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Vaccines Through Time: Conventional Foundations and Next-Gen Innovations–Part 1

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Abstract

Vaccination serves as a critical pillar of global public health, markedly decreasing infection rates and associated mortality. Traditional vaccine platforms such as live attenuated, inactivated, toxoid, and subunit vaccines have demonstrated effectiveness against pathogens like Mycobacterium tuberculosis and Plasmodium spp., yet they are constrained by antigenic variability, limited immune persistence, and complex production processes. Breakthroughs in synthetic biology, structural antigen design, and computational vaccinology have driven the development of advanced vaccine technologies, including mRNA-based vaccines, viral vectors, virus-like particles, and nanoparticle-based systems. These modern platforms enable precise antigen presentation, faster manufacturing, and broader protection against diverse and emerging variants. The mode of vaccine administration whether intramuscular, oral, intranasal, or transdermal also plays a pivotal role in shaping immune outcomes, influencing the magnitude, site-specificity, and duration of the response. Aligning delivery routes with innovative vaccine platforms has shown improved efficacy, particularly in enhancing mucosal immunity. Moreover, vaccination contributes to reducing antimicrobial resistance by lowering infection rates and minimizing antibiotic use. Innovations such as AI-assisted antigen prediction and self-replicating RNA platforms further expand the potential for long-lasting, cross-protective immunity. Despite these advancements, persistent challenges including reliance on cold-chain logistics, inequitable distribution, and public hesitancy remain significant. Future directions should focus on mucosal-targeted delivery, universal vaccine approaches, and decentralized distribution models to strengthen global health systems and pandemic readiness.

Keywords: Synthetic biology, structural vaccinology, immunoinformatics, vaccine delivery routes, mucosal immunity, antimicrobial resistance, AI-assisted antigen design

INTRODUCTION

Vaccination serves as a foundational element of preventive healthcare and is recognized globally as one of the most cost-effective and impactful strategies in public health [1]. Biotechnologically, vaccines are engineered biological agents that emulate pathogen-host interactions, thereby priming the immune system to recognize and neutralize infectious agents through the development of immunological memory. Their implementation has led to substantial declines in the prevalence of both endemic and emerging diseases, particularly in pediatric populations, improving survival metrics globally [1]. The inception of vaccination dates back to 1796 when Edward Jenner inoculated a young boy with cowpox, demonstrating immunity against smallpox. This milestone facilitated the creation of the first smallpox vaccine in 1798, eventually leading to the worldwide eradication of the disease by 1979 following extensive immunization efforts [2]. Traditional vaccine platforms–including live attenuated organisms, inactivated microbes, and purified antigenic

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components—have historically served as the backbone of immunization protocols, generating robust immune responses while maintaining safety profiles [1].

The introduction of routine immunization has been instrumental in combating vaccine-preventable diseases (VPDs), with marked reductions in childhood morbidity and mortality across varied epidemiological contexts [1]. Recognizing the strategic role of vaccines, the World Health Organization (WHO) incorporated lifelong immunization as a core target in the 2030 Sustainable Development Goals. Furthermore, the 2012 World Health Assembly endorsed the Global Vaccine Action Plan, which aimed to increase global vaccine coverage and eliminate diseases such as polio [2]. India's vaccine industry is projected to reach a market valuation of approximately US$1.31 billion by 2025, maintaining this value through 2029, supported by a compound annual growth rate (CAGR) of 0.09%. This growth is driven by enhanced health literacy, population expansion, and government-sponsored immunization campaigns. India's vaccination framework began with the Expanded Programme on Immunization (EPI) in 1978, focusing on six major VPDs. Later initiatives, including Mission Indradhanush (2014), Intensified Mission Indradhanush (IMI, 2017), and IMI 2.0 (2019), targeted underserved populations to improve vaccine coverage.

Integration of digital innovations like the electronic Vaccine Intelligence Network (eVIN) has enhanced cold chain management and streamlined vaccine logistics [3]. Nonetheless, immunization coverage remains inconsistent across different strata due to multiple influencing factors. Socio-demographic determinants such as maternal education level, the gender and birth order of children, urban-rural divides, and regional disparities contribute to uneven coverage. Operational barriers, including inadequate healthcare personnel in remote areas, cold chain breakdowns, underdeveloped surveillance systems, and limited laboratory infrastructure, further hinder vaccination delivery. Additionally, vaccine hesitancy–often fueled by cultural misconceptions, misinformation, and concerns regarding vaccine safety–remains a significant behavioral challenge.

Vaccine Efficacy and Route of Administration

The route by which a vaccine is delivered significantly influences immune activation by affecting how antigens are absorbed, distributed, and presented to the immune system. Selection depends on antigen stability, the desired immune response, and practical considerations. The main administration routes are intramuscular (IM), subcutaneous (SC), intradermal (ID), oral, and intranasal, each with distinct immunological and logistical features.

- *Intramuscular (IM) Injection:* Vaccines given intramuscularly are deposited into muscle tissue, which is rich in blood vessels, allowing fast absorption and sustained antigen presence. This method elicits strong antibody and cellular responses and is commonly used for vaccines such as influenza, hepatitis A and B, MMR, and COVID-19 (Pfizer-BioNTech, Moderna) [1]. Typical injection sites are the deltoid and thigh muscles. Local side effects like inflammation or bruising may occur.
- *Subcutaneous (SC) Injection:* Subcutaneous injections deliver vaccines beneath the skin into fatty tissue, resulting in slower absorption but prolonged immune stimulation. Vaccines for rabies, pneumococcal infections, and yellow fever often use this route. The upper arm and abdomen are typical injection sites [1]. This technique is straightforward and generally well tolerated.
- *Intradermal (ID) Injection:* Intradermal delivery targets the skin's dermis, which contains abundant antigen-presenting cells such as Langerhans cells, enabling potent immune responses with smaller antigen doses. While technically more challenging, this route is used in tuberculosis testing (Mantoux test) and for certain hepatitis B and rabies vaccines [1].
- *Oral Administration:* Oral vaccines activate mucosal immunity by delivering antigens to the gastrointestinal tract. They are easy to administer and suitable for mass vaccination programs. Although enzymatic breakdown and acidic pH reduce bioavailability to about 1–2%, proper formulation improves effectiveness [1]. Examples include vaccines against polio, rotavirus, and typhoid.

- *Intranasal Administration:* Administered via the nasal mucosa, intranasal vaccines stimulate both mucosal and systemic immunity, producing secretory IgA that protects against respiratory infections. This needle-free, self-administered method requires lower antigen doses and is well suited for children and low-resource environments [1]. Approved intranasal COVID-19 vaccines include Convidecia (China) and I NCOVACC (India).

The administration route plays a key role in determining vaccine efficacy by shaping how the immune system encounters the antigen as illustrated in Figure 1. Innovations in delivery, including needle-free and mucosal-targeted vaccines, are advancing global immunization strategies and improving readiness for future outbreaks.

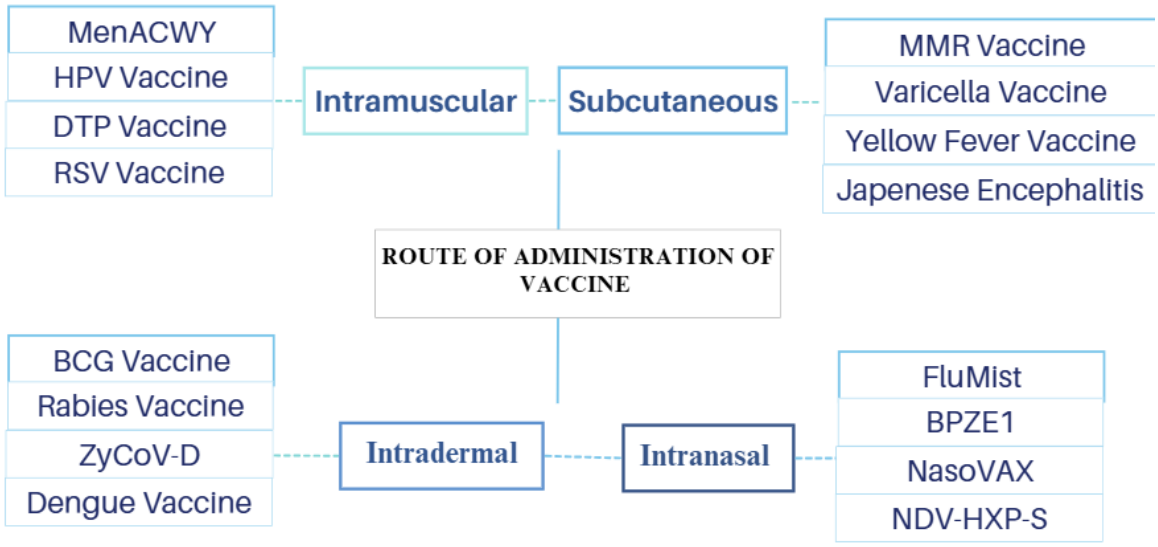

Figure 1. Comprehensive classification of vaccine delivery route.

THE MULTIFACETED IMPACT OF VACCINATION PROGRAMS: HEALTH, ECONOMY, ENVIRONMENT, AND PANDEMIC PREPAREDNESS

Vaccination programs are vital to global health and development, serving as more than just a shield against infectious diseases. They are key drivers of economic stability, educational attainment, and poverty reduction–especially in low- and middle-income countries. By improving overall population health, vaccines enhance cognitive development, workforce productivity, and environmental sustainability. In the face of evolving global health threats, vaccination remains a cornerstone for building resilience and achieving sustainable development goals worldwide.

Health and Immunological Benefits

Vaccines are powerful tools that offer protection against a wide range of infectious diseases by training the immune system to recognize and neutralize pathogens before they can cause illness. By inducing immunological memory, vaccines enable the body to mount a faster and stronger immune response upon future exposure to the same pathogen, providing long-term or even lifelong protection [4]. Administered to healthy individuals, vaccines not only protect the recipients but also reduce transmission across the broader population, contributing to herd immunity and lowering the overall risk of disease outbreaks. This proactive approach is especially critical in safeguarding children's health and cognitive development, as it prevents disease-related complications during early developmental stages [5].

Economic and Social Advantages

Beyond health benefits, vaccination significantly reduces economic burdens by minimizing medical expenditures such as consultation fees, medication costs, hospital admissions, and travel-related

expenses for treatment [6, 7]. This is particularly important in low- and middle-income countries like India, where approximately 51 million people live below the poverty line and often lack access to affordable healthcare [8]. Routine immunization supports health equity by providing preventive care regardless of financial status or geographic location, helping to break the cycle of poverty and illness [7].

Macroeconomic and Educational Benefits

Vaccination efforts deliver far-reaching benefits beyond health protection, particularly in low- and middle-income countries (LMICs), where healthcare expenses often fall directly on individuals. Prior to the COVID-19 pandemic, nearly one billion people faced catastrophic health costs, with another 500 million pushed into poverty during the pandemic [9, 10]. Vaccines help alleviate this burden–universal immunization against measles, rotavirus, and pneumococcal diseases in 41 Gavi-supported LMICs is estimated to save $4.6 billion and prevent financial hardship for 12.6 million people between 2016 and 2030 [11]. Globally equitable distribution of COVID-19 vaccines is also projected to generate $466 billion in economic returns by 2025 [12]. In addition to economic relief, vaccines foster long-term educational and cognitive development. By preventing early-life infections that can impair the immune system, such as measles, vaccines support healthier childhood development [13]. This translates into improved school attendance and academic outcomes. In LMICs, childhood vaccination correlates with an extra 0.2–0.3 years of education, with stronger effects among disadvantaged groups [14]. National immunization initiatives in countries like India, Mexico, and the U.S. have been associated with 1–13% higher lifetime earnings underscoring how stronger education and health literacy can build vaccine confidence and support more informed immunization decisions across populations [5, 15].

Environmental Impact

Vaccination programs contribute to environmental sustainability by influencing population dynamics. While they initially reduce child mortality, this often leads to lower fertility rates over time, as families gain confidence in child survival. For instance, Sweden's smallpox vaccination reduced birth rates by 1.16 per 1,000 population [16], and India's immunization campaign (1985–1990) led to a 2% drop in fertility demand [17]. These changes are closely linked to improved maternal health, female education, and greater economic opportunities. Vaccines also help lower the carbon footprint [18, 19]. While emphasizing the importance of greener production, distribution, and waste-management approaches to lessen the environmental impact of large-scale vaccination [20]. As child survival improves, families tend to have fewer children, easing population pressure. Additionally, healthier, vaccinated mothers pass on immunity to their children, reinforcing long-term reductions in infant mortality [21, 22]. By enhancing schooling and earning potential, vaccines raise the opportunity cost of childbearing, contributing to smaller family sizes and long-term environmental benefits [23].

Impact on Poverty Reduction

COVID-19 exposed how quickly infectious diseases can disrupt global systems. With the global population crossing 8 billion in 2022, rising human density, international travel, wildlife trade, and habitat loss have increased the chances of outbreaks [24]. Climate change has intensified this threat–around 58% of infectious diseases are worsened by environmental shifts that alter vector behavior and pathogen transmission [25]. Habitat disruption raises the risk of zoonotic spillovers [26], while warming in the Northern Hemisphere could expose 500 million more people to vector-borne diseases like Zika, chikungunya, and West Nile by 2050 [27]. To prepare for future pandemics, CEPI and the U.S. NIH are advancing prototype vaccines for virus families with human infection potential. These platforms aim to be rapidly adapted to unknown pathogens (termed "Disease X") and deployed within 100 days [28]. Strengthening mRNA and viral vector technologies, along with global vaccine safety infrastructure, is critical. Investment in early-warning systems for zoonotic diseases will further enable swift and effective outbreak responses.

Improved Pandemic Preparedness

The COVID-19 pandemic highlighted the vulnerability of modern society to infectious disease outbreaks. With the global population reaching 8 billion by November 2022, human density has

increased fivefold over the past century, contributing to more international air travel, intensified animal and plant trade, and greater habitat disruption–all factors that facilitate pathogen spread [24]. Around 58% of infectious diseases are exacerbated by climate change, which alters vector dynamics, such as mosquito spread, and impacts pathogen life cycles [25]. The risk of zoonotic diseases has also risen due to habitat disruption, increasing the likelihood of cross-species virus transmission [26]. Additionally, rising temperatures in the Northern Hemisphere are intensifying the spread of vector-borne diseases like Zika, West Nile, and chikungunya, potentially affecting 500 million more people by 2050 [27]. The Coalition for Epidemic Preparedness Innovations (CEPI) and the U.S. National Institute of Allergy and Infectious Diseases have developed a blueprint for pandemic preparedness, which includes creating prototype vaccines for viral families known to infect humans. These vaccines would be adaptable to new pathogens (referred to as Disease X), with the goal of having a vaccine ready for distribution within 100 days [28]. To support this, rapid-response vaccine platforms–such as mRNA and viral vector vaccines–need further development to ensure a timely and efficient response to future pandemics. Additionally, the establishment of global vaccine safety infrastructure is crucial to assess the safety and efficacy of these newly developed vaccines details are provided in a companion manuscript part II. Increased investment in surveillance and detection systems for zoonotic diseases, alongside preparedness for emerging pathogens, is necessary to ensure global health security and rapid containment of potential pandemics.

Combating Antimicrobial Resistance (AMR)

AMR is a growing global threat, driven by overuse of antibiotics and a lag in developing new drugs–only 13 were in late-stage trials by 2021, with no new classes in decades [29]. Vaccines offer a proactive solution by preventing infections and reducing the need for antibiotics. Immunization against *S. pneumoniae* and *Hib* has already led to sharp declines in both disease incidence and antibiotic use [30]. By lowering infection rates, vaccines reduce selective pressure on bacteria and limit the spread of resistant strains. For instance, pneumococcal vaccines have curbed multidrug-resistant *S. pneumoniae*. They also reduce bacterial colonization and horizontal gene transfer, key pathways for resistance spread in organisms like *E. coli* and *K. pneumoniae* [31]. Vaccines are especially crucial in LMICs and among vulnerable populations with limited access to antibiotics [32]. In agriculture, their use in livestock and aquaculture has cut down antibiotic use, lowering the risk of resistant bacteria entering the food chain [33]. By decreasing reliance on antibiotics, vaccines help preserve existing treatments, making them an essential tool in global AMR strategies [34].

Pathway to Sustainable Development and Global Resilience

Vaccination plays a transformative role in advancing public health, economic growth, and social equity, making it a cornerstone of sustainable development. By preventing infectious diseases, immunization reduces mortality, alleviates pressure on healthcare systems, and promotes long-term socioeconomic gains. Its impact extends beyond health–vaccines contribute to poverty reduction, gender equity, and educational attainment, all of which are foundational to global resilience. Vaccines align closely with multiple Sustainable Development Goals (SDGs). They support SDG 3 (Good Health and Well-being) by reducing disease burden and enhancing population-level immunity [35]. Through the prevention of illness-related financial shocks, particularly in low- and middle-income countries, vaccines contribute to SDG 1 (No Poverty) [36]. Equitable access to immunization promotes SDG 5 (Gender Equality) by improving health outcomes for women and children [37]. By safeguarding worker health and reducing absenteeism, vaccines drive SDG 8 (Decent Work and Economic Growth) [38]. They also enhance school attendance and cognitive development, reinforcing SDG 4 (Quality Education) [39]. Finally, large-scale immunization programs foster international collaboration and capacity-building, advancing SDG 17 (Partnerships for the Goals). Thus, vaccines are not only biomedical interventions but also strategic tools that strengthen global systems, reduce vulnerabilities, and accelerate progress toward a more equitable and sustainable future.

VACCINE EVOLUTION: FROM TRADITIONAL PLATFORMS TO NEXT-GEN INNOVATIONS

Vaccines are pivotal in directing the immune system to mount a targeted defense against infectious agents. Traditional platforms–including live-attenuated, inactivated, toxoid, and subunit formulations–have demonstrated enduring success in reducing disease burden. Yet, challenges such as time-intensive production, booster requirements, and limited suitability in certain populations can constrain their broader application during outbreaks. In response, advanced platforms have emerged that utilize molecular tools and computational modeling to streamline vaccine development. These include genetic vaccines encoding antigenic proteins, nanoscale delivery systems enhancing stability and targeting, plant-based oral vaccines promoting mucosal immunity, and AI-driven antigen selection for improved precision. Figure 2 presents a comparative flowchart of conventional and next-generation vaccine classes. While these innovations allow rapid and flexible responses to novel pathogens, they also introduce hurdles in terms of distribution logistics, manufacturing scalability, and long-term efficacy assessment. Together, these advances represent a shift toward more responsive and technologically integrated immunization strategies.

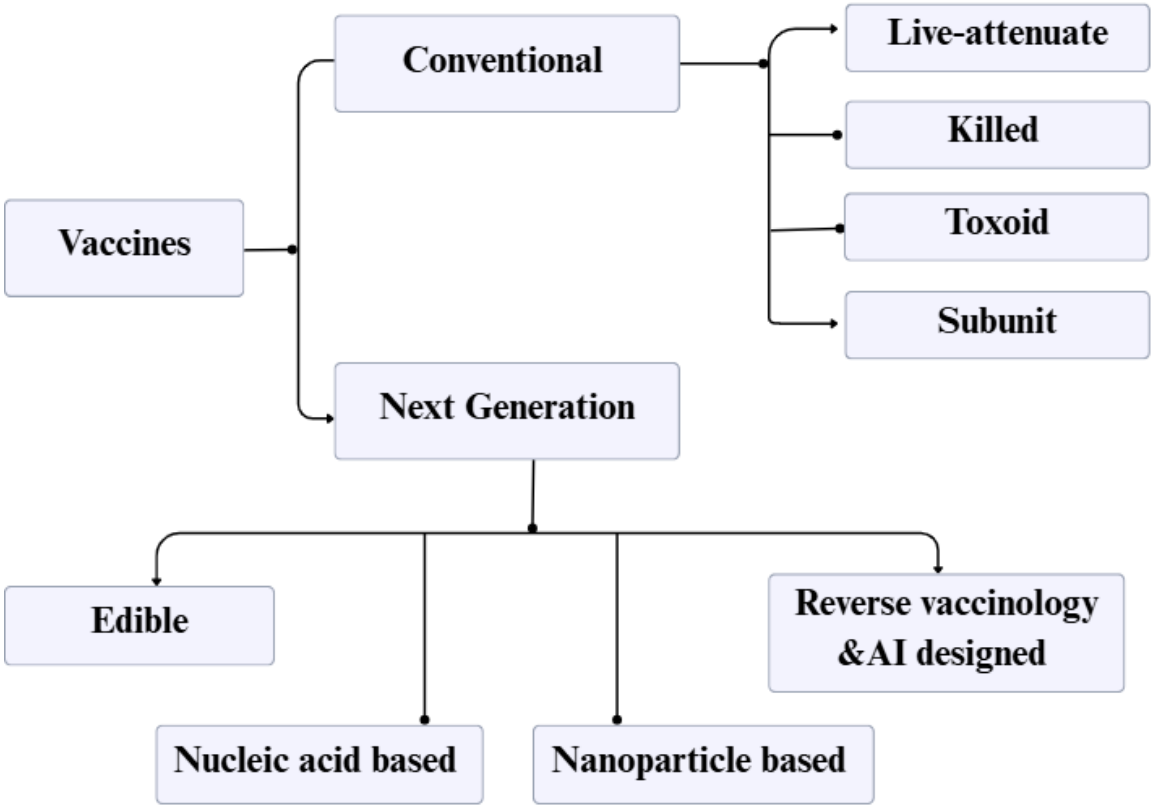

Figure 2. Structured breakdown of vaccine classes: conventional vs. next-gen vaccine.

Conventional vaccines are developed using traditional approaches such as weakened or inactivated pathogens, or purified components, to stimulate immune responses. They are reliable and widely used but often involve slower production and limited adaptability to emerging threats. The types of vaccines in this category are as follows

Live-Attenuated Vaccines

Live-attenuated vaccines are generated by weakening pathogens so they lose their disease-causing capacity but retain the ability to replicate and stimulate a robust immune response. This attenuation is often accomplished through serial passaging under selective conditions such as non-physiological temperatures or non-native hosts, promoting mutations that reduce virulence while preserving immunogenicity [40]. Modern approaches like increased replication fidelity and codon deoptimization

further enhance safety. For instance, Vignuzzi et al. showed that engineering poliovirus with a high-fidelity RNA-dependent RNA polymerase minimized the risk of reversion to its pathogenic form while sustaining effective immunogenicity [41]. Codon deoptimization–replacing codons with synonymous but suboptimal alternatives–reduces mRNA stability and translation efficiency, thereby limiting viral protein synthesis and replication capacity.

Immunologically, live-attenuated vaccines are highly effective because they mimic natural infection, leading to activation of innate immune sensors such as toll-like receptors (TLRs), which stimulate antigen-presenting cells and promote the release of cytokines like IL-12p40, IL-6, and IFN-α. These cytokines drive Th1-biased responses and support the formation of long-lived memory B and T cells. The yellow fever vaccine YF-17D exemplifies this mechanism, engaging TLR2, TLR7, TLR8, and TLR9 to elicit broad and durable immunity [41]. Such vaccines often do not require adjuvants and can provide lifelong protection with a single dose–as seen with the smallpox vaccine, which confers humoral immunity for up to 75 years and cellular immunity for over a decade. However, their use poses risks in immunocompromised individuals due to residual virulence, with reported cases of vaccine-derived poliovirus outbreaks and rabies reactivation. Additionally, production is complex and costly, requiring stringent biosafety protocols, skilled personnel, and extended timelines. Despite these limitations, live-attenuated vaccines remain foundational to global immunization programs, contributing to the eradication of smallpox and substantial control of poliovirus and Ebola virus infections.

Inactivated Vaccines/Killed Vaccines

Inactivated vaccines are formulated by destroying the replication capacity of pathogens while maintaining structural integrity of their antigens, allowing immune recognition without the risk of infection. This is commonly achieved using chemical agents such as formaldehyde, glutaraldehyde, or β-propiolactone, or through physical means like heat, ultraviolet, or gamma irradiation [40]. Formalin (a dilute form of formaldehyde) works by forming covalent bonds–such as methylol groups and methylene bridges–between nucleic acids and proteins. However, excessive concentrations or prolonged exposure can lead to epitope masking and protein aggregation, potentially reducing vaccine efficacy. Hence, careful optimization of inactivation parameters–concentration, time, and temperature–is necessary to preserve immunogenicity while ensuring safety [40, 41].

These vaccines are non-replicating, eliminating risks of reversion to virulence, making them safer than live-attenuated types. However, they predominantly trigger antibody-mediated responses, with limited activation of cellular immunity. To enhance effectiveness, they often require adjuvants, higher antigen doses, and multiple boosters. Despite these limitations, inactivated vaccines offer advantages such as thermostability, safety in immunocompromised individuals, and broad antigenic coverage. Examples include vaccines against poliovirus (IPOL®), hepatitis A (HAVRIX®, VAQTA®), and Japanese encephalitis (IXIARO®) [42]. A purified inactivated Zika virus vaccine (PIZV), prepared using 0.02% formaldehyde over 14 days and formulated with aluminum hydroxide, showed robust, dose-dependent neutralizing antibody responses and long-term protection in animal models, and advanced to phase 1 trials (NCT03343626) [43, 44]. This approach is also being explored for SARS-CoV-2 vaccine development [45, 46].

Toxoid Vaccines

Toxoid vaccines target bacterial exotoxins rather than the bacteria themselves. These vaccines are produced by chemically inactivating toxins–most effectively using formaldehyde–which eliminates toxicity while preserving the native antigenic structure required for immune recognition [40]. This method maintains the protein's secondary and tertiary conformations, ensuring stability and immunogenicity. In contrast, physical inactivation methods like heat or pH alteration can impair structural integrity and reduce immunogenic potential due to increased protein aggregation [40]. These vaccines primarily elicit a humoral immune response, generating neutralizing antibodies that block

toxin activity, limit tissue damage, and slow disease progression. $CD4^+$ T helper cell activation is crucial for B cell support and long-term antibody production. However, since toxoid vaccines do not clear the pathogen directly, elimination depends on innate immunity, antimicrobial therapy, or microbiota interactions. Examples include diphtheria, tetanus, and acellular pertussis (DTaP) vaccines such as Daptacel®, Infanrix®, and Kinrix® [47]. Toxoid vaccines are safe, stable, and suitable for large-scale use, as they contain no live agents. Adjuvants like aluminum salts are often added to boost efficacy but may cause local inflammation or, rarely, type III hypersensitivity reactions such as the Arthus reaction [47].

Subunit Vaccines

Subunit vaccines are formulated using specific antigenic components of a pathogen–such as proteins, peptides, or polysaccharides–capable of eliciting a targeted immune response without introducing the entire microorganism [48, 49]. These antigens can be obtained through the disassembly of viral or bacterial particles in culture or via recombinant DNA technology, resulting in recombinant subunit vaccines [50]. Unlike live-attenuated or inactivated vaccines, subunit vaccines do not pose a risk of infection or reversion to virulence, making them highly suitable for immunocompromised populations. Their defined composition enhances safety and manufacturing control; however, they often require adjuvants, multiple doses, and careful antigen selection to achieve sustained immunity [51]. The first recombinant subunit vaccine was introduced in the 1980s for hepatitis B and set a precedent for later vaccines targeting various pathogens. Approved examples include Engerix-B (Hepatitis B), Gardasil 9 (HPV), Flublok (Influenza), Shingrix (Herpes zoster) [52, 53], and Nuvaxovid (COVID-19) [54, 55]. There are different types of subunit vaccines, each categorized based on the nature of the antigenic component used and are as follow

- *Protein Subunit Vaccines:* These vaccines use isolated pathogen-derived proteins capable of inducing specific immune responses while excluding elements responsible for replication or pathogenesis [56, 57]. Recombinant protein subunits are expressed using heterologous systems such as yeast, baculovirus-insect cells [58, 59], or mammalian cultures, allowing high-yield and scalable production [60–62]. This approach has proven effective in vaccines against hepatitis B and HPV [63] and is being explored for highly variable viruses like HIV and Ebola [63, 64]. In COVID-19 vaccines, the spike protein or its receptor-binding domain is often used as the key immunogen [65].
- *Polysaccharide Vaccines:* These are composed of long-chain sugar molecules derived from the outer capsules of bacteria. They stimulate an antibody-mediated immune response by targeting surface polysaccharides without involving proteins [66, 67]. Examples include the pneumococcal and meningococcal polysaccharide vaccines, which protect against various serogroups including A, C, W-135, and Y [68]. The first Haemophilus influenzae type B (Hib) vaccine was also a polysaccharide-based formulation developed through NIH-supported efforts [69].
- *Conjugate Vaccines:* To enhance immunogenicity–especially in young children–polysaccharides are chemically linked to immunogenic protein carriers, converting the immune response from T-cell-independent to T-cell-dependent [70]. This conjugation improves memory B cell responses and results in more durable protection. Conjugate vaccines have been successfully implemented against Hib, Streptococcus pneumoniae, and Neisseria meningitidis.

Subunit vaccines represent a refined approach to immunization, offering high safety, precision, and compatibility with vulnerable populations. Their success in preventing viral and bacterial diseases, combined with adaptable production platforms, makes them a central strategy in both existing immunization programs and future vaccine development.

Next-generation vaccines are created using modern technologies like genetic material-based platforms, engineered delivery systems, and digital design tools to improve immune targeting and development speed. While they allow quick responses to new infections, their production often requires advanced resources and infrastructure. The types of vaccines in this category are as follows.

Edible Vaccines

Edible vaccines are developed by genetically engineering plants or animals to produce specific antigenic proteins that can trigger immune responses when consumed. These antigens are embedded within edible parts like fruits, leaves, or tubers and are capable of stimulating both mucosal and systemic immunity. Although labeled "edible," their effectiveness doesn't rely on taste or nutritional content [71]. This concept was initially proposed to improve vaccine accessibility in low-income regions. The first major breakthrough came in 1989 when plant-based antibody expression was demonstrated, followed by the successful production of antigens such as hepatitis B surface antigen (HBsAg) and heat-labile enterotoxin B subunit (LT-B) in the early 1990s [72–75]. A landmark study in 1998 confirmed the immunogenicity of these plant-derived vaccines, and by 2003, their role in inducing mucosal immunity was clearly established [76]. These vaccines primarily act through the gut-associated lymphoid tissue (GALT). Once ingested, antigens are taken up by M cells in the Peyer's patches and delivered to dendritic cells, which then activate T cells. This cascade promotes B cell activation and IgA production, providing localized mucosal defense. Goblet cells may also facilitate antigen transfer. However, repeated exposure to the same antigen can lead to immune tolerance via regulatory T cell activation, potentially reducing vaccine efficacy over time [77].

Edible vaccines have shown promise across various applications. In malaria studies, tomatoes expressing *Plasmodium* antigens (MSP4/MSP5) with cholera toxin B (CTB) prompted immune responses in mice. Carrots engineered to produce measles virus hemagglutinin elicited mucosal IgA responses. Transgenic potatoes expressing HBsAg demonstrated strong immune activation, particularly when the antigen was expressed in the roots. For autoimmune disorders, such as type 1 diabetes, potatoes producing insulin or glutamic acid decarboxylase (GAD) along with CTB showed improved glycemic regulation in animal models. To combat diarrheal diseases, crops like potato, corn, and tobacco have been modified to express bacterial antigens such as LT-B and CTB, successfully generating immune responses. Additionally, edible vaccines expressing anthrax protective antigen (PA) in crops like tobacco and spinach are being considered for use in veterinary immunization [77]. Overall, edible vaccines represent a safe, affordable, and needle-free alternative to conventional immunization. Their capacity to generate mucosal immunity, ease of distribution, and independence from cold-chain storage make them especially valuable for large-scale immunization programs in resource-constrained settings.

Nucleic acid Based Vaccines

Nucleic acid vaccines use genetic material from a pathogen to trigger an immune response, with the genetic material being either DNA or RNA. The concept of nucleic acid vaccines emerged in the 1990s. The first DNA vaccine-mediated immune response was observed when plasmid DNA encoding influenza A nucleoprotein led to a cytotoxic T lymphocyte (CTL) response. Similarly, in 1990, in vitro transcribed (IVT) mRNA vaccines were successfully tested in animal models, paving the way for further advancements. Nucleic acid vaccines have been extensively studied for their role in infectious diseases, cancer, autoimmunity, and allergies. These vaccines offer several advantages, including safety, effectiveness, and cost-efficiency, as they induce immune responses that specifically target the selected antigen without introducing the whole pathogen [78].

- *DNA Vaccines:* These vaccines are created by inserting the gene for an antigen into a bacterial plasmid, controlled by strong viral promoters like CMV. Once delivered into the body, the plasmid is taken up by host cells, leading to intracellular antigen expression. These antigens are presented via MHC class I and II molecules, activating both antibody-mediated and cell-mediated immunity. DNA vaccines are simple to design, inexpensive to produce, and thermally stable. They can be given multiple times with minimal side effects and include unmethylated CpG sequences that enhance immune activation by stimulating dendritic cells. However, their effectiveness in humans is often limited by low immunogenicity and inefficient transport into the nucleus. There are also concerns about potential integration into the host genome, though rare. New approaches like minicircle DNA vectors, which eliminate bacterial components, are being tested to enhance both safety and expression [78].

- *RNA Vaccines:* These vaccines utilize messenger RNA to deliver the genetic code for a viral or tumor antigen. Once inside the host cell, the mRNA is translated into a protein that the immune system recognizes as foreign. The mRNA structure includes a 5′ cap, untranslated regions (UTRs), a coding region (ORF), and a poly(A) tail, which together enhance stability and protein production. These vaccines are quick to manufacture, non-integrative, and do not carry the risk of altering host DNA. They eliminate the need for complex vectors and can be chemically modified to reduce innate immune activation while improving durability and translational efficiency. However, they are limited by their instability, short-lived antigen expression, and barriers to efficient cellular uptake, such as degradation by RNases and repulsion by cell membranes [78].
- *Self-Amplifying RNA Vaccines (saRNA):* saRNA vaccines are an enhanced version of RNA vaccines that include sequences from alphavirus replicons, enabling the RNA to self-replicate within cells. These replicons encode non-structural proteins (nsP1–nsP4), which form a replicase complex to amplify the RNA and extend antigen expression. A subgenomic promoter ensures high levels of antigen production. saRNA requires far smaller doses than traditional mRNA–effective responses in mice have been achieved with just 10 ng. These vaccines have shown strong potential in preclinical studies against diseases like COVID-19, Zika, rabies, HIV, and even in cancer therapy. While saRNA improves durability and potency, challenges include regulating replication and avoiding unintended RNA spread through extracellular vesicles. Still, it represents a powerful platform for long-term immunity with minimal dosing [79].

Nucleic acid vaccines, including DNA and RNA-based platforms, have transformed modern immunization by offering safe, rapid, and customizable solutions without using live pathogens. DNA vaccines provide long-term stability and can activate both arms of the immune system but face delivery and safety concerns in human applications. RNA vaccines, especially mRNA and self-amplifying RNA (saRNA), show high immunogenicity and fast production timelines, though they require optimized delivery systems due to their inherent instability. With ongoing innovations in molecular design and delivery technologies, these platforms are becoming increasingly effective. saRNA, in particular, holds promise for achieving strong immune responses at lower doses. As these vaccines continue to evolve, they are expected to play a central role in combating infectious diseases, cancer, and other emerging health challenges.

Nanoparticle Based Vaccines

Nanoparticle-based vaccines represent a sophisticated approach in immunology, utilizing nanoscale carriers to enhance the delivery, stability, and immunogenicity of antigens. These platforms enable controlled antigen release, targeted delivery to antigen-presenting cells, and the induction of both humoral and cellular immune responses. Commonly used nanoparticles include lipid nanoparticles (LNPs), as employed in mRNA COVID-19 vaccines; polymeric nanoparticles that provide sustained antigen exposure; inorganic particles such as gold and silica for their adjuvant properties; and protein-based nanoparticles like those in the Novavax vaccine. Their structural versatility and physicochemical tunability offer distinct advantages, including thermostability and potential for mucosal administration, which are critical in improving vaccine accessibility and efficacy against complex pathogens and emerging antibiotic resistance [80].

Virus-like particles (VLPs), a subclass of nanoparticle vaccines, are non-infectious nanostructures that mimic native viruses in size (20–200 nm) and morphology without containing any viral genetic material. These are produced by expressing structural proteins, typically capsid or envelope proteins, in heterologous systems such as yeast, insect, or mammalian cells, where they self-assemble into highly ordered particles. VLPs efficiently stimulate immune responses by promoting antigen uptake and presentation via MHC-II pathways and activating T-helper cells, which in turn drive B-cell differentiation and cytotoxic T-cell responses. Additionally, they may encapsulate immunostimulatory components such as single-stranded RNA that can activate Toll-like receptors (TLR7/8), thereby amplifying innate immune signaling [81]. The quadrivalent HPV vaccine Gardasil serves as a well-

established example of VLP-based immunization. It incorporates the L1 protein of HPV types 6, 11, 16, and 18, formulated with an aluminum-based adjuvant, and demonstrates high immunogenicity and safety. However, post-vaccination adverse events have been recorded, especially in younger populations. Diaz-Arévalo (2020) reported that these include mucocutaneous reactions (25%), rashes (22%), local injection site effects (20%), and serious reactions (7.5%) such as anaphylaxis, seizures, thrombocytopenia, and in rare instances, mortality [81]. Despite such events, nanoparticle and VLP-based platforms continue to offer tremendous promise for developing safe, effective, and customizable vaccines tailored to a range of infectious and non-infectious diseases.

Reverse Vaccinology & AI-Designed Vaccines

The integration of reverse vaccinology with artificial intelligence (AI) has fundamentally transformed the landscape of modern vaccinology. Reverse vaccinology employs genome-based bioinformatics approaches to identify potential vaccine antigens directly from pathogen sequences–eliminating the need for culturing. Unlike traditional immunological methods that typically focus on a limited number of dominant antigens, reverse vaccinology enables high-throughput screening of the entire proteome, uncovering conserved, non-cultivable, and subdominant antigens while filtering out human homologs to minimize autoimmunity risk. Its success is exemplified by the Bexsero vaccine against *Neisseria meningitidis* serogroup B, which was designed entirely through this approach [82, 83].

AI further augments this paradigm by introducing powerful computational frameworks for predicting antigenicity, immunogenicity, and structural stability. Machine Learning models like decision trees and random forests utilize biological data to predict vaccine-relevant features, while Deep Learning tools such as Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs) assist in accurate epitope prediction and protein modeling. Generative models like Variational Autoencoders (VAEs) and Generative Adversarial Networks (GANs) facilitate the design of novel immunogens, while molecular dynamics (MD) simulations and structure-activity relationship (SAR) models evaluate antigen-adjuvant compatibility and optimize immunogenic profiles [82].

The convergence of these technologies fosters rapid, cost-effective, and precision-guided vaccine development. AI-driven reverse vaccinology enables the rational design of vaccines tailored to elicit specific immune responses, with applications already seen in the development of vaccines against SARS-CoV-2, *Plasmodium*, and other complex pathogens. Additionally, AI contributes to clinical trial optimization by aiding in cohort selection, risk prediction, and real-time data monitoring. These advancements not only shorten development timelines but also enable the emergence of personalized and population-specific vaccines–laying the foundation for improved global epidemic preparedness and next-generation immunization strategies [84]. The Table 1 shows the AI tools and their associated models, highlighting their significance in the vaccine design process.

The convergence of reverse vaccinology and artificial intelligence (AI) is redefining the landscape of vaccine development. Reverse vaccinology, which traditionally involves screening pathogen genomes to identify potential antigens without culturing the organism, has been significantly enhanced by AI-driven models that bring greater speed, precision, and predictive power. Tools such as Vaxign-ML, which applies supervised machine learning to predict protective antigens based on genomic and proteomic features, and NERVE 2.0 [108, 109], which integrates multiple computational analyses to prioritize vaccine candidates, demonstrate the powerful synergy between these two approaches. AI amplifies the capabilities of reverse vaccinology by analyzing vast biological datasets, uncovering complex patterns, and optimizing antigen selection far beyond traditional methods. This integrated strategy not only accelerates vaccine design but also improves the likelihood of identifying highly immunogenic, safe, and broadly protective targets. As emerging infectious diseases continue to pose global threats, the synergy of reverse vaccinology and AI will be instrumental in advancing rapid vaccine development, supporting precision medicine, and strengthening global health preparedness [110].

Table 1. AI tools and models in vaccine design process.

S.N.	Step	Tool name	AI Method
1.	Genome Sequencing	Illumina [85], Oxford Nanopore [86], PacBio [87], VAX-seq [88], Visium SGE [89]	Sequencing + Spatial Transcriptomics (Deep Neural Networks)
2.	Generative Vaccine Design	VAXIGN-ML [90]	Generative Adversarial Networks (GANs) + Variational Autoencoders (VAEs)
3.	Antigen Candidate Prediction	Vaxign [91], Vaxijen [92], VacSol [93]	Machine Learning (ML) + Reverse Vaccinology
4.	Epitope prediction	NetMHCpan [94], BepiPred [94], ABCPred [95], SYFPEITHI [96], IEDB [97]	Neural Networks (NNs), Hidden Markov Models (HMMs), ML
5.	3 D structure Prediction	AlphaFold [98], RoseTTAFold [99]	Deep Learning (DL)
6.	Immune Stimulation	C-ImmSim [100]	Simulation + ML
7.	Codon Optimization	JCAT [101]	Genetic Algorithms (subset of ML)
8.	Adjuvant Screening and Candidate Optimization	Virtual Screening (AutoDock Vina [102], SwissDock [103])	AI-assisted Molecular Docking (Scoring Functions + ML Regression Models)
9.	Structural Validation and Stability Testing	Molecular Dynamics (GROMACS [104], AMBER [105])	AI-Augmented Molecular Dynamics Simulations (Reinforcement Learning)
10.	Adjuvant Safety and Efficacy Profiling	Structure- Activity Relationship (ADMETlab[106], pkCSM [107])	Graph Neural Networks (GNNs) + QSAR Modeling

CONCLUSION

The progression of vaccines from traditional methods to next-generation technologies marks a transformative shift in immunization, enhancing both effectiveness and accessibility. Conventional vaccines, such as live-attenuated, killed, toxoid, and subunit types, have been instrumental in controlling infectious diseases but face challenges like lengthy production, storage constraints, and safety concerns, particularly for immunocompromised individuals. To address these issues, modern vaccine approaches integrate breakthroughs in molecular biology, nanotechnology, and artificial intelligence. Nucleic acid-based vaccines, including mRNA and DNA vaccines, offer rapid development, precision, and strong immune responses, as demonstrated by their success against COVID-19. Edible vaccines, derived from genetically modified plants, present a needle-free alternative, making immunization more accessible. Nanoparticle-based vaccines improve antigen stability and targeted delivery, optimizing immune activation. Additionally, AI-driven reverse vaccinology accelerates vaccine discovery, significantly reducing development time while enhancing effectiveness. The transition to these innovative vaccine strategies represents a new frontier in disease prevention, enabling faster, more adaptable, and personalized immunization solutions. As research advances, these technologies will play a crucial role in responding to emerging health threats, improving global vaccine accessibility, and strengthening public health systems.

Future Directions

The advancement of vaccine development necessitates a synergistic integration of conventional and next-generation strategies to optimize immunogenicity, safety, and accessibility. While novel vaccine platforms enable rapid antigen design, enhanced stability, and targeted immune modulation, traditional vaccines, such as live-attenuated and inactivated formulations, remain indispensable due to their well-documented efficacy, durable immune responses, and cost-effectiveness. These conventional approaches continue to play a crucial role in disease prevention, particularly in low-resource settings where next-generation technologies may face logistical and infrastructural challenges. A hybrid framework incorporating adjuvant optimization, nanoparticle-based antigen delivery, and AI-driven epitope mapping can facilitate a more robust and adaptive immunization strategy. Furthermore, ensuring equitable distribution of advanced vaccines requires investments in scalable biomanufacturing, cold-chain infrastructure, and global supply chain resilience to bridge the accessibility gap. Future

research should focus on refining traditional vaccine platforms while integrating emerging biotechnologies to enhance antigen stability, immunogenicity, and cross-protection against evolving pathogens. By adopting a multidisciplinary approach that combines the reliability of conventional vaccines with the precision of next-generation innovations, the future of immunization can achieve greater efficacy, sustainability, and global health impact.

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