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Review on Approaches of A Reverse Vaccinology for Dangerous Pathogens of Animal

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Abstract
The traditional method of vaccine development includes pathogen culture in the laboratory, but this is not possible in the case of highly infectious pathogens that are hazardous to culture in the laboratory.Reverse vaccinology is regarded as a low-cost and effective method for screening the entire pathogen genome. The incorporation of the pangenome concept into the reverse vaccinology approach is critical in the search for broad-spectrum immunogenic targets and the analysis of closely related bacterial species.. The heart of reverse vaccinology is the selection of specific epitopes of interest capable of eliciting both cellular and humoral immune responses. Reverse vaccinology has also been used to combat viruses. The processes behind the development of protective immunity, and the principles that will direct the creation of the next generation of vaccines by using genomic techniques to investigate both the pathogen and the host. This review demonstrates the advancement of reverse vaccination, their applicability, and their limitations in the timely creation of effective vaccines against the most deadly infections.
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1. INTRODUCTION

Vaccines have become important instruments for combating infectious diseases and cancer (Plotkin,2003;Hussein,2015) Today, it is estimated that immunizations save between 2 and 3 million lives per year throughout the world. In the history of medicine, vaccines have been credited with many significant milestone victories, like the elimination of small pox in 1980 and the current elimination of polio in 2020. In 2019 (Razum et al), The easiest way to improve health is by administering biological preparations known as vaccines. Disease prevention is the most effective strategy for doing so (Sanou et al., 2012). A major step forward in the prevention of diseases for which there is no cure has been the development of vaccinations. The mortality rate for numerous diseases including smallpox, polio, measles, diphtheria, etc. was quite high in many nations, however thanks to the development ofvaccinations against various diseases has decreased to a negligible level. The use of vaccines has significantly lowered the death rate from some deadly diseases in many developing nations. Biological treatments called vaccines aid in strengthening an animal's immunity to a certain disease (Lara et al., 2011).

Depending on the pathogenecity of the microbes, vaccines can be prepared in a variety of ways. Edward Jenner established the concept of vaccination in 1796 by developing a vaccine against smallpox and preventing infection by isolating the materials from cows. In addition, he coined the term vaccine. Louis Pasteur established the principles of vaccinology after discovering that microorganisms cause infectious diseases. Salk and Sabin followed Pasteur's instructions. They developed a vaccine against polio using both killed and attenuated live polio virus. Measles is a highly contagious disease that mostly affects children. Rubella is another serious disease that causes serious birth defects in children (Rappuoli, 2007).

Hilleman used attenuated viruses to develop vaccines against measles, mumps, and rubella and focused on the development of vaccines against diphtheria, tetanus, N. meningitides, S. pneumonia, and other diseases (Lm, 2010). With the introduction of molecular biology and genetic engineering, the first vaccines against hepatitis B (Sollner et al., 2008) and Bordetella pertusis were developed (Bausri et al., 2012).

Since the beginning of the genomic era, new vaccine revolutions have occurred (Rinaudo et al., 2009). Shotgun sequencing has been used to provide complete genomic sequences of several pathogens. With the completion of the first living organism's sequence, genomic data was used for preparation. The genomic data was used to develop vaccines against the organism. An organism's complete genomic sequence is a reservoir of genes encoding proteins that can act as potential antigens and vaccine candidates. This novel approach to identifying proteins exposed on the surface by using the genome rather than the microorganism is known as "reverse vaccinology" (Rappuoli, 2000).

The idea behind reverse vaccinology is to select specific epitopes of interest that can elicit both cellular and humoral immune responses. Several criteria, such as sequence conservancy, binding affinity to MHC classes, allergen city, and so on, could be used to choose the peptide candidate. The purpose of this study was

- To look back at the evolution of vaccine development
- To review the state-of-the-art in reverse vaccinology.
- To examine the concept of reverse vaccinology and its significance in developing countries.

2. LITERATURE REVIEW

2.1. History of Vaccination

A vaccination is made up of antigens, which are immune-response-inducing chemicals that stimulate the body's white blood cells to produce specialized proteins called antibodies. This artificially induces the body to resist illness. Historically, the development of vaccines has been done empirically by isolating, inactivating, and injecting disease-causing microbes (or parts of them) (Rappuoli, 2014).Vaccination has been practiced for generations, around the year 1000, the ancient Chinese used one of the earliest accounts of vaccination, which involved inhaling dried powders made from the crusts of smallpox lesions (Xie and Zhang, 2000).

Immunization, derived from the Latin word immunis, which means "free of," was investigated in the late 18th century by the well-known physician Edward Jenner developed the first successful vaccine against smallpox in 1796 after demonstrating that infectious material from a woman with cowpox, when inoculated into the arm of a young boy, could prevent the young boy from contracting the life-threatening virus (Levine et al., 2010). (Table 1).

The application of powdered smallpox "crusts" and their insertion with a pin or other "poking" tool into the skin became customary about the 15th century. In the Middle East, the procedure—known as "variolation"—became highly popular. Oddly, these procedures were intended to maintain young women's appearance rather than to save lives.Lady Mary Montague, a stubborn aristocrat, introduced variolation to the West. She played a crucial role in marketing the procedure in Great Britain against significant opposition from the medical community due to both the process's stigma as a "Oriental" one and her femininity (Behbehani, 1983)

Genome sequencing revolutionized two decades ago, allowing for the discovery of novel vaccine antigens directly from genomic information. The process was dubbed "reverse vaccinology" to emphasize that vaccine design could begin with sequence information rather than growing pathogens (Rappuoli, 2000). Indeed, the first meningococcus B vaccine derived from reverse vaccinology has recently been licensed (Serruto et al., 2012; O'Ryan et al., 2014). (Table 1).

Today, a new wave of technologies in human immunology and structural biology provide molecular information that allows for the discovery and design of previously impossible vaccines against respiratory syncytial virus (RSV) and human CMV (HCMV), as well as the proposal of universal vaccines to combat influenza and HIV infections (Burton, 2002; Dormitzer et al., 2012; Haynes et al., 2012) (Table 1).

Table 1: Historical milestones monitoring the impact of new technology on vaccine research and development

Discover and design vaccines	Years	Technologies and description	References
Classical vaccinology	1796	Growth of microorganisms allows making killed and live-attenuated vaccines or to discover antigens used for subunit vaccines. Jenner starts growing cowpox in cows marking the beginning of vaccinology.	(Willis, 1997; Baxby, 1999)
	1995	The first sequencing of the entire genome from a bacterium	(Fleischmann *et al*., 1995).
	2002	Proposes to use human mAbs to design new vaccines	(Burton, 2002).
	2013	Graham and Kwong first report that RSV pre-fusion F antigen successfully derived from Structure-based design is protective in the animal model	(McLellan *et al*., 2013a).
Reverse vaccinology	2000	Genomics, high-throughput protein expression, and animal models:	(Pizza *et al*., 2000).
	2012	Vaccine antigens are discovered using the genomic information without the need for growing microorganisms. Antigens selected in silico are expressed and screened in animal models.	(European Medicines Agency, 2012).
	2008	Human mono clonals are used to identify protective antigens/epitopes.	(Dormitzer *et al*., 2008).

Source ;current author(mulatu.H. *et.al*.,2022)

2.2. Reverse Vaccinology versus Conventional Vaccinology

Reverse vaccinology is the use of genomic information with the aid of a computer to prepare vaccines without culturing microorganisms. The use of genetic engineering to produce vaccines was the first revolution in the field of vaccination. The pathogenic components of organisms were identified in this approach by laboratory culturing. However, it was not a very successful vaccine preparation strategy (Flower et al., 2010). To accomplish this, all pathogen genes that can efficiently act as candidate vaccines are studied; however, there are some limitations, such as the inability to predict polysaccharides and lipids, which are some vaccine active compounds. The conventional approach to vaccine development uses two methods: first, attenuation of pathogens by serial passages in vitro to obtain live-attenuated strains to be used as vaccines, and second, identification of protective antigens to be used in non-living, subunit vaccines (Rappuoli R et al.,1999).
The conventional and reverse vaccinology comparisons were given in (Table 2).

Name of Vaccine	Advantage	Disadvantage
Conventional vaccinology	Polysaccharides may be used as antigens Lipopolysaccharide,Glycolipids and other CD1-restricted antigens can be used	Long time required for antigen identification Antigenic variability of many of the identified antigens Antigens not expressed *in vitro* cannot be identified Only structural proteins are considered
Reverse vaccinology	Fast access to virtually every single antigen Non-cultivable microorganisms can be approached Non abundant and not immunogenicduring infection antigens can be identified Antigens that are transiently expressed during infection can be identified Antigens not expressed *in vitro* can be identified Non-structural proteins can be used	Non proteic antigens cannot be used (polysaccharide,lipopolysaccharides, glycolipids and other CD1-restricted antigens)

Table 2: A comparison of traditional and genomic techniques to vaccine development
Source: (Rappuoli *et al.,* 2012).

2.3. Reverse Vaccinology Modification

Reverse vaccinology is a method that identifies novel antigens by using the genome sequences of pathogens that are of interest to it, whether they are parasitic, bacterial, or viral. Experimental biology should then be used to confirm the antigens' activity (Rappuoli, 2001). Identification of vaccine candidates against serogroup B meningococci by the completion of whole-genome sequencing was one of the earliest uses of genomics in vaccination (reverse vaccination) (Pizza et al., 2000). Understanding and controlling infectious pathogens can be greatly aided by genome sequencing. Researchers can use this technology to identify target genes for drug discovery as well as reveal small genetic variations between strains of a specific organism to define its virulence and improve control methods.

The foundation of reverse vaccination is high throughput genomic sequence analysis. Scientists can limit correlations based on the diverse range of data bases thanks to the ongoing flow of fresh genomic sequence and functional annotation data from many taxonomic lineages, which enables the development of more trustworthy analytical and predictive methods. In order to identify numerous structural and functional signatures, such as ligand binding sites, sorting signals, protein domain profiles, various motifs with catalytic sites, and more, one of the most crucial methods was the alignment of multiple homologous sequences (Vivona et al., 2008).

Bioinformatic approaches have recently been used to uncover functional information and enable researchers to tackle biological and biotechnological problems that require the integration of diverse strategies of both in silico (on computer) and experimental evidences. Aside from data analysis, a variety of algorithmic approaches have been used to create novel tools. The functional potential of these in silico approaches has found a pattern in reverse vaccinology (Yasser and Amira, 2011)

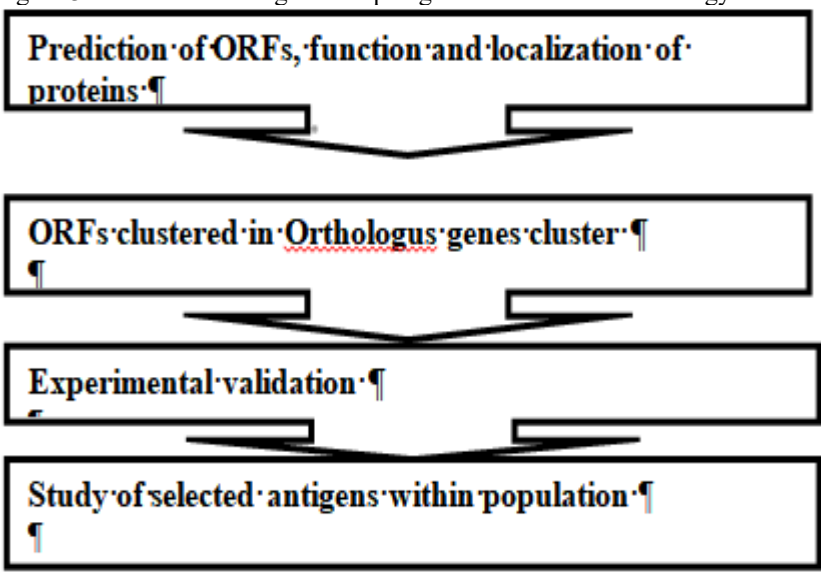
Reverse vaccinology represents a revolution in both immunology and biotechnology, demonstrating how integrating tools can solve biological problems such as vaccine design. However, when compared to traditional vaccine production protocols, reverse vaccinology represents a significant advancement. It makes use of the growing number of genome sequences for various organisms. The method predicts suitable candidate vaccine

molecules by using computer analysis of the genomic sequence. Unfortunately, the method does not provide conclusive evidence that the chosen antigens are immunogenic or protective. On the contrary, the approach enables the identification of novel protein antigens in addition to those discovered by traditional protocols (Yasser and Amira, 2011)

2.4. Pan Genomic Reverse Vaccinology

The genomes of different isolates of the same organism are compared using computer analysis in this approach. Streptococcus agalactica was the first pan genome approach used (Figure 4). (Lafebure and Stanhope, 2007; Zhao et al., 2012).

Figure 3: Process flow diagram for pan genomic reverse vaccinology.



2.5. Comparative Reverse Vaccinology

Reverse Vaccinology (RV) was first created, the prediction of potential vaccine candidates relied only on an in silico examination of the genome of a single strain. To this day, in silico analysis is still the key component of an RV even though selection criteria have been incorporated (Yongqun He et al., 2010). The pathogenic and non-pathogenic strains of a species are compared at the genetic level in this method. It discusses how various organisms' proteins differ from one another in structure.

2.6. Epitope Prediction Characteristics in Reverse Vaccinology

Antigenic determinants called epitopes are crucial for an organism's immunity. These can be seen on the outside of creatures that the antibody can recognize (Ansari and Raghava, 2010). Reverse vaccineology is the study of how computer programs can analyze genomes to anticipate the surface protein epitopes. The development of a potential vaccine depends heavily on the epitopes. B and T lymphocytes are the primary players in the immune system. The paratopes of an antibody can recognize the antigen's epitopes, and B cells are crucial in this process. Because of their interaction with the processed antigenic peptides, T cells can occasionally contribute to cell-mediated immunity (Saha and Raghava, 2007).

3. APPROACH OF REVERSE VACCINOLOGY

The ability to apply the reverse method of vaccine creation is made possible by the entire genome sequence of numerous bacteria, parasites, and viruses. Meningitides, listeriosis, malaria, endocarditis, etc. Among the pathogens that can be tackled by reverse vaccination, anthrax is undoubtedly one of the most representative. On the other hand, there is a long list of infections for which traditional methods of vaccine development have either failed or only offered partial relief. They include bacteria like Chlamydia, pneumococcus, streptococcus, staphylococcus, pseudomonas, Borrelia, Escherichia coli, gonococcus, typhoid, Brucella, Ricksettia, and bartonella (the genome sequences of the majority of these pathogens are almost complete and available on the website http://www.tigr.org), as well as parasites like Leish (Rappuoli, 2000).

4. CONCULISION AND RECCOMMENDATION

reverse vaccinology uses the entire protein repertoire of each pathogen **to select the best candidate vaccine antigens**. This allows the development of vaccines that were previously difficult or impossible to make and can

lead to the discovery of unique antigens that may improve existing vaccines. A vaccine molecule must offer broad-spectrum protection against several populations worldwide in order to be effective. Therefore, estimating the population fractions in the target endemic zones based on HLA genotypic frequencies is crucial for constructing an epitope-based subunit vaccination. Beginnings of reverse vaccineology have changed the paradigm of vaccine development from traditional culture-based methods to high-throughput genome-based technologies. The traditional method of developing vaccines involves growing pathogens in a lab, however this is impossible when dealing with viruses that are extremely contagious and dangerous to culture in a lab. The successful application of several pathogens' whole genomes, such as group B meningococcus, has been made possible by genome sequencing.

The entire genome sequence is required for the prediction of epitopes and other surface proteins, which is a critical component of reverse vaccinology for the development of a successful candidate vaccine. Advances in Recombinant DNA technology, Immunology, and Bioinformatics have significantly accelerated vaccine development in developed countries, while developing countries continue to lag in these areas. The development of a wide range of innovative vaccines using the increase in technologies will be part of future attempts to improve public health around the world.

The following recommendation is derived from the preceding conclusion;

- Developing countries must prioritize the development of capacity to design, manufacture, test, and deploy vaccines.
- To produce effective and safe vaccines, additional approaches to counteract the pathogens' high biological complexity, such as allowing the inclusion of multiple epitopes from multiple antigens, are required.
- Further Clinical applications, such as in cancer and autoimmunity, could benefit from methods to either trigger or modulate cellular immune responses.
- There is no single method that is universally applicable and successful; instead, we must integrate several equally valid, equally partial methods and benefit from their synergy.'

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