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Advancements in molecular techniques impacting the diagnostic landscape for dengue in the near future

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ABSTRACT

Denue viral epidemics have escalated worldwide, with over 100 million infections occurring annually. The spread of the mosquito vector is supported by the increase in urbanization and climate change with models predicting potential impact of 6.1 billion people by 2080. Dengue is a mosquito borne viral infection caused by dengue virus, the genus of which contains four serotypes. While this virus causes mild infections with flu-like illness, it can occasionally develop into a potential lethal complication called severe dengue. There is currently no specific treatment but early detection and access to proper medical care can lower the fatality rates to below 1%. Currently prevention and control depend on stringent effective vector control measures with the help of community participation. Out true understanding of the circulation, transmission dynamics, immunological interactions, and association of genetic and antigenic diversity with severity remains limited. Thus the approach to investigate dengue virus epidemiology, immunological interactions, and genetic diversity using highly specific diagnostic and pathogen sequencing methods is crucial and requires developing integrated, targeted and effective intervention strategies. This paper explores current and emerging molecular methods and their anticipated impact on the diagnostic landscape for dengue in the near future.

Introduction

Denue viral epidemics have escalated worldwide, with over 100 million infections occurring annually. The European Centre for Disease Prevention and Control (ECDC have reported over five million cases of dengue with 2000 related deaths since the beginning of 2024[1]. Regions hardest hit include the America, South-East Asia, and the Western Pacific with Asia bearing 70 % of the global disease burden in Bangladesh, Malaysia, Thailand, and Vietnam[2-4]. The spread of the mosquito vector is supported by the increase in urbanization and climate change with models predicting potential impact of 6.1 billion people by 2080[5]. WHO has introduced the Global Arbovirus Initiative (GLAI)to address this growing risks of arbovirus transmission[6]. Out true understanding of the circulation, transmission dynamics, immunological interactions, and association of genetic and antigenic diversity with severity remains limited. Thus, the approach to investigate dengue virus (DENV) epidemiology, immunological interactions, and genetic diversity using highly specific diagnostic and pathogen sequencing methods is crucial and requires developing integrated, targeted and effective intervention strategies. Dengue is a mosquito borne viral infection caused by dengue virus, the genus of which contains four serotypes. While this virus causes mild infections with flu-like illness, it can occasionally develop into a potential lethal complication called severe dengue. There is currently no specific treatment but early detection and access to proper medical care can lower the fatality rates to below 1%. Currently prevention and control depend on stringent effective vector control measures with the help of community participation.

Diagnosis

Dengue can be diagnosed clinically; however, laboratory tests are required to confirm the infection. Definitive diagnosis is important not only for clinical management of patients, but more so for interventions during outbreaks, epidemiological surveillance, and for vaccine development and monitoring. Development of assays is ongoing, and newer assays using more advanced technologies have been developed, though not fully validated. The main hurdle lies in the incompletely understood pathogenesis of dengue, with multiple sequential infections notwithstanding the issues with primary and secondary infection status which further complicate the diagnosis. Hence as has been said in many publications, for a diagnostic assay to be useful and effective, users need to have some degree of confidence in the test to improve disease management, especially in the acute early stage and for detecting signs of severity. An ideal dengue diagnostic test would be simple, rapid, with high sensitivity and specificity, preferably able to differentiate between primary and secondary infections, serotype the virus and most importantly of all be affordable. The optimal time frame for diagnosis would be from the onset to 10 days post-infection. In reality more than half only consult the physician when in dire situations, with many people in third world countries resorting to traditional healing. It is also important to note that 2% do not seroconvert and a high number are asymptomatic.

Diagnostic tools currently are mainly either serologically based, nucleic acid-based or antigen detection. A good understanding of clinical conditions of dengue patients is essential for appropriate usage of these tests. The highest confidence lies in the isolation of the virus, thus fulfilling Koch's postulate, detection by direct immunofluorescence (IF), genome amplification via PCR[7-10] or detection of viral antigens like NS1 using either ELISAs or rapid lateral flow tests[11-17]. However, more often than not, we tend to use tests out of convenience and hence look for antibodies and for these, diagnostically useful results are best obtained using paired samples. Currently, virus isolation, PCR and Direct IF are carried out only in reference and research laboratories. NS1 however, is more user-friendly and apart from ELISAs, rapid flow tests have been developed and some validated[18-22]. it is important to note that cross reactivity with other flaviviruses including West Nile virus (WNV), St. Louis encephalitis virus (SLE), Japanese encephalitis virus (JEV), Zika virus and

yellow fever virus (YFV) do occur and hence a review of the patient's past medical history, recent travel history, and vaccination record (especially yellow fever vaccination) is needed to determine the likelihood that the current acute febrile illness is actually due to an infection with dengue virus. In many instances, a paired sample is best utilized to make a diagnosis, especially for those who present late after onset of illness (> 5 days) as virus and viral antigens become undetectable, and in these instances, the tests need to be carried out on all viruses suspected. The current infection will be the only pair demonstrating a four-fold rise[18,19] .

Dengue virus induces the production of all classes of antibodies, primarily targeting the virus envelope proteins. The level of antibodies primarily depends on whether the individual has a primary or a secondary dengue infection[20,21]. In recent years, the development of rapid assays has allowed patient specimens to be tested in point-of-care situations. Many manufacturers of immunochromatographic tests (ICTs) claim the tests to be able to detect and differentiate between primary and secondary infections with dengue [23,24]. However, the diagnostic accuracy of such assays has not been reliably established because of the multiplicity of methodologies used in evaluation. Newer bio-diagnostics devices that can be quantitative and or qualitative, are the prototypes of the future rapid diagnostic test kits that will be commercialized if they have desirable traits such as the ability to be portable, automated and easily disposed of. A survey of the literature shows that most biosensors that are being developed for dengue use piezoelectric, optical and electrochemical methods[25]. More recently, nanoparticle beads, mass spectrometry as well and microsequencing have been utilized and appear promising[34-40]. Generally, biosensor kits need to be validated to be used as a rapid test for dengue. Future dengue assays have to be able to not only confirm the serotype of the virus but also have markers that are able to predict disease severity and indicate level of immunity/protection.

Biosensor assays maybe the solution but must be able to be made user friendly, cheap and be validated in different geographical regions as assays do not necessarily perform similarly in all the countries and need to be tweaked to perform for that region as where dengue is endemic there will be high pre-test odds for the presence of anti-dengue IgG or previous infection with other flaviviruses that may interfere with proper interpretation of test results. Newer platforms, the usage of smaller sample volumes, may assist towards improving the current dengue diagnostics to one that is definitive. These include: Next Generation Sequencing (NGS) technologies, Molecular Detection, Polymerase Chain Reaction (PCR) Based Tests, Isothermal Amplification Based Tests, CRISPR-Based Diagnostics, Microfluidics and Lab-on-a-Chip Technologies, Multiplexed kit based on Luminex technology, Genomics and Bioinformatics, Artificial Intelligence and Machine Learning and more importantly to have them as Portable Diagnostic Devices. Here we review these platforms as possible molecular techniques that may impact the diagnostic landscape.

Molecular Detection

DENV Nucleic acid tests (NATs) is considered as the gold standard for detecting DENV at an earlier stage of infection due to its high sensitivity[26]. These tests require expensive equipment and skilled personnel and cannot be performed in Point-of-Care (POC) settings. The viral genome can be detected within five to seven days of symptom onset. Many assays have been developed to provide semi-quantitative or qualitative detection. In RT-PCR, the viral RNA is extracted from various types of samples, transcripted to cDNA, then amplified and detected by various dyes, which is read by devices to determine positive and negative results[27]. They are able to target and differentiate all the serotypes to as low as 100 viral copies/mL in a single step. Others have been developed to detect and differentiate DENV from ZIKV, YFV, and Chikungunya virus[28] with no cross-reactivity. These are now automated using cartridges where samples are loaded and contain all the extraction, amplification and automatically provides the qualitative result with a lowest detection point of 1 and 10 PFU/mL for DENV-1,3 and DENV-

2,4, respectively[29]. Nucleic Acis Tests (NAT) assays approved by FDA include the CDC DENV-1-4 Real-Time RT-PCR Multiplex assay and Trioplex rRT-PCR Assay (for Dengue, Chikungunya, and Zika)[30] with varying sensitivities ranging from 95-100% [31].

The isothermal Amplification Based Tests amplifies using a single temperature and are useful in rural areas as a thermocycler is not required, thus reducing the costs. To date, this test has not been approved worldwide but the technology has been utilized in other platforms such as reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), Reverse Transcription Recombinase Polymerase Amplification (RT-RPA), and nucleic acid sequence-based amplification (NASBA). This technique uses 4-6 primers with 2 loop primers that can recognize at least 6-8 distinct targets of the viral genome [32,33]. Using just a water bath (60-65 C) targets that are amplified can be visualized with the naked eye and have been shown 98-100% sensitivity[34]. A single tube reaction method has been developed to obtain results within 30 minutes[35,36] with no false positives. The main advantages if these assays are the rapidity, cost-effectiveness, isothermal, highly sensitive, and specificity of 100%[37]. However, they are not quantitative. Similar to the RT-LAMP assay is the NASBA-based DENV detection assay requires sample pre-processing and cautious handling of the viral targets[38]. A novel isothermal amplification technique is RPA, which detects 1–10 DNA copies of the target within 20 min at a temperature range of 37–42 °C[39]. Developed by Teoh et al, it targets the 3′-UTR regions[40] and mobile units were developed in Thailand, and more recently, detection was carried out with lateral flow dipsticks with the capability of detecting 1 to 10^6 copies/μL for DENV-1[41]. Hence, this platform can be employed in resource-limited settings with high enough sensitivity.

Luminex Technology

Molecular assays have disadvantages, in terms of the need for agarose gels and with risks of sample cross-contamination. Real-time PCR techniques require fluorescence detection filters and hence there is a limit to the number of targets when multiplexing[42-44]. To this end RT-PCR/Luminex-based assays may be a useful alternative for multiplex diagnosis[45]. The Luminex xMAP technology was developed in the late 1990s and can now perform multiplexed biological assays, with the number of applications using this technology on the rise[46,47]. Briefly, this technology involves coupling capture probes with carboxylated microspheres, which are then hybridized to PCR amplicons in a 96-well plate, placed in a thermocycler, and then analysed in the MAGPIX Luminex® platform. Analysis using Luminex xPonent® for MAGPIX® software v. 4.2 enables identification of microspheres with conjugates in the samples. Using 10-fold serial dilutions of RNA copies of each reference DENV serotype, specificity can be evaluated by using RNA obtained from clinical samples confirmed by real-time RT-PCR amplification. These assays have usually been used to detect pathogens or protein markers implicated in several pathological conditions[43,48,49-51], but studies involving nucleic acid detection are still relatively limited. The use of the RT-PCR/Luminex technique might lead to significant improvements in the early diagnosis and treatment of acute DENV infections. However, because of the possibility of having different targets in different stages of infection, the diagnosis of DENV infection must still be performed by combining methods: considering the nature of the NS1 antigen, the presence of viral particles in the blood, and the levels of IgM and IgG antibodies against DENV[14,52]. In a study by Mauro Jorge Cabral-Castro (2016), which aimed to standardize a DNA hybridization assay based on the Luminex technology for detecting and serotyping dengue virus (DENV), the RT-PCR/Luminex assay correctly identified all 16 DENV isolates with a concordance of 86.7% with that of a semi-nested PCR. With this system, other etiologic agents, such as Chikungunya and Zika virus's targets can be added to obtain differential diagnosis and more accurate detection. Using the Luminex xMAP instruments, simultaneous detection and quantitation of up to 80 secreted proteins in a single well through Invitrogen ProcartaPlex assays. The versatility of this multiplex technology has been extended to gene

expression analysis through the Invitrogen QuantiGene Plex assays developed by Thermofisher. It is noted that the Luminex system exhibits comparable accuracy and sensitivity to traditional assays like ELISA and qPCR, but with significantly enhanced efficiency and throughput.

Lyudmyla et al (2019) used a Luminex direct hybridization assay (DHA) to discriminate DENV1–4, CHIKV, and ZIKV[53,54]. They developed a diagnostics panel based on reverse transcription-PCR amplification of viral RNA and an xMap Luminex architecture involving direct hybridization of PCR amplicons and virus-specific probes. The hybridization sensitivity on Luminex microspheres and PCR specificity of the multiplex assay were increased with two DNA innovations[55,56]. The assay was validated with infected Vero cells, mosquito pools, and mosquito saliva. The panel was able to discriminate above arboviruses with a limit as low as 10–20 genome equivalents for CHIKV, DENV1, DENV3, DENV4, or up to 80 genome equivalents for DENV2 and ZIKV. In the improved version of the Luminex instrument, the assay steps can be consecutively executed on Luminex platform, and all reactions performed automatically. Using a 96-plate format cost of a single assay is reduced.

CRISPR-Technology for Diagnostics:

Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR), is a highly variable locus consisting of short and identical repeated palindromic sequences that are naturally found in the genome of bacteria, separated by large spacer sequences[57]. First discovered in the bacterium *Escherichia coli* as a defence system against invasion of viral DNA into the genome, CRISPR/Cas has ushered in a new era of functional genetics and revolutionized the methodology of gene knockout with simplicity and rapidity, but it is also powerful for gene knock-in and gene modification[58]. Upon transcription to pre-crRNA, the CRISPR locus is then processed by CRISPR-associated (Cas) proteins to generate mature CRISPR guide RNA (gRNAs)[10]. These then induce indiscriminate cleavage of target nucleic acid thereby protecting the bacterial cell against any invading bacteriophage[57,59]. This genome editing technology is a technology for precisely targeted modification of endogenous genes in organisms[60]. A specific endonuclease is used to cut DNA strands to achieve the insertion, deletion, and replacement of specific target DNA sequences[61]. Using this, researchers can edit multiple specific sequences efficiently and economically and change or eliminate the molecular functions of target genes.

CRISPR-Cas-based diagnosis relies on the identification of viral nucleic acid sequences by gRNAs followed by non-specific cleavage of viral nucleic acid by Cas[62,63]. This reaction is usually coupled with probe molecules that generate a fluorescent/visual signal which is detected SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking), DETECTR (DNA endonuclease-targeted CRISPR transReporter), CARVER (Cas13-assisted restriction of viral expression and readout), PAC-MAN (Prophylactic Antiviral CRISPR in human cells), SHINE (Streamlined Highlighting of Infections to Navigate Epidemics), All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR) are examples of CRISPR-Cas molecular methods used in disease diagnosis[64,65].

In a study by Prajapati A, et al (2022)[57], DENV-specific CRISPR-Cas13 gRNAs were designed for the conserved and variable/hyper variable genomic regions among four DENV serotypes using bioinformatics tools. Based on SHERLOCK, Zhang's group combined Cas13a and Cas13b with other CRISPR-associated protein nucleases such as Cas12 and Csm6 to realize multiple pathogen detections, including dengue virus, which were measured quantitatively as low as 2aM. Myhrvold et al. also adopted HUDSON to pair with the CRISPR-Cas13a system to detect dengue virus[66] in order to optimize the unnecessary viral nucleic acid extraction step in both SHERLOCK and SHERLOCKv2 and dengue virus could be detected directly in patient samples within 2 hours. In addition to that, a sensitive electrochemical detection method was used to detect dengue virus with the aid of CRISPR-Cas13a-

assisted catalytic hairpin assembly[66]. With a detection limit of 0.78 fM, the proposed biosensor provides a simple and sensitive electrochemical method for dengue virus detection.

In recent years, application of the CRISPR/Cas system has revolutionized the field of POC nucleic acid diagnostics and genetic editing and here CRISPR/Cas13a stands out as the sole RNA-targeting CRISPR effector, with high signal amplification and falling under the Class II type VI-A CRISPR/Cas system[57]. Apart from this CRISPR-Cas12a is recognized for its unique to indiscriminately cleave any adjacent non-target single-stranded DNA (ssDNA) in closed proximity to the target DNA[67] and this platform is easy to fabricate, have ultra-high sensitivity, high specificity to single-base variation, and good portability making them suitable for POC diagnostics[64,68,69]. Cao et al., in their study used the activated Cas13a to cleave the crRNA of Cas12a for detection of dengue virus. They formulated a field-deployable diagnostic strategy for dengue virus based on Cas13a and Cas12a using Paper-based strips, utilizing Lateral Flow Strip Assays which successfully enabled sensitive and field-deployable diagnostics for dengue virus, demonstrating its potential utility in clinical diagnostics. Paper-based strips, utilizing Lateral Flow Strip Assays (LFSA), offer the advantages of ease of observation and cost-effectiveness[70,71], but the complexity of sequence design, sensitivity, and specificity deserve further improvement. Hence a Paper-based strip with high sensitivity, simplicity, and specificity should be developed for the detection of RNA.

Zhang et al (2025) developed a robust novel, rapid, and highly sensitive diagnostic method for universal DENV detection by integrating recombinase polymerase amplification (RPA) assay and CRISPR with its associated (Cas) protein 12a (CRISPR/Cas12a) system into a single step. They tested specificity against 26 virus-infected cell samples including Sendai virus, Herpes simplex virus-1, Parainfluenza A virus, and vesicular stomatitis virus tested in triple repetitions. No cross reactivity was observed, with different infection time and multiplicity of infection (MOI). Sensitivity was shown to be 91.7 copies/test at a 95% probability using recombinant plasmids containing all 4 Dengue serotypes. Their approach was then translated into a universal DENV RT-RPA-CRISPR/Cas12a-lateral flow dipstick (LFD) platform, which successfully identified all four serotypes of DENV with a sensitivity of approximately 250 copies/test[66]. In a another study by Jiaye Zhong et al (2025) who developed single-tube assay combining RAA, bit with CRISPR-Cas13a technology, they obtained a sensitivity of 10^3 copies/mL^{-1} with an average sensitivity of 95.8%. There was no or minimal cross-reactivity against three flaviviruses (Zika, West Nile, and Murray Valley encephalitis) of 96.6% in a One-step method[72]. Bardwaj, P. et al (2025) and Guozhen Tian et al (2024) combined both Cas13a and Cas12a in their assay and their technique, utilizing an activated Cas13a system cleaving the crRNA of Cas12a, triggered a cascade that amplifies the virus signal, achieving a low detection limit of 190 fM with fluorescence. Exhibited good sensitivity and may serve as a field-deployable diagnostic tool for dengue virus[73,74].

The above assays showed good analytical sensitivity and no cross-reactivity with other related aetiologies tested. These assays are comparable to RT-PCR, are user-friendly and appear as an ideal point-of-care test which drives field-deployment in reducing healthcare burden, providing differential diagnosis, and supporting initiating early and prompt treatment to patients in resource-poor countries.

Microfluidics and Lab-on-a-Chip Technologies:

MF researchers predicted that these technologies would bring about "lab-on-chip" devices that would revolutionize clinical diagnostics and translational research. Using technologies developed by the computer chip industry, MF devices can significantly reduce reagent usage, chemical reaction times, and cost on a single "chip," which, in turn, could enable novel POC and point-of-need solutions for clinical diagnostics. An integrated microfluidic system which utilizes virus-bound magnetic bead complexes for

rapid serological analysis of antibodies associated with an infection by the dengue virus was reported by Yu-Fang Lee et al (2009) where they integrated one-way micropumps, a four-membrane-type micromixer, two-way micropumps and an on-chip microcoil array in order to simultaneously perform the rapid detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) to dengue virus using magnetic beads which can capture antibodies in serum samples and these are then detected by fluorescence-labelled secondary antibodies. The entire magnetic complex sandwich is transported automatically into a sample detection chamber which captures the optical signals and analyzed by a real-time optical detection module. The entire process performed automatically on a single chip takes 30. Their assay has a detection limit of 21 pg. This has opened avenues for multiplexing enabling detection of not only dengue but other mosquito borne pathogens. Due to cost barrier to designing, prototyping, and manufacturing these devices at scale for widespread use this field has not yet fulfilled its potential in the research or clinical areas[75].

Genomics and Bioinformatics:

Genomics surveillance on Dengue viruses enables detection of naturally occurring mutations leading to genotypic variations or shifts in serotypes, which also do affect diagnostics. This is critical for monitoring the effectiveness of newly implemented control strategies[76,77] as well as the effectiveness of diagnostic assays. Apart from that, these analyses elucidate several important features of DENV such as rates and constraints of evolution, viral population sizes, selection pressures, and putative recombination [78]. To get better insights into the epidemic pattern of the disease, consistent monitoring in a given population is crucial, aiding in the prevention of this impending threat[79,80].

Voges et al developed and validated their technique (DengueSeq) to sequence the known diversity of dengue virus from clinical samples together with existing amplicon-based sequencing workflows to enhance the global capacity for whole-genome dengue virus sequencing. Using a broad panel of samples to represent the breadth of genetic diversity, different sample types, and a range of virus copies, they were able to estimate that the limit of detection to achieve at least 70% genome coverage ranged between 10^1-10^2 RNA copies/µL for all four serotypes. DengueSeq was as sensitive as other commonly used primer schemes for whole-genome sequencing of viruses of public health importance. This system also has an added benefit of reliably detecting multiple serotypes in the same sample. They recommend selection of samples with higher RNA copies to increase the chances of generating near-complete genomes and to use reference genomes for reference-based alignment of sequencing reads as this had an impact on genome coverage of highly divergent genomes[76].

In the search for biomarkers many researchers have integrated bioinformatics tools with genomic data to facilitate the identification of predictive biomarkers for dengue severity, aiding in patient management and treatment strategies. Dengue biomarker analysis faces significant challenges. Biomarkers such as NS1 antigen and IgM/IgG antibodies, because of the lack of specificity and cross-reactivity, overlap with other flaviviruses like Zika and chikungunya, leading to diagnostic inaccuracies[81]. These markers also vary across disease stages and are influenced by host factors such as age, immune status, and genetic background, making consistent detection difficult[82]. Omics technologies have improved our understanding of disease mechanisms and therapeutic targets[83] and relevant genes and their associations with diseases can be identified[84]. This computational method allows for early detection and proactive management of health risks by understanding the complex interaction between genes and disease pathways[85].

Li-Min Xie et al (2021) employed bioinformatics analysis to identify potential biomarkers related to dengue fever and identified twelve hub genes, including MX1, IFI44L, IFI44, IFI27, ISG15, STAT1, IFI35, OAS3, OAS2, OAS1, IFI6, and USP18. Using gene ontology (GO) and the Kyoto Encyclopedia of

Genes and Genomes (KEGG), pathway enrichment were carried out, and a protein–protein interaction (PPI) network was constructed to gain insight into the actions. With thus they predicted IFI44L and IFI6, as potential biomarkers with DENV infection, providing promising targets for the treatment of dengue fever to a certain extent[86]. Paul et al (2025) noted that differences in gene expression patterns were seen across datasets, possibly due to factors such as patient demographics, viral strain diversity, or dataset-specific biases. Integrating bioinformatics approaches they identified 20 potential biomarker genes. Further analysis indicated several genes were consistently dysregulated across multiple datasets, notably, AURKA, BUB1, BUB1B, BUB3, CCNA2, CCNB2, CDC6, CDK1, CENPE, EXO1, NEK2, ZWINT, and STAT1. These genes are implicated in various cellular processes, including cell cycle regulation, mitotic checkpoint control, and chromosomal segregation and functionally were associated with immune response to the virus, cell division, RNA processing, and immune responses[87,88]. However, experimental validation is lacking but the findings indicate the importance of these biomarkers in disease progression and highlight their potential in developing targeted diagnostic as well as therapeutic strategies for dengue fever.

NGS Platforms

Next-generation sequencing (NGS), in recent years has been widely used in infectious disease surveillance[89]. Two different strategies are utilized for molecular diagnosis of infectious diseases: metagenomic sequencing and targeted amplicon sequencing. With metaqgenomic sequencing the whole genome is sequenced usually for unknown targets and is expensive[90,91]. Target sequencing is used when pathogen is known and is more accurate[92]. This technique can now be directly applied to clinical samples is faster, less laborious, less time-consuming, and more flexible[92]. The main challenge here is the variation in quality of RNA and viral load[93] which can be addressed by adjusting amplicon sizes and increasing number of primers or using nested PCR[94,95].

Su et al (2022)[89] validated primer panels in a rage of DEN-positive clinical samples from as many different serotypes and genotypes as possible. Using 30 cycles for those samples with a C_T (Cycle Threshold) value below 30 and 35 cycles for those samples with a C_T value above 30, they validated 31 clinical samples and noted that all of samples had 100% coverage of the DENV genome ORF with mapping percentage of the downstream data ranging from 86.10% to 99.58%. The main limitation was potential for cross-contamination. Hence their study provided a universal, rapid, and effective tool for the integration of genomics with dengue surveillance, can be cost-effective, scalable system and adds little time and complexity as sample numbers increase. However, the panels need further validation with more clinical samples and using many other genotypes.

Recently, more and more studies have applied deep sequencing technique to obtain consensus sequences in the individual level. However, rich information embedded in output data has not been fully explored. Data deposited in Sequence Read Archive (SRA) under NCBI (National Centre for Biotechnology Information), for example, could therefore become a useful resource for researchers to address clinical or epidemiological questions. Applying deep learning technology on big data analysis will be crucial to elucidate underlying mechanisms by integrating intra-host sequencing data with a systemic collection of epidemiological data acquired.

Artificial Intelligence and Machine Learning:

AI can be applied to analyse vast amounts of epidemiological, clinical, and genomic data, assisting in predictive modelling for dengue outbreaks and improving diagnostic algorithms.

Artificial Intelligence (AI) technologies have emerged as promising tools in the medical field, revolutionizing the way diseases are detected, diagnosed, and treated. AI's ability to analyse complex datasets, including medical imaging, patient

records, and environmental data, makes it a valuable tool for detecting dengue outbreaks and supporting clinical diagnosis.

AI Techniques Used in Dengue Detection include Random Forest (RF) model, Support Vector Machines (SVM), Decision Trees (DT), Image-based Diagnosis, Neural Networks for Predictive Modelling (RRN, LSTM) or a combination of all named here. RF analyses historical case data, weather patterns and demographic data and has the ability to handle large data sets. SVM is used to analyze patient symptoms, laboratory results, and serological tests to differentiate between dengue and other febrile illnesses (Sajana et al., 2018). Decision trees enable interpretability for clinical diagnosis and outbreak prediction. Recurrent Neural Networks (RNNs) and Long Short-Term Memory (LSTM) networks have been employed to predict dengue outbreaks by modelling temporal data such as historical weather conditions and dengue cases. Several challenges exist, and these include data quality and availability, algorithm bias, infrastructure limitations, and regulatory and ethical issues. However, AI has the potential to enable better management of dengue outbreaks and reduce the disease burden[96].

Bohm BC et al (2024)[97] developed an intelligent system to detect dengue cases early[98]. Their aim was to use a combination of characteristic disease to guide clinical investigations[99]. They acknowledge certain limitations inherent to this research and that is the models were trained exclusively using data from two specific municipalities, rather than encompassing a broader national dataset. Hence plausible that the model's accuracy could be affected when extended to encompass data from other locations. Another notable limitation is linked to the reliance on secondary data sources. This reliance introduces a potential loss of information due to various factors, including incomplete forms, potential oversight of clinical signs within records, and the inability to incorporate additional variables. For instance, the consideration of a patient's history of virus exposure remains unaccounted for in their study. Their study showed that the decision tree model obtained the best compromise between predictive performance and computational complexity and recommends its implementation in mobile applications or on computers with basic configurations to prevent disease progression. More studies are however needed to refine these findings, to include national datasets from multiple locations representative of the country.

In a systematic review of 32 scientific papers by Giron et al (2025)[100], they identified 48 distinct machine learning and deep learning in terms of their predictive ability. The SVM emerged as the most prevalent algorithm (28.13%) followed by Random Forest (18.75%). PCA-SVM algorithm (poly-5), a variant of the PCA-SVM algorithm, had the most exceptional performance, attaining 99.52% accuracy, 99.75% sensitivity, and 99.09% specificity. However, larger data sets are required before any of these systems become robust in addressing the challenge of early diagnosis in patients with suspected dengue. The timely diagnosis of patients infected with dengue has significant potential to optimize treatment and significantly reduce mortality in the healthcare setting[101].

Conclusion

The diagnostic landscape for dengue virus infections is undergoing a significant transformation driven by rapid advancements in molecular diagnostic techniques, which are poised to significantly transform the landscape of dengue detection and surveillance in the near future. Traditionally, serology and viral culture, though still valuable, lack the sensitivity, specificity, or speed required for effective clinical and public health response. Emerging technologies such as real-time reverse transcription polymerase chain reaction have set new standards for early and accurate detection, enabling diagnosis during the acute phase of infection when viral loads are highest. Others include CRISPR-based diagnostics, isothermal amplification methods like LAMP, Microfluidics, next-generation sequencing, and point-of-care nucleic acid amplification tests offer

the potential for earlier, more accurate, and more accessible detection of dengue virus infections. These innovations enhance diagnostic sensitivity, and specificity, are faster, more cost-effective, and field-deployable and support rapid outbreak response and informed public health strategies. NGS has further been developed for dengue surveillance, allowing not only the identification of viral strains and serotypes but also the tracking of mutations, outbreak sources, and transmission patterns in real-time. Integrating molecular diagnostics into healthcare systems, especially in endemic and resource-limited regions, may enable closing existing diagnostic gaps. The tools, however, need to become affordable and user-friendly enabling easier interpretations required in any diagnostic setting. Overall, these advancements in molecular techniques hold great promise for improving the accuracy, speed, and accessibility of dengue diagnostics, ultimately leading to better disease management and control efforts. In conclusion, the ongoing evolution of molecular diagnostic technologies is set to play a critical role in the near future of dengue diagnostics. These advancements will empower healthcare providers with faster, more reliable tools, improve outbreak preparedness, and ultimately contribute to reducing the global burden of dengue.

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