capability to bind with toxin and to neutralize the harmful effects of normal exotoxin. The procedure for the preparation of toxoid vaccines was strictly regulated in order to attain the detoxification or inactivation devoid of extreme structural alteration to the antigenic epitopes (figure 8). The best examples for toxoid vaccine were against diphtheria and tetanus.

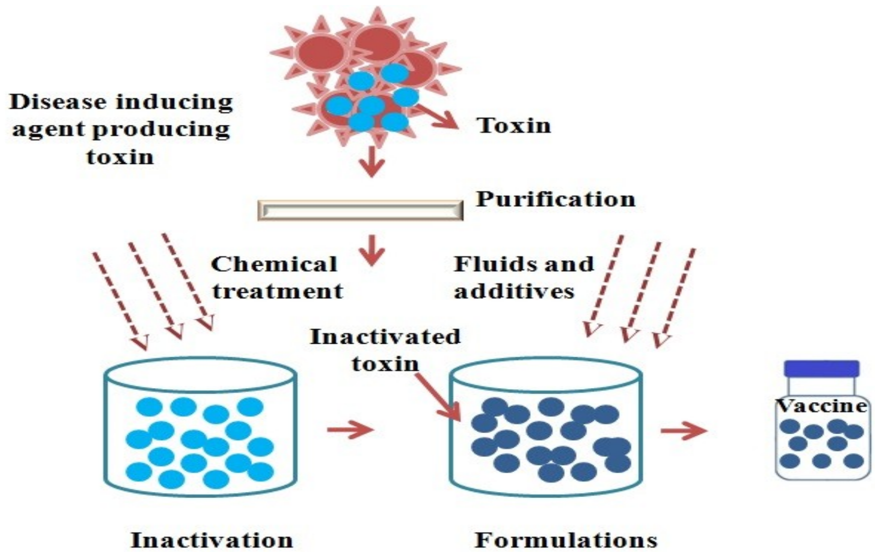


Figure 8: Toxoid vaccine

7. Role of adjuvant in vaccine

The chemical agents supplemented along with vaccine formulation to maintain and induce suitable protective immunological response against infections are the adjuvants. Adjuvant enhances the immunogenicity of the antigen, without acting themselves as antigen. The practice of using right adjuvant helps in vaccine formulation to trigger selectively an adaptive or innate immunity to achieve antigen specific immune responses. Thus adjuvant assists the proteins to turn into more effective vaccine by inducing protective, strong and durable immune response. The approved and licensed vaccine adjuvants are listed in table 2 [54].

Table 2: Approved and licensed vaccine adjuvants for human use.

Adjuvant	Year	Class	Description
Alum	1926	Mineral salt	Improves HI and Th2 response, used in more than 80% human vaccine
MF59	1997	Oil in water emulsion	Improves HI and CMI response, used in influenza vaccines
Virosomes	2000	Liposome	Improves HI and CMI responses, used in influenza and hepatitis A vaccine
AS03	2009	Oil in water emulsion	Improves HI and CMI responses, used in H1N1 pandemic
AS04		Alum- adsorbed TLR4 agonist	Improves HI and CMI response, used for HPV and HBV vaccines

The importance of the adjuvant is growing significantly with aging of the population. According to many experts, adjuvants would be an important component for widespread usage of vaccine in entire population since they can promote the immune response in vaccinated old people. Most commonly used adjuvants for human vaccines are aluminum salts, eliciting a complex mechanism to favor antibody induction. Currently, new forms of adjuvants have been proposed for different vaccines which mainly includes the bacterial products [heat labile enterotoxin B (LTB) subunit, cholera toxin B (CTB) subunit], viral products (viral-like particles), plant derived products (saponin derivative), oil-based emulsions, biodegradable particles (liposomes), synthetic and molecular adjuvants [55]. But safety is the primary consideration of the proposed adjuvants. Hence, while preserving the efficacy of an adjuvant it is essential to introduce a method to eliminate the reactive actions of an adjuvant. For the efficient use of adjuvants, they can be combined with particular route of delivery such as transcutaneous or intranasal, oral immunization for stimulating mucosal immunity.

Although diverse in composition and the capacity in stimulating immune system; virosomes, liposomes and ISCOMS can be assembled around the idea of a lipid vesicle to which both antigenic targets and immunomodulatory molecules can be substituted [56]. The ionic charge can be modified to requirements based on their lipid composition and production system, physical properties, size of the vesicle. The above mentioned criteria affect the capability of the delivery system to develop depot, which gets attached to antigen-presenting cells (APCs) and the antigen that loads to the delivery system [56]. In several means, these adjuvants enclosed with lipid and associated proteins resemble naturally enveloped bacteria or viruses. The virus-like particles (VLP) take this process a step advanced, where the lipids and antigenic target derives the pathogen directly thus arbitrating a delivery vehicle that be similar to a pathogen lacking the genes required to initiate the infections [57]. The accurate and acceptable combination of antigens and adjuvant concentration to optimize is a critical task for the subsequent downstream adaptive immune response in the development of any novel vaccine.

8. Vaccine production from plants

Vaccines stimulate the production of antibodies in both animals and humans to provide immune protection from severe disease conditions [58]. The non-availability of vaccines for lethal disease treatments has created problems and made directed the complete global focus towards developing vaccines that are easier, safer, and more efficient. Normally, there exist three types of vaccine production procedures particularly based on cells, eggs and developed using investigational-manufacturing systems. The most prevalent, influenza vaccine developed in 9 to 12 days old embryonated eggs [59, 60]. It is the most conventional method used over 6 decades and involved the injection of viral particle into the eggs and incubated further for the replication of viral particle. The antigen thus obtained after the purification procedure of the eggs contains vaccine viral particles which undergoes further procedures to attain the final product. But choosing the most suitable influenza virus strains with the purpose to get replicated for vaccine production is a great task. There remains a key restriction in this process as not all strains of influenza virus are potent to get replicated in embryonated eggs, therefore adversely affects the quantity of vaccine produced in the eggs [59].Latest advances in the methods of molecular biology have greatly supported in the expansion of new approaches for the development of subunit vaccine containing proteins from pathogenic bacteria, viruses or parasites.

The endeavor to develop plant vaccines in 1989 was made by Hiatt and coworkers [61]. Dr. Arntzen and his peers established the principle of using ‘transgenic plants’ for the manufacturing and delivery of subunit vaccines and well demonstrated that the drawbacks of conventional vaccines could be solved by this manufacturing definition. By the expression of surface protein antigen of *Streptococcus mutants* in tobacco plants, the initial progress of subunit vaccine was developed by them. Later they also demonstrated the production of hepatitis B and heat- labile toxin B in potato tubers and in potato plants. It was approved for the initial time that significant immunogenic responses can be achieved by means of edible vaccines in 1998 [61] according to National Institute of Allergy and Infectious Diseases (NIAID) by utilizing the perception that plants as a bioreactor. Subsequently, the plant based vaccines are generally easy to handle, complicated storage facility not requisite, production cost found to be economical, easy to increase the large scale production and therefore this technique can contribute to much faster and cheaper alternative for vaccine production [62-66]. Additionally, production of vaccines from the above mentioned method contributes an appropriate, needle free and easy administrable form of vaccines [65-67]. Plants have developed into a more precise promising system to express and produce an extensive range of functionally dynamic pharmaceutical proteins [68] of great significance to health care industry with benefit s over conventional bioreactors.

The common plant bioreactors thus used are tomato, tobacco, potato, rice and corn. So far, there are many transgenic plants used for the production of different types of vaccines, which includes vaccines of four different types mainly bacterial, viral, parasitic and immune-contraceptive vaccines [65] .Till now there are numerous plant originated vaccines produced but many at present are in the clinical trial phase itself due to the delay in getting approval. Generally two vaccines namely plant derived scFVmAB used in the production of a 'recombinant HBV' vaccine in Cuba and New castle disease virus (NDV) vaccine for poultry got license from US Department of Agriculture (USDA) [69]. But owing to the fact that plant based vaccines comes under the category of genetically modified crops there is no plant derived vaccine still received license from US Food and Drug Administration (FDA) [70].

9. Development of vaccine

For the identification of antigens that are appropriate for disease avoidance, detailed and thorough information of their biology, etiology and structural arrangement of the pathogen, communication with cellular receptors in host system and its disease inducing mechanism are essential. It is also important to know the route of entry and subsequent replication sites and cycles of the desired pathogen. Because knowing these details are crucial that different vaccination strategies might be implemented to protect against pathogens entering via different routes such as the respiratory (influenza, pneumococcus), gastrointestinal (Salmonella) or genital tracts (Herpes simplex virus [HSV] or human immunodeficiency virus [HIV]), or entering the bloodstream by injury/injection (hepatitis B/C) or mosquito bite (Malaria, failaria, dengue) [71-73].

Generally, less than one tenth of the vaccine candidates achieve licensure due to the high failure rate of the unpredictable nature of the biological organisms required for the vaccine production and the variability of how the human immune system will detect process and react to the vaccine antigen. Appropriate levels of immune response may be produced by some vaccine candidates but they may induce significant adverse reactions. But some may be safe but ineffective at preventing diseases. Although incorporating multiple antigens into one single vaccine, the challenges related with developing safe and effective vaccines are even greater. The continuous research towards the discovery of a new vaccine antigen and novel approaches to immunization usually take years for the fulfillment and cost millions of dollars. After successful discovery, to reach the final licensing point many improvements has to be conducted.

9.1 Pre-clinical stage of vaccine process

In the pre-clinical stage of the development, initial study is based on understanding the pathogen and disease condition mainly focused to resolve most appropriate vaccine characteristics concerning both potential antigens and the type of immunological response that the vaccine must exert to defend against infection by humoral and/or cell-mediated immunity [74]. During vaccine development, consistent manufacturing procedure that would ensure a product conformity from lot -to -lot all the way through clinical studies are followed and as well as on the market [75]. A number of *in vitro* and *in vivo* tests are executed to demonstrate potential immunogenicity of the purified antigen, by using suitable established animal models for this study. Initial toxicity evaluation and dose-response studies were also carried out.

9.2 Phase I: Clinical trials

The prime objective of phase I trials determines the safety of the candidate vaccine in dose-setting studies with a small group (i.e., tens to hundreds) of human volunteers [76].These study are either performed in open-label/blinded trials. The phase I trials are executed in healthy individuals; on the other hand, if the vaccine's target population varies such as infants, older adults, pregnant women are involved, phase I trials may be united with phase II trials as a time and cost-saving measure [77]. In this particular instance, a small group of participants are brought together to complete the phase I section of the trial, pursued by large number of participants in phase II trial.

9.3 Phase II: Clinical trials

The major aim of phase II trials is to assess the safety and tolerability of the vaccine in wider study population (hundreds to thousands) that display more immunogenicity by surrogate markers of the candidate vaccine [76]. To predict the vaccine's protective effect, the immunologic markers selected should be suitable to the preferred response. For example, cell mediated immunity represents an important role to prevent *Varicella zoster* reactivation. As a result, activated CD4+ T-cell incidents were assessed to evaluate immunogenicity of the newly developed vaccine candidate [78, 79].

9.4 Phase III: Clinical trials

Phase III vaccine trials, conducted particularly in large study group containing more than10,000 volunteers, multicentre, randomized and controlled trials participating at risk for the targeted disease condition[76].The vaccine efficacy (VE) is the primary outcome obtained from this controlled study is about the, which signifies the risk reduction (RR) in developing a predetermined result in the vaccinated population compared to the unvaccinated population:

$$\text{Vaccine efficacy} = [(1\text{-RR}) \text{ x}100\%]$$

The infection incidence (e.g., polymerase chain reaction to confirm varicella zoster virus) or some other neurological complication (e.g., postherpetic neuralgia) may be interpreted by the pre-specified result.

9.5 FDA licensure and phase IV

Finally, the FDA approves new vaccine candidates by the same method as similar to that of biological products. Initially, before commencing clinical trials an Investigational New Drug application (NDA) has to be proposed. After successful completion of phase III trials, a Biologic License Application (BLA) is send to FDA for review before commencement of vaccine to the market [80]. The FDA intends to make a decision on at least 90% of new BLAs within 10 months of approval as mandated by the goal set by the Prescription Drug User Fee Act (PDUFA) [79]. However, according to estimates, only 50% to 60% of new NDA's were accepted during the financial years 2010 to 2014. BLA will obtain approval upon initial application followed by the submission of the additional details provided as per requisition by the FDA. The time between application and approval may be as long as 2 years [80, 81]. After the vaccine is brought to market and administered, phase IV trials continue to wider population than in clinical trials to collect safety and efficacy results [80]. These studies are essential requisite for the FDA on a case-by-case basis, to describe additional safety and effectiveness in various sub-populations.

10. Delivery of vaccine

The first administration of vaccination were performed through scarification (ie, disruption of the epidermal layer of skin), but today's vaccination administered by means of hypodermic needle and syringe into muscle (i.m.), subcutaneous tissue (s.c.), or skin (i.d.) [82]. It can also be delivered through mucosal route i.e., orally or nasally, but particular formulations are required for the delivery route to avoid antigen degradation or inactivation. Due to the adverse and highly acidic location in which the vaccine must endure inside the gastrointestinal tract, oral administration is highly recommended to ensure adequate absorption and prevent low bioavailability [83, 84]. Based on the different availability of vaccine, different administration route is required based on the formulation of the vaccine, cellular uptake or tissue vascularity. Therefore, each administration route has its own benefits and drawbacks.

10.1 Intramuscular immunization

While considering the quickness and simplicity for vaccine delivery the most common route is by intra muscular or subcutaneous administration. By this method relatively large doses can be delivered in thigh muscle angled at 90 degree 'deltoid or anterolateral' where sufficient blood supply is seen. Majority of vaccines to date have been administered intramuscularly. Examples include DT, hepatitis A and B, influenza, HiB, HPV, pneumococcal, and meningococcal [85].Generally, i.m. or s.c. administrations have been recorded to be painful and less effective in arising broad immunogenicity consequently necessitating higher levels of vaccine immunogen levels compared to the skin-based immunizations[85-89].So that multiple applications are generally needed to evoke a strong immunological response.

10.2 Subcutaneous immunization

In comparison to intramuscular administration, subcutaneous injections are administered into adipose tissue (buttocks) at 45 degree angle. Subcutaneous injections can result in extended antigen retension because of the limited drainage and vasculature. Although the prolonged existence of antigen may lead to increased immunogenicity due to prolonged absorption, that may leads to an amplified number of incidence of local adverse reactions like granulomas and abcesses predominantly when co administered with adjuvants. Overall few vaccine are administered s.c. than i.m. they are varicella, Q-fever, IPV, and some MMR/MMRV vaccines [85]. Some vaccines such as pneumococcal, MMR, yellow fever and rabies can be administered either by s.c. or i.m. depending upon the manufacturer's instruction [85].

10.3 Cutaneous immunization

The first method of immunization was performed by scarification on the skin surface, accompanied by the topical administration of cow pox or vaccinia virus to cross react to provide protection against small pox. Mantoux on early 20th century was first to described the cutaneous immunization, which involves the introduction of substance with a needle parallel (<30 degrees) into the skin resulting in a bleb formation [90]. One of the most possible alternatives to conventional immunization is the intradermal injections as they take advantage of the skin's unique immune system to elicit a strong immunological response. While compared to i.m. immunization, i.d. demonstrated improved immunogenicity 5-10 folds much better against in-fluenza [86, 91-95],rabies [96] or HBV vaccines [97, 98] but difficulty to administer due to the thin layer of dermis. Only BCG immunizations are currently performed by i.d. Skin barrier disruption is considered as physical injury that induce local tissue damage or trauma to which immune system responds by releasing danger signals such as heat shock proteins, dsDNA, monosodium uric acid and other substances that set off triggers cascade of immunological reactions[99, 100]. Biolistic injections [101], electroporation [102-104], iontophoresis [105], ultrasound [106, 107] and tattooing devices [108-111] are instances in which cutaneous immunization technique were employed.

10.4 Mucosal immunization

Mucosal tissue immunization has benefit of accumulating the vaccine in or near vicinity to the primary site of infection, thus enhancing secretion of IgA by eliciting natural or humoral immune response [107]. The most important benefit of mucosal or specifically oral routes is that they are much easier to administer than any other parenteral administration method and are very less likely to transmit blood borne diseases. Though, several challenges connected to mucosal immunization however have yet to be resolved. For the successful antigenicity, it must withstand the low pH and enzymatic digestion in the gastrointestinal tract as well as need to enter the epithelial barrier [112]. This can be accomplished only by adapting, enhancing and improving the vaccine formulation.

11. Effectiveness and role of vaccines

Vaccines have made significant impact on public health-care system. Their impact on reducing mortality rate stood second only while considering the importance and provision of safe drinking water [112]. Individuals are given vaccine to protect them from several infections, but vaccination imparts a major role in shielding whole population from infectious disease exposure. The effectiveness and the level of vaccine coverage achieved in the given population are the two main important factors that contribute to the capability of a vaccine to eliminate or control disease progression. The response may differ to some extend from country to country. But FDA licensed vaccine are considered highly effective for preventing disease progression everywhere.

Vaccination programs protect people from infectious diseases both directly and indirectly. According to Haber when a population is infected direct protection occurs by lowering the possibility of vaccine recipients being infected or lessen the infectiousness of vaccinated individuals when a widespread of infection happens in a population [113] (figure 9). Indirect protection is attained by declining disease spreading within the population, thus reducing the disease transmission rate for both vaccinated and unvaccinated individuals. Vaccine effectiveness measures the defensive effects of vaccination by reducing the vaccinated individual's risk of infection compared to that of susceptible and non-vaccinated individual [114]. Greenwood and Yule in 1915 designed and calculated vaccine efficacy for the typhoid and cholera vaccines. Vaccine efficacy studies measures and ensures the several possible outcomes such as disease attack rates, medical visits, hospitalizations, and costs.

Vaccine effectiveness is the potentiality of the vaccine to prevent the outcome of interest in the real world. Vaccine effectiveness can be divided into- direct, indirect, total, and overall effects. The direct effectiveness compares the risk associated in the randomly selected individuals with vaccinated individual [113]. The indirect effect estimates of the dissimilarity in the degree of safety received by unvaccinated individual in the incidence or lack of a vaccination program. The total effectiveness covers the relative infection risk rate in vaccinated individual compared to non-vaccinated individual before the commencement of a vaccination program [115]. As a consequence, the overall effectiveness of vaccination demonstrates the outcome of the vaccination program as well as the influence of individuals who is vaccinated [116]. The disease transmission reduction rate for an average individual in a population with a specified degree of coverage of a vaccination program compared with average individual in an equivalent population without vaccination program is generally referred as vaccination program effectiveness [115,116]. Therefore the overall effectiveness is taken into account to estimate the influence of immunization programs at the population level and also it depicts the benefits attained by both immunized and non immunized individuals [117,118].

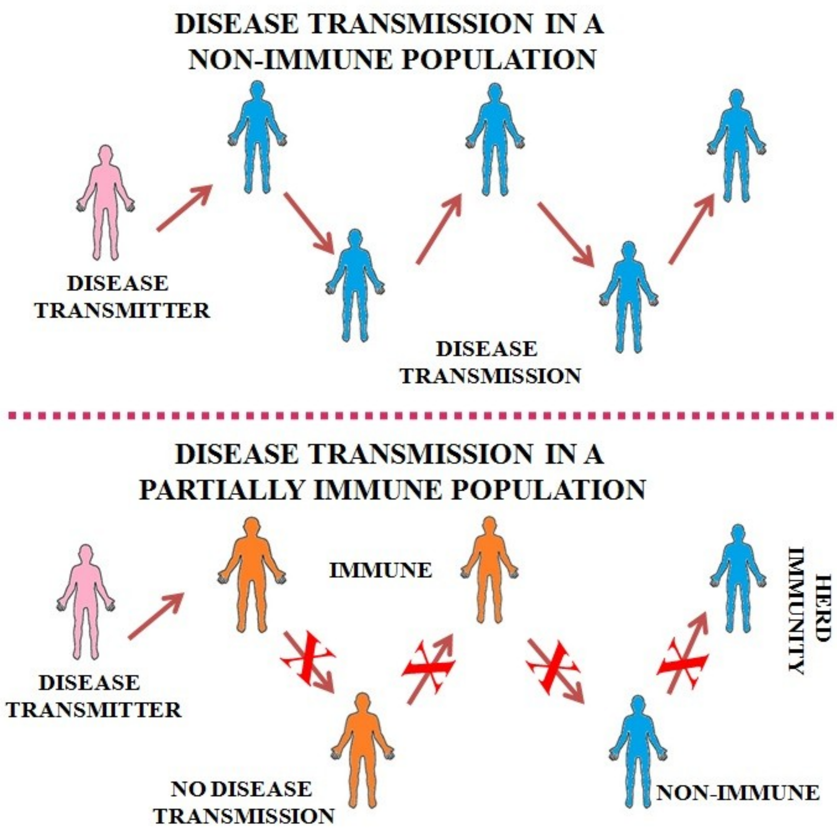


Figure 9: Herd immunity

12. Safety and Risks associated with vaccine therapy

The term 'safety' is related the nature of the damage or harm occurred to an individual. Second, while connected to vaccines, there is the risk occurring at the time of vaccination, weeks later or even years or decades later. There is a chance that the appearance of a disease state is mere accidental through vaccination rather than caused by vaccination which is an issue that is very hard to resolve to the satisfaction and justification of both injured personal and the vaccinators. With regard to the above concern, the each individual's immune mechanism system of varies and there is an unexpected chance or unusual situation in which vaccination causes the disease that is intended to avoid, or it can trigger allergic or adverse reactions as a result of defected immune system. When dealing with population or cohorts of millions of individuals it is difficult to assume that all the vaccine recipients will be unharmed as a result of vaccination instead we expect a lesser chance that a certain proportion could be discomforted due to the variation in immune response towards the vaccine. It must be considered in the combination with the 'benefits' that accrue after the use of vaccine not just the 'risks'. One of the greatest problem remains is that, people are more likely to project the disadvantages of the current injury as more persuasive and powerful than the observed benefits of the absence of disease to be expected in future. This would definitely distort the approval of the safety of a vaccine.

The vaccine safety is a key concern for the public, manufacturers, immunization providers, and vaccine recipient. The benefits of vaccination are indisputable. To maintain a public confidence in immunization program is critical for preventing a decline in the vaccination rates that leads to the outbreaks of diseases. The vast majority of vaccine related adverse effects are mild and are temporary. Naturally pain at the injection site and mild fever may occur. The mild or adverse reactions towards the vaccine are mainly due to the individual differences in the immune responses. There are government authorities that regulate the clinical developments of vaccine. Prior to the grant of a government license, a rigorous review of vaccine safety must be carried out. During an immunization program, the nature and incidence of the adverse events following immunization is monitored continuously.

Vaccines have been shown in human clinical trials to cause common side effects such as discomfort and inflammation at the site of injection, fatigue, malaise, and mild fever. Measurement of inflammatory cells at the site of injection, reduce food intake, loss of body weight, and changes in body temperature could be the mostly exhibited side effects in animals [119]. The adverse reactions after immunization are unexpected and undesirable. The each components present in vaccine may aid complications; it must be ensured that vaccine components do not pose a risk to vaccine safety either separately or in combination. Any adverse medical hazard that happens after immunization but not necessarily happen based on the vaccine side effects usually referred as Adverse Event Following Immunization (AEFI). These unexpected actions can be categorized into five based on the cause of the event. These events are associated with the vaccine products and quality defects, due to immunization mistake, because of over anxiety about immunization and some coincidental events. The adverse events due to vaccines occur only with a certain frequency. The frequent and minor reactions after vaccination usually exhibited are fever and malaise. The allergic reactions towards vaccine antigen or its component may cause unusual and serious reactions. Vaccine development has a lot of challenges to face, including the identification of safe and effective adjuvants, antigens and the most acceptable suitable delivery mechanism and it should be significant in balance with cost, risks and benefits [120].

13. Conclusion

Although the human vaccines has attained several benefits but their potentials for further impact is also significant. Scientific advancements can be implemented to accelerate production and simplify delivery of vaccines, but then socio and political commitment to immunization programs must be maintained to gain the full benefits of this incredible medical breakthrough. A newly emerged type of vaccine that has a higher therapeutic potential to treating variety of human and animal ailments is the plant based system generated vaccines. The advantages and benefits of plant derived vaccine can solve the obstacles that are faced by these fascinating biological products. As a result, it is expected that regulatory authorization will finally be granted to assist in the control of disease transmission globally. Vaccine development against more complicated infections like tuberculosis, malaria, and HIV has been challenging and difficult with few successes to date. The final success against these infections occurs only when combinations of vaccines are administered or each component has the ability to activate and stimulate different arms of the immune system. Vaccines are more likely be used to prevent or modulate the pathogenesis of some non-infectious diseases in the long run. A great advancement has already been attained with therapeutic cancer vaccines and has other possible probable targets including addiction, diabetes, hypertension, and also for Alzheimer’s disease. The scientific and medical groups are making serious efforts to mitigate Covid-19 pandemic and related waves of viral transmission by introducing preventative vaccines and re-purposing accessible drugs as possible therapies. This novel corona virus has consequently alarmed the scientific community to use alternative approaches to hasten the vaccine development process. The utmost goal is to provide economic vaccine that generates spontaneous, strong and extended immunity with least potential side effects, executed without the need for expensive cold chain system.

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15. Conflicts of interest

The authors declare that they have no conflict of interests.

16. Data Availability

The authors declared that the research data referred to correctly cited in the manuscript's reference section.

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