Abstract: Current review article focuses on dengue, which is one of the most fatal infectious illnesses and is considered to be a worldwide threat. The paper covers essential topics including an overview on neglected tropical diseases with specific emphasis on Dengue fever, mosquito’s cycle of life and mechanism of infection, adaptive response, and different stages in dengue immunopathogenesis. The current work is also dedicated to the thorough study of dengue outbreak across the globe with a narrowed study to tropical and subtropical regions. Moreover, this review article demonstrates the correlation between climate factors and dengue incidence. Furthermore, we present an overview of the detection strategies of dengue including the latest developments in commercial and non-commercial platforms. Several attempts in developing an effective vaccine to protect individuals from dengue infection and the stage of clinical trials are gathered in the present work as well. Future directions including bio-control are also discussed in this review article. In an overall view, effective management of Dengue is a multidisciplinary task that requires international involvement from different backgrounds and expertise to address this global concern. This review article briefly portrays some of these connecting areas across the disciplines while many other perspectives remain uncovered.

Keywords: Dengue fever, Dengue immunopathogenesis, Dengue outbreak, Effect of climate factors on Dengue, Detection platforms for Dengue, Bio-control of Dengue.

1. INTRODUCTION

Infectious illnesses are the products of pathogenic microorganisms including different categories of viruses, bacteria, parasites as well as fungi. These microorganisms may live within or on the surface of living beings spreading to other beings directly or indirectly [1]. Such infections can be contracted directly from one individual to another or transmitted via a medium. Consumption of contaminated meals or drinks or exposure to the surrounding microorganisms could be other causes of infection. Signs and symptoms can vary depending on the microorganism that has caused the infection. In almost all cases, fever and fatigue are considered to be the primary manifestations of such infections. Minor symptoms might be resolved by the home-therapies while severe signs and symptoms require immediate hospitalization.

Perhaps the main concern and unaddressed issue of tropical and subtropical regions are neglected tropical diseases (NTDs), which have a large family. World health organization (WHO) reports 18 most life-threatening NTDs categorized as follows: (i) protozoan diseases including chagas...
disease, human African trypanosomiasis, and leishmaniases; (ii) bacterial diseases including buruli ulcer, leprosy, and trachoma; (iii) helminth diseases including cysticercosis/taeniasis, dracunculiasis, echinococcosis, foodborne trematodiasis, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiases, and yaws and; (iv) viral diseases including Dengue, chikungunya, zika, and rabies. NTDs nearly affect 1.5 billion individuals in 149 countries across the globe, specifically among the underprivileged population [1]. NTDs typically spread in tropical climates with resource-limited medical setups while in other regions with better standards of life and hygiene, NTDs are almost wiped out [2, 3]. Among different NTDs, we review dengue fever (DF) as the focus of this review article due to its serious worldwide threats (Fig. 1A).

Similar to yellow fever, West Nile and Japanese encephalitis, DF belongs to *Flaviviridae* family. This mosquito-borne infection transmits to individuals via bite of *Aedes* mosquitos (Fig. 1B) [4].

WHO indicates over 50 million infection cases with Dengue virus (DENV) (Fig. 1C) in each year [1] though some approximations prove this estimation to be 100 million cases per year. DF is prevalent in tropical and subtropical zones. This fatal illness, however, can become a worldwide threat by those who contract the illness whilst traveling to different destinations around the globe [5]. There are four serotypes for DENV including DENV-1, DENV-2, DENV-3 and DENV-4. The fifth serotype has recently been reported as well (DENV-5). Infection with any of the serotypes does not create immunity to other serotype. While initial infection typically results in DF, the problem can develop into rather harsher phases including Dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS) [5-7]. Patients who have been infected with DENV more than once (other serotypes) are facing a higher risk for experiencing syndromes including DHF and DSS [5-7]. Common symptoms of DF include fever, nausea, vomiting, headache, skin rashes and pain in the muscles [8-10]. Manifestations when patients enter the severe stage of the disease usually involve a high fever, agitation, bleeding underneath the skin, sensitive skin (when experiencing DHF) and circulatory collapse (when experiencing DSS) [5-7]. DF is known as a self-limited debilitating febrile illness. In adults, it is accompanied by a range of symptoms such as headache, retro-orbital pain, myalgia, leukopenia, thrombocytopenia, and seldom hemorrhagic manifestations [11, 12]. DHF is characterized by fever, hemorrhagic manifestations, thrombocytopenia (less than 100,000 cells/mm³), and plasma leakage such as ascites, hemoconcentration, pleural effusion or hypoproteinemia [13]. There are four grades of DHF from which stages III and IV could result in lethal outcome as patients experience hypotensive shock amplified by cytokine storm that targets vascular endothelium increasing vascular permeability [12, 14]. Although there is immune cross-reactivity between DENV serotypes, partial protection occurs over a short period of time that might leave individuals fully susceptible to heterologous secondary dengue infection [15]. Previous dengue exposure is known to predispose individuals to a subsequent secondary infection with fatal results [16]. With a large number of world population facing the danger of this deadly infection, dengue is, without a doubt, one of the biggest threats to public health [4, 17]. Dengue detection in the primary stages is expected to diminish the mortality degree from 20% to less than 1% [8-10].

In this review article, the life cycle of dengue mosquito and the mechanism of infection are reviewed. The article has a specific emphasis on the global distribution of dengue fever and its different serotypes. Furthermore, the effect of environmental factors, in particular, climate change, on dengue outbreak is thoroughly discussed. The article also concentrates on the latest advancements in Dengue diagnosis with a major focus on commercialized and non-commercialized platforms for effective detection of Dengue infection. Moreover, the current review article summarizes the efforts in vaccine development that could grant human beings immunity against this worldwide threat.

### 2. MOSQUITO CYCLE OF LIFE AND MECHANISM OF INFECTION

These mechanisms of infection and replication lead to the pathogenesis of the DENV disease inside the mosquito and human. When the mosquito finished its life cycle, from egg
to larva and pupa, the adult female mosquito is able to contract the infection when biting a DENV infected person. Particularly in acute phases of Dengue when the virus is spread over the bloodstream causing viremia, the mechanism of transfer takes place easier. The mosquito that is carrying the infected blood starts a new infection cycle that lasts for 28 days. Inside the mosquito’s body, the virus infects midgut and salivary glands, while spending 8-10 days in incubation. After this extrinsic incubation in mosquito, the virus is ready to change the reservoir thus a new victim can be infected. Between 4-7 days from the infection, the patient would show clinical symptoms and possibly experiences an acute phase of the disease. Fig. (2) describes the cycle of infection from an infected person to a healthy individual.

3. DENGUE IMMUNOPATHOGENESIS

The immunological manifestations begin when DENV is inoculated into the dermis or epidermis. Occasionally the virus can also be injected directly into bloodstream. Resident immune cells such as Langerhans, dendritic cells (DC), and macrophages that are infected could transfer to the lymph nodes [19, 20]. Infected cells recruit more inflammatory cells that also get infected and migrate to lymph nodes, therefore, disseminating the infection. The interaction of DENV with DC is through recognition of the DC-specific ICAM3-grabbing non-integrin (DC-SIGN) that enables the infection [21-23]. Polymorphisms in this molecule may correlate with an increased change of Dengue infection as well as severity of the disease [24, 25]. A polymorphism is the occurrence of two or more genetically determined phenotypes in a population. For instance, a variation in a single nucleotide in a DNA sequence may result in a mutant protein with a missing or a completely different function. Other molecules described in this viral interaction are heat shock proteins (HSP)-90 and HSP70 as well as mannose receptor on macrophages or monocytes [26]. Also, monocytes change into CD14+CD16+ cells that are able to stimulate plasma-blast differentiation and antibody production [27].

The immune response is the primary defense strategy against pathogens and involves recognition of viral peptides.

Fig. (2). Cycle of Dengue infection from egg to larva, pupa, and adult mosquito: The image describes the process of incubation, infecting a healthy person, contracting the disease from an infected person and transferring it to another healthy person [18].
or RNAs with pattern recognition receptors (PRRs) in order to produce inflammatory and antiviral cytokines. PRRs that may have a role in DENV infection are Toll-like receptors (TLRs), particularly endosomal TLR-3s that recognize ssRNAs. After the interaction, the intracellular TLR signaling pathway induces the production of interleukin-8 (IL-8) and interferon (IFN)-α/β (type I IFNs). In addition, a different PRR such as retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5) could recognize dsRNA in the cytoplasm, to start producing IFN-α and synergize with TLR-3 signaling. The IFN is an efficient antiviral mechanism, which interferes with viral replication [14, 28, 29]. The type 1 IFN is regulated by interferon transcripational factors (IRF) 3 and 7 for which some studies in mice models demonstrated that the protective effect was related to the combined response from both genes [30]. Nevertheless, DENV has inhibition mechanisms, which includes the down-regulation of IFN production by non-structural proteins that cleave to a stimulator of interferon genes (STING) protein, referred to as MITA signaling pathway or signal transducer and activator of transcription 1 (STAT1) [31, 32]. Fig. (3) provides detailed information in regard to the immunopathogenesis of Dengue.

Another cytokine that is secreted during the acute phase is TNF-α. Yen et al. demonstrated for the first time that tissue infiltration with macrophages that secreted TNF-α produces endothelial damage and may contribute to plasma leakage [33]. A murine model of DHF has demonstrated that high viral titer, macrophage infiltration and production of TNF-α are key events to exacerbate the hemorrhage [34]. In the hemorrhagic tissue, the endothelial activation can over-express inducible nitric oxide synthase (iNOS) and oxygen species. It was observed that the mice lacking iNOS reduced the severity of hemorrhagic effects in DHF [33]. Also, viral NS1 can be recognized by TLR-4 on the endothelial cell surface, increasing the permeability [35, 36].

Bloodstream levels of TNF-α or NS1 have been proposed as biomarkers [33]. Late production of IL-10 anti-inflammatory cytokine and gene polymorphism also correlates with DENV severity, this molecule is secreted by mononuclear phagocytic system and some adaptive immune cells [37, 38]. The chemokines responsible for the recruitment of different inflammatory cells to infected tissues are IL-8 [39], C-C (cysteine-cysteine) motif chemokine ligand-2/monocyte chemotactic protein-1 (CCL2/MCP-1), and C-X-C (cysteine-amino-acid-cysteine) motif chemokine-10 also known as interferon gamma-induced protein-10 (CXCL10/IP10). These chemokines might be also involved in endothelial permeability. A higher serum concentration of CCL2 is related to hypotension, thrombocytopenia and hemorrhagic shock in patients with DHF in comparison to DF [40-42]. Moreover in vitro assays showed a contribution of CCL2 and TNF-α in tight junction disruption [42]. Although soluble chemokine receptors in the bloodstream are increased, there is no substantial evidence on their pathological role in Dengue infection. These receptors are believed to be released into the bloodstream by shedding from the cell membranes [42]. Many conditions may alter the level of chemokines and other soluble molecules; for example infection with human immunodeficiency virus (HIV) or varicella-zoster virus (VZV) could increase the level of chemokines and impact their physiological symptoms.

During the dissemination, there is an increase in the viral particles and the antigens in the blood stream. There are evidences for the existence of viral particles in some other sites of infection such as liver or even the central nervous system (CNS). However, there is no evidence for their productive replication in CNS or detectable antigens in the liver tissue during DHF/DSS [43]. Classical histopathological findings of the liver show the occurrence of Councilman bodies that represents the degeneration of the hepatocytes. These injuries are caused when DENV invades the hepatocyte inducing apoptosis. Kupffer cells form such injuries to engulf the apoptotic bodies. They need specific microenvironments and natural cell contacts with the virus to produce such bodies, though results show no active infection of kuffer cells. It is known that the attachment of DENV to the liver endothelial cells is mediated by L-SIGN and homolog of DC-SIGN [14, 44-46].

4. ADAPTIVE RESPONSE

The secondary or subsequent DENV infection implicates the adaptive immune response. In comparison to the innate response, the adaptive system has more specialized features and very specific antigen recognition. Moreover, adaptive cells have immunological memory leaving the cells prepared for a secondary exposure with a faster and more efficient response. This is the reason for the severe clinical manifestations in DENV. The adaptive immune system is characterized by the activation of TCD4 or TCD8 lymphocytes and B-lymphocytes. TCD4 cells are also known as helpers and are involved in B cell activation to produce specific antibodies. Meanwhile, TCD8 cells have cytotoxic mechanisms to kill infected cells. During Dengue infection, all these cellular and humoral compounds are activated by antigen presentation from DC or macrophages. After this presentation, the lymphocytes can proliferate, differentiate, and produce cytokines in order to clear the infection [47].

There are several associations with increased protection from severe disease including high polarization of TCD4 cells to CX3CR1+ . This chemokine receptor may lead to the migration of CX3CR1+ to the inflamed and infected tissues where they can exert effector functions [48]. While it is believed that TCD4 cells from healthy donors have an impaired response when exposed to DENV, they are known to produce less IL-2, downregulate IL-2 receptor, and are unable to proliferate [49]. De Matos et al., have suggested that a high proliferation of TCD8 might protect them against dengue by reducing viral reproduction. These authors found that in patients with DENV-2 there is an association between high amount of TCD8 effector memory subset and the late phase of the disease in which these cells produce a high amount of IL-2 and IFN-γ (IFN type II). Furthermore, data show an increase in other memory TCD8 cells due to DENV stimulation that could be related to the protection mechanism [50]. DENV severity and protection also has been attributed to human leukocyte antigen (HLA). This molecule is a very polymorphic receptor in DC and macrophages during the antigen presentation carrying DENV peptides [51-53].
Fig. (3). Immunopathogenesis during Dengue disease: Central circle indicates the viral entry and infection in human skin cells. The first immune cells to engulf the virus and be infected are Langerhans, macrophages and DC. These cells travel to the bloodstream and spread the infection through the lymph nodes spreading the infection into resident cells. Cytokine storm mediates inflammatory features in patients such as fever (TNF-α, IL-8, CCL-2, IL-6). IFNα/β can block viral replication, but is not very efficient during DENV disease. Some macrophages (MΦ) can differentiate and help to plasma blasts to produce antibodies. The virus disseminates to other organs like liver or brain. The adaptive system also activates, in a late phase of the disease, T CD4 cells downregulate the IL-2 and IL-2R levels, meanwhile the T CD8 cells proliferate and produce IFN-g in order to eliminate the virus. The B-lymphocytes can differentiate into plasmatic cells that produce IgG antibodies. The virus disseminates to other organs like liver or brain. The adaptive system also activates, in a late phase of the disease, T CD4 cells downregulate the IL-2 and IL-2R levels, meanwhile the T CD8 cells proliferate and produce IFN-g in order to eliminate the virus. The B-lymphocytes can differentiate into plasmatic cells that produce IgG antibodies. The virus disseminates to other organs like liver or brain. The adaptive system also activates, in a late phase of the disease, T CD4 cells downregulate the IL-2 and IL-2R levels, meanwhile the T CD8 cells proliferate and produce IFN-g in order to eliminate the virus. The B-lymphocytes can differentiate into plasmatic cells that produce IgG antibodies. The virus disseminates to other organs like liver or brain. The adaptive system also activates, in a late phase of the disease, T CD4 cells downregulate the IL-2 and IL-2R levels, meanwhile the T CD8 cells proliferate and produce IFN-g in order to eliminate the virus. The B-lymphocytes can differentiate into plasmatic cells that produce IgG antibodies. 

The B lymphocyte activation depends on TCD4 cells. The main function of B cells is to evolve into plasmatic cells (through the differentiation process) to produce antibodies. DENV-reactive antibodies (Abs) formed from a previous primary DENV infection are major threats for development of severe case of Dengue [54, 55]. Antibodies can neutralize virus by opsonization or kidnapping them in order to interfere the virus adhesion to the target cell. The specific epitope and adequate concentration of the neutralizing Ab are important to lead the viral clearance by the phagocytic system, for in some cases, the neutralization will be partial or sub-neutralizing. This non-neutralizing Abs allow the entrance of the virus more efficiently through Ab receptors. In the presence of sub-neutralizing DENV-specific Abs, a phenomenon occurs that is known as Ab-dependent enhancement of infection (ADE), which amplify the viral infection and exacerbate the disease [55-57]. In vitro assays indicate that neutralizing anti-DENV Abs could increase infection risk [58]. When protective antibodies are transferred to animal models of viremia, they convert Dengue-like manifestations into severe symptoms [59]. Cross-reactivity of subneutralizing anti-envelope Abs between DENV serotypes was demonstrated in secondary infection with a higher avidity that could be responsible for ADE [55, 57]. However, it should be noted that not all the patients experience secondary severe infection.
5. DENGUE OUTBREAK

5.1. Global Epidemic of Dengue

Dengue has been globally spread from the tropical and sub-tropical regions throughout the globe [60]. Most of the tropical and sub-tropical climates had reported dengue infection cases throughout past decades. The virus and the mosquito vectors are continuously increasing in the tropical/sub-tropical zones. Rapid urbanization, globalization, and ignorance towards implementing effective methods for mosquito control have led to an enlarged epidemic of Dengue in the previous few decades [60]. Global transportation, intracontinental air travel, and sea shipping are considered as the crucial factors contributing to Dengue spread. Some estimation indicates 390 million infection cases per year, from which 96 million cases have clinically manifested. Overall, Dengue and its known 5 serotypes have resulted in a spectrum of challenges ranging from public health issues to economic and environmental concerns [61]. Dengue burdens Asian and Latin America as DHF and DSS become the dominant causes of fatality in these regions. Due to the rapid evolution and expansion of DENV worldwide, the associated genotypes to virulence have scattered through South and South-east Asia as well as the Pacific and Americas [62]. Aedes albopictus can endure low-temperate climate, thus threatening Europe and North America with possible regional transmission [63].

The Dengue (DEN) or DEN-like epidemics crossed the world reaching to Africa and India, Oceania and Americas in the period between 1823 and 1916, while Dengue outbreaks were mainly observed in Southeast Asia after World War II. Philippines and Thailand were the zones recognized to have the severe DHF in the 1950s. The Asian origin of DENV-2 strain was reported in Cuba in 1981 with an increased harshness of symptoms in following outbreaks, resulting in the first DHF epidemic in the Americas [62]. While this disease is now widespread over 100 countries, WHO identified the Americas, South-East Asia, and Western Pacific as the highly affected regions [64]. Reported infections across the mentioned regions exceeded 1.2 million in 2008. In 2010, Puerto Rico reported the major epidemic in its history with 20 deaths and 5,382 confirmed infected cases. Approximately 2.4 million cases were infected in three highly affected zones in 2010, 2013 and 2015. Over the past few decades, there was an increased case of infection in the Caribbean, with the co-circulation of different serotypes [65]. This epidemic paradigm was found to be dramatically enhanced in 2016 with the largest outbreak worldwide in comparison to the previous years. The Americas reported more than 2.38 million cases, slightly less than 1.5 million cases reported only in Brazil. This statistic proves that the outbreak rate is nearly three times higher than that of 2014. The Dengue deaths were reported 1,032 in these regions. In 2016, more than 375,000 suspected infection cases were reported in the Western Pacific. Philippines and Malaysia reported 176,411 and 100,028 cases, respectively, in the same year. An outbreak was declared with more than 7,000 suspected in the Solomon Islands. In the African regions, Burkina Faso reported a localized outbreak of Dengue with 1,061 probable cases. According to WHO, each year the number of new cases arises across the globe. The phylogeographer analysis by Wei and Li in 2017 showed that Dengue transmitted via two main routes from South America to the Caribbean and from East and Southeast Asia to Puerto Rico. Furthermore, this study proved that viral phenotypes are associated with phylogeny in Nicaragua and Puerto Rico (P < 0.05) [62].

Growing number of DHF and DSS incidents were recognized in India indicating the risk of DENV infections concurrence. Reddy et al., in 2017 investigated the coexistence of Dengue infection in Northern Kerala (a Southwest state in India) between 2013 and 2015 [66].

Among the five serotypes, two serotypes are genetically different and are the common causes of Dengue widespread with severe consequences. In each serotype, the strain difference allows virus classification into separate groups based on their genetic characteristics as well as the geographical distributions. This phenomenon leads to identifying genotypes that control the outcome of the disease. The major contributor to the occurrence of DHF/DSS is the secondary infection with a serotype unlike that which caused the primary infection [62]. The consecutive infections by more than one DENV serotype are also known to result in DHF and DSS [66].

5.2. Outbreak of Dengue in Tropical Regions

The study by Collao et al. in 2015 determined the diversity of flavivirus mosquitoes present in Easter Island. This was the first study in Chile recognizing the presence of flavivirus in vectors in Easter Island [67]. This proved an urgent need for an antiviral approach or for an immediate disease management in order to control the outbreak. By describing a maculopathy and panuveitis case, Ooi et al., in 2016 provided warning on the chance ophthalmic complications in Australia particularly after several outbreaks in equatorial and across tropical Australia. Along with global warming, such complications occurrence may give rise to the distribution of Aedes aegypti throughout subtropical Australia [68]. Undurraga et al., in 2015 assessed the yearly economic burden of Dengue on Mexico in 2010 and 2011. Different sources of data such as the opinion of patients and information gathered from hospitals in regards to surveillance costs as well as other expenses declared by Mexican Ministry of Health were merged to study the economic consequences of Dengue outbreak in this country. By using Monte Carlo simulations, this sensitivity analysis with 95% certainty levels (CL) predicted that Mexico had in fact 139,000 (95% CL: 128,000-253,000) cases of observed symptoms and 119 (95% CL: 75-171) average death per year (in 2010 and 2011) as opposed to reported cases of observed symptoms (30,941) and death (59). The yearly budget spent on surveillance and vector control was reported to be USD 170 million (95% CL), or USD 1.56 (95% CL) per capita. From this total estimation, USD 87 million (95% CL) or USD 0.80 per capita (95% CL) relates to the actual illness. Subsequent factors, co-occurrence of contamination, the influence of Dengue on tourism, as well as the impact on public health further impose an additional financial burden on Mexico. This study shows that Mexico along with Panama, Puerto Rico, Nicaragua, and Thailand undergo Dengue burden more than any other country across the globe. It is worthwhile to know that during an epidemic episode, the economic challenges could
be even higher than expected due to the contributing elements [69].

A Study by Martins et al. (2014) investigated patients in acute phase of infection in widespread of Dengue in 2011 who participated in a clinical and virological descriptive analysis at center for tropical and infectious diseases in Manaus, Brazil. A total number of 677 patients were examined by RT-nested-PCRs for flaviviruses (DENV 1-4, Saint Louis encephalitis virus-SLEV, Bussuquara virus-BSQV and Ilheus virus-ILHV), alphavirus (Mayaro virus-MAYV) and orthobunyavirus (Oropouche virus-OROV). Different DENV serotypes were detected in 260 analyzed cases (38.4%). Thirteen patients were found to be co-infected with more than one DENV serotype and six patients (46.1%) were in the acute phase. Sequencing the nucleotide proved that DENV-1 belonged to genotype V, DENV-2 to the Asian/American genotype, DENV-3 to genotype III and DENV-4 to genotype II. The presented results should raise serious concerns for authorities in charge of public health specially when frequent widespread serotypes are concerned [70]. Another study by Mendez et al., in 2012 reconstructed the phylogenetic history of DENV-2 by the aim of a sequence examination from a 224 bp PCR-amplified sample that showed carboxyl terminus on envelope (E) gene extracted sera from 48 Colombian patients. The results proved the oldest examined sample to be of an American genotype origin (subtype V), while other strains which were gathered later in 1990 were of an American/Asian genotype origin (subtype IIIb). This genotype concurs with the first observed DHF cases in Colombia in late 1989 which was followed by an increase reports of DHF in the subsequent year. Developing into new clades, different families of American/Asian subtype rapidly grew and scattered across Colombia. A direct correlation with the increased death-rate, however, remained to be drawn [71].

5.3. Outbreak of Dengue in Subtropical Regions

For the first time, Reddy et al., in 2017 declared the simultaneous reports of Dengue infection in northern Kerala, India. A close correlation was found between DENV-1 of this study and a strain identified in Delhi (2006) and in South Korea (2015) epidemics [66]. Zhang et al., in 2017 have studies pregnant women in China concluding that pregnant individuals are more prone to developing acute phases than the non-pregnant ones. A recent study demonstrated that pregnant women who have experienced Dengue infection are liable to suffer from stillbirth, miscarriage, preterm birth, and low birth weight [74].

An investigation by Azhar et al., 2015 showed several epidemics of DENV serotypes 1, 2 and 3 in the western zones of Saudi Arabia since 1994. Some strains from Asian and African-American genotypes of DENV-1 were found to be circulating in Western parts of Saudi Arabia until 2006. Nonetheless, previous studies conducted in Saudi Arabia reported partial sequencing of the E-gene while the full genome sequencing remained undone. In 2011, the first full genome sequencing of DENV-1 strain was performed in Jeddah, Saudi Arabia. A close connection was found between DENV-1-Jeddah-1-2011 strain and D1/H/IMTSSA/98/606 sample that was obtained in 1998 in Djibouti, Africa, from the Asian genotype. Additional examinations revealed a high level of association between DENV-1-Jeddah-1-2011 strain and isolates of Jeddah and Somalia proposing a major outbreak during 2004-2006. These findings showed the possibility for the presence of Asian genotype strains in Saudi Arabia prior to 2004 (likely to be brought by African travelers), which remained circulating in a vast part of western Saudi Arabia for the following decade. This study draws a specific conclusion on the role of pilgrimage and the outbreak of Dengue from endemic areas to other parts of the world [72].

An Investigation on the epidemiology of Dengue in Nepal by Subedi & Taylor-Robinson in 2016 found that the native Dengue infections were first identified in Nepal, at the border with India and China. Consequently, a spectrum of infections was spread across the country not only covering low-altitude areas but also all-over higher altitudes including Kathmandu, the capital city. A range of actions was taken to tackle the outbreak of Dengue, nevertheless, the real measure of Dengue’s impact on Nepal remains unclear as majority of the studies were performed on privileged areas where Dengue occurs rather less [61]. The past epidemics, however, have confirmed that often the scale of the infection during the time of outbreak can surpass the capacity of the countries to manage the situation. Therefore, there is an urgent need to define effective strategies for understanding epidemiology, prevalence mapping, timely diagnosis, and surveillance.

5.4. Correlation between the Climatic Factor and Dengue Incidence

Dengue virus transmission primarily occurs through mosquito vector Aedes aegypti that is mainly influenced by the interaction between host-pathogen and environmental elements including precipitation, temperature, wind speed, and humidity [73, 74]. Researchers in endemic zones have attempted to develop predictive strategies based upon different key-important factors such as local climate and vector, which permit timely actions and interventions for addressing the issue [63].

Temperature changes and rainfalls play important roles and are known as the local climate factors for viral reproduction, adult mosquito breeding, growth rate, survival, and biting behavior [75, 76]. Several studies included the effect of El Niño (oscillation of a group of warm oceanic water in the central and east-central equatorial Pacific) as an interannual variation of Dengue incidence [77-81]. Dengue is recognized as a climate-responsive infectious illness. Therefore, the climate-based devices for early warning of Dengue can improve the surveillance and infection management [64].

5.5. Different Climate Factors Versus Dengue Incidence in Subtropical Regions

Hii et al., (2016) reviewed the present position of scientific investigations on climate, Dengue widespread, and climate-based early warning systems in Malaysia as one of the endemic countries. The review was dedicated to the relationship of the climate factors and Dengue by the aim of field studies, laboratory tests, and statistical modeling. PubMed, Scopus, EBSCO (MEDLINE), Web of Science, and WHO publications were the database for this review. All studies that included climate-related factors and their impact on the
infection, Dengue mosquitoes, DENV, as well as the latest advancements in Dengue-related climate elements were included in this comprehensive review. Based on this research, only a few studies have demonstrated the strong correlation between climate and Dengue in Malaysia [80]. An investigation by Sang and Chen (2015) used a minimum temperature of the region, and growing rainfall for prediction of the Dengue outbreak in four different areas of Guangzhou, China. The obtained data were categorized into seasonal and other environmental factors by the aim of a seasonal-trend decomposition procedure based on loess (STL). Resultant data between 2006 and 2012 were employed for analysis and the data obtained between 2013 to 2014 were used for validation. Factors including autocorrelation, seasonality, and long-term trend were studied in contrast to the minimum temperature and accumulative rainfall in the time of outbreak. Sang and Chen proposed that mentioned environmental and seasonal factors can result in a clear early warning to hamper the upcoming outbreaks [64]. Based on a study by Sun et al., (2017), essential spatial-temporal clusters were detected in Sri Lanka. The prediction of Dengue occurrences was possible by merging the past data with climate indicators. This prediction that showed the high risks Dengue incidences in hotspots during epidemics in contrast to the whole area has saved time as well as resources for taking effective action [60]. The average annual temperature for Sri Lanka ranges from 28°C to 30°C while the relative humidity may change from 60% to 90% depending on the seasons. The average yearly temperatures are mostly homogeneous in the low altitudes while it may swiftly increase in the higher altitudes. Raised rainfall level impacts the abun-
dance of vector by growing the number of immature mosqui-
tos hatching the eggs. Elevated humidity can help mosquitos survive even through the heavy precipitations reduce the survival chance for larvae and pupae. Therefore, Dengue transmission and outbreak highly correlate not only to the rainfall but also to humidity [60].

The correlation between Dengue occurrence and diurnal temperature ranges (DTRs) in Colombo district, Sri Lanka, were explored by Ehelepola & AK (2016). The weekly-basis Dengue incidences in Colombo between 2005 to 2014 were calculated and the daily highest and lowest temperatures from different weather forecast stations were collected and transferred into a weekly analysis. Studying DTR against Dengue growth has shown an inverse correlation for DTR > 7.5 °C while a direct correlation was observed for DTR < 7.5°C [81]. Hii et al., (2016) emphasized that climate change predictions for Malaysia prove a growing danger of Dengue widespread in the future. This finding suggested that it is essential for institutions in Malaysia to develop early warning systems for Dengue based on climatic factors in such tropical regions [80].

5.6. Different Climate Factors Versus Dengue Incidence in Tropical Regions

The study by Wiwanitkit in 2006 indicated that the prevalence of Dengue infection in Thailand might depend on rainfall, as this country is located in both tropical and sub-
tropical regions. Therefore, the surveillance and control of mosquito should be intensified during the period of high rainfall. However, the other confounding factors like ambi-
ent temperature and humidity, which also determine the transmission of Dengue should be considered, before concluding that the increased prevalence is only the result of rainfall alone [82]. Choi et al., (2016) reported a considerable correlation between temperature and precipitation and Dengue occurrence after analyzing several regions in Cambodia [83]. The authors also proved that the local factors play a great role in Dengue widespread and early warning systems installed in each region can serve a great purpose in preventing the outbreak. This study alongside many others prove that tackling the challenge of Dengue incident and outbreak could be a regional effort, and similar successful strategies in one location may result in failure in other destinations. Consequently, identifying endemic areas with comparable climates and characteristics as well as the past history of outbreaks in such areas is fundamentals for preventing future incidents [60]. Another study by Sharma et al., (2014) observed that DHF cases between 1998 to 2004 using Geographic Information System software (GIS). Spatial group of data was collected for each individual year using the Nearest Neighbor Index (NNI). The interaction between space and time and DHF incidence was analyzed using the Knox Test and the Nearest Neighbor Hierarchical (NNH) method was utilized to identify the DHF hotspots. Pearson r’significance test was used for measurement of space and time gaps. The results showed a decrease in the average gap between DHF incidence correlating with the activities that lead to an outbreak. The results also showed a declining trend in sequential gaps between DHF reports leading to the geographically spreading of the illness in the intervals of 2 years. This study has also shown a pattern that indicates Dengue moves from rural to urban districts that, in turn, results in outbreak due to the transportation. DHF, in specific, can become more localized that, in turn, would result in a harsher epidemic. During each Dengue outbreak, the infection increases with time which results in dramatic peaks in disease distribution over the given space. This finding facilitates the understanding of spatial and temporal algorithms of Dengue epidemics that allows taking preventive actions [65].

Araju et al., (2015) found that the high temperature of the land, moderate humidity, and poor vegetation in Sao Paulo, Brazil to have favored Dengue widespread. In this study, the recorded Dengue incidence between 2010 and 2011 was studied in respect to several environmental and socioeconomic factors. GIS, thermal remote sensing images, and census data were applied to categorize different urban areas based on the land surface temperature, the vegetation coverage, the density of population, the socioeconomic conditions, and the housing qualities. The analysis showed that in the major parts of the mapped regions (93%) the land surface temperature was above 28°C. It was also found that the Dengue occurrence considerably decreases in high vegetation coverage. A report has demonstrated the land surface temperature of above 32°C to have a stronger impact on Dengue growth than poor socioeconomic districts, highly populated neighborhoods, or slum-like zones. Further investigations proved the temperatures of 28°C to 32°C to be highly favorable for A. aegypti mosquito larval development, blood feeding, and oviposition suggesting temperature as the highest contributing factor to Dengue growth and outbreak among the rest among the rest [84]. Climate change and global
warming are the main contributors to the Dengue spread as well. Therefore, prevention and control of Dengue are essential and require international collaboration.

6. DENGUE DIAGNOSIS

Dengue diagnosis is possible by virus isolation, serological tests, or through molecular identification. An on-going Dengue infection can be detected by testing samples (serum or plasma, cerebrospinal fluid, or autopsy tissue specimens) within first 5 days from the start of viral symptoms via virus isolation or genome identification by reverse transcription-polymerase chain reaction (RT-PCR) [85].

Detection techniques such as one-step real-time RT-PCR or nested RT-PCR are commonly used in clinics and hospitals for Dengue detection, which typically concurs with the viremia and the febrile symptoms [86]. Detection confirmation can also happen via biorecognition of Dengue viral antigen (mainly NS1) or RNA by immunofluorescence or immunohistochemical analyses. A seroconversion from negative to positive presence of IgM antibody against Dengue can be another proof for the infection as well. Moreover, an increase in IgG antibody level in blood serum corresponds to both, acute and convalescent phases of Dengue infection in patients.

An IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) is a helpful technique for detection of the recent infection cases [86]. Identification of IgM in blood serum while having a negative RT-PCR result and no clear symptoms registered could be interpreted as a recent Dengue infection as IgM antibodies remain active in body for few months after the occurrence of infection.

The initial immune response after a Dengue incidence involves production of IgM and IgG antibodies against the proteins of enveloped virus. Depending on the past infection occurrence (primary or secondary with Dengue or other flavivirus infection), the immune response varies. A primary infection is characterized by a low titer antibody response with the appearance of IgM antibodies followed by low titer presence of IgG towards the end of one week. In a secondary infection, however, IgM antibody level rises rapidly while IgGs are detectable even at the onset of the fever, which their concentration dramatically increases over the following two weeks. According to the Pan American Health Organization (PAHO) guidelines, approximately 80% of all Dengue infections have measurable IgMs by day five of illness, which might remain detectable for over 90 days [87].

MAC-ELISA with sensitivity and specificity of approximately 90% and 98% respectively has become a vital tool for Dengue diagnosis. Nonetheless, considering the fatality of Dengue over the relatively short period, MAC-ELISA might not provide with timely information as it normally provides the results around day five of the illness when the antibody level reaches to the detectable phase. MAC-ELISA is commercially available in diagnostic laboratories. The assay is based on IgM antibodies capturing by using anti-human-IgM antibody on a microtiter plate. One of the limitations of this technique is the cross-reactivity among circulating flaviviruses.

PCR tests are used to identify the viral genome in blood serum. The virus could be isolated and sequenced for further analysis. Real-time RT-PCR assays were established for rapid diagnosis of Dengue with high sensitivity, and specificity. A positive PCR result is a strong proof of Dengue infection, which typically accompanies with the serotype identification as well. A negative result, however, does not necessarily correspond to the absence of infection. Patients, in such cases, are typically requested to submit another serum sample for serological assessment within the first week from the onset of symptoms.

Following the same strategy as that of MAC ELISA, IgG ELISA can be employed for detection of past Dengue infection cases. In general, IgG ELISA falls short when specificity within the flavivirus serocomplex families is concerned. A simple algorithm helps to distinguish between the primary and secondary Dengue infection: (i) a negative IgG presence in the acute phase along with a positive IgG level in the convalescent phase corresponds to the primary Dengue infection. (ii) a positive IgG presence in the acute phase along with a rise in IgG level in the convalescent phase attributes to a secondary Dengue infection.

Detection of NS1 protein of Dengue virus correlates with early diagnosis of the infection [37]. Dengue NS1 antigen can be detected in blood serum as early as the onset of the fever (day 1) and up to day 18 of the illness. The NS1 ELISA platform is commercially available for Dengue detection and has been thoroughly evaluated for its sensitivity and specificity. Due to the specificity of the assay, NS1 assay can be employed for differential diagnostics between the flaviviruses.

Plaque Reduction and Neutralization Test (PRNT) and the microneutralization PRNT are known as the most specific serological analyses determination of Dengue antibodies [88]. The PRNT tests are employed for infecting serotype identification in convalescent sera. PRNT assay quantifies the concentration level of the neutralizing antibodies in the serum of the infected patients measuring the level of protective antibodies against infecting agents in such individuals [88]. It is a biological assay based upon the principles of antibody-antigen interaction in inactivation process of the virus that is incapable of infecting or replicating. The microneutralization assay operates in the same fashion, though, instead of calculating the quantity of plaques-per-well, it uses a colorimetric measurement of the virus-induced cell lysis for determination of the end-point dilution. This assay was developed for high throughput testing purposes. Other techniques for IgM detection including capture ELISA, capture ultramicroELISA, dot-ELISA, and dipsticks have also been developed [87].

7. COMMERCIAL AND NON-COMMERCIAL STRATEGIES

Nowadays, a varied range of commercial platforms for Dengue detection is available in the market. Table 1 presents some of these commercial platforms employed to rapidly detect Dengue virus in human samples.

As can be observed, the measured target mostly involves antibodies against Dengue produced in the body or the NS1
Table 1. Commercial platforms for Dengue detection.

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Company</th>
<th>Target(s)</th>
<th>Format</th>
<th>Dengue Serotype</th>
<th>Conditions</th>
<th>Specifications</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmunoComb II Dengue IgM &amp; IgG</td>
<td>Orgenics RT/Inverness (Israel)</td>
<td>NS1 IgM IgG IgA</td>
<td>Solid phase ELISA</td>
<td>DENV serotypes not specified</td>
<td>Storage: 2-8 °C, Total test duration: 3h</td>
<td>10 μL of sample (serum/plasma) is required</td>
<td>[89]</td>
</tr>
<tr>
<td>Rapid test device</td>
<td>Abon Biopharma (China)</td>
<td></td>
<td></td>
<td></td>
<td>Storage: 2-30 °C, Total test duration: 10 min</td>
<td>10 μL of whole blood or 5 μL of serum/plasma is needed</td>
<td></td>
</tr>
<tr>
<td>OnSite Dengue IgG/IgM Combo</td>
<td>CTK RTIgM CTK Biotech (USA)</td>
<td></td>
<td></td>
<td></td>
<td>Storage: 2-30 °C, Total test duration: 2-5 min</td>
<td>5 μL of serum/plasma is needed</td>
<td></td>
</tr>
<tr>
<td>SD Bioline IgG/IgM</td>
<td>Alere (USA)</td>
<td></td>
<td></td>
<td></td>
<td>Total test duration: 15-20 min</td>
<td>Serum, plasma, or whole blood can be used</td>
<td>[90]</td>
</tr>
<tr>
<td>SD Bioline Dengue Duo kit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Covers detection from acute to convalescence phases of infection Serum, plasma or whole blood can be used</td>
<td>[91]</td>
</tr>
<tr>
<td>ASURE® Dengue IgA Rapid Test</td>
<td>MP Diagnostics, (USA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Serum, plasma or whole blood can be used, Needs 25 μL of sample</td>
<td>[92]</td>
</tr>
<tr>
<td>Dengue NS1 DetectTM Rapid test</td>
<td>InBios International, Inc (USA)</td>
<td></td>
<td>Lateral flow</td>
<td>DENV (1-4)</td>
<td>Storage: room temperature, Incubation: 30 min, 37 °C</td>
<td>Needs 50 μL of sample, as well as pipette and tubes</td>
<td>[93]</td>
</tr>
<tr>
<td>Hapalyse Dengue-M PA kit</td>
<td>Pentax (Japan)</td>
<td></td>
<td></td>
<td></td>
<td>Test duration: 90 min, Storage: 2-8 °C</td>
<td>Serum or plasma can be used, Test cannot be conducted at home, Small sample volume (1 μL) is needed</td>
<td>[94]</td>
</tr>
<tr>
<td>Dengucheck WB</td>
<td>Zephyr Biomedicals (India)</td>
<td></td>
<td></td>
<td></td>
<td>Storage: 4-30 °C, Total test duration: 15 min</td>
<td>Serum, plasma, or whole blood can be used</td>
<td></td>
</tr>
<tr>
<td>NS1 antigen strip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>It is a rapid diagnostic tests with a high level of accuracy (negative predictive value (NPV) of 95.2%)</td>
<td>[95]</td>
</tr>
<tr>
<td>Dengue Duo Cassette</td>
<td>Panbio diagnostics (Australia)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>The test offers single addition of sample, convenient and rapid analysis, and high sensitivity and specificity. It cannot be performed at home.</td>
<td>[94]</td>
</tr>
</tbody>
</table>

(Table 1 contd....)
glycoprotein, common to any type of Dengue virus. NS1 detection is crucial to detect Dengue at the initial stages, when the immune response may not have generated enough number of antibodies to be detected. The most powerful of these tests are those that are capable of measuring more than one target simultaneously since this introduces a chance for discrimination between the primary and secondary infections. The qualitative tests have the limitation as the negative results may omit a recent infection. Obviously, lateral flow assays are faster than 96-well plate assay formats. Most of these assays can detect all DENV serotypes and need strict storage conditions, which imply that if such conditions are not fulfilled, the test may fail.

Table 2 offers a selection of the latest (2017-2018) developments on Dengue diagnosis techniques, which were reported but are not yet commercially available. As the Table demonstrates, the electrochemical detection strategies are rather favorable as they eliminate the need for labeling. One of the disadvantages of these tests is that they might not identify all Dengue serotypes since some techniques are only

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Company</th>
<th>Target(s)</th>
<th>Format</th>
<th>Dengue Serotype</th>
<th>Conditions</th>
<th>Specifications</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panbio Dengue IgM capture kit</td>
<td></td>
<td>✓</td>
<td>96-well plate</td>
<td></td>
<td>Storage: 2-30 °C, Incubation: 130 min, 37 °C</td>
<td>Uses a single dilution of serum, It is economic since wells can break apart from each other to perform separate assays. Sensitivity: 94.7%, specificity 100%</td>
<td>[96]</td>
</tr>
<tr>
<td>Pathozyne Dengue IgM capture</td>
<td>Omega diagnostics (UK)</td>
<td>✓</td>
<td></td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 120 min, 37 °C</td>
<td>Needs 1 μL of serum</td>
<td>[94]</td>
</tr>
<tr>
<td>Dengue fever virus, IgM capture DsSelect</td>
<td>Focus Diagnostics (USA)</td>
<td>✓</td>
<td></td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 240 min, room temperature</td>
<td>Qualitative detection is performed only for serum</td>
<td>[94]</td>
</tr>
<tr>
<td>Pan-E (Early detection) ELISA</td>
<td>Panbio/Alere (USA)</td>
<td>✓</td>
<td>96-well plate</td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 120 min, room temperature</td>
<td>Time to test is ~3 hours, 75 μL of sample is needed</td>
<td>[89]</td>
</tr>
<tr>
<td>IgM Capture ELISA</td>
<td>InBios International, Inc. (USA)</td>
<td>✓</td>
<td></td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 120 min, room temperature</td>
<td>The test offers early detection but there is a chance for cross-reactive with other flaviviruses</td>
<td>[97]</td>
</tr>
<tr>
<td>Platelia™ Dengue NS1 Ag</td>
<td>Bio-Rad, (France)</td>
<td>✓</td>
<td></td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 90 min, 37 °C</td>
<td>One-step qualitative or semi-quantitative detection with the sample volume of 50 μL</td>
<td>[89]</td>
</tr>
<tr>
<td>DENV-JEV MACE</td>
<td>Venture Technologies Sdn Bhd (Malaysia)</td>
<td>✓</td>
<td></td>
<td></td>
<td>Storage: 2-8 °C, Incubation: 250 min, Room temperature or overnight at 4 °C</td>
<td>Time of the test: 2 days, 2 μL of sample is required</td>
<td>[89]</td>
</tr>
</tbody>
</table>

8. DEVELOPED VACCINES FOR DENGUE PROTECTION

An ideal vaccine should produce a life-long protective immune response, and high efficacy while having no adverse effects on body. In addition, a suitable Dengue vaccine must be effective for all four serotypes neutralizing Abs to avoid ADE by cross-reactivity or subneutralizing Abs. Several
Table 2. Latest non-commercial technologies for Dengue detection.

<table>
<thead>
<tr>
<th>Method of Detection</th>
<th>Result Interpretation Time</th>
<th>Tracer</th>
<th>Measured Particle</th>
<th>Dengue Serotype</th>
<th>Sample Volume</th>
<th>Limit of Detection</th>
<th>Remarks</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>105 min</td>
<td>FITC labeled secondary antibody</td>
<td>NS1</td>
<td></td>
<td>50 mL</td>
<td>15 ng/mL</td>
<td>High specificity and no cross-reactivity with Japanese encephalitis or Zika viruses, Successful evaluation with clinical samples</td>
<td>[98]</td>
</tr>
<tr>
<td></td>
<td>10-20 min</td>
<td>Functionalized single-walled carbon nanotube networks</td>
<td>NS1 in mosquito</td>
<td>DENV-2</td>
<td>10 mL</td>
<td>0.09 ng/mL</td>
<td>Affordable, label-free, highly sensitive</td>
<td>[99]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>10 min</td>
<td>Changes in the amperometry of quaternary alloy functionalized nanostructure</td>
<td></td>
<td></td>
<td>Few drops</td>
<td>17 nM</td>
<td>Inexpensive, fast, sensitive, low power consumption</td>
<td>[100]</td>
</tr>
<tr>
<td></td>
<td>2h</td>
<td>Cyclic and differential pulse voltammetry of ZnO/Pt-Pd nanocomposites</td>
<td>DNA sequence</td>
<td>DENV (1-4)</td>
<td>One drop</td>
<td>$4.3 \times 10^{-4}$ M</td>
<td>Since the DNA sequence is common to all four serotypes, this technique eliminates the need for serotype-specific analysis. However, DNA is susceptible to degradation, which can be a disadvantage of this method.</td>
<td>[101]</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>Changes in the amperometry of modified Mn2O3 electrospr nanofiber</td>
<td></td>
<td></td>
<td>Not available (immersion)</td>
<td>$120 \times 10^{15}$ M</td>
<td>The use of bandgap metal oxide material enhances the limit of detection down to unprecedented values</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>Single-walled carbon nanotube network functionalized with heparin</td>
<td></td>
<td>DENV-1</td>
<td>10 mL</td>
<td>$8.4 \times 10^{7}$ tissue culture infection dose/mL, (~8 Dengue virus capsids per chip)</td>
<td>Low cost, label-free, ultra sensitive</td>
<td>[103]</td>
</tr>
<tr>
<td>Microfluidic dielectrophoresis</td>
<td>5 min</td>
<td>Functionalized fluorescent beads</td>
<td></td>
<td>DENV-2</td>
<td>15 mL</td>
<td>$10^4$ plaque forming units/mL</td>
<td>Low sample volume, fast, reusable, serotype specific</td>
<td>[104]</td>
</tr>
<tr>
<td>Surface plasmon resonance</td>
<td>3 min</td>
<td>Gold modified nanoparticles</td>
<td></td>
<td></td>
<td>100 mL</td>
<td>$10^7$ tissue culture infection dose</td>
<td>Fast, simple procedures, detection prior to the onset of symptoms</td>
<td>[105]</td>
</tr>
<tr>
<td>Fluorescence/Colorimetric</td>
<td>24 hours</td>
<td>Fluorescence labels or enzyme activity in infected cells</td>
<td></td>
<td>DENV (1-4)</td>
<td>50 mL</td>
<td>&lt;10 focus forming units /well</td>
<td>Easier and faster than classical plaque assay, useful to test antiviral compounds</td>
<td>[106]</td>
</tr>
<tr>
<td>Photoluminescence</td>
<td></td>
<td>Unquenched quantum dots due to molecular beacons</td>
<td>RNA sequence</td>
<td></td>
<td>31-260 copies /mL</td>
<td>Ultrasensitive with discrimination among the serotypes</td>
<td></td>
<td>[107]</td>
</tr>
</tbody>
</table>
research groups described that the peptide sequences of DENV could induce neutralizing or subneutralizing Ab response, probing them on different models of infection (in silico, in vitro, and in vivo) [108, 109]. With obtained information for these tests, some vaccine candidates were developed and examined in clinical trials. In that regard, WHO published different guidelines for clinical evaluation of Dengue vaccines in endemic areas and on the quality, safety, and efficacy of live attenuated vaccines [110]. The successful trials for other flaviviruses live attenuated vaccines suggested that developing vaccine against Dengue is doable [111]. Table 3 summarizes different types of vaccines, the developer, the country, the characteristics of the vaccine as well as the status of approval. Sanofi Pasteur’s is a pharmaceutical industry to launch the first the U.S. Food and Drug Administration (FDA) approved the tetravalent Dengue vaccine (TDV).

For vaccines evaluation in respect to the efficacy, the quality, and the safety, several steps are necessary to be taken including preclinical investigations and phases I-IV evaluations. The aim of the preclinical phase is to prove the effect, toxicity and kinetic of the vaccine in cell culture, in silico and in animal models. There is a shortcoming for vaccine evaluation against Dengue in respect to animal models, as they do not closely represent the human disease. This disadvantage, to a great extent, introduces challenges for development and evaluation of the vaccine candidates. Macaque model passes through a short incubation period representing only the infection but no the disease. Other models such as AG129 IFN deficient mice develop only DHF. Meanwhile, the human models must be performed with caution and in highly monitored clinical conditions. After phase III, the FDA can approve the vaccine if it completes the WHO guidelines. Phase IV is a prospective assay that searches for new features in patients receiving the vaccine [112].

The protection of Dengue vaccine is typically measured by the ability of the vaccine in neutralization of the Ab titers (corresponding higher titers to better protection). Vaccine can also be analyzed based on class and subclass switching and avidity to E or membrane (M) proteins [113]. Overall, the current clinical trials for Dengue vaccines proved to be safe without showing disease enhancement in vaccinated patients. However prospective and long-term assays must be conducted in human subjects in order to analyze the entire immune activation and to ensure that immunity does not predispose severe Dengue symptoms. This long-term monitoring is recommended for at least 3-5 years after vaccination [114].

The currently approved vaccine (Sanofi-Pasteur vaccine) has shown to be safe when is administered to children living in Dengue endemic areas. One of these studies was performed in Latin American population, with 25 months of monitoring [141]. The efficacy percentage varies from one country to another. For instance, Brazil and Colombia had efficacy over 64%, which was considerable in comparison to Mexico with an efficacy of 31.3% [114]. This might be related to polymorphisms in HLA in different populations, which also presents an opportunity to advance rather more individualized approaches in the vaccine development. The protection with Sanofi-Pasteur vaccine also varies for different Dengue serotypes, with high protection against DENV3-4 serotypes, and low protection against DENV1-2 [142]. Further analysis in vaccinated patients is required to analyze the efficacy and the safety of vaccination based upon the countries.

9. FUTURE DIRECTIONS IN BIO-CONTROL OF THE MAJOR DENGUE VECTORS

Although numerous advances were made in the areas of epidemiology, pathogenesis and identification of the risk factors of Dengue, there is no certain treatment established for Dengue fever and its acute phases. Development of an effective vaccine or discovery of novel molecules/compounds to prevent or treat Dengue may open windows of opportunity to novel therapies for Dengue fever. Reviewing patient’s medical history, recent travels, and records of vaccination record can be of great help to determine the likelihood of infection with Dengue virus. Other interesting aspect could be the bio-control of the major vectors: Aedes aegypti and Aedes albopictus mosquitoes, using microorganisms or plant extracts.

One of the main areas for future research would be the study of monoclonal antibodies and development of antivirals based on nanoparticles [143, 144]. Nanotechnology is a promising research platform, which has a wide field of applications in bio-control of mosquito larvae. Some studies have shown that microorganisms have probed larvicidal activities against A. aegypti, specially the entomopathogenic fungus Beauveria bassiana and the bacterium Bacillus thuringiensis var. israelensis. In this perspective, different research groups have proposed the green-synthesis of silver nanoparticles with a potent control against larvae of A. aegypti [145, 146]. Alternatively, plant extracts could be interesting candidates due their bioactive compounds that are biodegradable, eco-friendly, and potentially suitable for the vector control.

Different strategies for the vector control of mosquitoes were introduced to prevent the transmission of DENV. The use of insecticides has been the base of most control programs, nevertheless their toxicity, the resistance of A. aegypti population to many chemical insecticides, and the detrimental environmental effects, made such efforts unsuccessful. A suitable choice of bio-control agent that is effective, environmental-friendly, and eco-bio-social-friendly remain a major challenge. Bio-control strategies of A. aegypti using larvicidal bacteria and their toxins hold potentials for the biological control of the vector; Bacillus thuringiensis svar. israelensis (Bti), Lysinibacillus sphaericus (Ls), Beauveria bassiana (Bb), and Wolbachia piipiensis were reported in previous studies [147-149]. The synergistic activities of the protein crystals of Bti, Cry4Ba, Cry4Aa, Cry11Aa, and Cyt1Aa were widely used in strategic programs for mosquito control. These proteins accumulate in the parasporal body and when ingested, they are proteolytically activated causing an osmotic lysis of the midgut thus resulting in larval death [150]. When toxins of L. sphaericus (BinA and BinB) are eaten by the mosquito, large cytoplasmic vacuoles are formed in the midgut, which causes larval death [150]. The study of García-Mungia et al. showed that the transmission of the soil fungus B. bassiana virgin males
of A. aegypti contaminated with conidia caused 90% mortality of the healthy female mosquitoes in two weeks and decreased fecundity in exposed females [148].

A new bio-control approach has been studied with the use of Wolbachia pipientis, gram-negative bacteria that limit the replication of DENV and also inhibits the reproduction of A. aegypti. Its function was attributed to its capacity to invade wild mosquito populations and cause cytoplasmic incompatibility and damage in the reproduction system of the host [151, 152]. Successful control of A. aegypti requires strategic multidisciplinary approaches including the development of biological larvicidal formulations together with environmental management and sustainable programs.

**CONCLUSION**

In this review article, we focus on Dengue as one of the most dangerous infectious diseases from the category of the neglected tropical diseases. Key important topics including the mosquito's cycle of life and mechanism of infection, the adaptive response, and different phases of Dengue infections are reviewed in a great detail. The global outbreak of Dengue with a major emphasis on tropical and subtropical regions is reviewed in the presented work as well. In that perspective, we demonstrate that there is a direct correlation between the climate factors and Dengue occurrence. The current article also presents an overview of the Dengue diagnosis reviewing the latest developments in commercial and non-commercial diagnosis platforms. Furthermore, several efforts made for the development of an effective vaccine against Dengue infection and the corresponding clinical trails are introduced here. Timely diagnosis of Dengue is expected to reduce the fatality rate to a considerable extent. In general, Dengue is known as an international concern that requires solid multidisciplinary strategies to control its outbreak and to reduce the impact of the infection on the well being of human beings.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

species is key to hemorrhage development. J. Virol., 2008, 82(24), 12312-12324.


Dengue Fever: A Worldwide Threat


[90] Adegoke, O.; Park, E.Y. Bright luminescent optically engineered core/alloy shell quantum dots: an ultrasensitive signal transducer


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