Epidemiology of Dengue in Mexico and Biotechnological Solutions: A Review.

Eduardo Garcia-Martinez [1†*] (0000-0001-7902-6040), Ahtziri Hernandez-Arce [1†] (0000-0003-1154-6669) , Isabel Zamarripa-Zercovitz [1†] (0000-0002-6899-0026) y Emma Herrera [2] (0000-0003-3261-0869).

[1] Biotechnology undergraduate student. Universidad Anáhuac México Campus Norte, Av. Lomas Anáhuac 46, Col. Lomas Anáhuac, C.P. 52786, Huixquilucan, Estado de México, México.
[2] PHD. In Science in Biomedicine and Molecular Biotechnology. Universidad Anáhuac México Campus Norte, Av. Lomas Anáhuac 46, Col. Lomas Anáhuac, C.P. 52786, Huixquilucan, Estado de México, México.

* eduardogmtkd@gmail.com
† These authors contributed equally.

ABSTRACT
Dengue disease is a public health matter in some regions of the world. Since it was first reported in Mexico in 1941 it has been a very complex and relevant problem to address. Antigenic diversity, genetic and immunological susceptibility are some factors that limit the development of an efficient treatment for the disease. The control methods used are vector and symptomatic ones. This review aims to discuss the epidemiology of Dengue in Mexico from 2000 to 2021 and the different biotechnological strategies that are in development. The findings show that Mexico had an increase in cases since 2019 which affected people of all ages. Current research has reasonable expectations for preventive care and therapeutic treatment that include vaccines, antivirals and monoclonal antibodies.

Key words: Dengue Virus, Dengue, Epidemiology, Mexico, Vaccines, Monoclonal Antibodies, Antivirals.

RESUMEN
El Dengue es un problema de salud pública en algunas regiones del mundo. Desde que se reportó por primera vez en México en 1941 ha sido un problema muy complejo y relevante de abordar. La diversidad antigénica, la susceptibilidad genética e inmunológica son algunos factores que limitan el desarrollo de un tratamiento eficaz para la enfermedad. Los métodos de control utilizados son el vectorial y el sintomático. Esta revisión tiene como objetivo discutir la epidemiología del Dengue en México del 2000 al 2021 y las diferentes estrategias biotecnológicas que se encuentran en desarrollo. Los hallazgos muestran que México tuvo un aumento de casos desde 2019 y que afecta a personas de todas las edades. La investigación actual tiene expectativas razonables para la atención preventiva y el tratamiento terapéutico que incluyen vacunas, antivirales y anticuerpos.

Palabras clave: Virus del Dengue, Dengue, Epidemiología, México, Vacunas, Anticuerpos Monoclonales, Antivirales.

INTRODUCTION

Dengue is a mosquito borne disease that represents a serious public health problem worldwide (Daep et al., 2014; WHO, 2020). The estimated incidence of Dengue is approximately 284 to 528 million infections per year, the average cost per case is around $84.73 USD for fatal cases and 70.10 USD for cases admitted to hospitals (Hasan et al., 2016). It is a viral infection caused by Dengue virus (DENV) that belongs to the family *Flaviviridae.* Its genome is composed of a single strand of positive RNA (ssRNA+) that goes from 9.2 to 11.0 kb in length. It has a single open reading frame that encodes for three structural proteins that form the capsid (C), envelope (E) and membrane (M), and seven nonstructural proteins which play a role in assembly and replication, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (*Dengue Viruses*, n.d.; ICTV, 2020).

Viral infection begins when the mosquito vector genus *Aedes* deposits the viral particles in the epidermis where cells such as keratinocytes and dendritic cells of the skin are permissive to infection. These migrate to lymphatic organs for viral replication in the cells' cytoplasm (Laureti et al., 2018; Lim et al., 2018). Upon arrival, the viral particle opens the nucleocapsid to emancipate the viral RNA, which takes over the host cell machinery in the rough endoplasmic reticulum to replicate, transcribe and translate its genetic information. Later, it becomes enveloped, matured, and converted into an infectious particle that is released and can now infect other cells in the host (*Dengue Viruses*, n.d.; Pierson & Diamond, 2020).

Mexico classifies Dengue infection severity in four main groups, being asymptomatic infections, undifferentiated fever, Dengue fever (DF) or non-severe cases, and Dengue hemorrhagic fever (DHF) which is characterized by increased capillary permeability and hypovolemic shock (Lim et al., 2018). The severity of disease depends on many factors that have not been fully understood (Martina et al., 2009). The knowledge gathered from outbreaks have revealed that antigenic diversity among serotypes is one of the most critical of them (Lan & Hirayama, 2011; Thanachartwet et al., 2015). This antigenic diversity allows classification of DENV in four serotypes (Yung et al., 2015). The exposure to one serotype confers partial protection to a secondary infection with other serotypes, which is called heterotypic infection (Bell et al., 2019; Dejnirattisai et al., 2016). Paradoxically, the heterotypic infection exponentially increases the risk of severe disease due to the development of ineffective and detrimental immunity. These processes are explained in the two more accepted theories called Antibody Dependent Enhancement (ADE) and Original Antigenic Sin (OAS) (Dejnirattisai et al., 2016; Rothman, 2011).

Currently there are no approved prevention methods for this viral agent, or treatments for this disease beyond those intended to relieve the symptoms of infection. However, multiple research projects and clinical trials are testing several molecules with therapeutic properties which can be classified as vaccines, drugs, and antibodies (Deng et al., 2020; Rajapakse et al., 2012; San Martín et al., 2012). The current review aims to discuss the epidemiology of Mexico from 2000 to 2021, including major outbreaks, incidence, and social context, as well as biotechnological strategies against Dengue.

MATERIALS AND METHODS

Epidemiology of Dengue in México

We conducted a literature and documentary search of available sources describing the epidemiology of Dengue in Mexico between 2000 and 2021, aiming to discuss the evolution over time. Number of cases, including both DF and DHF, as well as deaths and mortality rates per year, outbreaks, seroprevalence, serotypes distribution and other relevant information were compiled and analyzed.

The databases consulted included PubMed, Scientific Electronic Library Online (SciELO), PAHO/WHO database, Mexican academic and medical databases. The numbers of cases, deaths and serotype distribution were collected from PAHO dynamic figures, CENAPRECE bulletins and Ministry of Health Epidemiological week reports and were compared to find similarities, gaps and irregularities. Additionally, medical reports, guidelines and other documental resources provided by government institutions, health and academic sector and international organizations were included to better understand the sociopolitical context in this period aiming to discuss its implication in the progression of Dengue. Some of the keywords used to collect relevant information were “Dengue’, ‘epidemiology’, ‘Mexico’ and ‘outbreak’.

Biotechnological Solutions

Preclinical, Clinical, and Experimental studies were included for discussing the current status of biotechnological solutions against Dengue Disease. Vaccines, Monoclonal antibodies and Antiviral drugs were the approaches discussed in the present work.

Literature was collected from PubMed and Scholar Google from the period of 2014 up to date. The efficacy of the treatments was determined in terms of reduction of viral titers for RNA quantification, NS1 sera levels and mortality rates in case of animal experiments. Given the lack of preclinical and clinical studies in monoclonal antibodies-based therapy, experimental studies evaluating the efficiency for treating Dengue in murine and non - human primates were included.

RESULTS AND DISCUSSION

The Origins of a Public Health Problem

The first great Dengue epidemics date back to the 18th to 19th century in Asia, Africa and North America. This time period was characterized by spontaneous but large epidemics, and although there were no molecular tests for characterization, patients reported clinical pictures compatible with DF (San Martín et al., 2012). Later, the commercial, economic and ecological stage of the 20th century facilitated the dissemination and cocirculation of different serotypes of DENV worldwide (Warkentien, 2016). Moreover, severe and fatal cases of Dengue started to increase, DHF epidemics started in Manilla Philippines in 1954 (Warkentien, 2016), followed by Southeast Asia countries in the years 1958 - 1980 and South and Central Pacific in the same timeframe (Ivonne Torres-Galicia et al., 2014).

Due to the increasing threat of the *Aedes* mosquito as a vector of hemorrhagic diseases, the Pan American Health Organization (PAHO) made important efforts to control its spread during 1947 - 1970, achieving the eradication in more than 20 countries across the Americas (San Martín et al., 2012; Warkentien, 2016). However, gradual loss of both political and social interest in the disease, as well as flexibility in measures taken by PAHO, resulted in the deterioration of the program, reinfestation of lost territory and the greatest geographical extension ever (Ivonne Torres-Galicia et al., 2014; San Martín et al., 2012; Schneider et al, 2010). Consequently, this would cause an important increase in outbreaks throughout the Americas, and the beginning of a serious health problem that affects thousands of people to date (San Martín et al., 2012; Ivonne Torres-Galicia et al., 2014).

Mexico’s Epidemiology

First Dengue transmission reports in Mexico appeared in 1941, with an estimated incidence of 6,955 cases per 100,000 people (Ivonne Torres-Galicia et al., 2014; Schneider et al, 2010). In the next 2 decades, the country experienced low incidence and even eradication in 1963 mainly due to the PAHO campaigns (Schneider et al, 2010). However, the reintroduction of the viral agent in 1978 resulted in multiple outbreaks south of the Mexican territory (Schneider et al, 2010; Laredo-Tiscareño et al., 2012). Even though the following outbreaks of Dengue did not show an important increase in cases, the territorial extension of *Aedes* mosquito went up to 1100 m over the sea level and there was a reintroduction of different serotypes, which alarmed health experts (Subsecretaría de Salubridad DIGEPI, 1984; PAHO/WHO, n.d.; Koopman & Gómez - Dantés, 1986).

Mexico experienced the introduction of serotypes 1,2 and 4 in the mid-eighties (Schneider et al, 2010; Laredo-Tiscareño et al., 2012) and subsequently DENV 3 in 1994 (Ivonne Torres-Galicia et al., 2014; San Martín et al., 2012). As Haldstead's studies would demonstrate, cocirculation of different serotypes elevates the risk of Dengue complications (Halstead, 1981). The first consequences of this fact would be noticed in 1984, with the first deaths caused by Dengue (Koopman & Gómez - Dantés, 1986). DHF cases would also increase dramatically in the following years, reaching a peak in 1997 with 52,561 cases and 37 deaths (Fajardo-Dolci et al., 2012; Ivonne Torres-Galicia et al., 2014; PAHO/WHO, n.d.). Exposing these key events will help to understand the context of Dengue in the 21st Century, which is summarized in table 1.

Table 1. Epidemiology of Dengue in México

Year	Serotypes	Total Number of Cases	Incidence (Per 100,000 hab.)	Number of cases Regular Dengue	Number of cases Dengue Hemorrhagic Fever	Deaths	Mortality Rate (Per 100,000 hab.)
2000	DENV 1, 2, 3	21,665	21.9	21,615	50	0	0.00
2001	No information	6,019	6.0	5,828	191	0	0.00
2002	DENV 1, 2, 3	8,415	8.3	6,986	1,429	6	0.01
2003	No information	3,599	3.5	2,180	1,419	0	0.00
2004	DENV 1, 2, 3, 4	6,243	6.0	4,284	1,959	13	0.01
2005	DENV 1, 2, 3	12,607	11.9	8,352	4,255	0	0.00
2006	DENV 1	22,810	21.2	18,333	4,477	0	0.00
2007	DENV 1, 2, 3, 4	40,539	37.1	32,642	7,897	10	0.01
2008	DENV 1, 2, 3	25,040	22.6	18,926	6,114	24	0.02
2009	DENV 1, 2, 3, 4	238,289	212.0	227,015	11,374	96	0.09
2010	DENV 1, 2, 3	51,635	45.3	45,299	6,336	20	0.02
2011	DENV 1, 2, 3, 4	63,628	55.0	59,338	4,290	36	0.03
2012	DENV 1, 2, 3, 4	165,749	141.3	148,043	17,706	170	0.14
2013	DENV 1, 2, 3, 4	231,498	198.4	212,831	18,667	192	0.16
2014	DENV 1, 2, 3, 4	124,943	103.8	116,275	8,668	39	0.03
2015	DENV 1, 2, 3, 4	219,593	180.2	214,129	5,464	42	0.03
2016	DENV 1, 2, 3, 4	129,263	105.5	806	130,069	34	0.03
2017	DENV 1, 2, 3	89,892	72.0	89,518	375	34	0.03
2018	DENV 1, 2, 3, 4	78,621	62.3	77,763	858	45	0.04
2019	DENV 1, 2, 3, 4	268,458	210.4	264,898	3,560	371	0.29
2020	DENV 1, 2, 3, 4	129,639	93.6	119,581	1,058	79	0.06
2021	DENV 1, 2, 3, 4	20,894	16.0	20,761	133	8	0.01

*Data was obtained from PAHO (PAHO/WHO, n.d)

In the last two decades there have been five major outbreaks caused by Dengue, with the presence of the infected vector in 30 out of the 32 states of the country. The first major outbreak was in 2007, with around 40,539 registered total cases, with four serotypes present. The most affected state was Quintana Roo. Later in 2009, the second major outbreak was more severe with 238,289 reported cases in which 11,374 cases correspond to DHF, and the most affected state by this outbreak was Colima. The third major outbreak can be identified in 2013, with 231,498 reported cases, from which 18,667 are DHF, according to the PAHO. The fourth major outbreak was in 2015 with 219,593 total cases. And finally in 2019, the most recent major outbreak, the PAHO reported 268,458 cases, with the highest value for mortality rate of these two decades being 0.29, with 371 deaths (Ivonne Torres-Galicia et al., 2014; Secretaría de Salud, 2014). *(See table 1).*

The Dengue virus has been present in the last two decades in Mexico; it has shown an increase in the incidence in the Pacific and Gulf regions. In 2002, Mexico reported a new trend in which an increase of cases shifted towards pediatric and juvenile populations, age groups 15 to 24 years were the most susceptible to have DHF. The main states affected by this trend were Colima, Guerrero, Michoacan and Oaxaca. Dengue in children and juveniles represents a risky situation due to the clinical features and the early complications presented, which are mainly associated with a rapid and fulminant disease evolution that involves many organs and that could lead to a fatal outcome. Although there was an increase of infection in children and young people, it is not a specific age-related disease (Ivonne Torres-Galicia et al., 2014; Torres-Galicia et al., 2014).

From 2000 to 2013, the main approach to managing Dengue was focused on programs to control the vector by increasing the insecticide application coverage. This approach did not work out and the cases kept appearing and affecting young population. In 2013, the approach changed, and the strategies allocated resources to prevention (Secretaría de Salud, 2014). The new approach assigned brigades of health promotors and vector control by keeping risk factors under control in the most susceptible areas. The strategy also reduced the use of insecticides to contribute to the sustainability and avoid the vector resistance to insecticides. Since this program was modified, the country has invested in the development and introduction of a vaccine against Dengue fever, with the participation of the public and private sectors, to prepare Mexico to be one of the first countries to have a vaccine against Dengue (Secretaría de Salud, 2014).

Biotechnological Solutions

In the present day there is no specific treatment available for Dengue infection mainly because of controversial results reported in clinical trials of different therapeutic candidates (Rajapakse et al., 2012; Eerde et al., 2019). This lack of consistency in results may be a consequence of both viral and host factors that intervene in DENV infection (Thanachartwet et al., 2015). Genetic variability in terms of polymorphisms in Major Histocompatibility Complex (MHC), cytokine profile and response variations, cellular receptors and other immunological elements determine the clinical evolution of pathology (Thanachartwet et al., 2015; Lan & Hirayama, 2011). Moreover, viral load, Dengue serotype, subsequent infection and time period between infections also have a great impact on the outcome of pathology (Lan & Hirayama, 2011; Yung et al., 2015).

Despite the limitations previously exposed, numerous therapeutic and prophylactic strategies have been explored in recent years (Low et al., 2017; Thisyakorn & Thisyakorn, 2014). Biotechnology has greatly contributed to modern medicine by providing molecular diagnostic tools that allow medical personnel to precisely detect diseases and make smarter decisions (Afzal et al., 2016). It has made it possible to produce novel and more complex drugs for prevention, combat and even eradication not only infectious diseases such as polio and smallpox, but also non transmittable diseases like cancer (Afzal et al., 2016; Sarthak Aggarwal, 2021). Within the vast market of Biopharmaceutical products, therapeutic proteins represent the most relevant products due to the great demand and potential applications (Schillberg et al., 2019). For infectious diseases management and control, the current arsenal is conformed mainly by vaccines, antibodies and antiviral molecules (Afzal et al., 2016). These last products could generate a direct or indirect inhibitory effect in the vital process of virus life cycle (Low et al., 2017). Additionally, biotechnology allowed to detect, isolate and take the production to an industrial scale of molecules from living organisms and development of antiviral drugs (Obi et al., 2021).

Vaccines

The development of Dengue vaccine candidates has advanced over the last decade because researchers and pharmaceutical companies have invested resources to create several vaccine candidates involving various approaches. Referring to replicating viral vaccines which are created by reducing the virulence of the pathogen without compromising its viability, through attenuation by cell cultures or mutagenesis, and the formation of chimeric-like viruses, these vaccines are robust, have broad immunity and are long lasting, but can present genetic instability and a possibility of reversion. On the other hand, there are non-replicating viral vaccines, which include DNA vaccines, inactivated virus vaccines, subunit protein vaccines and virus-like particles; these vaccines have reduced reactogenicity and balanced immune response, but are less broad, potent and durable (Khetarpal & Khanna, 2016)(Redoni et al., 2020). A listing of the current vaccine candidates in different phases of clinical trials is shown in table 2.

Table 2. *Developing Vaccines for Dengue Infection*

Vaccine Name	Type	Clinical Trial Phase
Dengvaxia	Live attenuated virus	Licensed
TDV	Live attenuated virus	III
TDV 003/005	Live attenuated virus	III
TDENV-PIV	Inactivated vaccine	II
D1ME100	DNA vaccine	I
TVDV	DNA vaccine	I
DEN-80E	Subunit vaccine	I
TLAV/TPIV	Heterologous prime/boost	I
DEN 1/2 chimeric virus	Live attenuated virus	Preclinical
HR-Tet	Live attenuated virus	Preclinical
ChinDENV	Live attenuated virus	Preclinical
Purified psoralen inactivated virus	Inactivated vaccine	Preclinical
cEDIII	Subunit vaccine	Preclinical
YF17D-D2/pE1D2	Heterologous prime/boost	Preclinical
VEE-VRP	Virus like particle	Preclinical
DSV4	Virus like particle	Preclinical
DENV-2 VLP	Virus like particle	Preclinical
MV-DEN	Viral Vector	Preclinical

(Khetarpal & Khanna, 2016; Redoni et al., 2020)

In the present day there is only one vaccine that is licensed in Mexico and over 20 Dengue endemic countries, which is the Chimeric Yellow Virus Dengue Vaccine (CYD-TDV), commercialized under the name Dengvaxia created by Sanofi Pasteur. It is a tetravalent live-attenuated yellow fever virus vaccine 17D, aimed to provide a balanced immunity against all of the four serotypes of Dengue. It has an age indication limited to persons of 9-45 years of age, because other ages present a relative risk of Dengue-related hospitalization during clinical trials. Additionally, this vaccine appears to act like a primary natural infection in people who have not been infected, also known as naïve individuals. These individuals are at a higher risk of developing secondary-like infection which is associated with more severe disease; because of that, it can only be applied to people who have already been infected with Dengue (Khetarpal & Khanna, 2016; Redoni et al., 2020).

Dengvaxia and most of the current vaccine candidates lead to the generation of cross-reactive antibodies, which is an important problem with Dengue infection, so research has to continue for vaccine candidates. Then, the ideal vaccine has to provide protective immunity from DENV infection regardless of serotype, age and previous infection (Redoni et al., 2020).

Monoclonal Antibodies

Monoclonal Antibodies (mAb) - based therapy represents an alternative to vaccines. This passive immunization approach has shown successful reduction of viral load and pathology resolution against some other agents such as RSV, Ébola virus and recently SARS - CoV2 (Hu et al., 2019). However, DENV mAb therapy is limited by similar factors to vaccine approach, including cross reactivity, ADE and neutralizing capacities (Hooft van Huijsduijnen et al., 2020).

Both structural (E, prM, C) and nonstructural (NS1, NS3, NS5) DENV proteins exert humoral responses (Hurtado Monzón et al., 2020). For that reason, many DENV directed mAbs have been characterized and studied such as those directed to the envelope protein (Hurtado Monzón et al., 2020). Envelope protein is the main antibody target due to its role in attachment, internalization, and interaction with host cells (Hooft van Huijsduijnen et al., 2020). Envelope directed mAbs that have already been proved *in vivo* are listed in table 3. Multiple studies showed that in terms of neutralizing capacity, serotype specific mAbs presented the highest grade (Natali et al., 2021). In vivo assays with Human mAb (HmAb) 1F4 and 5J7 demonstrated that structure of epitopes plays a crucial role in molecular recognition and neutralization of virus (Fibriansah et al., 2014; Young et al., 2020). Particularly, those Abs directed to DIII in E protein, are the most potent, serotype - specific antibodies reported (Budigi et al., 2018; Natali et al., 2021). However, these kinds of Abs are not easily found in human sera. In order to improve its serotype spectra and neutralizing capacity, two mAbs have been produced. Firstly, VIS513 is an engineered, humanized mAb that is able to potently neutralize DENV avoiding ADE (Budigi et al., 2018), and relieve symptoms of severe disease (Ong et al., 2017). Secondly, m366.6 is a variant of m366 which underwent an affinity maturation process for augmented affinity to 4 serotypes (Hu et al., 2019). For that reason, DIII directed mAbs are promising therapeutic options for Dengue (Budigi et al., 2018).

Table 3. *Therapeutic Monoclonal antibodies candidates against dengue infection*

mAB	Serotype Specificity	Target Molecule	Biological Activity	Host of Isolation
1F4	DENV 1	Envelope (D I and DI - DI hinge)	Significant reduction of viral RNA copy number in infected mice (Fibriansah et al., 2014; Young et al., 2020)	Human
5J7	DENV 3	Envelope (Quaternary structure)	Reduction of an average of 10 fold virus titers in in AG129 mice at nanomolar concentrations (Young et al., 2020)	Human
747(4)B7	DENV 1	Envelope Dimer Epitope (EDE2)	Prevented Aedes mosquitoes from acquiring DENV 1 from plasma (Tuan Vu et al., 2019)	Human
753(3)C10	DENV 1, 4	Envelope Dimer Epitope (EDE1)	Blockade of DENV 1, 4 transmission to Aedes mosquitoes (Varadarajan, Srinivasan, Maity & Ghosh, 2016).	Human
VIS513	DENV 1 -4	Envelope (Domain III)	Reduction of DENV titers and protects from both primary and secondary antibody enhanced infection (Budigi et al., 2018).	Engineered
m366.6	DENV 1, 3, 4 DENV 2 (Partial protection)	Envelope (Domain III)	Protection from lethal DENV 1 - 4 infection in mouse model (Hu et al., 2019)	Human
3G9	DENV 3	Fusion Loop Epitope (Envelope DII)	Strong neutralization and prolongation of survival of mice after a lethal DENV challenge (Kotaki et al., 2021).	Human
1G5 - LALA	DENV 1	Envelope	Protection against DENV 1 infection in murine model with no ADE (Xu et al., 2017).	Human
prM - AID	DENV 1	prM antibodies	Reduction in viral titers, IL - 10 and ALT in mice challenged with DENV1 (Wang et al., 2017)	Mouse
SIgN - 3C - LALA	DENV 1 - 4	Envelope Dimer Epitope (EDE)	Decrease viremia of 4 serotypes in adult infected mice (Lu et al., 2018).	Human
1C19	DENV 1 - 4	bc Loop (Envelope DII)	Recognizes an adjacent site of FL and potently neutralizes all serotypes (Smith et al., 2013).	Human
D23-1G7C2	DENV 1 - 4	Envelope	Broadly neutralizes 4 serotypes with no ADE in mice (Ramadhany et al., 2015).	Human
N297Q - B3B9	DENV 1 - 4	Fusion Loop Epitope (Envelope DII)	Cross - neutralizing activity to all serotypes with no ADE in mice (Injampa et al., 2017).	Human
1H7.4	DENV 2	NS1	100 % of survival in mice receiving sublethal dose of DENV plus NS1 (Beatty et al., 2015)	Mouse
2E8	DENV 1 - 4	NS1	Reduction of viral titers and NS1 levels in mouse sera. Reduction of DENV - induced prolonged bleeding time in mouse (Xu et al., 2017).	Mouse

Despite high neutralization activity, limited pan - serotype recognition of specific mAbs and the relatively high risk for complication of disease via ADE made researchers look for other options (Fibriansah et al., 2014). Complex epitopes such as the quaternary Envelope Dimer Epitope (EDE) have been demonstrated to stimulate the production of antibodies (Kotaki et al., 2021) with high diverse neutralization and cross reactivity among serotypes (Dejnirattisai et al., 2014; Varadarajan, Srinivasan, Maity & Ghosh, 2016). Unfortunately, the presence of diverse affinity antibodies in primarily infected patients appeared to reduce the efficiency (Varadarajan, Srinivasan, Maity & Ghosh, 2016) of EDE directed mAbs 747(4)B7 and 753(3)C10 (Tuan Vu et al., 2019). Although the mechanism of decreased efficiency remains unclear, it is hypothesized that antibodies with diverse neutralization capacities compete with each other for epitopes (Varadarajan, Srinivasan, Maity & Ghosh, 2016). These results suggest that other criteria for further mAb target selection need to be considered such as degree of conservation or immunodominance. Fusion Loop Epitope is a glycerine rich, hydrophobic sequence located in the distal end of DII (Klein et al., 2013). It is implicated in critical processes of infection such as dimerization of E protein and pH dependent membrane fusion (Costin et al., 2013). Additionally, this linear epitope is immunodominant and highly conserved among DENV serotypes and even other flaviviruses which make it an interesting candidate (Costin et al., 2013; Rouvinski et al., 2017). Several groups have characterized and produced FLE antibodies, reporting a broad cross reactivity between serotypes (Deng et al., 2011; Smith et al., 2013). Unfortunately, most of these anti FLE mAbs showed neutralization values considerably lower compared to those mAbs directed to E dimers or quaternary viral structures and potently induce ADE (Kotaki et al., 2021, Smith et al., 2013). This poor neutralization capacity seems to be caused by suboptimal exposition of the FLE region in mature DENV particles (Rouvinski et al., 2017). Interestingly, Tsai et. al reported that FLE from patients with a secondary infection potently neutralize DENV, while those form patients with primary infection show weak neutralization capacity (Tsai et al., 2013). These findings prompted new research such as the one conducted by Kotaki et. al, in which anti FLE 3G9 mAb underwent an affinity maturation process and Fc modification (Kotaki et al., 2021). These optimizations increment 3G9 potency and reduce the risk of ADE (Kotaki et al., 2021).

ADE is theorized to occur due to the suboptimal neutralization of DENV that facilitate the entry to dendritic cells via FcγR, resulting in the exacerbation of pathology (Dejnirattisai et al., 2016). Consequently, mAbs therapeutic candidates should avoid or even reduce the risk of ADE *in vivo*. Several strategies to overcome this problem have been proposed. mAb - Fc modification by deletion of glycosylation sites such as N297Q-B3B9 (Injampa et al., 2017) or induction of mutations (SIgN-3C - LALA, IG5 - LALA) reduced ADE and seemed to be decisive in the performance of the therapy (Xu et al., 2017; Lu et al., 2018). Alternatively, the administration of anti-idiotypic antibodies specific to prM mAb dramatically reduced ADE effect in mice challenged with DENV1 (Wang et al., 2017).

Challenges in development of structural proteins directed mAbs that efficiently treat Dengue make researchers explore nonstructural proteins as potential therapeutic targets because of the lack of ADE induction and high degree of conservation (Wan et al., 2014). NS1 is a multifunctional protein that plays crucial roles in both viral cycle and pathogenesis (Nasar, Rashid and Iftikhar, 2019). This is the only protein constitutively secreted by infected host cells (Chen, Lai and Yeh, 2018), and serum concentration of NS1 is correlated to disease severity (Nasar, Rashid and Iftikhar, 2019). Some pathogenic roles attributed to this protein are coagulation cascade disruption, vascular leakage, thrombocytopenia, proinflammatory factors production, etc (Nasar, Rashid and Iftikhar, 2019). NS1 mAb have demonstrated multiple therapeutic mechanisms including the reduction of viral titers, reduction in DENV replication via complement-dependent cytotoxicity (CDC), cross reactivity (Wan et al., 2017) and reduction of NS1 induced symptoms (See table 3).

mAbs represents an interesting alternative/complement to vaccination, with promising results such as those previously presented. This kind of therapy may reduce the risk of severe dengue and disease transmission (Sultana et al., 2009). However, mAbs approach faces important barriers including cross reactivity, potency and the risk of ADE as well as lack of experimental models that mimics human pathogenesis of Dengue (Kotaki et al., 2021; Chokephaibulkit et al., 2020). Limited capability in mounting full immune responses, lower infection efficiency, high technical and economic requirements and lack of clinical manifestations are some reasons why mAbs and other therapeutic candidates cannot be carried to Clinical Trials (Chokephaibulkit et al., 2020; Zompi & Harris, 2012).

Antivirals

Currently, there are no approved or available antiviral drugs for the treatment of Dengue in any of its clinical manifestations. The only treatment available is supportive fluid therapy for severe Dengue and anti-inflammatory drugs to treat the symptoms of mild Dengue. The development of antiviral drugs effective against Dengue has been a big challenge. The main objective of an antiviral drug for Dengue is to be able to reduce the viral load in the first 70 hours in order to prevent the progression to a more severe clinical manifestation. One of the limitations of drug development is the fact that the antiviral should inhibit all four DENV serotypes (Anasir et al., 2020).

Different studies have been made to understand and detect new antiviral metabolites in natural sources that are able to target viral proteins (*See table 4).* There are many different approaches to the development of antiviral drugs against Dengue. One of the most advanced approaches is the drug repurposing for Dengue therapy, in which many studies are currently in clinical trials. Approved and used antiviral drugs for other viral and non-viral diseases is the fastest and less expensive strategy to identify a treatment for Dengue. Despite the effort, this strategy did not show promising results against Dengue. Some of the drugs that were evaluated in clinical trials include chloroquine, celgosivir, ribavirin, prednisolone, and lovastatin *(See table 5)* (Sagaya Jansi et al., 2021).

Table 4. *Therapeutic compounds candidates against dengue infection from natural and chemical sources*

Compound	Organism	Target molecule	Biological Activity	Reference
7-O-Methyl-glabranine	*Tephrosia madrensis*	-	The flavonoid showed a dose dependent inhibitory effect in vitro on dengue-2 virus. The study showed a 70% inhibition at a concentration of 25 µM.	Sagaya et al. 2020
Brefeldin A	*Penicillium sp. FKI-7127*	-	Brefeldin A can inhibit the four serotypes of dengue virus. It showed an early antiviral effect on the life cycle of dengue.	Raekiansyah et al. 2017
Fucoidan	*Cladosiphon okamuranus*	-	The polysaccharides demonstrate an inhibitory effect on in vitro study of 80% on DENV2. In the serotypes 3 and 4 are moderately susceptible and for the serotype 1, fucoidan did not present and effect. There is no establish molecular mechanism of the inhibitory effect yet.	Teixeira et al. 2014
Narasin	*Streptomyces aureofaciens*	-	The polyether showed that it has a 50% inhibitory effect against all four DENV serotypes. It has a minimal cytotoxicity. Inhibits post-entry stages of viral replication by disrupting viral protein synthesis.	Teixeira et al. 2014
ST-610	-	NS3 Helicase	Benzoxazole inhibitor. Potent inhibitor of DENV 2 in the viral titer reduction assay. It showed to be non-toxic against DENV 1-4 on in vitro studies.	Tian et al., 2018; C.M. Byrd, 2013
MLH40	-	Entry inhibitor, protein M-E interaction	Peptidyl inhibitor. Active against DENV 1-4. It showed an IC50 value of 31.41 µM against DENV2.Inhibits the viral entry.	Tian et al., 2018; A. Panya, 2015
ST-148	-	Capsid	Inhibites the entry and assembly or release of the virions by enhancing capsid-protein interaction and causing structural rigidity	Tian et al., 2018; P.Scaturro, 2014
Peptide-conjugated phosphorodiamidate morpholino (PPMO)	-	Viral genome, targets 5' terminal nucleotide and 3' cyclization sequence regions (3'CS)	Reduction of viral titers of DENV2. The 3'CS targeted PPMO inhibited the replication of all four serotypes of DENV.inhibited DENV replication by interfering with both mRNA transcription and protein translation machinery.	Panda et al., 2021

Table 5. Antivirals candidates for repurposing strategy against dengue infection

Compound	Biological Activity and Target molecules	Study characteristics	Clinical trial results
Chloroquine	Inhibits DENV entry and the replication in vitro and in vivo. Inhibit the fusion between virus and host membrane.	Placebo vs Chloroquine, 307 participants on the clinical trial.	There was no change in the viremia and NS1 levels
Prednisolone	Reduce and prevent the development of more severe clinical manifestations or complications Literature supports the modulation of the function of endothelial glycocalyx and its anti inflammatory properties.	Placebo vs prednisolone. 225 participants of ages from 5 to 20.	No significant change in clinical or virological end points.
Lovastatin	Showed an moderate inhibitory effect in DENV2 replication. In animal models study that used mice it protected them from DENV2 infection. It is a cholesterol synthesis inhibitor, which allows to limit membrane mobilization needed for the viral replication	Placebo vs lovastatin, 300 participants .	Efficacy was not address. There is no beneficial effect on the clinical manifestations or viremia levels of DENV.
Balapiravir	Inhibit DENV4 RNA synthesis on in vitro studies. It is supposed to be an inhibitor of the NS5.	Placebo vs balapiravir, 64 participants of ages from 18 to 65 years old.	No changes in immunological and virological end points..
Ribavirin	Showed synergistic effects when CM-10-18 is present, both are able to suppress DENV2 replication in vitro and on murine model.	Phase 2 clinical trail multicenter, to evaluate safety. Practical randomized controlled clinical research. 300 participants	

For example, chloroquine is being evaluated due to its ability to inhibit endosomal acidification, which interferes with the fusion of the viral and endosomal membranes. *In vitro* studies showed that chloroquine inhibited DENV1 replication in THP cells and DENV2 replication in U937 and Vero cells. In animal model studies, chloroquine was able to reduce viremia in monkeys infected with DENV2. Even do, the previous promising results of the *in vitro* studies, the chloroquine was not able to reduce viremia and NS1 antigen in Dengue patients (Sagaya Jansi et al., 2021).

Different approaches are being studied that are focused on targeting the viral proteins or the targeting of the viral genome to inhibit the replication of the virus *(See table 4)*. The main idea of using oligonucleotides is to target viral genomes, in order to inhibit the replication or translations, known as silencing of the viral genome expression. The oligonucleotides interact with specific regions of the target genome and downregulate the gene expression or disrupt the expression.

This strategy designs and uses different types of RNA or DNA molecules like siRNA, miRNA, CRISPR, ribozyme, among others (Panda et al., 2021).

Another strategy is to design antivirals that target viral proteins, in which the main focus is to use inhibitors to the different proteins that allow Dengue to replicate. The main candidate targets are NS3/NS2B protease, NS3 helicase, NS5 polymerase RNA-dependent, non-enzymatic NS4B, and the E glycoprotein. Each of these protein targets plays an important role in the replication of the virus and could help reduce the viremia in patients with Dengue. The inhibitors of each protein are being studied and still have some limitations to overcome before their approval and use in clinical trials (Anasir et al., 2020; Tian et al., 2018).

CONCLUSION

Dengue disease represents a complex health problem in Mexico. In the last century, the incidence in cases have shown irregular patterns. The country has experienced 5 major outbreaks of Dengue, in which people of all ages have been affected. Given the susceptibility of the general population and increasing cases in 2019, the problem must be urgently addressed. Epidemiology surveillance is crucial for guiding health authorities to take pertinent actions of prevention, monitoring and control. As the PAHO program for controlling vector previously demonstrated, coordinated efforts of governmental institutes, private companies and population could help to reduce the risk of transmission. Unfortunately, the tools we currently have are limited due to the percentage of asymptomatic cases and low comprehension of molecular and immunological mechanisms of pathology.

In addition to epidemiology, our arsenal against Dengue is focused on preventive and therapeutic strategies. To date, vaccines, monoclonal antibodies, and antiviral drugs represent the most promising prophylactic and therapeutic approaches. Although preliminary *in vitro* and *in vivo* have shown hopeful results, remarkable obstacles need to be overcome. ADE, cross reactivity genetic susceptibility and the lack of an animal model that fully mimics pathogenesis of Dengue are some factors that hinders the development of definitive treatment and conduction of preclinical and clinical trials.

It is a mandatory impulse, innovation and improvement in preventive, diagnostic, and therapeutic tools. Experimental results would help us to fully understand the disease and mechanisms that lead to resolution or exacerbation, as well as finding an area of opportunity to protect thousands of people worldwide.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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