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Review on Approaches to Reverse Vaccinology Against Dangerous Pathogens in Animals1

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ABSTRACT
Vaccines have been recognized as major and effective tools For controlling disease impact, vaccinology is a field with great opportunity. Contribution of vaccines towards societal development by improvement of health status and increasing life-expectancy has been paramount. The conventional way of vaccine development includes culturing of pathogens in laboratory but this is not possible in case of highly infectious pathogens that are hazardous to culture in laboratory. The concept of reverse vaccinology is based on selecting specific epitope of interest that are capable of provoking cellular as well as humoral immune response which is the heart of reverse vaccinology. Some approaches against viruses have also been done by reverse vaccinology. Applying genomic approaches to study both the pathogen and host will ultimately increase our fundamental understanding of pathogen biology, mechanisms responsible for the development of protective immunity, and guide next-generation vaccine design. This review paper show development of reverse vaccinology, their relevance, and limitations in the timely development of useful against the most dangerous pathogens.
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1. INTRODUCTION

Vaccines have emerged as major tools for the control of infectious diseases and cancer (Plotkin,2003;Hussein,2015) Currently vaccines are known to save at least 2-3 million lives annually worldwide. The eradication of small poxin1980 and the recent elimination of polio in 2020 are monumental landmark achievements attributed to vaccines in the history of Medicine.(Razum et al, 2019). Disease prevention is the most effective approach for health and can easily be achieved by administration of the biological preparation called vaccines (Sanou *et al*., 2012). Development of vaccines has proved a milestone in prevention of diseases for which the cure is not available. In many countries mortality rate for various diseases like small pox, polio, measles, diphtheria etc (CDC, 2013) was very high but due to invention of vaccines against these diseases, it has fallen to negligible. In many developing countries vaccines have played an important role in decreasing the mortality rate due some major killer diseases. Vaccines are biological preparations that are helpful in improving the immunity of an animal against a particular disease (Lara *et al*., 2011).

Vaccines can be prepared by various means depending on the pathogenecity of microbes. The concept of vaccination was given by Edward Jenner in 1796 by developing vaccine against smallpox and averting the infection by isolating the materials from cow. He also introduced the term *vaccine.* When it was found that microorganisms are the cause of infectious diseases, Louis Pasteur gave the rules of vaccinology. The rules given by Pasteur were followed by Salk and Sabin. They prepared the vaccine against polio that is killed and attenuated live polio virus as a vaccine respectively. Measles is a severely communicable disease that mainly infects the children. Rubella is another serious disorder that causes severe child birth defects (Rappuoli, 2007).

Hilleman developed vaccine against measles, mumps and rubella (Lm, 2010) with the help of attenuated viruses and focused light on development of vaccine against diphtheria, tetanus, *N. meningitides*, *S. pneumonia* and so on.There have been many innovations in the field of vaccines, the first being against hepatitis B (Sollner *et al*., 2008) and *Bordetella pertusis* with the introduction of the molecular biology and genetic engineering (Bausri *et al*., 2012).

The start of genomic era new revolutions have been taking place in the field of vaccines (Rinaudo *et al.*, 2009). The application of shotgun sequencing has been introduced in giving the whole genomic sequences of several pathogens. With the completion of the sequence of the first living organism, the genomic data was used for the preparation of the vaccines against the organism. The complete genomic sequence of an organism is the reservoir of genes encoding the proteins that can act as potential antigens that can be used as vaccine candidates. This technique of identifying the proteins that are exposed on the surface by using genome instead of the microorganism, this novel approach is known as “reverse vaccinology” (Rappuoli, 2000).

The concept of reverse vaccinology is based on selecting specific epitope of interest that are capable of provoking cellular as well as humoral immune response. The peptide candidate could be selected based on several criteria including, sequence conservancy, binding affinity to MHC classes, allergen city, etc. The aim of

the present work was

- To over eview the evolution of vaccine development
- To review the state of the art of Reverse vaccinology
- To review the idea of the Reverse vaccinology and its importance in Developing country

2. LITERATURE REVIEW

2.1. History of Vaccination

The concept of vaccination has been around for centuries. One of the first documented accounts of immunization was practiced by the ancient Chinese around AD 1000, by inhaling dried powders derived from the crusts of smallpox lesions (Xie and Zhang, 2000). Around the 15th century, a practice of applying powdered smallpox "crusts" and inserting them with a pin or "poking" device into the skin became commonplace. The process was referred to as Variolation and became quite common in the Middle East. Oddly, these practices were not meant to save lives but to preserve the beauty of young women. Variolation was brought to the West by a tenacious aristocrat, Lady Mary Montague, who played a critical role in promoting the process in Great Britain, despite a great deal of resistance from the medical establishment, both because Variolation was considered an "Oriental" process and because of her gender (Behbehani, 1983). These initial empirical observations gave rise to the origin of vaccination. Immunization, derived from the Latin word *immunis* meaning "*free of*," was investigated by the well-known physician Edward Jenner in the late 18th century. Jenner in 1796 created the first successful vaccine against smallpox after showing that infectious material from a woman with cowpox, when inoculated into the arm of a young boy, could prevent the young boy from acquiring the life- threatening virus (Levine *et al*., 2010) (Table 1).

Smallpox was the first disease scientists tried to prevent by intentionally inoculating individuals at risk with the infecting agent (Cook, 2007). Almost a century later, Louis Pasteur in 1885, a world- renowned French chemist and biologist, also considered the "father of immunology," became involved in the practice of immunization, and became known for his principles of "isolate, inactivate, and inject" (Rappuoli, 2007). Pasteur is particularly renowned for his work on the vaccine for anthrax (a bacterial infection that was decimating sheep herds at the time) and rabies (a highly contagious viral infection that attacks the central nervous system). Pasteur was able to produce an attenuated form of the virus, which he then used for immunization (CDC, 1985) (Table 1).

A vaccine is comprised of antigens (molecules that trigger an immune response) that artificially induce the body to resist infection by stimulating the body's immune system (white cells) into producing specialized proteins known as antibodies. Traditionally, vaccines have been developed empirically by isolating, inactivating and injecting the microorganisms (or portions of them) that cause disease (Rappuoli, 2014). Two decades ago, genome sequencing revolutionized this process, allowing for the discovery of novel vaccine antigens starting directly from genomic information. The process was named "reverse vaccinology" to underline that vaccine design was possible starting from sequence information without the need to grow pathogens (Rappuoli, 2000). Indeed, a vaccine against meningococcus B, the first deriving 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 the fields of human immunology and structural biology provide the molecular information that allows for the discovery and design of vaccines against respiratory syncytial virus (RSV) and human CMV (HCMV) that have been impossible thus far and to propose universal vaccines to tackle influenza and HIV infections (Burton, 2002; Dormitzer *et al*., 2012; Haynes *et al*., 2012) (Table 1).

Table 1: Historical milestones tracking the impact of new technologies on vaccine discovery and design

Discover and design vaccines	Year	Technologies and descriptions	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).

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2.2. Reverse Vaccinology against Conventional Vaccinology

Reverse vaccinology relies on the genomic information to identify relevant protein antigens and the design of algorithm for mapping potential B and T cell epitopes for diagnostic or vaccine purposes (Sette A, and Rappuoli R, 2010). The use of genomic information with aid of computer for the preparation of vaccines without culturing microorganism is known as reverse vaccinology. The first revolution in field of vaccination is the use of genetic engineering to produce vaccines. In this approach the pathogenic components of organisms were identified by culturing in laboratory. But it was not a very successful approach for vaccine preparation (Flower *et al*., 2010).

The genome sequences provide at once all protein antigens that the pathogen can express at any time. This approach contains genome sequences, computer analysis and prediction of epitope / antigen and candidate vaccine. By this approach one may discover a new antigen that can work on a different pattern. High throughput screening is required for the production of feasible candidate vaccine. For achieving this, all genes of pathogens are studied that can efficiently act as candidate vaccine but there are some limitations that it can't predict polysaccharides, lipids which are some active compounds of vaccine. The comparisons of the conventional and reverse vaccinology were given in (Table 2).

Table 2:- Comparison of Conventional and Genomic approaches to vaccine development

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)

Source*:* (Rappuoli *et al.,* 2016).

2.3. Alteration in Reverse Vaccinology

Genome sequencing is a powerful tool for understanding and controlling infectious pathogens. Using this technology, researchers can identify target genes for drug discovery and reveal small genetic variations between strains of a specific organism to define its virulence and improve the method of control. The approach of reverse vaccinology uses the genome sequences of viral, bacterial or parasitic pathogens of interest as starting material for the identification of novel antigens, whose activity should be subsequently confirmed by experimental biology (Rappuoli, 2001). One of the earlier applications of genomics to vaccinology (reverse vaccinology) had been the identification of vaccine candidates against serogroup B meningococcus by the completion of the whole-genome sequencing (Pizza *et al*., 2000). They had cloned the open reading frames (ORFs) that encode putative virulence factors and surface-localized proteins of meningococcus. Several hundred ORFs (350 surface-exposed protein coding frames) were cloned into expression vectors, purified and used to immunize mice. The antibodies binding properties to the products of ORF were analyzed using fluorescent activated cell sorter (FACS) analyses and Enzyme linked immune sorbent assay (ELISA). The primary vaccine candidates were then tested *in vitro* and/or animal models to provide an insight on the protective efficacy. Twenty nine of these surface-exposed proteins were found to be bactericidal. (Yasser and Amira, 2011).

Reverse vaccinology is based on the high throughput analyses of genome sequences. With continuous flow of new genomic sequence and functional annotation data from different taxonomic lineages permits scientists to confine correlations depending on the wide range of data bases, enabling the design of more reliable analytical and predictive tools. One of the most important tools is the alignment of multiple homologous sequences that permitted the identification of large number of structural and functional signatures including ligand binding sites, sorting signals, protein domain profile, different motifs with catalytic sites and more (Vivona *et al*., 2008).

The prediction-driven experiments may imply functions for disease gene products (Emes and Ponting, 2001, Vacca *et al*., 2001). Later, more complex, genome-wide analyses have led to the identification of proteomic

complements that underlie regulatory pathways or interaction network organization in model organisms (Carpi *et al*., 2002, Li *et al*., 2006). More recently, bioinformatic approaches are 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. Besides data analyses, a variety of algorithmic approaches have been used to develop novel tools. The functional potential of these *in silico* approaches has found its pattern in reverse vaccinology (Yasser and Amira, 2011)

Reverse vaccinology involves the *in silico* screening of a pathogen entire genome to identify genes encoding proteins with the attributes of good vaccine targets. This reverse approach takes advantage of the increasing availability of whole pathogen genome sequences, either single pathogenic isolate or pan-genomes (the genomic information from several isolates) of a pathogenic species. The main attraction of RV lays in its applicability to any pathogen with WGS data and to which antibody-mediated immunity for protection against disease is crucial. Its use in the discovery of candidate antigens comprising vaccines targeting other bacterial pathogens, including the multidrug-resistant Acineto bacter baumanii, has been demonstrated (Talukdar *et al*., 2014; Chiang *et al.*, 2015; Meunier *et al*., 2016). Indeed, the genome sequence provides an exhaustive catalogue of virtually all protein antigens that the pathogen can express at any time. Reverse vaccinology thus begins with bioinformatics analysis to identify antigens *in silico* that are then tested experimentally. This sequence is a reversal of the standard workflow in which analysis requiring culturing the organism comes initially and bioinformatics analysis subsequently. This approach, used originally against meningococcus, allows fast identification of candidate antigen as target for vaccination and provides new solutions for those vaccines that have been difficult or impossible to develop (Rappuoli, 2001; Sette and Rappuoli, 2010).

Several curated databases are now developing that provide comprehensive information about experimentally validated antigens, e.g. Protegen (Yang *et al*., 2011), IEDB (Zhang *et al*., 2008), AntigenDB (Ansari *et al*., 2010).

Reverse vaccinology presents a revolution in both immunology and biotechnology and shows how a biological problem like designing a vaccine could be solved by applying integrating tools. However, reverse vaccinology presents a huge advance compared to the conventional vaccine production protocols. It takes advantage of the growing number of genome sequences available for many organisms. The approach uses computer analysis of the genomic sequence to predict suitable candidate vaccine molecules. Unfortunately, the approach does not provide certain evidence that the selected antigens are either immunogenic or protective. On the contrary, the approach permits the identification of novel protein antigens besides the antigens discovered by the traditional protocols (Yasser and Amira, 2011)

2.4. Pan Genomic Reverse Vaccinology

In this approach the genome of the different isolates of same organism is compared with each other by using computer analysis. The first pan genome approach was done against *Streptococcus agalactica* (Figure 4) (Lafebure and Stanhope, 2007; Zhao *et al*., 2012).

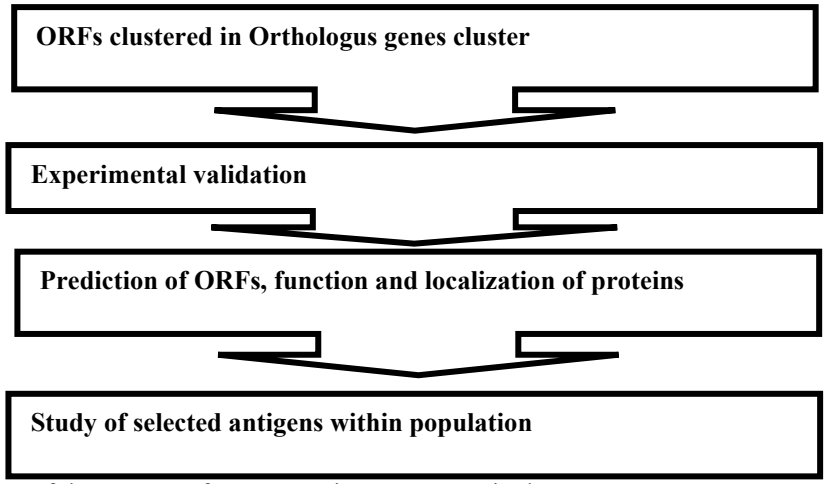
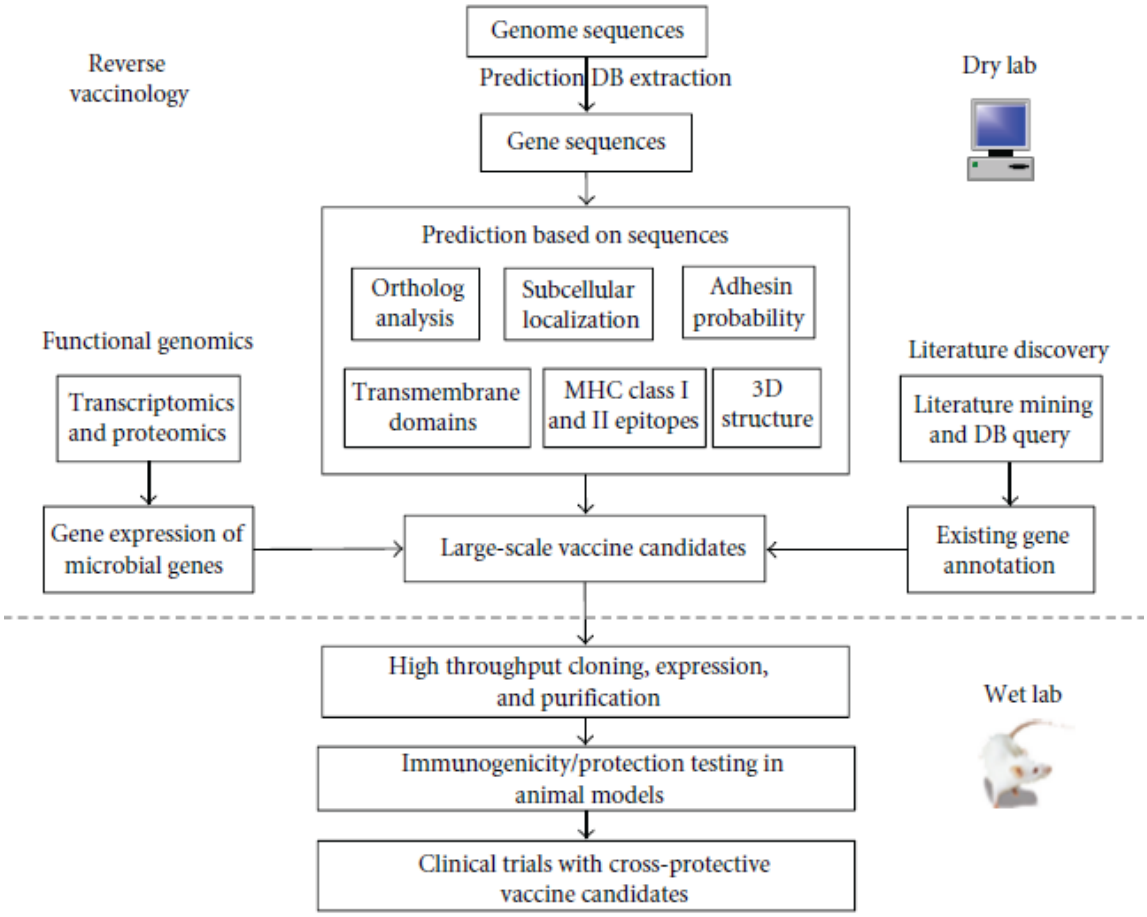


Figure 3:- Flow chart of the process of pan genomic reverse vaccinology

2.5. Comparative Reverse Vaccinology

Initially, when Reverse Vaccinology (RV) was developed, prediction of putative vaccine candidates was based solely on *in silico* analysis of the genome of a single strain. Now that selection criteria have been implemented, however, *in silico* analysis remains the central step in an RV (Yongqun He *et al.,* 2010) (Figure 4). In this approach the pathogenic and non-pathogenic strains of one species are compared at their genetic level. It deals

with the differences in structure of proteins of different organisms.



Source: (Yongqun He *et al.,* 2010)
Figure 4: A schematic demonstration of integrative reverse vaccinology strategy towards vaccine development.

2.6. Character of Epitope Prediction in Reverse Vaccinology

An epitope is an antigenic determinant that plays an important role in immunity of an organism. These are present on the surface of organisms that can be detected by the antibody (Ansari and Raghava, 2010). Reverse vaccinology deals with computational analysis of genome that can be used for the prediction of the epitopes that are surface proteins. So the epitopes play an important role in development of a candidate vaccine. The major role played in immune system is by B and T lymphocyte. B cells are important in recognizing the epitopes of the antigens that can be identified by the paratopes of antibody. In some cases, T cells play a role in cell mediated immunity as the processed antigenic peptides interact with the T cell when they are presented in context of T cell. So the prediction of the epitopes of T and B cell plays an important role in determination of the candidate vaccine. The epitope prediction plays an important role in designing of epitope based vaccine (Saha and Raghava, 2007).

I. **T-Cell Epitope Mapping and Prediction**

One of the problems facing traditional vaccines is the lack of a broad cell-mediated immune response against variable pathogens (Yasutomi *et al*., 1993; Carruth *et al*., 1999; Shapiro, 2013). Humoral immunity may help prevent infection, but to date only a limited number of antibodies with neutralizing capability have been identified for viruses such as HIV. The induction of cell-mediated immune responses with a large repertoire of immune specificities has emerged as an essential characteristic for the clearance or control of hypervariable viral infections such as HCV and HIV (Yasutomi *et al*., 1993; Cristillo *et al*., 2006; Azizi *et al*., 2007).

T cell recognizes the antigenic peptides only when they are presented by MHC I or II, with the help of the CD4 and CD8 molecule. Given the importance of T-cell responses in controlling viral infections, the larger number of T-cell epitope mapping and prediction algorithms available today comes as no surprise (De Groot and Berzofsky, 2004; De Groot, 2006). One of the more comprehensive programs seems to be EpiMatrix from EpiVax Inc (Sirgkyj *et al.,* 2011).

II. B-Cell Epitope Mapping and Prediction

The antigen antibody interaction plays an important role in immunity, binding takes place at antigenic determinant also known as B-cell epitopes.

The B-cell epitopes are defined by a specific surface region of an antigenic protein, and may be divided into two different types of epitopes: linear epitopes and conformational epitopes (Davies and Flower, 2007).

The linear epitopes are short peptides while conformational epitopes composed of amino acid folded in 3-dimensional protein structure (El-Manzalawy and Honaver, 2010). The mapping of the B cell epitopes can be done by various techniques. The focus of the scientist is only on the determination of Linear B cell epitope (Flower, 2007).

The propensity value of amino acid plays an important role in determination of its position in B cell epitopes. It was introduced by Hopp and Woods. They utilized the Levitt hydrophobicity scale for the determination of the propensity value for each amino acid (El-Manzalawy and Honaver, 2010). Today, several tools are available for the prediction of linear B cell epitopes such as PREDITOPE (Odorico and Pellequer, 2003), PEOPLE (Alix, 1999), BEPITOPE (Odorico and Pellequer, 2003) and BcePred with the determination of the propensity value, ABCpred uses the machine learning based method for the prediction of the Linear B cell epitopes (Saha and Raghaya, 2006)

The conformational B cell epitope prediction can be done by Sequence based prediction method, Structure based prediction method and Mimotpoe analysis based prediction method are known (El-Manzalawy and Honaver, 2010). DiscoTope is used for the determination of the conformational B cell epitope prediction. PEPITOPE uses combination of propensity value and half sphere exposure value of amino acid residues (El-Manzalawy and Honaver, 2010).

B-cell epitope-mapping algorithms include 3DEX (3D-Epitope-Explorer) (Schreiber *et.al,* 2005), CEP (conformational epitope predictor) (Kulkamikale *et.al*., 2005) and DiscoTope (Kolaskar and kullamikale, 1999). 3DEX software is designed to allow the localization of linear peptide sequences within the three-dimensional structure of a protein. CEP predicts epitopes of proteins with known structures using accessibility of residues and spatial distance cutoffs to predict antigenic determinants, conformational epitopes and sequential epitopes (Kolaskar and kullamikale, 1999; Kulkamikale *et al*., 2005) DiscoTope was designed specifically for the prediction of conformational B-cell epitopes (Ansari and Raghava, 2010).

Developments in B cell epitope prediction include; Prediction of the protective linear B cell epitopes (Sollner *et al*., 2008), hybrid and consensus prediction of B cell epitopes, improved conformational B cell epitope prediction, critical assessment of B cell epitope prediction (El-Manzalawy and Honaver, 2010), immune epitope database and analysis resources with the help of these databases one can easily identify and predict the B cell epitope very correctly (Kim *et al*., 2012).

3. APPROACH OF REVERSE VACCINOLOGY

The complete genome sequence of many bacteria, parasites and viruses means that the reverse approach to vaccine development can be put into practice. Such as Meningitides, Listerosis, Malaria, Endocarditis, .Anthrax are perhaps some of the most representative among those that can be approached by reverse vaccinology. However, the list of the pathogens where the conventional approaches to vaccine development have failed or provided only partial solutions is extensive. Among these we can list bacteria such as *Chlamydia*, pneumococcus, *Streptococcus*, *Staphylococcus*, pseudomonas, *Borrelia*, *Escherichia coli*, gonococcus, typhoid, *Brucella*, *Ricksettia* and *Bartonella* (the genome sequences of most of these pathogens are about to be completed and available on the website http://www.tigr.org), and parasites such as *Leishmania* and many others (Rappuoli, 2000).

4. CONCULISION AND RECCOMMENDATION

Vaccines can be prepared by various means depending on the pathogenecity of microbes. For effective vaccination, a vaccine molecule must provide broad-spectrum protection against different populations around the world. Thus, in designing an epitope-based subunit vaccine, it is important to estimate the fractions of the population in the target endemic zones based on HLA genotypic frequencies.The Beginnings of reverse vaccinology have shifted the paradigm of vaccine development from conventional culture-based methods to high-throughput genome based approaches. The conventional way of vaccine development includes culturing of pathogens in laboratory but this is not possible in case of highly infectious pathogens that are hazardous to culture in laboratory. The sequencing of the complete genome of many pathogens, such as group B meningococcus, has allowed the successful application of reverse vaccinology where conventional approaches have failed. The genome sequences of a large number of isolates can be screened for homology. The whole genome sequence is required for the prediction of epitopes and other surface protein; which is the important part of reverse vaccinology for the designing of a successful candidate vaccine. Advances in Recombinant DNA technology, Immunology and Bioinformatics have considerably accelerated vaccine development in advanced

countries, while the developing countries are still lagging behind in these domains. From above conclusion the following recommendation is forwarded;

- The need to build capacity to design, manufacture,test and deploy vaccines is a priority in developing countries.
- Further approach to counteract the high biological complexity of the pathogens by allowing inclusion of multiple epitopes from multiple antigens is needed to produce effective and safe vaccines.
- Collaboration of many partners is necessary.No one method is universally applicable and successful; rather we need to integrate several equally-valid, equally-partial methods and draw from their synergy.
- Vaccine value in a world of limited resources cost effectiveness becomes important in decision making Health economics not able to assign the right value to vaccine.

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ABBREVIATIONS

CAD	Computer Aided Design
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CDC	United States center for disease control and prevention
DNA	Deoxy riboneuclotide acid
E. coli	Escherchia coli
ELISA	Enzyme linked immune sorbent assay
FACS	Fluorescent Activated Cell Sorter
HCMV	Human Cytomegalovirus
HCV	Hepatitis C virus
HIV	Human Immune Deficiency Virus
IEDB	Immune Epitope Data Base
L. monocytogenes	Wisteria monocytogenes
LCV	Leukocytoclastic Vasculitis
LPXTG	Leu-Pro- X-Thr-Gly
MenB	Meningtidis B
MHC I	Major histocompatibility one
MHC II	Major Histocompatibility two
N. meningtidis	Niesseria meningtidis
NIH	National Institutes of Health
S. pneumonia	Streptococcus pneumonia
ORF	Open Reading Frame
PSORTb	Protein subcellular localization to improve
RSV	Respiratory Syntial Virus
RV	Reverse vaccinology
TMHMM	Tied Mixture Hidden Markov Model
WGS	Whole genome sequence