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"In Silico Strategies and Comparative Genomics in Arboviruses: A Comprehensive Review of Therapeutic and Vaccine Target Discovery"

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ABSTRACT

Arboviruses, including Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV), are a concern as significant global health crises due to their rapid spread, complex vector-host interactions, and lack of targeted therapeutics or licensed vaccines. This RNA virus is spread by Aedes mosquitoes and shows high genetic variability with a range of clinical complications. Conventional laboratory approaches for drug and vaccine discovery are time-consuming and very expensive, thus leading to a move towards computational (in silico) strategies. This review explains the genomic architecture and biological insights of DENV, CHIKV, and ZIKV which highlighting their evolutionary relationships, transmission dynamics, and clinical relevance. We discuss the power of comparative genomics in identifying conserved, essential, and non-host homologous genes that serve as potential therapeutic and vaccine targets. A systematic in silico pipeline—encompassing genome annotation, essential gene identification, host non-homology analysis, subcellular localization prediction, druggability and immunogenicity assessment, epitope modeling, and network analysis—is presented. To identify promising candidates for downstream validation and clinical development, we can use integrated computational methods. Our review emphasizes the need for an integrated, comparative genomic approach to overcome the challenges posed by viral diversity and co-circulation, ultimately guiding effective therapeutic and vaccine design against these high-burden arboviral diseases.

Keywords: Arbovirus; Dengue (DENV); Chikungunya (CHIKV); Zika (ZIKV); In Silico Analysis, Vaccine Target; Epitope Prediction, Immunoinformatics.

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Introduction

ARthropod-BOrne VIRUS is shortened to Arbovirus. Arthropod-borne pathogens continue to pose a serious hazard to both human and animal health, as seen by the rise in arboviral infection emergence and reemergence over the past few decades. Dengue, chikungunya, Zika, and other infections are not restricted to developing or tropical areas of the planet; they are happening all over the world. Vectors of haematophagous insects, including sandflies, tick flies, and mosquitoes, transmit infections caused by arboviruses. As accidental hosts, humans only become infected when a vector feeds on their blood; they do not participate in the virus's spread. The individual in pain receives supportive treatment, and only a limited amount of precaution can be taken. An important factor in the massive expansion of arboviruses has been the increase in global temperatures, as well as human invasion and domestication of newer geographic areas. Arboviruses have an extremely complicated life cycle since they require several hosts to complete it, including vertebrate hosts and the main arthropod vectors (Sukhralia et al. 2019). Arboviruses spread by mosquitoes can cause unexpected clinical consequences and are difficult to diagnose. The three most significant arbovirus-caused diseases in the world, particularly in tropical and subtropical areas, are dengue, chikungunya, and Zika. Humans contract these diseases from daytime-biting Aedes aegypti and Aedes albopictus mosquitoes. The spread of Aedes aegypti is the main cause of the growing number of dengue and chikungunya cases in India. Controlling dengue outbreaks efficiently requires a quick and precise diagnosis (Soni et al., 2023). The present scenario is intricate, as there is no vaccine available for Zika and chikungunya, nor is there a targeted treatment for the three arboviruses (Paixão, Teixeira, & Rodrigues, 2018). Mosquitoes possess the capability to transmit arboviruses by obtaining a viremic blood meal from an infected host. Upon ingestion of the blood, the virus enters the mosquito's midgut and infects the epithelial cells, which may subsequently facilitate the dissemination of the virus to the mosquito’s internal tissues and organs. Following the extrinsic incubation period (EIP), the virus may migrate to the salivary glands, culminating in the transmission of the virus to a new host through the mosquito's saliva. The efficiency with which the virus crosses anatomical barriers, such as the midgut and salivary glands, is influenced by multiple genetic and biological factors that govern the mosquito's antiviral immune response. It is noteworthy that geographic populations of the same mosquito species may exhibit differences in their immunological backgrounds, resulting in variable susceptibilities to the transmission of arboviruses (Mariconti et al., 2019).

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Arboviruses and Their Genomic Insights

Pegivirus, Pestivirus, Hepacivirus, and Flavivirus are the four genera that are currently recognized within the family (Simmonds et al., 2017). Pestiviruses cause serious illness in domestic ungulates like cattle and pigs (de Oliveira et al., 2020), and the Hepacivirus genus includes the blood-borne hepatitis C virus (HCV), a major cause of chronic liver disease in human populations worldwide (Manns et al., 2017; Pierson & Diamond, 2020). Pegiviruses are not known to be associated with disease. Furthermore, viruses in the genus Flavivirus, such as dengue viruses 1–4 (DENV 1–4) and the Zika virus, are spread between vertebrates by blood-feeding arthropod vectors (such as mosquitoes and ticks) and create widespread outbreaks that infect millions of people annually (Bamford et al., 2022). Dengue and Zika viruses are among the more than 50% of viruses in the genus Flavivirus, family Flaviviridae, that infect humans. Small, spherical, and 40–50 nm in size, flavivirus virions have lipid envelopes that carry single-stranded, non-segmented RNA. The envelope protein of flaviviruses has common group epitopes that can cross-react in serological testing, and their genomes are roughly 11,000 bases long (Paixão, Teixeira, & Rodrigues, 2018).

The prevalence of viral-related sequences found in the genomes of many creatures that have been sequenced thus far indicates that viral integrations of DNA viruses and retroviruses are a widespread occurrence (i.e., genetic code from retroviruses constitutes roughly 8% of the human genome). Integration potentials were presumed low for these non-retroviral RNA viruses as they did not encode an equivalent to the reverse transcriptase coding nor the necessary integration machinery to facilitate transfer into DNA genomes efficiently. There is increasing evidence in the literature of genome integrations from non-retroviral RNA viruses, supporting the genesis of integrations from single-stranded (positive and negative) and double-stranded RNA viruses in somatic and germline host cells. Arboviruses, or viruses carried by arthropods, and insect-specific viruses, or ISVs, are examples of RNA viruses. The Asian tiger mosquito, Aedes albopictus, and the yellow fever mosquito, Aedes aegypti, are two eukaryotic species whose genomes have been shown to contain sequences from NRVs. For several epidemiologically significant arboviruses, such as the Zika virus, Chikungunya virus, and Dengue viruses (DENVs), these two species serve as the main vectors (Pischedda, 2021).

The most significant viral disease that arthropods can transmit to humans is dengue. An estimated 2.5 billion individuals reside in regions where dengue is a possible illness. Given the rise in chikungunya-related chronic joint illnesses, congenital anomalies, Guillain-Barré syndrome, and other autoimmune symptoms, a global alert has been issued for Zika (Beltrán-Silva et al., 2018). Here we review recent advancements in our understanding of the biology of DENV, ZIKV, and

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CHIKV, and highlight areas of knowledge that have inhibited the development of efficacious vaccines and therapeutics.

Dengue: Dengue is a viral disease characterized by fever and a range of symptoms that can vary in severity. It is a significant cause of illness and death in many regions where it is endemic. Annually, around 390 million people are infected with dengue, and approximately 96 million of these individuals exhibit clinical symptoms of varying severity. Tragically, up to 20,000 of those infected may die from the disease (Beltrán-Silva et al., 2018; Bhatt et al., 2013). Dengue virus (DENV) is classified in the family Flaviviridae and the genus Flavivirus. There are four serotypes of the virus, DENV-1, DENV-2, DENV-3, and DENV-4. Although these serotypes are antigenically similar, they differ enough that infection with one does not provide long-term cross-protection against the others. A person infected with one dengue serotype gains lifetime immunity to that specific serotype. The genome of the virus consists of a single RNA chain, which contains a nucleocapsid surrounded by structural proteins M and E, as well as seven non-structural proteins. Among these non-structural proteins, NS1 interacts with the host immune system and triggers T cell responses. This immune response has been utilized as a diagnostic marker for dengue infection (Beltrán-Silva et al., 2018; World Health Organization et al., 2009).

Chikungunya: Chikungunya (CHIK) fever is a viral illness that begins suddenly, typically with high fever, skin rashes, and joint pain, often followed by long-lasting rheumatic issues (Beltrán-Silva et al., 2018). The virus belongs to the Alphavirus genus within the Togaviridae family. Structurally, it consists of a capsid, a phospholipid envelope, and a single-stranded RNA genome. The Alphavirus genus includes 28 recognized viruses, such as O'nyong-nyong, Ross River, and Mayaro viruses. Chikungunya virus (CHIKV) is classified into four genotypes: East-Central-South African (ECSA), West African, Asian, and Indian Ocean lineages (Paixão, Teixeira, & Rodrigues, 2018; Pialoux et al., 2007; Nunes et al., 2015).

Zika: Zika virus infection is a viral exanthema, like dengue and chikungunya, most commonly presenting with acute febrile illness, exanthematous rash, and non-purulent conjunctivitis. The vast majority of people who get infected with the virus will end up with no symptoms at all, and if symptoms do occur, they're usually mild and self-resolving. Zika virus (ZIKV) is a single-stranded RNA virus made up of 10,794 nucleotides, or 3,419 amino acids. It is a member of the Flavivirus genus of the Flavivirus family. The virus has been found in many different mosquito species, such as Aedes apicoargenteus, Aedes luteocephalus, Aedes aegypti, Aedes vittatus, and Aedes furcifer (Beltrán-Silva et al., 2018).

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Role of Comparative Genomics in Target Discovery

Finding therapeutic targets has usually meant doing experiments in a wet lab, which can take a lot of time, money, and sometimes aren't very accurate. Nonetheless, due to progress in bioinformatics, chemoinformatics, and several omics technologies, in silico (computer-based) methods have gained significant prominence (Wooller et al., 2017; Dai & Zhao, 2015). These computational methods use big data and analytical tools to greatly reduce the number of possible targets, which speeds up the drug discovery process, shortens the time it takes to develop drugs, and lowers the costs of experiments (Zhang et al., 2022).

Since there are currently no major specific treatments or preventive measures for arboviral illnesses, it is essential to develop targeted vaccines or drugs for these viruses. A promising approach to achieve this is through comparative genomics, where the genomes of various viruses are studied to identify common targets. Previous comparative genomics studies have been conducted on viruses such as the foot-and-mouth disease virus, Ebola virus, and Epstein-Barr virus to understand their mechanisms of action in humans (Sukhralia et al., 2019).

Comparative genomics makes use of genome database resources and software to uncover pathogens' fatal flaws that impact their growth and reproduction in the host, including genes crucial to the pathogens' survival, growth, and vital functions, in order to identify therapeutic targets (Zhang & Ren, 2015). Furthermore, by comparing the genomes of pathogens and hosts, comparative genomics can also eliminate homologs, preventing the host from experiencing harmful side effects from newly developed medications, thus improving the success rate of drug creation (Zhang et al., 2022; Zhang & Ren, 2015). Two crucial traits are present in therapeutic targets found using comparative genomics techniques. The efficacy of the newly developed medication is ensured by the fact that the chosen targets significantly affect a few crucial physiological processes of the infection. The safety of the pharmacological effects of new drugs can be improved by avoiding any harmful side effects that may occur when the drug interacts with the target by comparing the protein sequences of the host and possible therapeutic targets to see if there is homology (Dutta et al., 2006). Additionally, research is being conducted to explore the dynamics of mosquito genomes. While individual studies on different serotypes of dengue have been carried out, such as DENV-1, DENV-2, and DENV-3, the issue of heterotypic infection remains unresolved until all serotypes are studied comparatively. By employing comparative genomics, researchers may not only gain insights into the evolution of the dengue virus but also pave the way for the development of a single drug or vaccine that is effective against all serotypes

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for human immunization (Sukhralia et al., 2019). This methodology aids in the identification of non-host homologous proteins, thereby minimizing off-target effects in humans (Wei et al., 2002).

In Silico Tools and Strategies:

- **Analysis of Genome retrieval and Annotation:** For genome retrieval, the National Center for Biotechnology Information (NCBI) and the Virus Pathogen Resource (ViPR) databases are used to obtain the full genomes of the target pathogens. For genome annotation, RAST, Prokka, or VIGOR tools are used. Pan-genome analysis tools like Mauve, OrthoFinder, or Roary can be used to perform pan-genome analysis and identify core, accessory, and unique genes.
- **Identify the Essentiality of Genes:** To identify which genes are essential for pathogen survival, so that we, compare bacterial/viral genes against essential gene databases such as DEG or CEG.
- **Analysis for host's non-homologous proteins:** For the analysis of the host's non-homologous proteins, we compare the host proteome with the pathogen proteome by using the BLASTp tool and filter the highly homologous proteins.
- **Prediction of subcellular localization:** In which we can predict either the proteins are cytoplasmic, membrane-bound, or secreted. Tools like PSORTb, CELLO, and TMHMM are used.
- **Evaluate the ability of Druggability and Antigenicity:**
  - For Drug Targets, ProtParam, DrugBank, PubChem, Drug-Likeness, and SwissADME tools are used. To analyze the three-dimensional structure of target proteins, tools like SWISS-MODEL or AlphaFold are used. To identify druggable pockets, DoGSiteScorer or Q-site finder is used.
  - For Vaccine targets, IEDB, NetCTL, and VaxiJen are used to predict B-cell and T-cell epitopes. Similarly, ToxinPred, AllergenFP or AllerTop, and VaxiJen can be used to evaluate toxicity, allergenicity, and antigenicity, respectively.
- **Perform Molecular Docking and Epitope Modeling:**
  - For Drug target discovery, HADDOCK or SwissDock, or AutoDock Vina is used.
  - For Vaccine target discovery, epitope modeling, and Immune system simulation by using such as C-ImmSim or SIMMUNE is used.
- **Analysis of the network and pathways:** To obtain information about how specific targets interact within host-pathogen networks and to confirm their roles in key pathways, we use STRING, Cytoscape, and KEGG for this analysis.

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- **Validation and Ranking:** Organize targets based on literature evidence, druggability/immunogenicity scores, essentiality, and uniqueness. The highest-ranked candidates will then be sent for validation both in vitro and in vivo.

Conclusion

Arboviruses such as dengue, chikungunya, and Zika represent an all too urgent and growing threat to global health, especially in tropical and subtropical developing countries. Their increasing worldwide burden poses one of today's most urgent public health crises, exacerbated by the absence of effective vaccines or strong, targeted antiviral therapies. This review explains the complex biology and genetic features of DENV, ZIKV, and CHIKV viruses, and also their transmission and pathogenesis. Researchers are using comparative genomics and in silico methods to discover a powerful, cost-effective, and time-efficient way to discover new therapeutic and vaccine targets. These druggable and immunogenic candidates can be identified through genome annotation, essential gene prediction, host non-homology analysis, and structural modeling to ensure off-target effects are minimized. Further, findings of conserved genomic features across virus strains help to guide the design of broad-spectrum anti-viral treatments. Such computational techniques increase the rate at which scientific research can proceed through the early stages of drug and vaccine development while requiring fewer costly experimental trials. We still require further in vitro and in vivo validation of these in silico predictions to ensure that they perform effectively in the real world. We would be able to speed up target discovery and generate resistance against these emerging arboviruses by integrating omics data, AI-generated modeling, and surveillance of vector populations in real time. To better control and prevent arboviral infections, researchers and public health partners must continue to translate genomic discoveries to clinical use.

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